Rosiglitazone prevents the impairment of human islet function induced by fatty acids: evidence for a role of PPARγ2 in the modulation of insulin secretion

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Incubation of isolated rodent (3, 17, 50, 51, 53) or human islets (27, 52) in the presence of high FFA concentration has been consistently shown to impair glucose-mediated insulin release and cause apoptosis. The deleterious effect of FFA on insulin release has been confirmed in perfused-pancreas studies as well (36). In vivo studies have led to more controversial results. Paolillo et al. (31) reported increased insulin secretion after short elevation of plasma FFA but an inhibitory effect after more prolonged Intralipid infusion. Similarly, Carpentier et al. (11) observed impaired glucose-stimulated insulin secretion in the presence of 1 mM plasma FFA. On the other hand, other authors were not able to confirm a deleterious effect of high plasma FFA on insulin secretion (5, 10, 19). More recently, Jensen et al. (22) have reported that short-term (24-h), low-grade Intralipid infusion was associated with reduced peripheral insulin action but increased insulin secretion, although the possibility that over a longer period β-cell exhaustion could ensue was not ruled out. Not only does controversy exist about the impact of increased plasma FFA concentration on insulin secretion in vivo, but also uncertainties remain concerning the intimate mechanism(s) responsible for the FFA effects on the human islet. Moreover, different fatty acids (i.e., saturated vs. unsaturated) are known to exert divergent effects on the β-cell (6, 20, 28).

Expression of peroxisome proliferator-activated receptors (PPARs) has been demonstrated in rat pancreatic islets (8). Interestingly, in the islets of normal rats, PPARα is expressed to a higher extent than PPARγ (54), whereas this proportion is inverted in islets from obese Zucker diabetic fatty (ZDF) rats. The balance between PPARα and PPARγ activities plays a major role in determining cell fat storage (2). More recently, PPARγ expression has been proven in normal human pancreatic islet cells as well (15). Because PPARγ is thought to stimulate fatty acid uptake and their intracellular binding and esterification (24, 37), a role of these nuclear receptors in mediating the FFA effects on insulin secretion was postulated. Therefore, an experimental design was developed (1) to evaluate the expression of PPAR isoforms in isolated human islets compared with adipose tissue and pancreatic acinar cells, 2) to assess the effect of 24-h culture in the presence of high glucose and/or FFA concentrations on PPAR expression and insulin release, and 3) to determine the effects of concomitant incubation with the PPARγ agonists rosiglitazone and 15-deoxy-

Since the appreciation that chronic exposure to glucose impairs glucose-stimulated insulin secretion (43), lipotoxicity has been described to account for the alteration of β-cell function associated with elevation of plasma free fatty acids (FFA) (23).

expression of peroxisome proliferator-activated receptors (PPARs) is a subgroup of the superfamily of nuclear receptors, with three distinct main types: α, β, and γ (subdivided into γ1 and γ2). Recently, the presence of PPARγ has been reported in human islets. Whether other PPAR types can be found in human islets, how islet PPAR mRNA expression is regulated by the metabolic milieu, their role in insulin secretion, and the effects of a PPARγ agonist are not known. In this study, human pancreatic islets were prepared by collagenase digestion and density gradient purification from nonobese adult donors. The presence of PPAR mRNAs was assessed by RT-PCR, and the effect was evaluated of exposure for up to 24 h to either 22 mM glucose and/or 0.25, 0.5, or 1.0 mM long-chain fatty acid mixture (oleate to palmitate, 2:1). PPARβ and, to a greater extent, total PPARγ and PPARγ2 mRNAs were expressed in human islets, whereas PPARα mRNA was not detected. Compared with human adipose tissue, PPARγ mRNA was expressed at lower levels in the islets, and PPARβ at similar levels. The expression of PPARγ2 mRNA was not affected by exposure to 22 mM glucose, whereas it decreased markedly and time dependently after exposure to progressively higher free fatty acids (FFA). This latter effect was not affected by the concomitant presence of high glucose. Exposure to FFA caused inhibition of insulin mRNA expression, glucose-stimulated insulin release, and reduction of islet insulin content. The PPARγ agonists rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J2 prevented the cytostatic effect of FFA as well as the FFA-induced changes of PPAR and insulin mRNA expression. In conclusion, this study shows that PPARγ mRNA is expressed in human pancreatic islets, with predominance of PPARγ2; exposure to FFA downregulates PPARγ2 and insulin mRNA expression and inhibits glucose-stimulated insulin secretion; exposure to PPARγ agonists can prevent these effects.

pancreatic islets; peroxisome proliferator-activated receptors; polymerase chain reaction; messenger ribonucleic acid; lipotoxicity; gluco- cocotoxicity; prostaglandin; free fatty acids

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Δ12,14-prostaglandin J2 (PGJ2) on PPAR expression and insulin secretion.

MATERIALS AND METHODS

Human islet preparation. Pancreatic islets were prepared by collagenase digestion and density gradient purification, as previously reported in detail (29, 35). For the purpose of this study, 13 human pancreata (from donors aged 25–68 yr) were used with the approval of the local ethics committee. After isolation, islets were cultured in M-199 culture medium as previously described (29, 35) for 3–5 days before experiments were carried out. Within this period, aliquots of ~500 hand-picked islets were cultured free floating for 24 h in M-199 culture medium under the following conditions: 1) control M-199, 2) M-199 containing 22 mmol/l glucose and/or 0.25, 0.5, or 1.0 mmol/l long-chain fatty acid mixture (oleate to palmitate 2:1; Sigma Chemicals, St. Louis, MO), 3) M-199 containing 1.0 mmol/l long-chain fatty acid mixture plus 15 μg/ml rosiglitazone, or 10 μM PPARγ2, and 4) M-199 containing 1.5 μg/ml rosiglitazone or 10 μM PPARγ2. The FFA mixtures were prepared as described in detail previously (4, 26, 35). The final pH of all media was buffered at pH 7.4.

Intracellular triglyceride content. Triglyceride content in human islets was determined in three different experiments before and after 24-h incubation with 1.0 mM FFA mixture, rosiglitazone (1.5 μg/ml), and FFA plus rosiglitazone as well as in control islets. Aliquots of 500 cultured islets were selected under microscope from each pool of human islets. Very large and very small islets were excluded in an effort to minimize intergroup size differences. Islets were washed twice with Hanks’ balanced salt solution and suspended in 50 μl of 2 mol/l NaCl, 2 mmol/l EDTA, and 50 mmol/l sodium phosphate buffer (pH 7.4). After 1–2 min of sonication, 10 μl of the homogenate were mixed with 10 μl of tert-butyl alcohol and 5 μl of Triton X-100-methyl alcohol mixture (1:1 by volume) for lipid extraction. Measurements were made in duplicate using the Sigma Triglyceride (GPO-Trinder) kit.

Glucose-stimulated insulin release. After a 24-h incubation, islet glucose-stimulated insulin secretion was determined as previously described. Briefly, following a 45-min preincubation period at 33 mmol/l glucose, batches of ~30 islets of comparable size were kept at 37°C for 45 min in Krebs-Ringer bicarbonate solution (KRB) and 0.5% albumin, pH 7.4, containing 3.3 mmol/l glucose. At the end of this period, the medium was completely removed and replaced with KRB containing 16.7 mmol/l glucose. After an additional 45-min incubation, the medium was removed. Samples (500 μl) from the different media were stored at ~2°C until insulin concentrations were measured by immunoradiometric assay (Pantec Forniture Bio- mediche, Turin, Italy). Total insulin content was measured after acid-alcohol extraction, as previously reported (29, 35).

Islet mRNA expressions. mRNA expression of the different PPARs and insulin was measured by reverse transcription reaction followed by polymerase chain reaction (RT-competitive PCR) or real-time quantitative RT-PCR. Total RNA was extracted from human pancreatic islets, adipose tissue, or acinar cells by use of the SV Total RNA Isolation System (Promega). Total RNA was quantified by absorbance at A260/A280 nm (ratio >1.65) in a Perkin-Elmer spectrophotometer, and its integrity was assessed after electrophoresis in 1.0% agarose gels by ethidium bromide staining.

For RT-PCR, each mRNA first-strand cDNA synthesis was performed from 1 μg of total RNA in the presence of dNTP mix (final concentration 200 μM) and 200 μM of a thermostable reverse transcriptase (Superscript II, Gibco-BRL). After the reaction, the RT fluid was added to a PCR mix (5 μl buffer 10x, 0.2 μM of Deoxynucleotide Mix, 500 pmol of specific sense and antisense primers, and 2 U of Taq polymerase) in a final volume of 25 μl. The tubings were subjected to 35 cycles of amplification, including denaturation for 60 s at 95°C, hybridization for 60 s at 52–60°C for the varying PPARs, and elongation for 90 s at 72°C.

For real-time quantitative RT-PCR, the genes of interest were obtained from GenBank (National Center of Biotechnology Information), the cDNA synthesis was performed from 2 μg of total RNA, and the primer and probe sequences were designed using Primers Express Software (PE Applied Biosystems). To avoid amplification of genomic DNA, the primer-probe sets were designed to span exon-exon borders. The probes were labeled with FAM at the 5’ end and TAMRA at the 3’ end. PCR amplifications were performed in a total volume of 25 μl containing 2 μl of cDNA sample, 200 nmol/l of each primer, 100 nmol/l of corresponding probe, and 12.5 μl of TaqMan Universal PCR Master Mix. For each reaction, the polymerase was activated by preincubination at 95°C for 10 min. Amplification was then performed by 40 cycles of switching between 95°C for 15 s and 60°C for 60 s. The PPARγ2 and human insulin cDNA quantity of each cDNA sample was normalized to the housekeeping gene for β-actin.

According toRefs. 1, 16, 42, and 47, the primers (sense and antisense) for RT-PCR described below were used to generate human PPARα, -β, total γ, -γ2, human insulin, and -β-actin cDNAs. For PPARα: 5’-CCA GGA TTT AGG AGC CTG TCC-3’ and 5’-AAG TTC TAC AAG TAG GCC AGC-3’ (492 bp); for PPARβ: 5’-AAC TGC AGA TGG GCT GTG AC-3’ and 5’-GGC TAT AGG TCG TAT CAC ATC-3’ (484 bp); for total PPARγ: 5’-TGT CTC AGT AAT GGA AGA CGA CC-3’ and 5’-GGCA TTA GAG TAC CCC ACC AC-3’ (474 bp); for PPARγ2: 5’-GGC ATT CCT TCA ATG ACA-3’ and 5’-GCAA TTA GAG TAC ACC CCC AC-3’ (380 bp); for human insulin: 5’-GGG TTT GTG AAC CAA CAC CTG-3’ and 5’-GTT GCA GTA GTT CTC CAG CTC-3’ (261 bp); for β-actin: 5’-ACC AAC TGG GAG AGT GAG-3’ and 5’-CGT GAG GAT CCT CAT GAG GTA AGT C-3’ (354 bp). For real-time RT-PCR, the primers (sense and antisense) and probes described below were used to generate human PPARγ2 and human cDNAs. For PPARγ2: 5’-GAA CCA CGG CAC CAT GAT G-3’ (sense), 5’-TGA AGC ACT GGA AGT CGA ACA ACA C-3’ (antisense) and 5’-CTG TGG CCA CAC ACT GAT CCA GCC G-3’ (probes) (100 bp); for human insulin: 5’-TGCC TTT GTG AAC CAA CAC CTG-3’ and 5’-GTT GCA GTA GTT CTC CAG CTC-3’ (261 bp); for β-actin: 5’-ACC AAC TGG GAG AGT GAG-3’ and 5’-CGT GAG GAT CCT CAT GAG GTA AGT C-3’ (354 bp). The probe and primer sequences for human β-actin were obtained from PE Applied Biosystems (Pre-Developed TaqMan Assay Reagents Control Kit). The final PCR products were analyzed on a 1–1.5% nondenaturing agarose gel.

To determine whether islet incubation in the presence of PPARγ agonists can induce fibroblast differentiation into adipocytes, expression of fatty acid-binding protein (FABP4) mRNA was determined in human islets as well as in human adipocytes by use of the primers 5’-TCC AGT GAA AAC TTG GAT TAT-3’ (sense) and 5’-ACG CAT TCC ACC ACC AGT TTA TCA-3’ (antisense).

Statistical analysis. Data are presented as means ± SD. Statistical analysis was performed by the ANOVA test, followed by Bonferroni correction as appropriate.

RESULTS

PPAR mRNAs are expressed in isolated human islets. As shown in Fig. 1, mRNA expression for PPARβ, total PPARγ, and PPARγ2, but not PPARα, was detected during 10 experiments performed in isolated human islets. Compared with human adipose tissue, total PPARγ and PPARγ2 mRNAs were expressed at lower levels in the islets (Fig. 1). Only PPARβ mRNA was found in pancreatic acinar tissue (not shown). Because PPARγ2 mRNA accounted for most of total PPARγ mRNA in islets, we decided to determine whether high glucose or fatty acid concentration in the culture medium could simultaneously alter the expression of this specific isoform and glucose-mediated insulin release.
FFA, but not high glucose concentration, modulate mRNA expression of PPARγ2. Human islets from five pancreases were used to assess the effect of high FFA or glucose concentration on the mRNA expression of PPARγ2. Twenty-four-hour incubation of human islets in the presence of high glucose concentration did not cause any major change in the expression of PPARγ2 mRNA (Fig. 2). On the other hand, inhibition of PPARγ2 mRNA expression occurred after exposure to FFA (Fig. 2). Addition of glucose to FFA did not prevent the decline in PPARγ2 mRNA expression noted with FFA alone (not shown).

The effects of 0.5 and 1.0 mmol/l FFA on PPARγ2 mRNA expression were evaluated as a function of time as well, and results are shown in Fig. 2. With 0.5 mmol/l FFA, inhibition of PPARγ2 mRNA expression was complete after the eighth hour. When the FFA concentration was doubled, a quicker inhibition occurred (Fig. 2).

PPARγ2 agonists, rosiglitazone and PGJ2, prevent lipotoxicity in isolated human islets. To explore the role of PPARs in the impairment of human islet function, human islets from four different pancreases were used to perform experiments with rosiglitazone and PGJ2. As illustrated in Figs. 3 and 4, incubation of human islets in the presence of the two agonists was associated with increased PPARγ2 mRNA expression. Moreover, both rosiglitazone and PGJ2 were able to completely prevent the suppression of PPARγ2 mRNA expression induced by 1.0 mM FFA. The changes in PPARγ2 mRNA expression under the different experimental conditions were paralleled by similar variations in the insulin mRNA expression: reduced by FFA and recovered by concomitant human islet incubation with 1.0 mM FFA and rosiglitazone or PGJ2 (Figs. 3 and 4).

In control islets, acute elevation of glucose concentration in the medium was associated with an increase in insulin release from 2.2 ± 1.5 to 4.7 ± 1.5% of insulin content. In contrast, elevation of fatty acids in the medium resulted in a lower basal insulin release (data not shown). Table 1 summarizes the results of insulin release as well as total insulin content in the different experimental condition explored. The addition to the medium of the two PPARγ2 agonists rosiglitazone and PGJ2 did not alter glucose-mediated insulin release nor total insulin content (Table 1 and Fig. 5). As already observed, incubation of human islets in the presence of 1.0 mM fatty acid was associated with a marked impairment of insulin release (0.6 ± 0.05 and 0.9 ± 0.6% of islet content for rosiglitazone or PGJ2 experiments, respectively; P < 0.01 vs. control) and significant impoverishment of islet insulin content (Table 1).

When the two PPARγ2 agonists were coincubated with 1.0 mM fatty acids, insulin release at low glucose concentration was no more different compared with control islets (data not shown). In addition, insulin release in response to high glucose and insulin content increased significantly with no difference compared with control islets (Fig. 5 and Table 1).

Rigoslitazone reduces triglyceride content in human islets. Triglyceride content was determined in human islets exposed for 24 h to 1.0 mM fatty acids, rosiglitazone (1.5 μg/m) and the combination of the two. Compared with control islets (1.1 ± 0.4 μg/ml), fatty acids cause an increase in triglyceride content (2.1 ± 0.8 μg/ml, P < 0.05), whereas a decrease (0.3 ± 0.1 μg/ml, P < 0.05) followed incubation in the presence of rosiglitazone. When coincubated, rosiglitazone completely
prevented triglyceride accumulation in the isolated islets (0.8 ± 0.4 μg/ml).

To assess whether changes in lipid metabolism could be due to differentiation of fibroblasts present within the islet into adipocytes FABP4 mRNA expression was determined under the aforementioned experimental conditions (Fig. 6). The level of expression normalized by β-actin mRNA was not modified by fatty acids (0.059 ± 0.004), rosiglitazone (0.061 ± 0.002), or the combination of the two (0.024 ± 0.006) compared with control islets (0.077 ± 0.001). In all conditions, moreover, the level of expression was at least 10-fold lower compared with adipose tissue (0.743 ± 0.085).

DISCUSSION

The present study strongly supports a role for PPARγ2 in the modulation of insulin secretion from isolated human islets. In particular, our results suggest that PPARγ2 may prevent the lipotoxic effect on the β-cell exerted by increasing the fatty acid concentration in culture medium.

Our finding confirms that the mRNA for PPARγ is expressed in human pancreatic islets (21) and provides evidence for expression of PPARβ mRNA as well. In contrast, by using specific primers previously employed with other human tissues (1, 16, 42, 47), we could not detect PPARα mRNA expression, suggesting a tissue-specific distribution of the PPAR subtypes. No specific assay was performed in the present study to determine the contribution of endocrine cells compared with the nonendocrine component. Nonetheless, contamination by adipose cells is unlikely, given the different pattern of expression of the PPAR subtypes in the islets compared with adipose tissue (Fig. 1). The same was true by comparison with pancreatic acinar cells (data not shown). Therefore, as indicated by Dubois et al. (15), PPARγ appears to be preferentially localized in the endocrine cells of pancreatic islets.

Interestingly, we observed that FFA, but not glucose, induced a dose- and time-dependent decrease of PPARγ2 mRNA expression. PPARs are fine modulators of intracellular lipid
metabolism. PPARγ (and in particular the γ2-isofom) plays a major role in stimulating the uptake of fatty acids, their intracellular binding, and their esterification, whereas PPARα promotes β-oxidation (23, 24, 37). Therefore, the ratio between these two PPAR subtypes is likely to affect the metabolic status of the cell, in particular lipid accretion vs. lipid depletion (14, 23, 24, 37). In turn, this is likely to affect insulin-secretory capacity. Triglycerides are of importance for the stimulus-secretion coupling in the β-cells, and a lipid-derived signal, possibly provided by hormone-sensitive lipase, may participate in the modulation of insulin secretion (30, 32). On the other hand, chronic elevation of the fatty acids leads to islet triglyceride accumulation and increased production of fatty acyl-CoA derivatives, such as ceramide, causing adverse effects on insulin secretion and β-cell survival (lipotoxicity) (17, 23, 27, 36, 40, 50, 51, 52).

Our finding that human islets express PPARγ2 mRNA to a greater extent than PPARα mRNA could be interpreted as a condition favoring lipid accumulation and, hence, lipid-induced damage. From this point of view, the inhibition of PPARγ2 mRNA expression in response to FFA but not glucose elevation represents an intriguing finding, as it may reflect a defensive adaptation to lipid accretion inside the cell. A down-regulation of PPARγ2 mRNA after exposure to its agonists has also been observed in brown adipocytes (46). These findings suggest the presence of feedback between the mRNA expression of PPARγ2 and at least some PPAR ligands.

With such a scenario in mind, we explored the possible link between PPARγ2 mRNA expression and glucose-mediated insulin release. To this purpose both a natural (PGJ2) and a synthetic (rosiglitazone) PPARγ ligand were employed. Twenty-four-hour incubation with the two agonists did not affect insulin release, total insulin content, and insulin mRNA expression. This finding is in agreement with previous reports obtained both in human islets (15) and in lean wild-type (+/+ ) ZDF rats (39), although more recent studies have reported a dose-dependent effect of rosiglitazone on insulin release from rat pancreatic islets (48). On the other hand, a marked reduction in both basal and glucose-stimulated insulin release was observed after a 24-h incubation of human islets in the presence of 1 mM fatty acids. In agreement with previous findings (7), elevation of fatty acids not only affected glucose-mediated insulin release but also led to a significant reduction in total insulin content. The inhibition of insulin release was well expected on the basis of the lipotoxicity concept (17, 23, 27, 36, 40, 50, 51, 52).

Although the increased availability of fatty acids may be associated with specific metabolic alteration at the β-cell level that might account for defective insulin secretion, other mechanisms could be invoked. This was also suggested by the suppression of PPARγ2 mRNA expression in the presence of fatty acid-enriched medium, whereas concomitant incubation with rosiglitazone and PGJ2 was associated with prevention of the suppression of PPARγ2 mRNA expression, insulin release, and islet insulin content. These results are very much in line with those obtained in islets from obese homozygous (fa/fa) ZDF rats, where troglitazone restored β-cell insulin secretion pattern by lowering islet fat content (38).

A possible limitation of our study could be the difficulty in separating a toxic vs. a functional effect of increased FFA availability due to the low albumin-to-FFA ratio we have employed. In a separate set of experiments, islets (n = 3) were incubated in the presence of medium enriched with more physiological albumin-to-FFA mixture (6% human albumin:1 mM FFA). For the sake of discussion, data are presented in Table 2. At variance with the original set of data, no suppression of insulin release was found at 3.3 mM glucose. Nonetheless, glucose-stimulated insulin release was suppressed by 53% in the presence of high FFA concentration, similarly to what observed in the original set of studies. It is of note that, even assuming a toxic effect, the effects of PPARγ agonists are retained, probably due to prevention of triglyceride accumulation.

In summary, although PPAR agonists may not have a direct effect in modulating insulin secretion, they may preserve the β-cell under condition of an excess of fatty acid availability, and therefore preserve the β-cell from lipotoxicity. This conclusion is supported by recent studies by Finegood et al. (18), showing that rosiglitazone treatment hampers the loss of β-cell mass in obese ZDF rats by maintaining β-cell proliferation and preventing net β-cell death.

### Table 1. Effects of 24-h incubation of human islets with FFA (1.0 mM rosiglitazone (1.5 μg/ml), PGJ2 (10 μM), rosiglitazone plus FFA, or PGJ2 plus FFA on glucose-stimulated insulin release and insulin content

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<thead>
<tr>
<th>Agonist</th>
<th>FFA</th>
<th>FFA + Agonist</th>
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<tr>
<td>Rosiglitazone</td>
<td>4.7±1.5</td>
<td>4.4±1.2</td>
</tr>
<tr>
<td>PGJ2</td>
<td>3.8±1.7</td>
<td>2.9±1.3</td>
</tr>
<tr>
<td>Control</td>
<td>187±24</td>
<td>170±23</td>
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Values are means ± SE. FFA, free fatty acids; PGJ2, 15-deoxy-Δ12,14prostaglandin J2. *P < 0.05 vs. control, agonist alone, and FFA + agonist.

![Fig. 5. Glucose-mediated insulin release (%total insulin content) from human islets after 24-h incubation with rosiglitazone (1.5 μg/ml), PGJ2 (10 μM), FFA (1.0 mM), and FFA + rosiglitazone or PGJ2. Data are presented as percent change from control islets. *P < 0.05 vs. all conditions. Results represent the means of 4 experimental series from 4 different pancreases.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00332.2004)
The mechanism(s) triggered by PPARγ2 were not investigated in this study. In fat-laden pancreatic islets from obese ZDF rats, troglitazone has been shown to exert a lipopenic action that was accompanied by a >30-fold increase in glucose-mediated insulin release and a doubling of the response to arginine (39). Such a lipopenic effect is confirmed in the present study. In fact, rosiglitazone alone did reduce the triglyceride content in isolated human islets. Moreover, rosiglitazone completely prevented the triglyceride accretion associated with islet incubation in the presence of high fatty acids. In vivo, this effect is likely to be amplified because of the ability of glitazones to prevent the spillover of lipids from adipose tissues into nonadipose tissues. The changes occurring in islet triglyceride content cannot be taken as direct evidence for a β-cell triglyceride accumulation. One could argue that fibroblasts within the islet may differentiate into adipocytes in response to PPARγ activation leading to accumulation of triglyceride and diversion of fatty acids from β-cell metabolism. According to this view, a lipogenic rather than a lipopenic effect would be responsible for the rescue of β-cell function in the presence of fatty acid abundance. Our results, however, dismiss this hypothesis on the basis of the absence of any transcription of FABP4 mRNA (Fig. 6), a marker for adipocyte differentiation.

The modulation of insulin secretion by FFA excess is unlikely to be mediated exclusively by suppression of PPARγ, since other compounds, not necessarily affecting these nuclear receptors, also have been shown to protect human islet function from FFA insult. In a recent study (25), addition of 2.4 μg/ml metformin to a high-FFA medium also resulted in a preservation of insulin release. Although metformin does not interact with PPARs, a common pathway of lipid metabolism may be activated by both metformin and rosiglitazone. The work by Unger and colleagues (43–45) has clearly shown a lipopenic effect of troglitazone in the β-cell. In our hands (25), metformin prevented the accumulation of triglycerides. More recently, Cnop et al. (13) have suggested that FFA can cause the death of normal rat islet cells through a nitric oxide-independent mechanism, suggesting that accumulation of triglyceride may serve as a cytoprotective mechanism against FFA-induced apoptosis by preventing a cellular rise of toxic FFA-derived moieties. Therefore, we cannot exclude the possibility that the final mechanism involved in prevention of islet function (i.e., insulin release) may be mediated through the activation of metabolic pathways that reduce the intracellular formation of toxic compounds.

Our results may lead to a paradox because PPARγ activation appears to exert a lipogenic effect in adipocytes but a lipopenic action in human islets. A possibility to reconcile this paradox may rely on different molecular mediators. For instance, white adipose tissue does not express PPARγ coactivator-1α (PGC-1α) (33), whereas the β-cell and brown adipose tissue do so. Forced expression of PGC-1α in white adipose tissue is associated with mitochondrial biogenesis (34). Recent data suggest that this coactivator is responsible for the activation of thermogenesis and oxidative metabolism in both brown fat and muscle (41). The PGC-1-dependent program includes both mitochondrial biogenesis and tissue-specific expression of uncoupling proteins. Recent work from Spiegelman et al. (49) has shown that the transcriptional coactivator PGC-1α mRNA and protein levels are significantly increased in islets from multiple animal models of diabetes and that adenovirus-mediated expression of PGC-1α also produces inhibition of glucose-stimulated insulin secretion. Thus one may postulate that, by suppressing PPARγ, fatty acid reduces β-oxidation in the β-cell, whereas treatment with the agonists restores the protection via PGC-1α.

In summary, our unique data, obtained with studies performed in isolated human islets, indicate that PPAR agonists such as rosiglitazone, initially proposed as insulin sensitizers, may exert a more comprehensive action, including a direct effect at the level of the β-cell. This effect is of particular interest because it seems to be operative mainly under conditions of islet fat accretion or increased fatty acid availability. A disturbance of fatty acid metabolism characterizes type 2 diabetes (21) and exerts a lipotoxic effect both in peripheral tissues and on the pancreatic β-cell. It is this simultaneous effect on both sites that may account for improvement of insulin sensitivity and insulin secretion that has been documented with glitazone treatment in impaired

Table 2. Insulin release (μU/ml) in 3 different experiments performed in the absence or presence of 6% human albumin: 1 mM fatty acid mixture

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<tr>
<th>Glucose</th>
<th>3.3 mM</th>
<th>16.7 mM</th>
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<tr>
<td>Control islets</td>
<td>9.9±1.0</td>
<td>24.8±5.9</td>
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<tr>
<td>1 mM FFA (6% human albumin)</td>
<td>12.5±4.9</td>
<td>11.7±2.4*</td>
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Values are means ± SE. *P < 0.05 vs. control islets.

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Fig. 6. Expression of fatty acid-binding protein (FABP4) mRNA in control human islets and in islets incubated for 24 h with rosiglitazone (1.5 μg/ml), FFA (1.0 mM), rosiglitazone + FFA, and in human adipose tissue. The figure is representative of 3 experiments.
glucose tolerance patients (12) and in women with previous gestational diabetes (9).

GRANTS

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