BMB
Reports

Inhibition of glutamate dehydrogenase and insulin secretion by KHG26377 does not involve ADP-ribosylation by SIRT4 or deacetylation by SIRT3

Eun-A Kim[1,#], *Seung-Ju Yang*[2,#], *Soo Young Choi*[3], *Woo Je Lee*[4,*] *& Sung-Woo Cho*[1,*]

[1]Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul 138-736, [2]Department of Biomedical Laboratory Science, Konyang University, Daejeon 302-718, [3]Department of Biomedical Science, Research Institute for Bioscience and Biotechnology, and Medical & Bio-material Research Center, Hallym University, Chunchon 200-702, [4]Department of Internal Medicine, University of Ulsan College of Medicine, Seoul 138-736, Korea

We investigated the mechanisms involved in KHG26377 regulation of glutamate dehydrogenase (GDH) activity, focusing on the roles of SIRT4 and SIRT3. Intraperitoneal injection of mice with KHG26377 reduced GDH activity with concomitant repression of glucose-induced insulin secretion. Consistent with their known functions, SIRT4 ribosylated GDH and reduced its activity, and SIRT3 deacetylated GDH, increasing its activity. However, KHG26377 did not affect SIRT4-mediated ADP-ribosylation/inhibition or SIRT3-mediated deacetylation/activation of GDH. KHG26377 had no effect on SIRT4 protein levels, and did not alter total GDH, acetylated GDH, or SIRT3 protein levels in pancreatic mitochondrial lysates. These results suggest that the mechanism by which KHG26377 inhibits GDH activity and insulin secretion does not involve ADP-ribosylation of GDH by SIRT4 or deacetylation of GDH by SIRT3. [BMB Reports 2012; 45(8): 458-463]

INTRODUCTION

Mammalian glutamate dehydrogenase (GDH) (EC 1.4.1.3) is a mitochondrial enzyme that reversibly catalyzes deamination of L-glutamate to 2-oxoglutarate using either NAD^+ or $NADP^+$ as a coenzyme (1). It has been reported that allosteric activation of GDH causes insulin release, suggesting that GDH may play a role in the pathophysiology of pancreatic β cells (2). The expression levels of GDH correlate with the rate of glutamate-stimulated exocytosis in β cells (3). Moreover, overexpression of constitutively activated GDH induces insulin secretion through enhanced glutamate oxidation (4). Also, GDH overexpression in rat islets potentiates insulin release at high glucose concentrations (5). In transgenic mice lacking GDH specifically in β cells, the resulting decrease in insulin secretory capacity decreases plasma insulin levels and diminishes the response to both feeding and glucose load (6). Inhibition of GDH activity has been shown to decrease insulin release (7), whereas activating mutations in GDH are associated with a hyperinsulinism syndrome (8, 9). Many mutation sites are located in the GTP-binding site and likely act by interfering with GTP binding (10, 11). Therefore, compounds that specifically target GDH could be therapeutically useful if they also inhibited the hyperinsulinism mutant forms of GDH (12).

It is known that GDH is regulated by reversible ADP-ribosylation (13). Although many researchers have studied ADP-ribosylation of mitochondrial proteins (13-17), the identity of proteins involved remained unknown until Herrero-Yraola *et al*. (13) showed that GDH is an actual target of this modification. SIRT4 is a mitochondrial enzyme that uses NAD^+ to ADP-ribosylate and downregulate GDH activity (18). Interestingly, small interfering RNA (siRNA)-mediated knockdown of SIRT4 enhances insulin secretion in β cells (19). SIRT4 is one of seven sirtuin protein family members (SIRT1-SIRT7) that share a catalytic domain with Sir2 (silent information regulator 2), an NAD^+-dependent protein deacetylase that controls longevity in lower eukaryotes. Another sirtuin family member, SIRT3, is a major mitochondrial deacetylase that controls the reversible lysine acetylation status of mitochondrial proteins (20, 21). GDH is a substrate of SIRT3, which can deacetylate and thereby stimulate GDH enzymatic activity (20, 21). The N- and C-terminal regions of SIRT3 influence its activity against GDH and peptide substrates, indicating that these regions play roles in regulation and substrate recognition (21).

Our recent study showed that KHG26377 (2-cyclopropylimino-3-methyl-1,3-thiazoline hydrochloride) inhibits GDH activity

*Corresponding authors. Woo Je Lee, Tel: +82-2-3010-5882; Fax: +82-2-3010-4278; E-mail: wjlee@amc.seoul.kr, Sung-Woo Cho, Tel: +82-2-3010-4278; Fax: +82-2-3010-4278; E-mail: swcho@amc.seoul.kr
#These authors contributed equally to this article.
http://dx.doi.org/10.5483/BMBRep.2012.45.8.040

Received 23 February 2012, Revised 2 March 2012, Accepted 12 March 2012

Keyword: ADP-ribosylation, Deacetylation, Glutamate dehydrogenase, Hyperinsulinism, Thiazole derivatives

and downregulates insulin secretion in pancreatic islets (22). However, its mechanisms of action have not yet been elucidated. In the current study, we have further investigated the regulatory mechanisms underlying the effects of KHG26377 on GDH activity.

RESULTS AND DISCUSSION

Effects of KHG26377 on GDH activity and insulin secretion *in vivo*

A number of recent studies have used inhibitors to decrease GDH activity in pancreatic islets (7, 12, 22-26). Upon glucose stimulation, GDH inhibition results in both diminished insulin release and decreased cellular glutamate levels (26). Green tea polyphenols inhibit GDH and reduce insulin release when pancreatic islets are stimulated with glutamine and BCH (2-amino-2-norbornane carboxylic acid), although not upon glucose stimulation (12). We previously reported the loss of GDH activity following perfusion of islets with KHG26377, a thiazole derivative, *in vitro* (22). KHG26377 also reduced the amount of insulin released and caused a rightward shift in the concentration dependence of glucose-induced insulin secretion in islets (22). To further investigate the mechanism and effects of KHG26377 on GDH activity and insulin secretion *in vivo*, we injected mice intraperitoneally with different concentrations of KHG26377. In agreement with our previous *in vitro* results (22), GDH activity was markedly reduced in a concentration-dependent manner in the KHG26377-treated group (Fig. 1A). KHG26377 injection also repressed glucose-induced insulin secretion *in vivo* (Fig. 1B), supporting the idea that KHG26377 inhibits insulin secretion by repressing GDH activity. Enzyme kinetic studies were performed to provide a more detailed comparison. As shown in Table 1, V_{max} was significantly reduced (up to 35%) in pancreatic islets from KHG26377-treated mice. However, no significant differences in K_m values for substrate and coenzyme were shown between the KHG26377-treated group and the control group (Table 1).

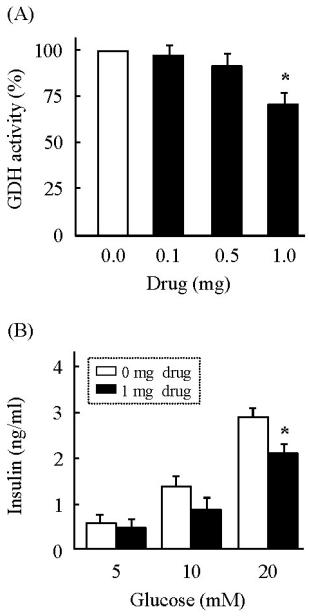

Fig. 1. Effects of KHG26377 on GDH activity and insulin secretion *in vivo*. (A) KHG26377 inhibits GDH activity *in vivo*. GDH activity was measured in isolated pancreatic islets from control and KHG26377-treated mice (n = 3 each). (B) KHG26377 downregulates insulin secretion *in vivo*. Insulin secretion was measured in isolated pancreatic islets from control and KHG26377-treated mice (n = 3 each) stimulated with glucose (5-20 mM). In this and all other figures, values are expressed as means ± standard errors of the mean (SEMs). *Significantly different between the two groups ($P < 0.05$).

Effects of KHG26377 on recombinant SIRT4-mediated ADP-ribosylation and inhibition of bovine GDH activity

Because KHG26377 did not inhibit GDH activity directly (data not shown), we surmised that some other mechanism plays a major role in the regulation of insulin secretion. Numerous reports have shown that GDH is a key enzyme in the control of insulin secretion in pancreatic β cells; moreover, mutations of GDH are associated with a hyperinsulinism syndrome (7-9). In addition to being modulated by allosteric regulation, GDH is known to be regulated by reversible cysteine-specific ADP-ribosylation in mitochondria (13, 25). According to these reports, ADP-ribosylation substantially inhibits GDH catalytic activity, and ADP-ribosylated GDH can be reactivated by an Mg^{2+}-dependent mitochondrial ADP-ribosylcysteine hydrolase (13, 25). Cys-119 might have an important role in the regulation of hGDH isoenzymes by ADP-ribosylation (25). SIRT4, the mammalian Sir2 homolog, was revealed as a mitochondrial ADP-ribosyltransferase that modifies GDH, and thereby affects insulin secretion and amino acid metabolism in the pancreas (18, 19, 27). In particular, it has been shown that glucose-stimulated insulin secretion is increased by SIRT4 downregulation and decreased by SIRT4 over-expression (19). Mono-ADP-ribosylated proteins can be generated by two mechanisms: through direct enzymatic transfer of ADP-ribose from

Table 1. Effects of KHG26377 on GDH activity *in vivo*

Parameters	Control	KHG26377
V_{max} (units/mg protein)	0.65 ± 0.07	0.42 ± 0.05*
$K_{m(NADH)}$ (μM)	33.05 ± 1.02	33.34 ± 0.97
$K_{m(Ammonia)}$ (mM)	7.01 ± 0.69	6.81 ± 0.75
$K_{m(Glutamate)}$ (mM)	6.53 ± 0.29	6.10 ± 0.40
$K_{m(\alpha\text{-Ketoglutarate})}$ (mM)	2.16 ± 0.36	1.99 ± 0.29

GDH activity was measured in isolated pancreatic islets from control or KHG26377 (1 mg)-treated mice. Values are expressed as means ± standard errors of the mean (SEMs). *Significantly different between the two groups ($P < 0.05$).

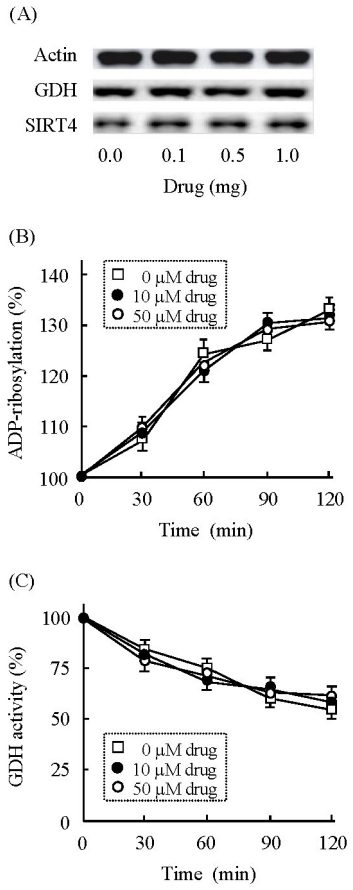

Fig. 2. Effects of KHG26377 on recombinant SIRT4-mediated ADP-ribosylation and inhibition of bovine GDH activity. (A) Effects of KHG26377 on the expression of GDH and SIRT4 protein in isolated pancreatic islets from control and KHG26377-treated mice. Western blotting was performed with anti-GDH and anti-SIRT4 antibodies. (B, C) Purified bovine brain GDH was ADP-ribosylated by recombinant SIRT4 (2 μg) with 0.1 mM NAD^+ in the presence and absence of different concentrations of KHG26377. Samples were withdrawn at the indicated times for assay of ADP-ribosylation (B) and GDH activity (C). Results are expressed as a percentage of the respective control.

NAD^+, and via a noncatalytic reaction with free ADP-ribose (15). According to Haigis *et al*. (18), SIRT4 does not function as an NAD^+ glycohydrolase to cleave NAD^+, but instead likely ADP-ribosylates GDH through direct transfer of ADP-ribose from NAD^+. Thus, it is reasonable to speculate that SIRT4 might also play a role in KHG26377-induced GDH inhibition and insulin repression. To test this possibility, we compared the protein expression level of GDH and SIRT4 in isolated pancreatic islets from control and KHG26377-treated mice by Western blotting. Our results, however, showed that SIRT4 and GDH protein expression were both unchanged in isolated pancreatic islets from KHG26377-treated mice (Fig. 2A), suggesting that KHG26377 does not in itself affect SIRT4 protein expression.

We next investigated whether ADP-ribosylation of GDH by recombinant SIRT4 was affected by KHG26377 in the presence of NAD^+ *in vitro*. Recombinant SIRT4 showed ADP-ribosyltransferase activity toward purified bovine brain GDH (Fig. 2B), an activity that was inhibited by nicotinamide (data not shown). These results are consistent with previous reports by Haigis *et al*. (18). However, KHG26377 showed no effects on the ADP-ribosylation of GDH (Fig. 2B). Since ADP-ribosylation is known to inhibit GDH activity (13), we further determined whether SIRT4 inhibited GDH activity in the presence of NAD^+. After a 2-h incubation, an aliquot of the reaction was removed for measurement of GDH activity. SIRT4 reduced the enzymatic activity of GDH by up to 40% (Fig. 2C). Moreover, this inhibitory effect of SIRT4 was lost in the absence of NAD^+ or in the presence of nicotinamide (data not shown), suggesting that ADP-ribosylation by SIRT4 is required for GDH inhibition. Once again, KHG26377 did not affect SIRT4-mediated inhibition of GDH activity (Fig. 2C). These results demonstrate that the reduction in GDH activity and subsequent insulin secretion by KHG26377 *in vivo* (Fig. 1) was not directly related to the ADP-ribosylase activity of SIRT4, although we cannot exclude the possibility that another mitochondrial ADP-ribosyltransferase is affected by KHG26377.

Effects of KHG26377 on recombinant SIRT3-mediated deacetylation and activation of GDH

Reversible deacetylation by SIRT3 is also known to regulate GDH. SIRT3 is a soluble mitochondrial protein that controls global mitochondrial protein acetylation levels. Schlicker *et al*. (21) identified GDH as one target of SIRT3. Although the functional significance of GDH acetylation is unclear, chemical acetylation has been shown to reduce the enzymatic activity of GDH (20, 21). In this study, we investigated whether the decrease in GDH activity induced by KHG26377, shown in Fig. 1, was related to SIRT3-mediated deacetylation. In these experiments, we measured total GDH, acetylated GDH, and SIRT3 protein expression in pancreatic mitochondrial lysates from control and KHG26377-treated mice. Pancreatic mitochondrial lysates were immunoprecipitated with an anti-GDH-conjugated resin, and immunoprecipitates were probed with anti-acetyl-lysine antibodies to detect acetylated GDH (Ac-GDH) and with GDH antibodies to detect total GDH (t-GDH). SIRT3 in pancreatic mitochondrial lysates was also detected by immunoblotting with an anti-SIRT3 antibody. KHG26377 treatment did not change the levels of total GDH, acetylated GDH, or SIRT3 proteins in pancreatic mitochondrial lysates (Fig. 3A and B).

It was previously shown that GDH is acetylated in a feeding-dependent manner (28). It is also known that GDH and SIRT3 are co-localized in the mitochondrial matrix (29), and that GDH from SIRT3 knockout mice is hyperacetylated com-

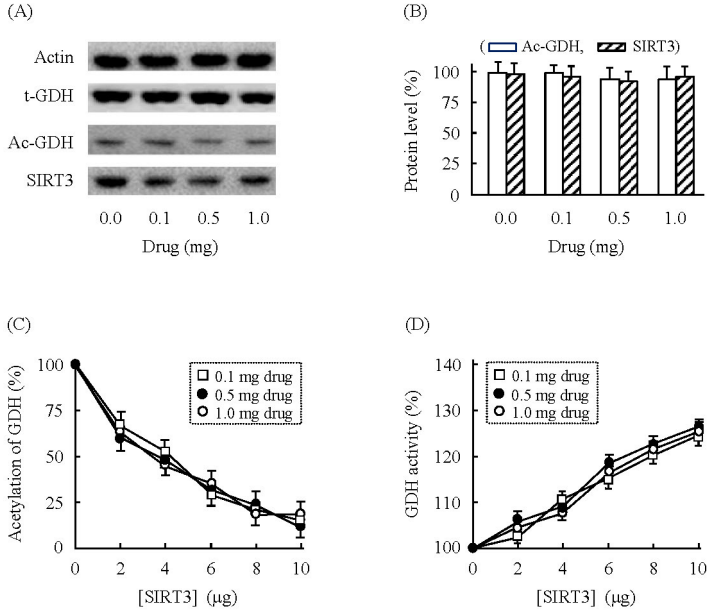

Fig. 3. Effects of KHG26377 on recombinant SIRT3-mediated deacetylation and activation of GDH. (A) Effects of KHG26377 on the expression of total GDH, acetylated GDH, and SIRT3 protein in pancreatic mitochondrial lysates from control and KHG26377- treated mice. Pancreatic mitochondrial lysates were immunoprecipitated with anti-GDH-conjugated resin, washed, and immunoblotted with anti-acetyl-lysine antibodies to detect acetylated GDH (Ac-GDH) and with anti-GDH antibodies to detect total GDH (t-GDH). Pancreatic mitochondrial lysates were also probed for SIRT3 by immunoblotting with anti-SIRT3 antibodies. (B) Densitometric analysis of Ac-GDH and SIRT3 expression levels shown in (A). (C, D) Effects of KHG 26377 on recombinant SIRT3-mediated deacetylation (C) and activation (D) of GDH obtained from control and KHG26377-treated mice. Results are expressed as a percentage of the respective control.

pared to protein from wild-type mice (20). Moreover, GDH isolated from mammalian mitochondria in its partly acetylated form has been tested as a SIRT3 substrate *in vitro* in an ELISA system using an antibody specific for acetylated lysine (21). Using such an ELISA, we found that recombinant SIRT3 deacetylated GDH isolated from mitochondria (Fig. 3C). However, KHG26377 had no effect on SIRT3-mediated deacetylation of GDH (Fig. 3C). We next tested the effect of deacetylation on GDH activity and found that the deacetylation of GDH caused by incubation with recombinant SIRT3 and NAD^+ increased GDH activity by up to 26% in a concentration dependent manner (Fig. 3D). Once again, KHG26377 had no effect on SIRT3-mediated activation of GDH (Fig. 3D).

The results presented here show that intraperitoneal injection of mice with KHG26377 significantly reduces GDH activity with concomitant repression of glucose-induced insulin secretion. These results are consistent with our previous studies showing that KHG26377 inhibits GDH activity and down-regulates insulin secretion in cultured islets (22). However, despite the fact that GDH is a known substrate of SIRT3 and SIRT4 (18-21), the effects of KHG26377 on GDH activity and insulin secretion *in vivo* do not involve the regulation of GDH through SIRT4-mediated ADP-ribosylation or SIRT3-mediated deacetylation. The actual regulatory mechanism involved in KHG26377-induced GDH inhibition remains to be elucidated.

MATERIALS AND METHODS

Materials

Insulin, dexamethasone, NAD^+, NADH, α-ketoglutarate, and L-glutamate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Insulin-free bovine serum albumin fraction, guinea pig anti-porcine insulin serum, and rabbit anti-guinea pig globulin serum were from the Cell Signalling Technology (Beverly, MA, USA) Fetal calf serum and Dulbecco's Modified Eagle's Medium were obtained from Gibco-BRL Life Technologies (Rockville, MD, USA) All other chemicals and solvents were of reagent grade or better. Bovine brain GDH and anti-GDH monoclonal antibodies were produced in our laboratory as previously described (23, 24). Recombinant human SIRT3 was purchased from Cayman Chemical (Ann Arbor, MI, USA), and recombinant human SIRT4 was purchased from Sigma-Aldrich. Antibodies against SIRT3 and SIRT4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibodies against acetyl-lysine were purchased from Cell Signaling Technology (Danvers, MA, USA). KHG26377 was synthesized as described previously (22).

Animal experiments

Three-month-old C57BL/6 mice (Harlan Sprague Dawley, Indianapolis, IN, USA) were housed in a pathogen-free facility under an approximate 12-h light/dark cycle with *ad libitum* access to food and water. The study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Asan Institute for Life Sciences, Asan Medical Center, which abides by the Institute of Laboratory Animal Resources (ILAR) guide. Mice were injected intraperitoneally with different doses of KHG26377. After 10 days, mice were sacrificed and organs were harvested and homogenized. Mitochondrial extracts were prepared by a modification of a previous method

(7). Islets were isolated from the pancreas by standard collagenase digestion and separated from the pancreas by centrifugation on a Ficoll gradient. Only islets that were undamaged and completely free of acinar and connective tissue were used. In experiments, isolated islets were cultured for 1 h in RPMI 1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in an atmosphere of humidified 95% air and 5% CO_2 at 37°C. Insulin secretion was stimulated by incubating islets in Krebs-Ringer bicarbonate solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM potassium phosphate, 1.16 mM magnesium sulfate, and 25 mM $NaHCO_3$, pH 7.4) containing different concentrations of glucose in the absence and presence of KHG26377 (1 mg) at 37°C for 1-2 h. Total islet insulin was measured in islets treated with ethanol overnight at −20°C. GDH activity was measured spectrophotometrically in both the direction of reductive amination of 2-oxoglutarate and in the direction of oxidative deamination of glutamate (23). For kinetic studies, K_m and V_{max} were calculated by linear regression of double-reciprocal plots and are expressed with standard errors.

ADP-ribosylation of GDH

Isolated pancreatic islets from control and KHG26377-treated mice were lysed in 0.1 ml of PRO-PREP protein extraction solution, and the lysate was incubated on ice with intermittent vortexing, followed by centrifugation at 14,000 rpm for 15 min at 4°C. The crude extracts from each group were diluted with loading buffer, boiled, and loaded onto 12% sodium dodecyl sulfate-polyacrylamide gels. Samples were electrophoresed and then transferred to a nitrocellulose membrane for Western blotting using monoclonal antibodies against GDH and SIRT4.

ADP-ribosylation of GDH was investigated with and without KHG26377 (10 μM and 50 μM), as described previously (25), by incubating 1 μg of recombinant SIRT4 with bovine brain GDH in 0.1 mM NAD^+, 50 mM MOPS-KOH (pH 7.5), 10 mM DTT, and 2 mM EDTA at 30°C. After incubation at 30°C for the indicated times, aliquots were withdrawn for measurement of remaining GDH activity, as described above. Non-enzymatic ADP-ribosylation was studied under the same conditions using ADP-ribose instead of NAD^+. For the analysis of ADP-ribosylated GDH, 0.1 mM [^{32}P]NAD^+ (10 μCi/ml) was used instead of NAD^+ and the reaction was stopped at the indicated times by acetone precipitation. The pellets were analyzed by Western blotting using anti-GDH antibodies, and incorporation of radiolabel into GDH was analyzed by autoradiography and densitometry.

Deacetylation of GDH

Pancreatic mitochondrial lysates were lysed for 30 min at 4°C in ice-cold NP-40 buffer (50 mM Tris [pH 8.0], 150 mM NaCl, 1% NP-40) containing complete EDTA-free protease inhibitors (Roche Molecular Biochemicals, Indianapolis, IN, USA) and 1 mM DTT. The lysate was clarified by centrifugation for 15 min at 4°C at 14,000 rpm in a tabletop centrifuge and immunoprecipitated with resin-conjugated anti-GDH. Immunoprecipitates were washed and incubated at 4°C for 3 h with primary anti-acetyl-lysine antibodies, for detection of acetylated GDH (Ac-GDH), and with anti-GDH antibodies, for detection total GDH (t-GDH). After washing three times in NP1 buffer (1% NP-40, 300 mM NaCl, 0.5 mM EDTA, 50 mM Tris-HCl, pH 7.4), immunoprecipitates were analyzed by Western blotting. Pancreatic mitochondrial lysates were also probed for SIRT3 by immunoblotting with anti-SIRT3 antibodies.

For the measurement of deacetylation of GDH by SIRT3, the acetylated GDH obtained from each group was bound to 96-well microliter plates by incubating at 4°C overnight. After washing with TBST (50 mM Tris [pH 7.4], 150 mM NaCl, and 0.1% Tween-20), plates were incubated for 1 h at room temperature with blocking buffer (TBST buffer containing 3% powdered skim milk). Plates were washed with TBST and Tris-buffered saline, and incubated with varying amounts of recombinant SIRT3 and 0.5 mM NAD^+ at 37°C for 45 min. The plates were washed again with TBST and Tris-buffered saline. Acetylated lysines were detected by adding a polyclonal anti-acetyl lysine antibody (1 : 1,000 dilution) and incubating for 2 h at room temperature. After washing with TBST, aliquots were withdrawn for the detection of remaining acetylated lysines with horseradish-peroxidase-conjugated anti-rabbit IgG and 3,3',5,5'-tetramethylbenzidine. After 5 min, the reaction was stopped with 1 M H_2SO_4 and optical density at 450 nm was measured with a microplate reader. For each aliquot, GDH activity was also measured as described above.

Statistical analysis

Statistical analyses were performed using Student's *t*-test or, where applicable, ANOVA followed by Student's *t*-test. Unless otherwise specified, each experimental point represents the mean of triplicate determinations on separate preparations, and standard deviations are indicated by error bars in each figure.

Acknowledgements

This work was supported by Bumsuk Academic Research Fund in 2010.

REFERENCES

1. Katagiri, M. and Nakamura, M. (2003) Reappraisal of the 20th-century version of amino acid metabolism. *Biochem. Biophys. Res. Commun.* **312**, 205-208.
2. Sener, A. and Malaisse, W. J. (1980) L-Leucine and a non-metabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* **288**, 187-189.
3. Høy, M. H., Maechler, P., Efanov, A. M., Wollheim, C. B., Berggren, P. O. and Gromada, J. (2002) Increase in cellular glutamate levels stimulates exocytosis in pancreatic β-cells. *FEBS Lett.* **531**, 199-203.
4. Anno, T., Uehara, S., Katagiri, H., Ohta, Y., Ueda, K.,

Mizuguchi, H., Moriyama, Y., Oka, Y. and Tanizawa, Y. (2004) Overexpression of constitutively activated glutamate dehydrogenase induces insulin secretion through enhanced glutamate oxidation. *Am. J. Physiol. Endocrinol. Metab.* **286**, E280-E285.
5. Carobbio, S., Ishihara, H., Fernandez-Pascual, S., Bartley, C., Martin-Del-Rio, R. and Maechler, P. (2004) Insulin secretion profiles are modified by overexpression of glutamate dehydrogenase in pancreatic islets. *Diabetologia* **47**, 266-276.
6. Carobbio, S., Frigerio, F., Rubi, B., Vetterli, L., Bloksgaard, M., Gjinovci, A., Pournourmohammadi, S., Herrera, P. L., Reith, W., Mandrup, S. and Maechler, P. (2009) Deletion of glutamate dehydrogenase in β-Cells abolishes part of the insulin secretory response not required for glucose homeostasis. *J. Biol. Chem.* **284**, 921-929.
7. Bryla, J., Michalik, M., Nelson, J. and Erecinska, M. (1994) Regulation of the glutamate dehydrogenase activity in rat islets of Langerhans and its consequence on insulin release. *Metabolism* **43**, 1187-1195.
8. Stanley, C. A., Lieu, Y. K., Hsu, B. Y., Burlina, A. B., Greenberg, C. R., Hopwood, N. J., Perlman, K., Rich, B. H., Zammarchi, E. and Poncz, M. (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N. Engl. J. Med.* **338**, 1352-1357.
9. Yorifuji, T., Muroi, J., Uematsu, A., Hiramatsu, H. and Momoi, T. (1999) Hyperinsulinism-hyperammonemia syndrome caused by mutant glutamate dehydrogenase accompanied by novel enzyme kinetics. *Hum. Genet.* **104**, 476-479.
10. MacMullen, C., Fang, J., Hsu, B. Y., Kelly, A., de Lonlay-Debeney, P., Saudubray, J. M., Ganguly, A., Smith, T. J. and Stanley, C. A. (2001) Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase. *J. Clin. Endocrinol. Metab.* **86**, 1782-1787.
11. Smith, T. J., Peterson, P. E., Schmidt, T., Fang, J. and Stanley, C. A. (2001) Structures of bovine glutamate dehydrogenase complexes elucidate the mechanism of purine regulation. *J. Mol. Biol.* **307**, 707-720.
12. Li, C., Allen, A., Kwagh, J., Doliba, N. M., Qin, W., Najafi, H., Collins, H. W., Matschinsky, F. M., Stanley, C. A. and Smith, T. J. (2006) Green tea polyphenols modulated insulin secretion by inhibition glutamate dehydrogenase. *J. Biol. Chem.* **281**, 10214-10221.
13. Herrero-Yraola, A., Bakhit, S. M. A., Franke, P., Weise, C., Schweiger, M., Jorcke, D. and Ziegler, M. (2001) Regulation of glutamate dehydrogenase by reversible ADP-ribosylation in mitochondria. *EMBO J.* **20**, 2404-2412.
14. Zolkiewska, A., Nightingale, M. S. and Moss, J. (1992) Molecular characterization of NAD: arginine ADP-ribosyltransferase from rabbit skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11352-11356.
15. Jorcke, D., Ziegler, M., Herrero-Yraola, A. and Schweiger, K. (1998) Enzymic, cysteine-specific ADP-ribosylation in bovine liver mitochondria. *Biochem. J.* **332**, 189-193.
16. Masmoudi, A. and Mandel, P. (1987) ADP-ribosyl transferase and NAD glycohydrolase activities in rat liver mitochondria. *Biochemistry* **26**, 1965-1969.
17. Frei, B. and Richter, C. (1988) Mono(ADP-ribosylation) in rat liver mitochondria. *Biochemistry* **27**, 529-535.
18. Haigis, M. C., Mostoslavsky, R., Haigis, K. M., Fahie, K., Christodoulou, D. C., Murphy, A. J., Valenzuela, D. M., Yancopoulos, G. D., Karow, M., Blander, G., Wolberger, C., Prolla, T. A., Weindruch, R., Alt, F. W. and Guarente, L. (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β cells. *Cell* **126**, 941-954.
19. Ahuja, N., Schwer, B., Carobbio, S., Waltregny, D., North, B. J., Castronovo, V., Maechler, P. and Verdin, E. (2007) Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J. Biol. Chem.* **282**, 33583-33592.
20. Lombard, D. B., Alt, F. W., Cheng, H., Bunkenborg, J., Streeper, R. S., Mostoslavsky, R., Kim, J., Yancopoulos, G., Valenzuela, D., Murphy, A., Yang, Y., Chen, Y., Hirschey, M., Bronson, R., Haigis, M., Guarente, L., Farese, R., Weissman, S., Verdin, E. and Schwer, B. (2007) Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol. Cell. Biol.* **27**, 8807-8814.
21. Schlicker, C., Gertz, M., Papatheodorou, P., Kachholz, B., Becker C. and Steegborn, C. (2008) Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. *J. Mol. Biol.* **382**, 790-801.
22. Yang, S. J., Hahn, H. G., Choi, S. Y. and Cho, S.-W. (2010) Inhibitory effects of KHG26377 on glutamate dehydrogenase activity in cultured islets. *BMB Rep.* **43**, 245-249.
23. Cho, S.-W., Lee, J. and Choi, S. (1995) Two soluble forms of glutamate dehydrogenase isoproteins from bovine brain. *Eur. J. Biochem.* **233**, 340-346.
24. Choi, S. Y., Hong, J. W., Song, M.-S., Jeon, S. G., Bahn, J. H., Lee, B. Y., Ahn, J.-Y. and Cho, S.-W. (1999) Different antigenic reactivities of bovine brain glutamate dehydrogenase isoproteins. *J. Neurochem.* **72**, 2162-2169.
25. Choi, M. M., Huh, J. W., Yang, S. J., Cho, E. H., Choi, S. Y. and Cho, S.-W. (2005) Identification of ADP-ribosylation site in human glutamate dehydrogenase isozymes. *FEBS Lett.* **579**, 4125-4130.
26. Yang, S. J., Huh, J. W., Kim, M. J., Lee, W. J., Kim, T. U., Choi, S. Y. and Cho, S.-W. (2003) Regulatory effects of 5'-deoxypyridoxal on glutamate dehydrogenase activity and insulin secretion in pancreatic islets. *Biochimie* **85**, 581-586.
27. Argmann, C. and Auwerx, J. (2006) Insulin secretion : SIRT4 gets in on the act. *Cell* **126**, 837-839.
28. Kim, S. C., Sprung, R., Chen, Y., Xu, Y., Ball, H., Pei, J., Cheng, T., Kho, Y., Xiao, H., Xiao, L., Grishin, N. V., White, M., Yang, X. J. and Zhao, Y. (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol. Cell* **23**, 607-618.
29. Onyango, P., Celic, I., McCaffery, J. M., Boeke, J. D. and Feinberg, A. P. (2002) SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13653-13658.