Metformin Restores Insulin Secretion Altered by Chronic Exposure to Free Fatty Acids or High Glucose

A Direct Metformin Effect on Pancreatic β-Cells

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Because metformin affects glucose and free fatty acid (FFA) metabolism in peripheral insulin target tissues, we investigated the effect of this drug in restoring a normal secretory pattern in rat pancreatic islets whose function has been impaired by chronic exposure to elevated FFA or glucose concentrations. We cultured rat pancreatic islets with or without FFA (2 mmol/l oleate/palmitate 2:1) or high glucose (16.7 mmol/l) concentrations in the presence or absence of metformin (0.25–12.5 μg/ml) and then measured insulin release, glucose utilization, glucose, and FFA oxidation. When compared with control islets, islets exposed to high FFA or glucose concentrations showed an increased basal and a decreased glucose-induced insulin release. In islets cultured for an additional 24 h with FFA or glucose in the presence of metformin (2.5 μg/ml), both basal and glucose-induced insulin secretions were restored. Both glucose utilization and glucose oxidation were altered in islets pre-exposed to high FFA or glucose concentrations. In particular, regarding control islets, glucose utilization was increased at 2.8 mmol/l glucose and decreased at 16.7 mmol/l glucose; glucose oxidation was similar to control islets at 2.8 mmol/l glucose but decreased at 16.7 mmol/l glucose. In contrast, oleate oxidation was increased in islets pre-exposed to FFA. All of these abnormalities were reversed in islets cultured for an additional 24 h with high FFA or glucose concentrations in the presence of metformin (2.5 μg/ml). In conclusion, our data show that metformin is able to restore the intracellular abnormalities of glucose and FFA metabolism and to restore a normal secretory pattern in rat pancreatic islets whose secretory function has been impaired by chronic exposure to elevated FFA or glucose levels. These data raise the possibility that, in diabetic patients, metformin (in addition to its peripheral effects) may have a direct beneficial effect on the β-cell secretory function. *Diabetes* 49:735–740, 2000

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BSA, bovine serum albumin; FFA, free fatty acid; KRBB, Krebs-Ringer bicarbonate buffer.

The biguanide metformin is widely used in Europe, Canada, and more recently the U.S. for the treatment of type 2 diabetic patients. Its antihyperglycemic effect is not a consequence of insulin secretion stimulation but rather is an effect on peripheral tissues that makes them more sensitive to insulin action. In vitro and in vivo studies indicate that the glucose-lowering effect of metformin is mainly the consequence of decreased hepatic glucose output and increased peripheral glucose uptake and utilization (1–8).

At the cellular level, metformin has different effects on glucose and free fatty acid (FFA) metabolism. In muscle, adipose tissue, and liver, metformin increases glucose oxidation but decreases FFA oxidation (9,10). However, whether metformin also affects glucose and FFA oxidation in other tissues, including the pancreatic β-cells, is unknown. This may be a relevant issue because chronic elevation of glucose or FFAs is known to inhibit insulin secretion in isolated pancreatic islets (11–21), and these abnormalities occur frequently in diabetic patients and negatively affect β-cell function. Moreover, experiments in isolated rat islets indicate that a glucose-FFA cycle (termed the Randle cycle [22]) with a reciprocal relationship between glucose and FFA metabolism is operative in the β-cell (23). When excess FFAs are present, FFA oxidation increases, and glucose oxidation is reduced. Because glucose oxidation plays an important role in glucose-induced insulin release, inhibition of glucose oxidation will then impair insulin secretion.

We investigated the direct effect of metformin in restoring insulin secretion in isolated pancreatic islets chronically exposed to FFAs or glucose and studied whether this effect of metformin was related to changes in islet glucose and FFA oxidation.

RESEARCH DESIGN AND METHODS

Materials. Crude collagenase was obtained from Boehringer Mannheim (Mannheim, Germany). Culture medium CMRL-1066, heat-inactivated fetal calf serum, glutamine, and gentamycin were obtained from Gibco (Glasgow, U.K.). All other chemicals were of analytical grade. [U-^{14}C]glucose, [5-^{3}H]glucose, [1-^{14}C]palmitic acid and [1-^{14}C]oleic acid were purchased from Du Pont-NEN (Boston, MA).

Islet preparation and culture conditions. Pancreatic islets were isolated by the collagenase method from fed male Wistar rats weighing 200–250 g

injected with 0.2 ml i.p. of a 0.2% pilocarpine solution 2 h before being killed by decapitation. With this technique, 300–400 islets were isolated from each pancreas (24). Purified islets were first cultured overnight at 5.5 mmol/l glucose in CMRL-1066 medium and then either for 24 h at 5.5 or 16.7 mmol/l glucose or for 48 h in a culture medium containing 2% bovine serum albumin (BSA) (FFA free) with or without 2 mmol/l long-chain FFAs (oleate/palmitate 2:1) as described by Lee et al. (25). In some experiments, islets were then cultured for an additional 24 h in the presence or absence of metformin.

Insulin secretion. At the end of the culture, the islets were washed twice in Krebs-Ringer HEPES buffer (115 mmol/l NaCl, 5.4 mmol/l KCl, 2.38 mmol/l $CaCl_2$, 0.8 mmol/l $MgSO_4$, 1 mmol/l Na_2HPO_4, 10 mmol/l HEPES, 0.5% BSA, pH 7.35). Groups of 5 purified islets were then incubated with 2.8–16.7 mmol/l glucose (30 min, 37°C), and then insulin was measured in the medium using radioimmunoassay. Results are expressed as insulin released in the medium (picograms · islet^{-1} · 30 min^{-1}).

Glucose utilization. The islet utilization of glucose was determined by measuring the formation of ^3H_2O from [5-^{3}H]glucose as previously described (26). In brief, groups of 15 islets were incubated in 40 µl of Krebs-Ringer bicarbonate buffer (KRBB) containing 2 µCi D-[5-^{3}H]glucose at glucose concentrations ranging from 2.8 to 16.7 mmol/l. The incubation was carried out in a plastic cup inside an airtight sealed 20-ml glass scintillation vial that contained 500 µl distilled water. After 2 h at 37°C, the reaction was stopped by adding 0.5 mol/l HCl (100 µl injected through the rubber seal). Scintillation vials were then incubated overnight at 37°C, and water radioactivity was measured. Under these experimental conditions, the recovery from the known amount of ^3H_2O was fairly constant and ranged from 50 to 60%.

Glucose oxidation. Glucose oxidation was determined by measuring the formation of $^{14}CO_2$ from [U-^{14}C]glucose (27). Groups of 15 islets were incubated in a plastic cup in 100 µl of KRBB (118 mmol/l NaCl, 4.8 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 25 mmol/l $NaHCO_3$) supplemented with 10 mmol/l HEPES (pH 7.4) containing 3 µCi D-[U-^{14}C]glucose (specific activity, 302 mCi/mmol) plus nonradioactive glucose to a final concentration of either 1.5 or 16.7 mmol/l. The cups, which were suspended in standard 20-ml glass scintillation vials, were gassed with O_2:CO_2 (95:5%) and were capped airtight. The vials were then shaken continuously at 37°C for 120 min. The islet metabolism was stopped by injecting 100 µl 0.05 mmol/l antimycin A (dissolved in 70% ethanol) into the cup. This was immediately followed by an injection of 250 µl hyamine hydroxide (Du Pont-NEN) into the vials. $^{14}CO_2$ was liberated from the incubation medium by a subsequent injection into the cup of 100 µl 0.4 mmol/l Na_2HPO_4 solution adjusted to a pH of 6.0. After 2 h at room temperature (to allow the liberated $^{14}CO_2$ to be trapped by the hyamine hydroxide), the cup was removed, and 10 ml of a scintillation fluid was added to each flask. Radioactivity was measured in a liquid scintillation counter.

FFA oxidation. FFA oxidation was measured by a modification of the method described by Chen et al. (28). In brief, groups of 30 islets were placed in a plastic cup connected to a rubber stopper in 0.2 ml of KRBB/10 mmol/l HEPES, pH 7.4, containing 5.5 mmol/l glucose, 2% BSA, 0.8 mmol/l L-carnitine, and 0.125 mmol/l [1-^{14}C]oleic acid (0.4 µCi). The assembly was sealed into a 20-ml liquid scintillation vial (previously gassed with O_2:CO_2 [95:5%]) and shaken for 2 h (100 oscillations/min) in a 37°C water bath. Islet metabolism was terminated by injection of 0.1 ml of 6% (wt/vol) $HClO_2$ into the cup, after which 0.3 ml methanolic hyamine hydroxide (1 mol/l) was injected into the vial to collect the $^{14}CO_2$. After a further 2 h of shaking, the cup was removed, and the $^{14}CO_2$ content of the vial was measured by liquid scintillation counting. Control incubation-lacking islets were run with each series. $^{14}CO_2$ production was linear for at least 2 h.

FFA oxidation was also measured at isotopic equilibrium by adding 1-[^{14}C]palmitate (3 µCi/ml) to the culture media during the culture with FFAs and metformin. After 3 days in culture, the islets were washed 3 times with KRBB/10 mmol/l HEPES (pH 7.4) and were then incubated for 2 h in a plastic cup containing 0.2 ml of the same buffer with 0.8 mmol/l L-carnitine, 2 mmol/l FFA, 0.6 µCi of 1-[^{14}C]palmitate, and 2.8 or 16.7 mmol/l glucose. The cups, which were suspended in standard 20-ml glass scintillation vials, were gassed with O_2:CO_2 (95:5) and capped airtight. The vials were then shaken continuously at 37°C for 120 min. Islet metabolism was terminated as described above.

Triglyceride measurement. Triglycerides were measured as described by Shimabukuro et al. (29). Groups of 200 islets were washed twice with Hanks' balanced salt buffer and were suspended in 50 µl buffer (2 mol/l NaCl, 2 mmol/l EDTA, 50 mmol/l sodium phosphate, pH 7.4). After sonication, 10 µl of the homogenate was mixed with 10 µl tert-butyl alcohol and 5 µl Triton X-100/methyl alcohol mixture (1:1 vol/vol). Triglyceride content was measured with the triglyceride GPO-Trinder (Sigma, St. Louis, MO) kit.

Statistical analysis. Statistical significance was assessed by Student's *t* test for unpaired comparisons.

RESULTS

Insulin release. In control rat islets, insulin release in the presence of a nonstimulatory glucose concentration (2.8 mmol/l) was 37 ± 6 (means ± SE) pg · islet^{-1} · 30 min^{-1} (n = 8) and increased significantly in response to 16.7 mmol/l glucose (848 ± 79 pg · islet^{-1} · 30 min^{-1} [n = 8]) (Fig. 1). In islets preexposed for 48 h to FFA, as expected, basal insulin secretion was significantly increased (185 ± 25 pg · islet^{-1} · 30 min^{-1}, P < 0.01), and glucose-stimulated insulin release was markedly reduced (402 ± 35 pg · islet^{-1} · 30 min^{-1}, P < 0.01) (Fig. 1). When these islets were cultured for an additional 24 h with FFA in the presence of metformin, a clear reversal of the insulin release pattern to control conditions was observed. The effect of metformin was maximal at a concentration of 2.5 µg/ml (Fig. 2). Basal insulin release was reduced and glucose-stimulated insulin release was increased compared with islets further cultured in the presence of FFA alone (Figs. 1 and 2). Similar data were obtained in islets exposed to high glucose concentrations (Table 1). Therefore, all of the experiments on glucose and FFA metabolism were performed at this metformin concentration. In contrast, the drug (0.25–12.5 µg/ml) did not affect insulin release in control islets (Fig. 2).

Glucose utilization. Glucose utilization increased from 19.3 ± 1.7 pmol · islet^{-1} · 120 min^{-1} at 2.8 mmol/l glucose to 102.0 ± 7.9 pmol · islet^{-1} · 120 min^{-1} at 16.7 mmol/l of glucose (n = 5) in control islets. In islets cultured in the presence of 2 mmol/l FFA, glucose utilization at 2.8 mmol/l glucose was increased compared with control islets, and the response to 16.7 mmol/l glucose was decreased (Table 2). When these islets were cultured for an additional 24 h in the presence of both FFA and metformin (2.5 µg/ml), glucose utilization at 2.8 and 16.7 mmol/l glucose was again similar to control islets (Table 2). Similar data were obtained in islets cultured with 16.7 mmol/l glucose: glucose utilization at 2.8 mmol/l was increased, but the value measured at 16.7 mmol/l glucose was significantly reduced compared with control islets

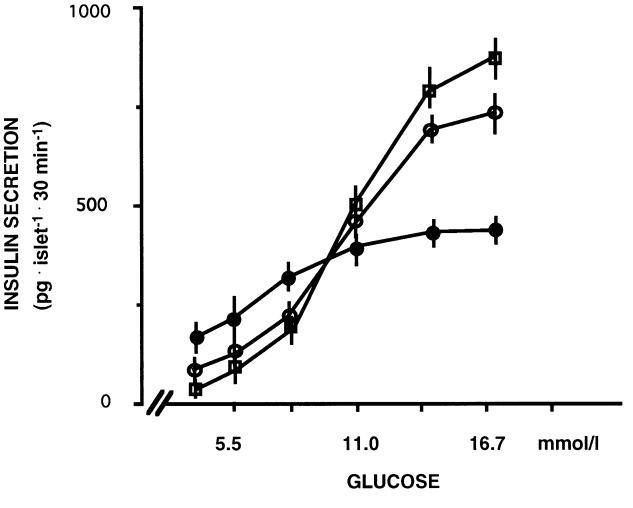

FIG. 1. Glucose-induced insulin release in control pancreatic islets (□) in islets exposed for 48 h to 2 mmol/l FFA (●) or in islets exposed for 48 h to 2 mmol/l FFA and then for an additional 24 h to 2 mmol/l FFA and 2.5 µg/ml metformin (○). Data are means ± SE of 8 separate experiments. Islet insulin content was 52 ± 4 ng/islet in control islets and 39 ± 3 and 41 ± 3 ng/islet in islets cultured with FFA and FFA plus metformin, respectively (n = 8, P < 0.05 vs. control islets).

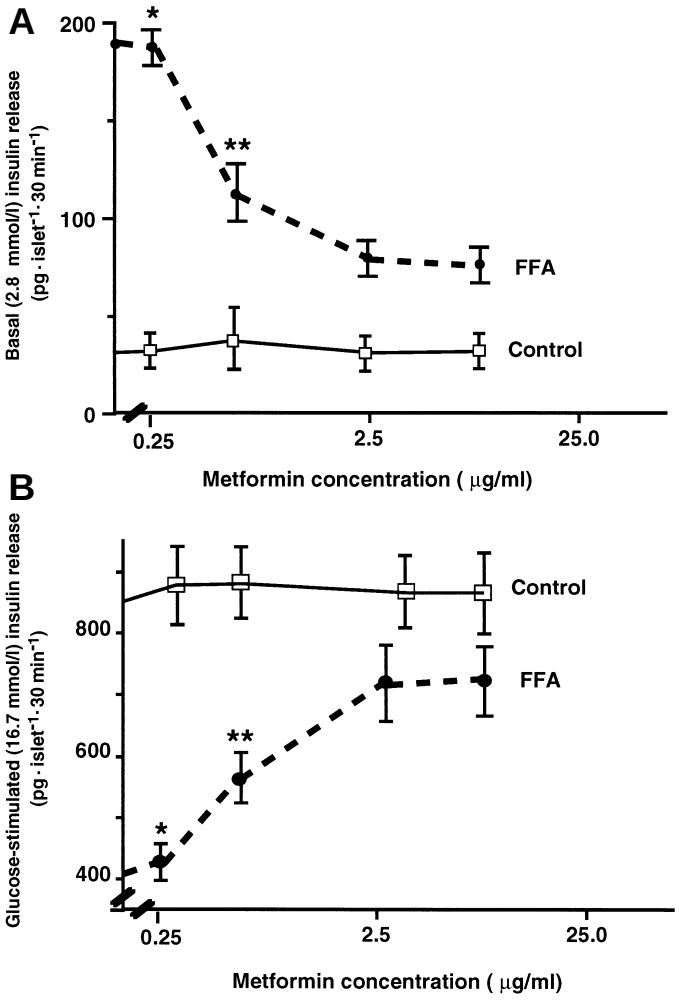

FIG. 2. Effect of metformin on basal (*A*) and glucose-stimulated (*B*) insulin release in rat pancreatic islets cultured with (●) or without (□) 2 mmol/l long-chain FFAs (oleate/palmitate 2:1). Data indicate insulin released in the medium, are expressed as pg · islet^{-1} · 30 min^{-1}, and are means ± SE of 8 separate experiments. *P < 0.01; **P < 0.05.

(Table 2). Additional 24-h incubation with metformin restored both alterations (Table 2). In contrast, metformin (2.5 μg/ml) did not significantly affect glucose utilization in control islets (Table 2).

TABLE 1
Basal (2.8 mmol/l) and glucose-stimulated (16.7 mmol/l) insulin release from rat pancreatic islets cultured either with or without glucose (16.7 mmol/l) and metformin (2.5 μg/ml) for the indicated periods

Culture conditions	2.8 mmol/l	16.7 mmol/l
Control	29 ± 15	978 ± 89
16.7 mmol/l glucose for 24 h	314 ± 31*	562 ± 28†
16.7 mmol/l glucose for 48 h	335 ± 22*	549 ± 33†
16.7 mmol/l glucose for 48 h + metformin (2.5 μg/ml) for 24 h	109 ± 26	911 ± 68

Data are means ± SE and indicate insulin released in the medium expressed as picograms per islet per 30 min. *P < 0.01; †P < 0.05 vs. control islets.

TABLE 2
Glucose utilization at 2.8 or 16.7 mmol/l glucose in rat pancreatic islets cultured either with or without 2 mmol/l FFA or 16.7 mmol/l glucose and 2.5 μg/ml metformin as indicated

Culture conditions	2.8 mmol/l	16.7 mmol/l
Control	19.3 ± 1.7	102.0 ± 7.9
Control + metformin (2.5 μg/ml) for 24 h	18.9 ± 2.0	106.4 ± 3.8
FFA for 72 h	43.6 ± 2.9*	71.4 ± 6.4*
FFA for 72 h + metformin (2.5 μg/ml) for 24 h	22.9 ± 2.3	94.9 ± 5.5
16.7 mmol/l glucose for 48 h	47.2 ± 2.8*	66.3 ± 4.4*
16.7 mmol/l glucose for 48 h + metformin (2.5 μg/ml) for 24 h	20.6 ± 3.3	99.2 ± 4.0

Data are means ± SE of 5 separate experiments and are expressed as picomoles per liter of glucose utilized per islet per 120 min. *P < 0.05 vs. control islets.

Glucose oxidation. In islets cultured with FFA, glucose oxidation at 2.8 mmol/l glucose was similar to that observed in control islets, but the value observed at 16.7 mmol/l glucose was significantly reduced (Table 3). Similar data were obtained in islets cultured with 16.7 mmol/l glucose: glucose oxidation at 2.8 mmol/l was unchanged, but the value measured at 16.7 mmol/l glucose was significantly reduced compared with control islets (Table 3). When metformin (2.5 μg/ml) was added for 24 h to islets cultured in the presence of FFA or high glucose concentrations, the glucose oxidation rate at 2.8 mmol/l glucose was slightly higher than that observed in control islets, although the difference was not significant (Table 3). In these islets, glucose oxidation at 16.7 mmol/l glucose was similar to that in control islets (Table 3). Metformin (2.5 μg/ml) did not significantly affect glucose oxidation in control islets (Table 3).

FFA oxidation. In control islets, FFA oxidation measured at nonisotopic equilibrium was 0.74 ± 0.06 pmol · islet^{-1} · 120 min^{-1} (n = 5). In islets cultured for 48 or 72 h with FFA, oleate oxidation increased to 1.09 ± 0.1 and 1.12 ± 0.2, respectively (P < 0.01 for control islets). In islets pre-exposed for 48 h to FFA and then cultured for an additional 24 h with FFA

TABLE 3
Glucose oxidation at 2.8 or 16.7 mmol/l glucose in rat pancreatic islets cultured either with or without 2 mmol/l FFA or 16.7 mmol/l glucose and 2.5 μg/ml metformin as indicated

Culture conditions	2.8 mmol/l	16.7 mmol/l
Control	14.3 ± 1.8	61.1 ± 2.9
Control + metformin (2.5 μg/ml) for 24 h	16.9 ± 2.0	66.4 ± 3.9
FFA for 72 h	13.9 ± 1.9	39.8 ± 3.4*
FFA for 72 h + metformin (2.5 μg/ml) for 24 h	16.9 ± 2.3	65.9 ± 4.5
16.7 mmol/l glucose for 48 h	12.8 ± 2.5	30.7 ± 2.9*
16.7 mmol/l glucose for 48 h + metformin (2.5 μg/ml) for 24 h	15.8 ± 2.4	59.3 ± 2.8

Data are means ± SE of 5 separate experiments and are expressed as picomoles per liter of glucose oxidized per islet per 120 min. *P < 0.01 vs. control islets.

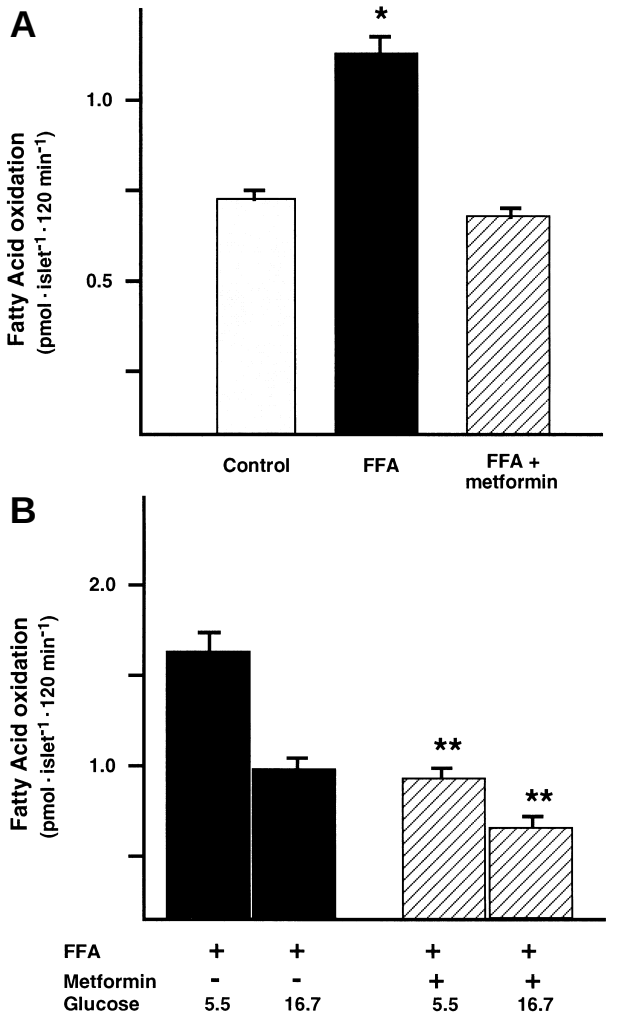

FIG. 3 FFA oxidation in rat pancreatic islets exposed for 48 h to 2 mmol/l FFA and then for an additional 24 h to 2 mmol/l FFA and 2.5 µg/ml metformin. *A*: [1-^{14}C]oleic acid was added at the end of the culture period. *B*: The labeled tracer was present during all cultures with FFAs and metformin. Data are means ± SE of 5 (*A*) and 4 (*B*) separate experiments. **P* < 0.01; ***P* < 0.05.

and metformin (2.5 µg/ml), oleate oxidation was similar to that in control islets (0.69 ± 0.08) (Fig. 3).

To better characterize the relationship between FFA and glucose oxidation and the role of endogenous fat oxidation, FFA oxidation was also measured at isotopic equilibrium by adding 1-[^{14}C]palmitate (3 µCi/ml) to the culture media during the culture with FFAs and metformin. Also under these experimental conditions, both at low and high glucose concentrations, metformin reduced FFA oxidation in islets cultured with 2 mmol/l FFA (Fig. 3). Metformin (2.5 µg/ml) did not significantly affect FFA oxidation in control islets (data not shown).

Triglyceride content. In control islets, triglyceride content was 12.3 ± 2 ng/islet (n = 5). In islets cultured for 72 h with FFA, triglyceride content was increased (53.1 ± 6 ng/islet, P < 0.001 vs. control islets). In islets pre-exposed for 48 h to FFA and then cultured for an additional 24 h with FFA and metformin (2.5 µg/ml), triglyceride content was intermediate (29.5 ± 2 ng/islet, P < 0.05 vs. control and FFA alone).

DISCUSSION

This study tested the hypothesis that metformin can modify the metabolic and secretory abnormalities induced in isolated rat pancreatic islets by chronic exposure to elevated FFA or glucose levels and investigated the possible mechanisms. Our results show that, when metformin is added to the culture medium, despite the persisting elevated FFA or glucose concentrations, all of the metabolic abnormalities are reversed, and both basal and glucose-induced insulin release are restored to the pattern observed in control islets. These data suggest, therefore, that metformin may also have a direct effect on insulin secretion in addition to its well-known effects on the peripheral insulin target tissues.

A beneficial effect of metformin on glucose desensitization has been recently observed in human islets (30). In our experiments, metformin restored basal and glucose-induced insulin release in 2 experimental models, respectively, of lipotoxicity and glucotoxicity. In both models, the effect of metformin was likely because of the improvement of β-cell glucose metabolism induced by the drug. In particular, our data have shown that glucose oxidation was impaired in islets cultured at high FFA or glucose concentrations and that metformin was able to restore it. Because glucose oxidation likely plays a central role in glucose-induced insulin release, all factors affecting it will in turn affect insulin release.

The inhibition of glucose-induced insulin release by high FFA levels has been related to the glucose-FFA cycle (Randle cycle) with the reciprocal relationship between glucose and FFA oxidation. This mechanism plays a major role in the development of insulin resistance in peripheral tissues when FFAs are increased (31). Increased FFA oxidation may impair glucose metabolism by several mechanisms, in particular by inhibition of pyruvate dehydrogenase activity. These abnormalities, which were first described in heart muscle and liver, were recently observed also in pancreatic islets and were implicated as the main cause of the FFA-induced secretory defect (32,33). An effect of metformin in peripheral tissues is to reduce FFA oxidation, and we now show that this effect occurs also in β-cells. However, in our experimental model of lipotoxicity, the respective values of FFAs and glucose oxidation (a factor of 60 in the relative rate of fat and glucose oxidation) refute the notion that the inhibition of glucose oxidation is only because of the increased FFA oxidation (Randle cycle). In addition, our data show that islets cultured at high glucose concentrations have impaired glucose oxidation and that metformin restores both glucose oxidation and insulin release. Thus, the most likely explanation for our findings is that the chronic exposure to high FFA or glucose levels impairs a common pathway of regulation of the oxidative glycolysis that metformin is able to reverse.

Previous data from Liu et al. (34) have already shown shared biochemical properties of glucotoxicity and lipotoxicity. Moreover, a defect in oxidative glycolysis has been already reported in 2 animal models of hyperglycemia and β-cell desensitization to glucose: the neonatal streptozotocin model and GK rats (35). In these animals, the β-cell secretory defect primarily results from a severe decrease in the mitochondrial oxidative catabolism of glucose-derived pyruvate that coincides with a lower ATP/ADP ratio in glucose-stimulated islets, altered closing of the ATP-responsive K^+ channels, and hence diminished insulin release.

Metformin also decreased the elevated triglyceride content of islets cultured in the presence of FFA, although the mechanism of this effect is not clear. In an animal model of genetically determined type 2 diabetes (the ZDF rat) in vivo, diabetes develops around 10 weeks of age, and the animal has elevated FFA levels, insulin resistance, and defective insulin secretion (36). In these animals, β-cell deficiency has been related to a high triglyceride content in islets that appears at the onset of hyperglycemia (37) and results from an increased β-cell esterification capacity, which is the consequence of a defect in the leptin receptor (38,39). In this animal model of hyperglycemia and hypertriglyceridemia, the addition of metformin to the food delays the onset of diabetes, decreases islet triglyceride levels and elevated basal insulin secretion, and increases the ability of the β-cells to respond to glucose stimulation (40).

In conclusion, our in vitro data show that metformin is able to restore the intracellular abnormalities of glucose and FFA metabolism and to restore a normal secretory pattern in rat pancreatic islets whose secretory function has been impaired by chronic exposure to elevated FFA or glucose levels. These models reflect the secretory changes that occur in most type 2 diabetic patients in whom hyperglycemia and hypertriglyceridemia impair β-cell function, which causes glucotoxicity and lipotoxicity. Our data indicate that metformin may have a direct effect on metabolism and function of β-cells exposed to elevated lipid or glucose concentrations. Because hyperglycemia and hypertriglyceridemia with elevated FFA levels are common findings in poorly controlled diabetes, these data raise the possibility that, in these patients, metformin (in addition to its peripheral effects) may have a direct beneficial effect on β-cell secretory function.

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