Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion

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Leclerc, Isabelle, Wolfram W. Woltersdorf, Gabriela da Silva Xavier, Rebecca L. Rowe, Sarah E. Cross, Greg S. Korbutt, Ray V. Rajotte, Richard Smith, and Guy A. Rutter. Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion. Am J Physiol Endocrinol Metab 286: E1023–E1031, 2004. First published February 10, 2004; 10.1152/ajpendo.00532.2003.—Metformin, a drug widely used in the treatment of type 2 diabetes, has recently been shown to act on skeletal muscle and liver in part through the activation of AMP-activated protein kinase (AMPK). Whether metformin or the satiety factor leptin, which also stimulates AMPK in muscle, regulates this enzyme in pancreatic islets is unknown. We have recently shown that forced increases in AMPK activity inhibit insulin secretion from MIN6 cells (da Silva Xavier G, Leclerc I, Varadi A, Tsuboi T, Moule SK, and Rutter GA, Biochem J 371: 761–774, 2003). Here, we explore whether 1) glucose, metformin, or leptin regulates AMPK activity in isolated islets from rodent and human and 2) whether changes in AMPK activity modulate insulin secretion from human islets. Increases in glucose concentration from 0 to 3 and from 3 to 17 mM inhibited AMPK activity in primary islets from mouse, rat, and human, confirming previous findings in insulinoma cells. Incubation with metformin (0.2–1 mM) activated AMPK in both human islets and MIN6 β-cells in parallel with an inhibition of insulin secretion, whereas leptin (10–100 nM) was without effect in MIN6 cells. These studies demonstrate that AMPK activity is subject to regulation by both glucose and metformin in pancreatic islets and clonal β-cells. The inhibitory effects of metformin on insulin secretion may therefore need to be considered with respect to the use of this drug for the treatment of type 2 diabetes.

5′-adenosine monophosphate-activated protein kinase; human islets of Langerhans; MIN6 cells

5′-AMP-Activated protein kinase (AMPK) is a multisubstrate, heterotrimeric serine/threonine protein kinase consisting of one catalytic α-subunit and two regulatory β- and γ-subunits (10, 47). AMPK activity is regulated allosterically by AMP and through reversible phosphorylation at Thr172 of the α-subunit (16–18, 22, 56) by the upstream kinase LKB1 (derived as a code name for the Peutz-Jeghers syndrome causative gene (3), also termed STK11 (for serine/threonine kinase 11) (24)). AMPK is a sensor of cellular energy charge that is activated by ATP depletion and the consequent increase in intracellular AMP (26). AMPK activation results in the inhibition of ATP-consuming pathways such as fatty acid and cholesterol biosynthesis by phosphorylation of acetyl-CoA carboxylase (ACC) and hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) (5) respectively, and promotes ATP production by stimulating fatty acid oxidation (26).

Metformin is a widely used anti-diabetic agent whose cellular mechanism of action was, until recently, obscure. However, Zhou et al. (58) showed that metformin activates AMPK in rat hepatocytes and skeletal muscle, an effect that may account for the effect of metformin on increased muscle glucose transport, decreased hepatic glucose output, and beneficial blood lipid profile (12). Metformin action on insulin release in vitro is more controversial, however (33, 44). In vivo, metformin decreases plasma insulin levels in diabetic subjects (12) and in patients with polycystic ovary syndrome (37). Up to now, these actions of metformin have generally been explained by an increase in peripheral insulin sensitivity and, hence, a decrease in blood glucose levels rather than a direct inhibition of pancreatic insulin release.

Leptin is secreted by adipocytes and stimulates fatty acid oxidation (38) and glucose uptake into muscle cells (25, 34) and prevents accumulation of lipids in nonadipose tissues (50). AMPK has recently been identified as the principal mediator of the effects of leptin on fatty acid metabolism in skeletal muscle (35), although this does not seem to be the case in heart muscle (1). In pancreatic islet β-cells, the effects of leptin are controversial, since it has been shown to have no effect, to inhibit, or to stimulate insulin secretion (28).

AMPK is now considered as a potentially interesting pharmacological target for the treatment of type 2 diabetes (41), since activation of the enzyme has been shown to decrease gluconeogenesis and to increase muscle glucose transport, both in vitro (19, 29, 52) and in vivo (23). Although we (8) and others (43) have previously shown that AMPK activity is regulated by glucose in clonal pancreatic β-cell lines, nothing is known about the regulation of this enzyme by glucose, or by metformin or leptin, in intact rodent or human islets of Langerhans. In this report, we tested the hypothesis that AMPK activity is regulated by these agents in primary islets and is involved in the regulation of insulin release. We show that glucose inhibits AMPK activity in islets isolated from three distinct species, namely mouse, rat, and human. In contrast, metformin stimulates AMPK activity and inhibits glucose-stimulated, but not KCl-induced, insulin secretion in both human and MIN6 cells (da Silva Xavier G, Leclerc I, Varadi A, Tsuboi T, Moule SK, and Rutter GA, Biochem J 371: 761–774, 2003).

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human islets and MIN6 β-cells. Leptin, however, has no effect on AMPK activity or insulin secretion in MIN6 cells or rat islets.

MATERIALS AND METHODS

Materials. Collagenase (type V), Histopaque-1077, -1083, and -1119 solutions, and metformin were from Sigma. Mouse recombinant leptin was from Sigma and Calbiochem. 5-Aminoimidazole-4-carboxamide riboside (AICAR) was from Toronto Research Chemicals (Toronto, ON, Canada).

Animals. Wild-type CD-I mice (20–25 g) and Wistar rats (150–200 g) were used for islet isolation and killed by cervical dislocation immediately before the islet isolation procedure (see Cell culture and islet isolation). All animal procedures were in accordance with the British Home Office Animals (Scientific Procedures) Act, 1986.

Antibodies. Guinea pig anti-insulin antibody was from Dako. Sheep anti-AMPK-α1 and -α2, and rabbit anti-AMPK-β1/2 antibodies were kindly provided by Dr. D. Carling (MRC Clinical Sciences, London, UK). Sheep anti-phospho-AMPK (Thr172) antibody was a generous gift of Prof. D. G. Hardie (Dept. of Biochemistry, University of Dundee, Scotland, UK). Rabbit anti-phospho-ACC (Ser79) antibody was purchased from Upstate Biochemicals (Lake Placid, NY).

Cell culture and islet isolation. MIN6 cells were used between passages 18 and 30 and grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 25 mM glucose and supplemented with 15% heat-inactivated fetal calf serum (FCS), 4 mML-glutamine, 100 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere at 37°C with 5% CO2 unless specified otherwise. Mice and rats were killed by cervical dislocation, and collagenase [0.5 mg/ml in Hanks’ balanced salt solution (HBSS)] was injected into the pancreatic duct (2.5 ml/mouse, 8 ml/rat). The distended pancreata were then incubated in a shaking water bath at 37°C in 0.5 mg/ml collagenase in HBSS for 10–20 min, and the islets were recovered by Histopaque density gradient centrifugation. Rodent islets were ≥85% pure as assessed by dithizone staining and were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS and antibiotics. Human islets were obtained from cadaveric donors’ pancreata as described (45). Unless otherwise stated, human islets were of >75% purity, with most of the remaining cellular contamination amylase positive, and were cultured in DMEM containing 25 mM glucose supplemented with 15% FCS and antibiotics for 1–7 days before experiments.

Adenoviruses. Adenoviruses encoding for enhanced green fluorescent protein (eGFP) only, hereafter named pAd-GFP (null), constitutively active AMPK (pAd-AMPK-CA), and dominant negative AMPK (pAd-AMPK-DN), have been described elsewhere (9). AMPK adenoviruses also express eGFP under a distinct cytomegalovirus promoter. Islets were infected at a multiplicity of infection of 100 viral particles/cell.

Immunohistochemistry. Paraffin-embedded rat pancreas slice sections on glass slides were dewaxed, rehydrated, and incubated for 6 min in 10% H2O2 to block endogenous peroxidase activity. The

Fig. 1. 5′-AMP-activated protein kinase (AMPK)-α subunit expression in whole rat pancreas and in dissociated human islets. A: immunohistochemistry for AMPK-α1 and -α2 subunits and insulin was performed on consecutive rat pancreata slices (see MATERIALS AND METHODS). Left: anti-insulin staining for islet localization; right, anti-AMPK-α1 and -α2 subunits staining as indicated. Black arrows indicate red blood cells. B: human dissociated islet cells were costained with anti-insulin (left, green signal) and anti-AMPK-α1 or -α2 subunits (right, brown signal), as described in MATERIALS AND METHODS. Scale bars, 50 μm (A) or 30 μm (B). Note that the fields selected in B were enriched in cells displaying strong staining for AMPK and likely to correspond to acinar (amylose-positive) cells in the preparation used.

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sections were then first incubated for 15 min in 3% BSA to block nonspecific binding and then for 30 min with guinea pig anti-insulin (α1, 1:500; α2, 1:100), washed three times in PBS, and incubated for 20 min with anti-guinea pig or anti-sheep biotinylated secondary antibodies (1:200) and stained using the avidin-biotin complex (ABC) method with peroxidase and diaminobenzidine (DAB) as the chromagen (Vector Laboratories, Burlingame, CA). Dissociated human islet cells (~50% pure) were double-stained using the ABC-DAB method for AMPK staining (as above) and FITC-conjugated secondary antibodies for insulin (1:200).

**AMPK activity assay.** MIN6 cells were cultured in 12-well plates in experimental conditions, washed twice in ice-cold PBS, and scraped into 200 μl of ice-cold lysis buffer [in mM: 50 Tris-HCl (pH 7.4, 4°C), 250 sucrose, 50 NaF, 1 Na pyrophosphate, 1 EDTA, 1 EGTA, 1 DTT, 0.1 benzamidine, and 0.1 PMSF, 5 μg/ml soybean trypsin inhibitor, and 1% (vol/vol) Triton X-100]. Extracts were centrifuged (13,000 g, 5 min, 4°C), and protein concentration was determined using a bicinchoninic acid protein assay reagent from Pierce. AMPK activity was determined using 5 μg of whole extract and the synthetic peptide SAMS (HMRSAMSLHLVKKR) as substrate (8). Islets were cultured in experimental conditions, and batches of 100 islets were lysed in 25 μl of ice-cold lysis buffer and centrifuged as above. Results are expressed in picomoles of 32 P incorporated per microgram of protein per minute (pmol·μg⁻¹·min⁻¹) or as a percentage of control conditions.

**Western blot analysis.** MIN6 cells or mouse islets were cultured and lysed as for AMPK activity. Fifty micrograms of whole cellular extracts were denatured for 5 min at 100°C in 2% SDS and 5% β-mercaptoethanol, resolved by 10% SDS-PAGE, and transferred to PVDF membranes before immunoblotting, as described in Ref. 31. Sheep anti-phospho-AMPK antibody was used at a dilution of 1:500, rabbit anti-phospho-ACC antibody was used at 1:125 dilution, and rabbit anti-AMPK-β1/2 was used at 1:5,000 dilution. Intensities were measured by digital scanning of gels and quantified using ImageJ (ImageJ@list.nih.gov).

**Insulin secretion assay.** MIN6 cells seeded in 12-well plates and preincubated as indicated in the figure legends were then incubated for 30 min in 1 ml of Krebs-HEPES-bicarbonate (KHB) solution [in mM: 130 NaCl, 3.6 KCl, 1.5 CaCl₂, 0.5 MgSO₄, 0.5 KH₂PO₄, 2 NaHCO₃, 10 HEPES, and 0.1% (wt/vol) BSA; pH 7.4] at 37°C containing the indicated glucose concentration in the presence or absence of other additions as given. Human and rat islets were either left uninfected or infected with null, AMPK-CA, or AMPK-DN adenoviruses, incubated for 48 h in RPMI 1640, and then divided in groups of five islets per condition and incubated for 20 min in 1 ml of KHB solution at the indicated glucose concentrations. Total insulin content was extracted into 1 ml of acid-ethanol-Triton solution [1.5% (vol/vol) HCl, 75% (vol/vol) ethanol, 0.1% (vol/vol) Triton X-100]. Secreted insulin and total insulin were measured using radioimmunoassay by competition with 125I-labeled insulin (Linco Research, St. Charles, MO).

**Statistics.** Data are given as means ± SE of at least three independent experiments. Comparisons between means were performed with Student’s t-test for paired data by use of Microsoft Excel software.
RESULTS

AMPK-α subunits are expressed at low levels in the endocrine pancreas. Immunostaining for the catalytic AMPK-α1 and -α2 subunits was performed on whole rat pancreata sections (Fig. 1A) and on dissociated human islet cells (Fig. 1B). For the whole rat pancreata staining, consecutives slices were examined. The first slices (Fig. 1A, left) were stained with anti-insulin antibody to localize the islets of Langerhans, and the second slices (Fig. 1A, right) were stained with anti-AMPK antibodies directed against the α1- or α2-subunits as indicated. Although staining for each isoform was evident in islets of Langerhans, the level of expression of AMPK subunits was lower than in the surrounding exocrine tissue or than in red blood cells (black arrows). Staining for AMPK-β1/2 subunits in mouse pancreas slices showed the same, relatively low level of expression in islets compared with exocrine tissue or red blood cells (not shown). Similarly, in dissociated human islet cells (Fig. 1B) co-stained with anti-insulin and anti-AMPK-α1 or -α2, insulin-positive cells (in green; Fig. 1B, left) were less intensely stained for AMPK (in brown; Fig. 1B, right) than the other cell types, which were mainly amylase positive (45).

AMPK activity is inhibited by glucose in intact rodent and human islets of Langerhans. To determine whether AMPK activity in primary islets was subject to regulation by changes in cellular energy status, as previously demonstrated in insulinoma cells (9, 43), we examined the activity of the enzyme at different extracellular glucose concentrations. Rat islets were incubated for 60 min (Fig. 2A) or overnight (16 h; Fig. 2B) in the presence of 0, 3, or 17 mM glucose or 17 mM glucose plus the cell-permeant activator AICAR (4 mM) (7), as indicated. Mouse islets (Fig. 2, C and E) were incubated overnight in 3 or 17 mM glucose, and human islets (Fig. 2D) were incubated overnight in 0, 3, or 17 mM glucose or 17 mM glucose plus 4 mM AICAR. AMPK activity decreased as the glucose concentration increased over the physiological range from 0 to 17 mM, and incubation with AICAR reactivated AMPK in the presence of high glucose concentration. Figure 2E shows that inhibition of AMPK activity in mouse islets with increased glucose concentration from 3 to 17 mM was due to dephosphorylation of the AMPK-α subunit.

AMPK overexpression inhibits insulin secretion from human pancreatic islets. Our previous work in MIN6 cells (9, 48) has shown that the forced activation of AMPK inhibits insulin secretion. To determine whether this phenomenon also obtains in primary human islets, we infected with adenoviruses containing the C-terminal region of the constitutively active AMPK-CA (the primary sequences of AMPK-α1 and -α2 are identical over the region used here, amino acids 1–312) or AMPK-DN (Fig. 3, B and C). Because confocal microscopy revealed that cells in the central core of large (>150 µm) mouse (Fig. 3A, bottom) or human (not shown) islets were infected (i.e., expressed eGFP) poorly (13), we performed all subsequent adenoviral experiments on islets of <100 µm in diameter to achieve 60% infection efficiency or better (Fig. 3A, top).

Figure 3B shows AMPK activity in uninfected human islets or in islets infected with null, AMPK-CA and AMPK-DN adenoviruses. No difference in AMPK activity was apparent after adenoviral infection with the null virus compared with noninfected islets. In contrast, infection with AMPK-CA increased measured AMPK activity by ~25% (n = 3 separate experiments, P < 0.05) although the infection with AMPK-DN decreased it by ~10% (n = 3, P < 0.05). Figure 3C shows the secretion of insulin from human islets after adenoviral infection with null, AMPK-CA, or AMPK-DN adenoviruses. The overexpression of AMPK-CA decreased insulin release, whereas the overexpression of AMPK-DN increased insulin secretion from the islets. It should be noted that the more substantial activation of insulin secretion (~25%; Fig. 3C) compared with the inhibition of AMPK (~10%; Fig. 3B) likely reflects the facts that the virus efficiently infects only ~60% of cells even within the small (<100 µm) islets used (Fig. 3A), corresponding to the most glucose-responsive β-cells at the
Metformin stimulates AMPK phosphorylation and inhibits glucose-stimulated insulin secretion from pancreatic MIN6 β-cells and human islets. Because metformin has been shown to activate AMPK in other tissues (35, 58), we explored the regulation of AMPK activity by this agent in MIN6 β-cells and human islets. After a 16-h incubation, metformin increased the activity of AMPK in MIN6 cells in a dose-dependent manner at both 3 and 17 mM glucose (Fig. 4A). The metformin doses used were higher than steady-state plasma levels of metformin (2), but studies in rat have shown that tissue levels are severalfold higher than in plasma (53), in part due to the substantial accumulation of the drug by mitochondria (see DISCUSSION). Furthermore, it is recognized that metformin actions in vitro require high doses and are slow in onset due to low rates of plasma membrane transport (2, 58). Correspondingly, a shorter incubation period of 60 min with metformin did not increase AMPK activity, whereas the cell-permeant AMP analog AICAR increased it approximately twofold under these conditions (Fig. 4B).

The stimulation of AMPK activity by metformin was accompanied by increased phosphorylation of the AMPK-α subunits on Thr172 as well as increased phosphorylation of the downstream target ACC on Ser79 (Fig. 4C), with no changes in the total amount of either protein as assessed by immunoblotting using antibodies to nonphosphorylated epitopes of either protein (Leclerc I, unpublished data). Thus quantitation of data from two separate experiments revealed an increase in the amount of AMPK phosphorylated at Thr172 in the presence of metformin of 59 and 66% at 3 and 17 mM glucose, respectively. In separate experiments, phospho-ACC (Ser79) immunoreactivity in extracts of cells incubated at 17 mM glucose

![Graph A: AMPK activity (% of 3 mM glucose) vs. Metformin (mM)]

![Graph B: AMPK activity (% of control) vs. Metformin 1 mM and AICAR 1 mM]
was 72.4 ± 1.1% of that measured in cells maintained at 3 mM glucose (means ± SE; n = 3 experiments, *P < 0.001), in line with previous results (9). Whereas 1 mM metformin had no significant effect on phospho-ACC levels at 3 mM glucose, the presence of the drug increased ACC phosphorylation at Ser79 to 118 ± 10% of basal values (n = 3 experiments, *P < 0.05) at 17 mM glucose.

Because activation of AMPK via the use of AICAR or by overexpression of a constitutively active form of the kinase inhibits glucose-stimulated insulin secretion in MIN6 cells (9), we postulated that metformin may also reduce secretion. As shown in Fig. 4D, incubation of MIN6 cells with 1 mM metformin for 16 h blunted insulin secretion compared with control cells at both 3 and 17 mM glucose. Metformin also stimulated AMPK activity and inhibited insulin secretion from human islets, as shown in Fig. 5, A and B, respectively.

To determine whether the loss of responsiveness to glucose caused by metformin may be due to a general decrease in the viability of cell or islet preparations, we examined the impact of the drug on the nutrient-independent stimulation of secretion elicited by a depolarizing concentration of KCl. As an argument against any loss of cell viability as the underlying cause, metformin had no impact on the stimulation of secretion provoked by 30 mM KCl from either MIN6 cells (Fig. 4D) or human islets (Fig. 5C).

Leptin does not activate AMPK activity in MIN6 cells. Mouse recombinant leptin failed to stimulate AMPK activity in MIN6 β-cells at either 3 or 17 mM glucose concentrations (Table 1).

**DISCUSSION**

AMPK activity is regulated by glucose in primary rodent and human islets. It is now well established that glucose inhibits AMPK activity in several insulinoma β-cell lines (8, 43). A concern, however, has been that the regulation of the enzyme may be a feature peculiar to immortalized, clonal β-cells. Indeed, AMPK activity is inhibited by glucose in mhAT3f hepatoma cells (30) and by insulin in Fao hepatoma cells (54) but is completely insensitive to these agents in primary rat or mouse hepatocytes (14, 30, 55) (Leclerc I and Kahn A, unpublished observations). Here, we examined this question in four distinct insulin-secreting cell preparations. First, we used the relatively well-differentiated β-cell line MIN6 (36) to assess the regulation and role of changes in AMPK activity in β- vs. other islet cell types. Second, human islets were employed (1) because these could be obtained in large quantities, facilitating biochemical measurements of changes in AMPK activity by direct phosphotransfer (SAMS peptide) assay and (2) because they allowed the assessment of the role of AMPK activity changes in islets from humans. Third, primary rat or mouse islets, which could be isolated in much smaller numbers than human islets but were of more reproducible quality (i.e., glucose responsiveness), allowed a large series of experiments to be performed on both AMPK activity and insulin secretion. In most respects, the regulation and role of AMPK in each preparation were essentially similar, demonstrating the conservation of this mechanism in three different mammalian species. However, we did note that absolute levels of AMPK catalytic subunits, as assessed by immunocytochemical or biochemical assay, were somewhat higher in the MIN6 cell line than in either human or rodent islets (Leclerc I, unpublished data).

The present study thus demonstrates for the first time that AMPK activity is regulated by glucose, over the physiological range of concentrations, in primary rat and human islets of Langerhans (Fig. 2). It should be stressed that the demonstra-
tion of these changes required close attention to several aspects of the islet isolation and incubation protocols. First, any contamination of fresh rat islet preparations with exocrine tissue tended to markedly increase the measured AMPK activity, which was not suppressible by glucose. This is consistent with the higher levels of the enzyme in the exocrine tissue (Fig. 1). However, maintenance of islets in culture for ≥3 days before the assay was found to overcome this problem, presumably by allowing time for contaminating both exocrine tissue and the hypoxic central cores of large islets (51), to necrose (26).

Our previous studies (9, 48) suggest that inhibition of AMPK may play an important role in the activation of insulin secretion at elevated glucose concentrations. The present work shows that changes in AMPK activity are also likely to modulate insulin secretion from intact human islets. Thus adenoviral overexpression of AMPK-CA and AMPK-DN inhibits and stimulates, respectively, insulin release from human islets (Fig. 3C). What mechanisms may underlie the effect of AMPK activation to inhibit insulin secretion? Increases in glucose concentration are believed to activate insulin release by enhancing ATP synthesis (27) and closing ATP-sensitive K$^+$ (KATP) channels (4). Subsequent depolarization of the plasma membrane (21) and the opening of voltage-sensitive (L-type) Ca$^{2+}$ channels (42) cause insulin-containing vesicles to fuse at the plasma membrane (40). In addition, KATP channel-independent “amplifying” effects may also contribute to the stimulation of release independently of changes in intracellular Ca$^{2+}$ concentration (20). In MIN6 cells, AMPK-CA appears to inhibit insulin release by interfering with both glucose metabolism (9) and the recruitment of insulin-containing vesicles to the plasma membrane (48). Future studies will be required to determine whether similar mechanisms are operative in primary β-cells and islets.

**Metformin activates AMPK in islets.** Metformin is a drug widely used to treat type 2 diabetes. Although its precise molecular mechanism of action has remained elusive for decades (41), an action to inhibit complex I of the respiratory chain and, hence, mitochondrial respiration and ATP synthesis, has been proposed (41). Although metformin is generally considered to have no direct effect on the pancreatic islet β-cell, an early report demonstrated a dose-related inhibition of glucose-stimulated insulin secretion and insulin biosynthesis by biguanides (44). Moreover, basal and glucose-stimulated insulin plasma levels are consistently decreased in metformin-treated patients (11), an observation usually attributed to the increase in peripheral insulin sensitivity (41). Here, we show that metformin activates AMPK activity in MIN6 cells and human islets of Langerhans and inhibits insulin release. It is likely that metformin, which is positively charged at physiological pH, needs to accumulate inside mitochondria as much as 1,000-fold (39) to exert these actions. Thus, although relatively high concentrations of metformin were used in the present studies, much lower concentrations, close to those likely to be found in blood plasma (2), may be sufficient to activate islet cell AMPK and inhibit insulin secretion after extended incubations.

**AMPK activity is insensitive to leptin in β-cells.** The reported effects of leptin (57) on the pancreatic β-cell are complex and controversial (28). Because leptin has recently been shown to activate AMPK in skeletal muscle (35), it seemed important here to determine the effects of this hormone on AMPK activity and insulin secretion in pancreatic β-cells. We did not, however, detect any significant increase in AMPK activity in response to leptin after incubation of MIN6 cells at either low (3 mM) or high (17 mM) glucose (Table 1) or in rat islets incubated overnight in the presence of 10 nM leptin (not shown). These results are reminiscent of the reported absence of leptin-mediated increase in AMPK activity in isolated working rat hearts (1). These data are consistent with the view that leptin signaling might stimulate AMPK activity only in the presence of the γ3-subunit, which appears to be expressed exclusively in skeletal muscle (1, 6).

In conclusion, extensive clinical studies (15) have demonstrated that metformin treatment leads to a reduction of diabetes-related end points, diabetes-related death, and all-cause mortality compared with conventional therapy. Furthermore, metformin can delay the onset of overt diabetes in the Zucker diabetic fatty rat by increasing insulin sensitivity (46). In contrast, the present and previous (8, 9) data suggest that sustained activation of AMPK in the pancreatic β-cell may be detrimental for the maintenance of β-cell phenotype. Correspondingly, a significant decline of β-cell function with time in type 2 diabetes (49) and a better preservation of β-cell function with diet alone (32) have both been reported. Together, these observations suggest that the impact of metformin on insulin secretion should be considered in selecting an appropriate therapy for individual diabetics. The present findings also suggest that drugs able to activate AMPK by stimulating leptin signaling, a mechanism apparently absent in β-cells but operative in skeletal muscle (1), may be of greater value for the treatment of type 2 diabetes than those that act through less specific mechanisms.

**GRANTS**
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### Table 1. Effects of leptin on AMPK activity in MIN6 pancreatic β-cells

<table>
<thead>
<tr>
<th>Glucose Concentrations, mM</th>
<th>Control</th>
<th>Leptin, 20 nM</th>
<th>Leptin, 100 nM</th>
<th>Control</th>
<th>Leptin, 100 nM</th>
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<tr>
<td></td>
<td></td>
<td>16-h Incubation: % of 3 mM Control</td>
<td>45-min Incubation: % of 3 mM Control</td>
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<td></td>
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<tr>
<td>3</td>
<td>100.0±2.4</td>
<td>102.5±7.3</td>
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<td>75.5±15.1</td>
<td>50.4±3.3</td>
<td>55.2±0.9</td>
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Data represent means ± SE of ≥3 independent experiments. AMPK, 5′-AMP-activated protein kinase. MIN6 cells were incubated either overnight in DMEM containing 3 or 17 mM glucose and the indicated leptin concentrations or for 45 min in Krebs-HEPES-bicarbonate buffer containing 3 or 17 mM glucose in the presence or absence of 100 nM leptin. Cell lysis and AMPK activity assays were performed as described in MATERIALS AND METHODS.
REFERENCES


