Endocrine, *vol. 26, no. 1, 71–77, February 2005* 0969–711X/05/26:71–77/$30.00

Further Evidence for the Regulation of Acetyl-CoA Carboxylase Activity by a Glutamate- and Magnesium-Activated Protein Phosphatase in the Pancreatic β Cell

Defective Regulation in the Diabetic GK Rat Islet

Rengasamy Palanivel,* Rajakrishnan Veluthakal,* Phillip McDonald, and Anjaneyulu Kowluru

Department of Pharmaceutical Sciences, Wayne State University and β Cell Biochemistry Laboratory, John D. Dingell VA Medical Center, Detroit, MI 48201

We previously identified and characterized a glutamate- and magnesium-sensitive PP2A-like phosphatase (GAPP), which dephosphorylated and activated acetyl-CoA carboxylase (ACC) in the islet β cell. Herein, we studied potential regulatory mechanisms by which GAPP is activated by glutamate and magnesium, and also quantitated the degree of activation, by glutamate- and magnesium, of ACC in normal rat islets and islets derived from the diabetic Goto–Kakizaki (GK) rat, a model for type 2 diabetes in humans. Our findings indicate that magnesium, but not glutamate, specifically activates the post-translational carboxylmethylation (CML) of the 36 kDa catalytic subunit of GAPP. Okadaic acid (OKA), which inhibits GAPP-mediated activation of ACC, also reduced the magnesium-stimulated CML of the catalytic subunit of GAPP in all the β cell preparations studied. These data suggest that the CML step may be necessary for magnesium- and glutamate-mediated activation of ACC. We also observed a marked attenuation in magnesium- and glutamate-facilitated activation of ACC activity in islets derived from the GK rat. Together, our findings raise an interesting possibility that inhibition of GAPP-catalyzed inactivation of ACC (and subsequent reduction in the generation of long-chain fatty acids) could contribute toward the abnormalities in insulin secretion demonstrable in this animal model for type 2 diabetes.

Key Words: Insulin secretion; acetyl-CoA carboxylase; protein phosphatase; glutamate; carboxylmethylation.

Introduction

The molecular and cellular mechanisms underlying nutrient-induced insulin secretion from the pancreatic β cell remain partially understood. Previous data, however, favor the postulation that phosphorylation and dephosphorylation of endogenous proteins play modulatory roles in β cell stimulus–secretion coupling *(1–5)*. The phosphorylation status of proteins is regulated by a balance of activities of protein kinases and phosphatases. Although several earlier studies, including our own, examined the roles for protein kinases in insulin secretion, very little has been studied with regard to the localization and regulation of protein phosphatases in the pancreatic β cell *(1–7)*.

The protein phosphatase 2A (PP2A) is a major serine–threonine phosphatase, which has been implicated in the regulation of many cellular events, including insulin secretion from the pancreatic β cell *(7–11)*. Typically, PP2A core enzyme is a dimer consisting of a 36-kDa catalytic subunit (PP2Ac) and a 65-kDa regulatory subunit. This dimer reacts with one of the third variable component, structural B subunit, culminating in the formation of the PP2A holoenzyme. Even though the precise function of the variable subunit(s) is unclear, it is speculated that it influences substrate specificity and/or subcellular localization of a given PP2A *(12–14)*. Reversible post-translational modifications of PP2Ac, including phosphorylation and carboxylmethylation (CML) have been described as regulatory mechanisms for PP2A in multiple cell types, including the pancreatic islet *(11,15–18)*. The phosphorylation of tyrosine^{307} in the catalytic subunit has been shown to inhibit the phosphatase activity *(19,20)*. Moreover, our recent findings in the β cell suggested that methylation of the carboxy terminal leucine^{309} residue in the catalytic subunit leads to a significant increase in the catalytic activity of PP2A, presumably via increased holoenzyme assembly *(11)*. Recent data from Sjoholm and co-workers *(6,21–23)* and from our own laboratory *(24)* have suggested important regulatory roles for PP2A in nutrient-induced insulin secretion. Together, these data indicated that inhibition of protein phosphatase activation leads to stimulated

*These authors contributed equally to this work.

Received January 5, 2005; Revised February 2, 2005; Accepted February 2, 2005.

Author to whom all correspondence and reprint requests should be addressed: Anjan Kowluru, PhD, Department of Pharmaceutical Sciences, 3601, Eugene Applebaum College of Pharmacy, Wayne State University, 259, Mack Avenue, Detroit, MI 48202. E-mail: akowluru@med.wayne.edu

insulin secretion, presumably by retaining key exocytotic proteins in their active phosphorylated state *(6,21–24)*.

Interestingly, protein phosphatases may also positively modulate functions of several key proteins that are involved in the intermediary metabolism of the islet β cell. For example, the catalytic function of ACC, a lipogenic enzyme, is regulated by phosphorylation–dephosphorylation *(25,26)*. ACC is inactivated by phosphorylation at multiple serine residues [−79, −1200, and −1215] by AMP kinase *(27,28)*. It is reactivated upon dephosphorylation by a PP2A-like activity. Using isolated hepatocytes, Gaussin et al. *(29)* described a predominantly cytosolic, magnesium- and glutamate-activated protein phosphatase that dephosphorylates and activates ACC. Based on its subcellular distribution and sensitivity to inhibitors, GAPP appears to be similar to PP2A. Although such regulatory mechanisms for ACC activation have been described in other cell types, it is not clear whether similar mechanisms are operable in the islet β cell for the regulation of ACC. Along these lines, we have recently characterized a glutamate- and magnesium-sensitive protein phosphatase 2A (PP2A)-like phosphatase, which catalyzed the dephosphorylation and activation of ACC in the islet β cell *(30)*. Based on further experimental evidence, we have implicated this phosphatase in insulin secretion *(30)*. As a logical extension to our above studies *(30)*, in the present report we studied: (a) the glutamate- and magnesium-mediated activation of ACC in multiple insulin-secreting cells, including normal rat islets; (b) potential effects of glutamate and magnesium on the CML of PP2Ac to determine whether such a post-translational modification step is involved in GAPP-mediated activation of ACC; and (c) the degree of activation and functional status of glutamate- and magnesium-mediated activation of ACC in islets derived from the GK rat, an animal model for type 2 diabetes in humans.

Results

Glutamate and Magnesium Activate ACC in Insulin-Secreting Cells

Our earlier studies demonstrated significant activation of ACC activity by glutamate and magnesium in lysates derived from normal rat islets and human islets *(30)*. We also reported that such a stimulation of ACC activity by magnesium and glutamate is mediated via activation of an OKA-sensitive PP2A-like phosphatase, which dephosphorylated ACC *(30)*. Data in Fig. 1 demonstrate that a combination of glutamate and magnesium markedly increased the activity of ACC in lysates derived from RIN, HIT, INS, and βTC3 cells. Such an activation by magnesium and glutamate ranged between 4.5- and 7.5-fold in these cells.

Magnesium, But Not Glutamate, Increases the Carboxylmethylation of PP2Ac in INS Cells and Normal Rat Islets

Using OKA, which specifically inhibits the PP2A family of protein phosphatases in n*M* concentrations, we have

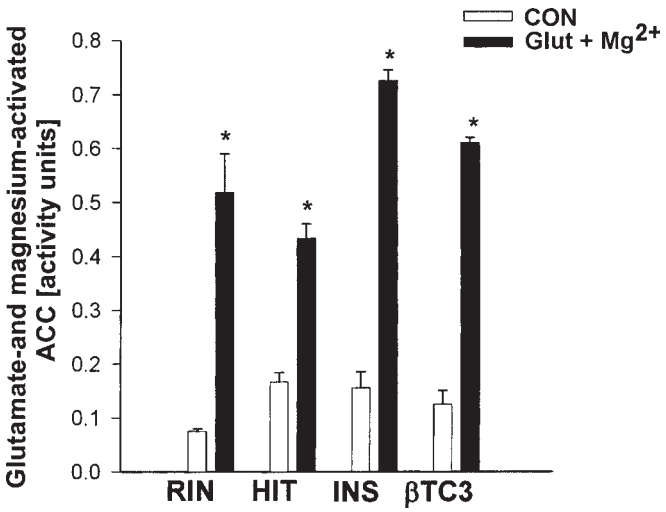

Fig. 1. Glutamate- and magnesium-mediated activation of ACC in insulin-secreting cells. ACC activity was assayed in lysates from various insulin-secreting cells (RIN, HIT-T15, INS, and βTC-3). Glutamate (50 m*M*) and/or magnesium (10 m*M*) were included in the assay mixture. ACC activity units were expressed as n moles of malonyl–CoA formed per minute per mg β cell protein. Data in open bars represent control values, and those in solid bars represent values obtained in the presence of glutamate and magnesium. Data are mean ± SEM from two independent experiments carried out in triplicate. *Represents $p < 0.05$ vs control values obtained in the absence of glutamate and magnesium.

reported earlier that glutamate- and magnesium- mediated activation of ACC involves the intermediacy of a PP2A-like protein phosphatase *(30)*. Further evidence for such a formulation was obtained by immunoneutralization studies in which preincubation of β cell lysates with an antiserum directed against PP2Ac markedly reduced the ability of glutamate and magnesium to stimulate the ACC activity. Together, these data pointed out that the putative phosphatase might belong to the PP2A subfamily. Data from multiple laboratories *(31,32)*, including our own *(11)*, demonstrated that the catalytic subunit of PP2A (PP2Ac) undergoes post-translational methylation at its carboxyl terminal leucine and that such a signaling step promotes the formation of holoenzyme assembly and subsequent increase in the catalytic activity of this protein. Therefore, in the next series of experiments, we examined whether glutamate- and magnesium-mediated activation of ACC involves their regulation of the CML of PP2Ac.

Data in Fig. 2A indicate no significant effect of glutamate on the CML of PP2Ac. In contrast, magnesium markedly (nearly 2.5-fold) stimulated the CML of PP2Ac. Furthermore, a combination of glutamate and magnesium exerted no additional stimulatory effects on the CML of PP2Ac beyond the effects seen in the presence of magnesium alone. In addition, a profound inhibition in the CML of PP2Ac was seen in the presence of OKA under all experimental conditions

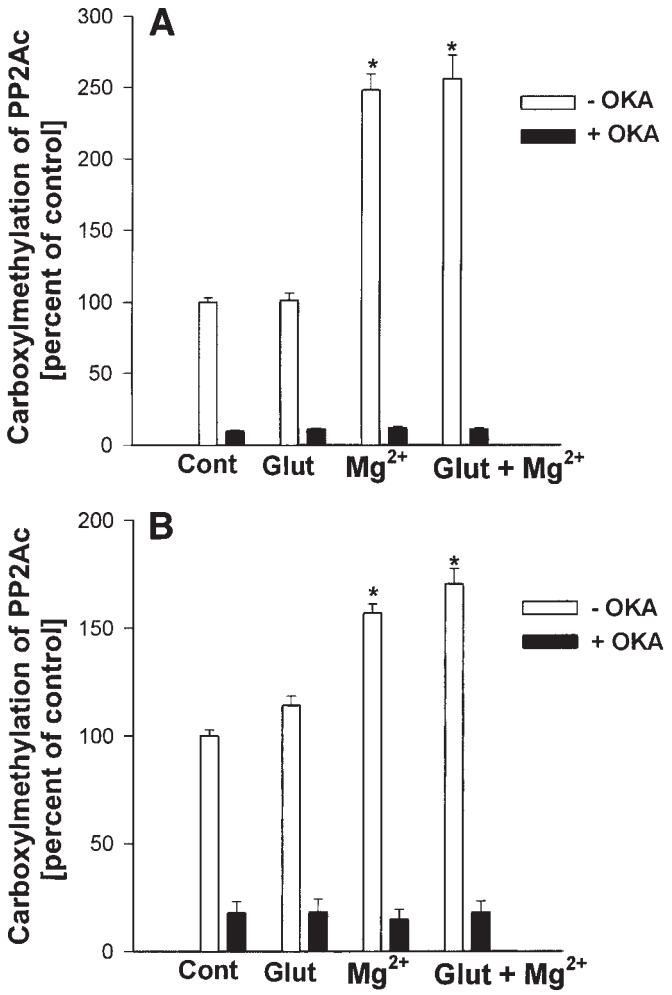

Fig. 2. Magnesium, but not glutamate, stimulates the carboxylmethylation of PP2Ac in INS-1 cells (**A**) and normal rat islets in an OKA-sensitive manner (**B**). The carboxylmethylation of the 36-kDa catalytic subunit was measured in lysates from INS-1 cells (**A**) or normal Sprague–Dawley rat islets (**B**) using ^{3}HS-adenosylmethionine as the methyl donor. Degree of the carboxylmethylation was determined by vapor-phase equilibration assay (see Methods for additional details). Magnesium (10 m*M*), glutamate (50 m*M*), and/or OKA (50 n*M*) were present as indicated. Data are expressed as percentage of control and are mean ± SEM from three individual experiments in triplicate. *Represents $p < 0.05$ vs control.

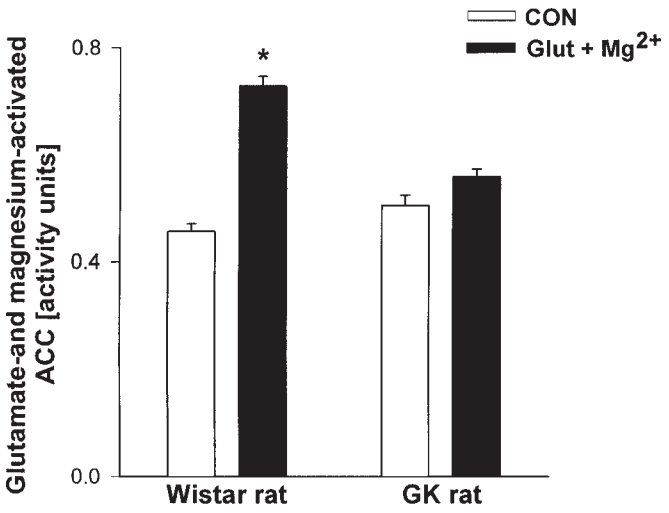

Fig. 3. Glutamate- and magnesium-mediated activation of ACC is markedly reduced in islets derived from the GK rat. ACC activity was assayed in lysates derived from Wistar (control) and GK rats as described under Methods. Glutamate (50 m*M*) and/or magnesium (10 m*M*) were included in the assay mixture. ACC activity units were expressed as *n* moles of malonyl–CoA formed per minute per mg islet protein. Data in open bars represent control values and those in solid bars represent values obtained in the presence of glutamate and magnesium. Data are mean ± SEM from three individual determinations. *Represents $p < 0.05$ vs control values obtained in the absence of glutamate and magnesium.

studied. These findings further strengthen our formulation that the C-terminal domain of PP2Ac is critical for GAPP function because OKA binds specifically to this region, thereby impeding the CML step *(9,11)*. It may also be pointed out that OKA markedly reduced glutamate- and magnesium-mediated stimulation of ACC activity under identical conditions in which it inhibited the CML of PP2Ac (Fig. 2A). These data further support the hypothesis that CML of the catalytic subunit of GAPP may be necessary for its activation and subsequent dephosphorylation of ACC. Virtually identical effects of magnesium and glutamate were seen on the CML of PP2Ac in lysates derived from normal rat islets (Fig. 2B). Together, our findings indicate that one of the primary effects of magnesium, but not glutamate, include activation of the CML of the catalytic subunit of GAPP. These findings also support earlier reports of divergent mechanisms of regulation of ACC activation by glutamate and magnesium in other cell types (see Discussion below).

Glutamate- and Magnesium-Mediated Activation of ACC is Markedly Reduced in Islets Derived from the Goto–Kakizaki Rat

Together, our above findings indicate that glutamate and magnesium activate ACC, which catalyzes the formation of malonyl–CoA, a precursor in the biosynthesis of long-chain fatty acids, which have been implicated in physiological insulin secretion *(33,34)*. Therefore, in the current study, we examined the functional activation of ACC by glutamate and magnesium in islets derived from the GK rat, a model for type 2 diabetes to determine whether insulin secretory abnormalities demonstrable in these animals are attributable at least in part to potential defects in glutamate- and magnesium-mediated activation of ACC. Data in Fig. 3 demonstrate significant stimulation by magnesium and glutamate of ACC activity in islet lysates from the control Wistar rats. However, glutamate and magnesium failed to augment ACC activity in islet lysates from the GK rat, suggesting potential abnormalities in GAPP-mediated ACC activation in GK islets.

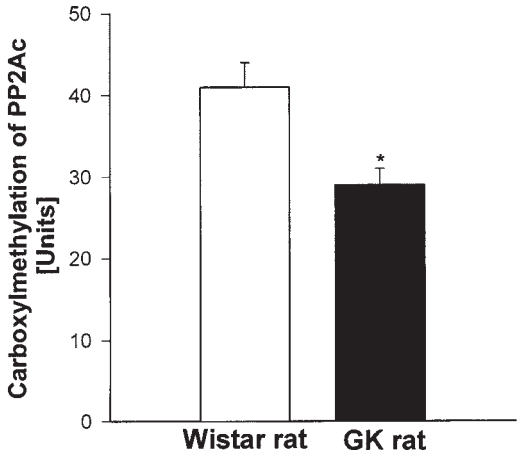

Fig. 4. The CML of PP2Ac is reduced in islets derived from the GK rat. The CML of PP2Ac was quantitated in lysates from Wistar and GK islets using [^{3}H]SAM as the methyl donor (see Methods for additional details). Relative degree of incorporation of methyl groups into the carboxyl terminal leucine of PP2Ac was quantitated by base-labile methanol assay. Data are expressed as units; defined as the amount of base-labile methanol released per μg islet protein. They represent mean ± SEM from three individual measurements. *Represents $p < 0.05$ vs control values obtained using Wistar islet lysates.

The CML of the Catalytic Subunit of PP2A Is Also Markedly Reduced in Islets Derived from the Goto–Kakizaki Rat

In the next series of experiments, we asked if the reduction in glutamate- and magnesium-stimulated ACC activity in the GK islets is due to alterations in the CML status of the catalytic subunit of PP2A in the GK islet. To examine this relative degree of CML, PP2Ac was quantitated in lysates from Wistar and GK islets using [^{3}H]SAM as the methyl donor (see Methods for additional details). Data in Fig. 4 indicate a marked reduction (30%) in the CML of PP2Ac in the GK islet. Together, our findings suggest potential alterations in glutamate-and magnesium-mediated functional activation of ACC in the diabetic GK islet. It appears that the CML status of the catalytic subunit of the putative phosphatase could contribute to such a functional defect in the diabetic islet (see below).

Discussion

One of the major aims of the current study was to understand potential regulatory mechanisms involved in glutamate- and magnesium-mediated activation of ACC activity in the islet β cell. Salient features of our current study are (a) glutamate- and magnesium-mediated activation of ACC is demonstrable in all insulin-secreting cells studied; (b) the mechanism of activation of ACC by magnesium, but not glutamate, might involve the post-translational CML of the catalytic subunit of GAPP, which we have shown to dephosphorylate and activate ACC; and (c) glutamate- and magnesium-mediated activation of ACC is markedly reduced in islets derived from the GK rat, an animal model for type 2 diabetes in humans.

Using HIT-T15 cells, we demonstrated previously *(30)* that a combination of glutamate and magnesium stimulates a PP2A-like protein phosphatase activity, and that such a phosphatase mediates the dephosphorylation and activation of ACC. Such a phosphatase was sensitive *(30)* to classical inhibitors of PP2A, such as OKA and microcystine *(9,11, 35)*. Furthermore, an antiserum directed against PP2Ac significantly attenuated GAPP-mediated activation of ACC *(30)*. Together, these data suggested that GAPP represents a PP2A-like phosphatase. The current study not only examined its regulation by post-translational CML, but also identified a potential defect in this activity in islets harvested from the diabetic GK rat.

Several previous studies *(31,32)*, including our own in the pancreatic β cell *(11)*, suggested important roles for post-translational CML of the catalytic subunit in the functional regulation of PP2A-like protein phosphatases. Although activation of its catalytic activity *per se* by CML is still debated *(17,36,37)*, it is widely believed that this modification facilitates the formation of PP2A holoenzyme *(17,38)*. In the present study, we have provided evidence to indicate that magnesium, but not glutamate, stimulates the CML of PP2Ac. Even though our earlier studies *(30)* in β cells have demonstrated significant potentiating effects of glutamate on GAPP-mediated activation of ACC, a combination of glutamate and magnesium had no appreciable effects on the CML of PP2Ac. These findings raise an interesting possibility that glutamate could exert regulatory effects on ACC at a step distinct from the CML, including direct allosteric effects on ACC. Such a formulation is compatible with earlier data from Boone's laboratory, which suggested that glutamate might induce ACC activation through complementary actions of phosphatase activation as well as a direct allosteric ligand for dephosphorylated ACC *(39)*. Additional studies are required to further verify these postulations in isolated β cells.

Our current findings also suggest significant abnormalities in the glutamate- and magnesium-mediated activation of ACC in islets derived from the GK rat. We observed very little stimulation of ACC in the presence of magnesium and glutamate in GK islets. To the best of our knowledge, this study is the first to demonstrate potential defects in PP2A-like enzymes in the diabetic β cell. It remains to be verified whether the abnormalities in such an activation step, which is mediated by GAPP, lie at the level of PP2A subunit expression or at the level of CML of PP2Ac in diabetic rat islets. Based on the evidence that we described herein (Fig. 4), it is likely that the CML status of PP2Ac could contribute toward the observed defect in glutamate- and magnesium-mediated activation of ACC in the GK islet. Future studies will need

to examine whether such reductions are attributable to decreased expression and/or functional activation of leucine carboxylmethyltransferase in the diabetic β cell.

Considerable degree of controversy still exists with regard to a "second messenger" role[s] for glutamate in physiological insulin secretion *(40,41)*. Recent data from MacDonald's laboratory estimated *(42)* intracellular glutamate levels to be around 10 m*M* (recalculated based on estimated intraislet volume of 1 nL and a protein concentration of 0.5 µg/islet). Furthermore, it has been shown that exposure of isolated islets to stimulatory glucose concentrations had minimal effects on intracellular glutamate levels *(42)*, albeit data from other laboratories suggest the contrary *(43)*. Nonetheless, estimated intracellular glutamate levels exceeded 80–100 m*M* in islets exposed to glutamine in the absence or presence of leucine (recalculated based on data given in ref. *42*). Our current findings of regulation of GAPP-mediated activation of ACC raise an interesting loci for glutamate's action in isolated β cell. We also suggest that it is not constitutively active in the islet, but requires an appropriate signal, including magnesium-mediated activation of its CML, for full functional activation of GAPP. Also, it should be kept in mind that such an activity is also finely controlled by other factors endogenous to the islet β cell, including the membrane-associated, heat-resistant, and trypsin-sensitive factors that we have identified to be present in the β cell membrane fraction *(30)*.

In conclusion, our current studies indicate that glutamate- and magnesium-mediated (i.e., GAPP-catalyzed) activation of ACC is operable in all insulin-secreting cells studied. Furthermore, our data suggest that activation of GAPP by glutamate and magnesium may, in part, be due to activation of its post-translational CML. Based on earlier data, we suggest that the role of glutamate is rather bimodal, in that it activates the phosphatase activity and also serves as a direct allosteric ligand for the dephosphorylated ACC as originally proposed by Boone et al. *(39)*. Finally, our data suggest a significant functional impairment in glutamate- and magnesium-mediated activation of ACC in the diabetic islet, which, in turn, could lead to an inhibition of biosynthesis of malonyl–CoA, a precursor for long-chain fatty acids, and subsequent insulin secretion. It is important to note that we observed comparable levels of ACC activity in the control and GK rat islets, and its ability to undergo activation by glutamate and magnesium, however, was markedly reduced in the GK islet. At least based on the information that we have at hand, it seems likely that the reduction in the activation of ACC in the diabetic islet may be due to alterations in the complexation of ACC with its putative phosphatase holoenzyme complex; such an interaction might well be under the control of the CML status of its catalytic subunit. Future studies will need to address these aspects governing potential interactions and cross-talk between ACC and its kinase-phosphatase cascade.

Materials and Methods

Materials

[^{14}C]Bicarbonate (50 mCi/mmol) and adenosyl-L-[^{3}H-methyl]methionine ^{3}H[SAM] were from NEN Life Science Products (Boston, MA). Okadaic acid was from Biomol (Plymouth Meeting, PA). Acetyl–CoA was from Sigma (St. Louis, MO). All other reagents used in this study were of highest purity available.

Sources of Clonal β Cells and Isolation of Cytosolic Fractions

HIT cells (passage 72) and RIN cells were purchased from American Type Culture Collection (Manassas, VA, USA). INS-1 cells were provided by Dr. GuoDong Li (Cardiovascular Research Institute, National University of Singapore, Singapore). βTC3 cells were provided by Dr. Shimon Efrat (Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel). Clonal β cells were cultured as previously described *(44,45)*. The cytosolic fractions from insulin-secreting cells were obtained by centrifugation of homogenates at 105,000*g* for 90 min (Ultima TL-100, Beckman, Palo Alto, CA) as described in refs. *46* and *47*.

Isolation of Islets from Control and Diabetic Rats

Pancreatic islets were isolated from male Sprague–Dawley rats (200–250 g body weight) by collagenase digestion method as we previously described *(48)*. Islets were manually picked twice under stereomicroscopic control to avoid any contamination by exocrine acinar tissue. GK rats were generously provided by Dr. Robert Farese (Tampa, FL). They were provided to our laboratory (at the Veterans Affairs Medical Center, Madison, WI) at the age of 8 wk and were then housed at our animal care facility. Offspring were allowed to reach the ages of 8–14 wk, when they were studied in parallel with age- and sex-matched Wistar rats (Harlan, Indianapolis, IN). As indicated in (ref. *48*) islets were isolated by collagenase digestion from control (Wistar) and diabetic (GK) rats. In brief, pancreases were inflated with collagenase solution (1 and 2 U/mL for Wistar and GK rats, respectively) in Hanks' balanced salt solution supplemented with 1% fetal bovine serum. After digestion with collagenase, islets were washed twice, passed through mesh (92-µm pore size), and then purified on a Ficoll gradient. Islets were then hand picked twice under stereomicroscopic control, to exclude extraislet debris. Also, as indicated in our earlier studies *(48)*, we made certain to isolate islets with grossly normal size and appearance to minimize any extrinsic artifacts of the isolation procedure and abnormal pancreatic morphology. Islets from control Wistar and diabetic GK rats were homogenized in a buffer consisting of 230 m*M* mannitol, 70 m*M* sucrose, and 5 m*M* HEPES, pH 7.4, containing 1 m*M* EGTA, 1 m*M* DTT, and 2.5 µg/mL each of leupeptin and pepstatin. All the experimental protocols involving the use of isolated islets from control Wistar rats and the

diabetic GK rats were approved by the appropriate Animal Care and Use Committee.

Assay of Acetyl–CoA Carboxylase

ACC activity was assayed in β cell lysates by the [^{14}C]bicarbonate fixation assay as we described in ref. *30*. The reaction mixture [total volume; 200 μL] consisted of 60 m*M* HEPES, pH 7.5, 1.2 m*M* DTT, 300 μ*M* acetyl–CoA, 3 m*M* sodium citrate, fatty-acid-free bovine serum albumin (1 mg/mL final concentration), and β cell protein, as indicated in the text. Magnesium and glutamate were included in the appropriate concentrations noted in the text. The reaction was initiated by the addition of [^{14}C]sodium bicarbonate (18 m*M* final, 2 μCi per tube) and was carried out at 37°C for 30 min. The reaction was terminated by the addition of perchloroacetic acid. The contents of the tube were mixed vigorously and left on ice for 15 min, then spun at 9300*g* for 8–10 min in an Eppendorf centrifuge. Next, 200 μL of the supernatant was transferred into a scintillation vial and dried under mild heat. Residue in the vial was dissolved in 400 μL distilled water, and radioactivity was quantitated by scintillation spectrometry. The specific activity of ACC was expressed as nanomoles of malonyl–Co A formed per min/mg protein.

Carboxylmethylation of PP2Ac

The reaction mixture (100 μL) consisted of 100 m*M* Tris-HCl, pH 7.5, 1 m*M* EDTA, 4 m*M* DTT, and β cell protein (25–30 μg). The reaction was initiated by the addition of ^{3}HSAM (100 μCi/mL; 0.7 μ*M*) and continued for 60 min at 37°C as indicated in the text. The reaction was terminated by the addition of SDS-PAGE sample buffer, and labeled proteins were separated by SDS-PAGE and identified by base-labile methanol release assay (see below).

Vapor-Phase Equilibration Assay

The α-carboxyl methyl group on the leucine-309 residue of modified PP2Ac is base labile *(18,31,49,50)*. To quantify this labeling, individual lanes of dried gels were cut in to slices (3–5 mm) and placed in 1.5 mL Eppendorf tubes (without caps) containing 350–500 μL of 1 *N* NaOH. Tubes were then placed in 20 mL scintillation vials containing 5 mL scintillant (Ultima Gold, Packard Instrument Co, Meriden, CT), and the vials were then capped and left at 37°C overnight to facilitate the base-catalyzed hydrolysis of methyl esters and the equilibration of released [^{3}H]methanol. After the incubation, the tubes were gently removed from the vials, and the sides of the tubes were rinsed (into the vials) with an additional 2 mL scintillant; the radioactivity was quantitated by scintillation spectrometry *(51,52)*.

Other Methods

Protein was assayed by a dye-binding method as described in ref. *53*. Significance of differences between the control and experimental groups was determined by Student's t-test. A p value <0.05 was considered significant.

Acknowledgments

These studies were supported by grants from the Department of Veterans Affairs (Merit Review and REAP awards), the National Institutes of Health (DK-56005), and the American Diabetes Association. A.K. is the recipient of a Research Career Scientist award from the Medical Research Service of the Department of Veterans Affairs. Experiments relating to abnormalities in GAPP function in GK rat islets were performed during A.K.'s stay at the VA Medical Center in Madison, WI. The authors thank Hai-Qing Chen and Sarah Durr for help with cell cultures, and Mary Rabaglia for isolation of islets from the control Wistar and diabetic GK rats.

References

1. Jones, P. M. and Persaud, S. J. (1998). *Endocrine Rev.* **19,** 429–461.
2. Ascroft, S. J. H. (1994). *Diabetologia* **37,** s21–s29.
3. Lester, L. B., Faux, M. C., Nauert, J. B., and Scott, J. D. (2001). *Endocrinology* **142,** 1218–1227.
4. Lang, J. (1999). *Eur. J. Biochem.* **259,** 3–17.
5. Kowluru, A. and MacDonald, M. J. (1984). *Arch. Biochem. Biophys.* **231,** 320–327.
6. Sjoholm, A., Honkanen, R. E., and Berggren, P. O. (1993). *Biosci. Rep.* **13,** 349–358.
7. Ammala, C., Eliasson, L., Bokvist, K., et al. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 4343–4347.
8. Ammon, H. P., Heurich, R. O., Kolb, H. A., et al. (1996). *Naunyn-Schmiedeber's Arch. Pharmacol.* **354,** 95–101.
9. Ratcliff, H. and Jones, P. M. (1993). *Biochem. Biophys. Acta* **1175,** 188–191.
10. Tamagawa, T., Iguchi, A., Uemura, K., Miura, H., and Nonogaki, K. (1992). *Endocrinol. Jpn.* **39,** 325–339.
11. Kowluru, A., Seavey, S. E., Rabaglia, M. E., Nesher, R., and Metz, S. A. (1996). *Endocrinology* **137,** 2315–2323.
12. Strack, S. (2002). *J. Biol. Chem.* **277,** 41525–41532.
13. Fellner, T., Lackner, D. H., Hombauer, H., et al. (2003). *Genes Dev.* **17,** 2138–2150.
14. Leulliot, N., Quevillon-Cheruel, S., Sorel, I., et al. (2004). *J. Biol. Chem.* **279,** 8351–8358.
15. Chen, J., Martin, B. L., and Brautigan, D. L. (1992). *Science* **257,** 1261–1264.
16. Chen, J., Parsons, S., and Brautigan, D. L. (1994). *J. Biol. Chem.* **269,** 7957–7962.
17. Tolstykh, T., Lee, J., Vafai, S., and Stock, J. B. (2000). *EMBO J.* **19,** 5682–5691.
18. Xie, H. and Clarke, S. (1994). *J. Biol. Chem.* **269,** 1981–1984.
19. Cohen, P. (1989). *Ann. Rev. Biochem.* **58,** 453–508.
20. Wera, S. and Hemmings, B. A. (1995). *Biochem. J.* **311,** 17–29.
21. Sjoholm, A., Honkanen, R. E., and Berggren, P. O. (1995). *Endocrinology* **136,** 3391–3397.
22. Sjoholm, A., Berggren, P. O., and Honkanen, R. E. (2001). *Biochem. Biophys. Res. Commun.* **283,** 364–368.
23. Sjoholm, A., Lehtihet, M., Efanov, A. M., Zaitsev, S. V., Berggren, P. O., and Honkanen, R. E. (2002). *Endocrinology* **143,** 4592–4598.
24. Palanivel, R., Veluthakal, R., and Kowluru, A. (2004). *Am. J. Physiol. Endocrinol. Metab.* **286,** E1032–E1041.
25. Witters, L. A., Moriarity, D., and Martin, D. B. (1979). *J. Biol. Chem.* **254,** 6644–6649.
26. Denton, R. M. (1986). *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **20,** 293–341.
27. Davies, S. P., Sim, A. T., and Hardie, D. G. (1990). *Eur. J. Biochem.* **12,** 183–190.

28. Hardie, D. G. and Carling, D. (1997). *Eur. J. Biochem.* **246,** 259–273.
29. Gaussin, V., Hue, L., Stalmans, W., and Bollen, M. (1996). *Biochem. J.* **15,** 217–224.
30. Kowluru, A., Chen, H. Q., Modrick, L. M., and Stefanelli, C. (2001). *Diabetes* **50,** 1580–1587.
31. Favre, B., Zolnierowicz, S., Turowski, P., and Hemmings, B. A. (1994). *J. Biol. Chem.* **269,** 16311–16317.
32. Bryant, J. C., Westphal, R. S., and Wadzinski, B. E. (1999). *Biochem. J.* **339,** 241–246.
33. Prentki, M. and Corkey, B. E. (1996). *Diabetes* **45,** 273–283.
34. Prentki, M. (1996). *Eur. J. Endocrinol.* **134,** 272–286.
35. Li, M. and Damuni, Z. (1994). *Biochem. Biophys. Res. Commun.* **202,** 1023–1030.
36. De Baere, I., Derua, R., Janssens, V., et al. (1999). *Biochemistry* **38,** 16539–16547.
37. Zhu, T., Matsuzawa, S., Mizuno, Y., et al. (1997). *Arch. Biochem. Biophys.* **339,** 210–217.
38. Wu, J., Tolstykh, T., Lee, J., Boyd, K., Stock, J. B., and Broach, J. R. (2000). *EMBO J.* **19,** 5672–5681.
39. Boone, A. N., Chan, A., Kulpa, J. E., and Brownsey, R. W. (2000). *J. Biol. Chem.* **275,** 10819–10825.
40. Moriyama, Y. and Hayashi, M. (2003). *Trends Pharmacol. Sci.* **24,** 511–517.
41. MacDonald, M. J. and Fahien, L. A. (2000). *J. Biol. Chem.* **275,** 34025–34027.
42. MacDonald, M. J. (2003). *Biochim. Biophys. Acta* **1619,** 77–88.
43. Wollheim, C. B. and Maechler, P. (2002). *Diabetes* **51(Suppl. 1),** S37–S42.
44. Zhang, H. J., Walseth, T. F., and Robertson, R. P. (1989). *Diabetes* **38,** 44–48.
45. Asfari, M., Janjic, D., Meda, P., Li, G., Halban, P. A., and Wollheim, C. B. (1992). *Endocrinology* **130,** 167–178.
46. Kowluru, A., Rabaglia, M. E., Muse, K., and Metz, S. A. (1994). *Biochem. Biophys. Acta* **1222,** 348–359.
47. Kowluru, A., Seavy, S. E., Rabaglia, M. E., and Metz, S. A. (1995). *Biochem. Pharmacol.* **49,** 263–266.
48. Metz, S. A., Meredith, M., Vadakekalam, J., Rabaglia, M. E., and Kowluru, A. (1999). *Diabetes* **48,** 1754–1762.
49. Lee, J. and Stock, J. (1993). *J. Biol. Chem.* **268,** 19192–19195.
50. Xie, H. and Clarke, S. (1994). *Biochem. Biophys. Res. Commun.* **203,** 1710–1715.
51. Kowluru, A., Seavey, S. E., Li, G., et al. (1996). *J. Clin. Invest.* **98,** 540–555.
52. Kowluru, A., Li, G., and Metz, S. A. (1997). *J. Clin. Invest.* **100,** 1596–1610.
53. Kowluru, A., Seavey, S. E., Rhodes, C. J., and Metz, S. A. (1996). *Biochem. J.* **313,** 97–107.