Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 350 (2006) 68–73
BBRC
www.elsevier.com/locate/ybbrc

Marked increase of insulin gene transcription by suppression of the Rho/Rho-kinase pathway ☆

Yumiko Nakamura [a], Hideaki Kaneto [a,*], Takeshi Miyatsuka [a], Taka-aki Matsuoka [a], Munehide Matsuhisa [a], Koichi Node [b], Masatsugu Hori [a], Yoshimitsu Yamasaki [a]

[a] *Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Japan*

Received 30 August 2006
Available online 12 September 2006

Abstract

The hallmarks of type 2 diabetes are pancreatic β-cell dysfunction and insulin resistance. It has been suggested that Rho/Rho-kinase is a mediator of insulin signaling, and thereby involved in the development of insulin resistance, regulation of insulin action, and glucose homeostasis, but the role of Rho/Rho-kinase in β-cells remained unknown. The aim of this study was to examine the possible role of Rho/Rho-kinase in β-cell function. Immunostaining showed that RhoA was expressed in mature β-cells, with higher expression observed in β-cells of diabetic C57BL/KsJ-db/db mice compared to non-diabetic mice. In addition, to examine the functional role of Rho/Rho-kinase in β-cells, we evaluated the effect of Rho-kinase inhibitors on insulin biosynthesis. Northern blot analysis showed that insulin mRNA levels were markedly increased by Rho-kinase inhibitors, Y-27632 and fasudil, in β-cell-derived HIT-T15 cells. Furthermore, using the luciferase reporter gene assay, insulin promoter activity was also dramatically increased by Y-27632, which was associated with an increase in the insulin mRNA level. These results suggest that suppression of Rho/Rho-kinase increases insulin promoter activity, which leads to an increase in insulin mRNA level. Taken together, Rho/Rho-kinase is activated in β-cells under diabetic conditions and suppression of the Rho/Rho-kinase pathway increases insulin gene transcription. These results imply that Rho/Rho-kinase activation is involved in the suppression of insulin expression found in diabetes and that suppression of the Rho/Rho-kinase pathway could be a useful tool to augment insulin gene transcription.

Keywords: Diabetes; Insulin; Rho-kinase

Rho-kinase is a serine/threonine kinase which is activated by the small G protein Rho. It is well known that Rho/Rho-kinase is involved in various cellular functions such as actin cytoskeleton organization, cell adhesion, cell motility, and vascular, and smooth muscle contraction [1,2]. It has been suggested that suppression of the Rho/Rho-kinase pathway could be a novel therapeutic strategy for various cardiovascular diseases such as hypertension and arteriosclerosis [3,4]. Regarding a role of Rho/Rho-kinase in diabetes, it has been suggested that Rho/Rho-kinase is a mediator of insulin signaling via interaction with insulin receptor substrate-1 (IRS-1) and thereby involved in the development of insulin resistance, regulation of insulin action, and glucose homeostasis [5–9]. It was shown that activation of the Rho/Rho-kinase pathway increased IRS-1 serine phosphorylation, leading to inhibition of downstream insulin signaling by blocking IRS-1 tyrosine phosphorylation in vascular smooth muscle cells [6]. On the other hand, it was shown that suppression of the Rho/Rho-kinase pathway in mice caused insulin resistance by reducing insulin-stimulated glucose uptake in skeletal muscle cells *in vivo* [8]. These results suggest that the Rho/Rho-kinase pathway is an important mediator of insulin signaling.

☆ *Abbreviations:* IRS-1, insulin receptor substrate-1; PDX-1, pancreatic and duodenal homeobox factor-1; PBS, phosphate-buffered saline; ABC, avidin–biotin complex.

* Corresponding author. Fax: +81 6 6879 3639.
E-mail address: kaneto@medone.med.osaka-u.ac.jp (H. Kaneto).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.08.192

The number of patients with type 2 diabetes is markedly increasing worldwide, and nowadays type 2 diabetes is recognized as the most prevalent and serious metabolic disease. Insulin plays a crucial role in maintaining blood glucose levels by facilitating glucose uptake into muscle and adipose tissue and suppressing gluconeogenesis in the liver. Normal β-cells can compensate for insulin resistance by increasing insulin secretion or β-cell mass, but insufficient compensation leads to the onset of glucose intolerance. Chronic hyperglycemia causes impairment of insulin biosynthesis and secretion, and once hyperglycemia becomes apparent, insulin biosynthesis and secretion are gradually decreased, eventually resulting in deterioration of β-cell function. This process is called ''β-cell glucose toxicity'' and is clinically often observed in type 2 diabetic subjects. β-Cell glucose toxicity is often accompanied by reduction of expression and/or activity of pancreatic transcription factors PDX-1 (pancreatic and duodenal homeobox factor-1) and MafA. It is well known that PDX-1 and MafA are important transcription factors for the insulin gene. PDX-1 is expressed in the pancreas and duodenum [10–12] and plays a crucial role in pancreas development, β-cell differentiation, and maintenance of mature β-cell function by regulating several β-cell related factors. MafA controls β-cell specific expression of the insulin gene through a *cis*-regulatory element called RIPE3b, and functions as a potent transactivator for the insulin gene [13–15].

Pancreatic β-cell dysfunction and insulin resistance are the hallmarks of type 2 diabetes, but a role of Rho/Rho-kinase in β-cell function remained unknown. In this study, we show that Rho/Rho-kinase is activated under diabetic conditions and that suppression of the Rho/Rho-kinase pathway markedly increases insulin gene transcription.

Materials and methods

Preparation of pancreas sections and immunohistochemical analysis. Diabetic C57BL/KsJ-db/db mice were purchased from Clea Japan (Tokyo, Japan). The mice were allowed free access to food and water in a specific pathogen-free environment. For the preparation of pancreas sections mice were anesthetized using pentobarbital sodium, a midline abdominal incision was made, and pancreata were removed and fixed overnight in a solution of 4% paraformaldehyde. Fixed tissues were processed routinely for paraffin embedding, and 5 μm sections were prepared and mounted onto slides. Before incubation with each antibody, the mounted sections were rinsed 3 times with phosphate-buffered saline (PBS). For immunohistochemistry, the avidin–biotin complex (ABC) method was performed using the Vectastain Elite ABC Kit (Vector Laboratories). The mounted sections were incubated overnight at 4 °C with a rabbit anti-RhoA antibody (Santa Cruz Biotechnology) at a dilution of 1:100, followed by incubation for 30 min with biotinylated anti-rabbit IgG antibody (Vector Laboratories) at a dilution of 1:200. Sections were then incubated with ABC reagent for 30 min and positive reactions were visualized by incubation with peroxidase substrate solution containing 3, 3′-diaminobenzidine tetrahydrochloride (DAB) (Zymed Laboratories), and the nuclei were counterstained with hematoxylin. Double immunofluorescence was performed by using rabbit anti-RhoA and guinea pig anti-PDX-1 primary antibodies (kindly provided by Dr. Christopher V.E. Wright, Vanderbilt University School of Medicine) at a dilution of 1:50 and 1:250, respectively. Secondary antibodies were anti-rabbit and anti-guinea pig antibodies used at a dilution of 1:200 (Molecular Probes). Fluorescent images were obtained using DXM1200F (Nikon Instech).

Cell culture. HIT-T15 cells (American Type Culture Collection No. 1777) were grown in RPMI-1640 medium containing 11.1 mmol/l glucose, 10% FCS, 100 μg/ml streptomycin, and 100 U/ml penicillin at 5% CO_2 and 37 °C. The HIT-T15 cells used in this study were between passages 80 and 100.

Northern blot analysis. Total RNA was isolated from HIT-T15 cells, and Northern blot analyses were performed. Six micrograms of total RNA was size-fractionated and transferred to a Hybond-N^+ membrane (Amersham Biosciences). The insulin probe was labeled with [α-^{32}P] dCTP using the Rediprime labeling system kit (Amersham Biosciences). After overnight hybridization with a ^{32}P-labeled probe at 42 °C, the membrane was washed twice with 1× SSPE (180 mmol/l NaCl, 10 mmol/l sodium phosphate, and 1 mmol/l EDTA, pH 7.4) and 0.1% SDS at 42 °C. The membrane was exposed on Kodak XAR film with an intensifying screen at −80 °C.

Gene transfection and luciferase assay. The plasmids used in the reporter gene analyses were pA3InsLuc containing a 326-bp 5′-flanking sequence of the human insulin gene and the rat insulin II promoter-reporter (luciferase) plasmid containing a 238-bp 5′-flanking sequence of the rat insulin II promoter region [16].

Twenty-four hour before transfections, HIT-T15 cells were replated in 12-well tissue culture plates. Cells were transfected with each reporter plasmid (0.5 μg) by the lipofection method using LipofectAMINE reagent (Invitrogen, Tokyo, Japan) under the conditions recommended by the manufacturer. The luciferase activity from phRL-TK served as an internal transfection control. The preparation of cell extracts and luciferase measurements were carried out 48 h after transfection according to the manufacture's protocol (Promega, Madison, WI.).

Isolation of nuclear and whole cell extracts. HIT-T15 cells were cultured in 60-mm diameter culture dishes until ∼80% confluency. For isolation of whole-cell extracts, cells were collected into microtubes, centrifuged for 20 s in a microcentrifuge, and resuspended in 200 μl of 10.0 mM Hepes, pH 7.9, containing 10.0 mM KCl, 1.5 mM $MgCl_2$, and 0.5 mM dithiothreitol. After incubation at 4 °C for 15 min, the cells were lysed by passing 10 times through a 22-gauge needle. Next, the cells were centrifuged for 20 s in a microcentrifuge, and the supernatant (cytoplasmic fraction) was removed and frozen in small aliquots. The pellet, which contained the nuclei, was resuspended in 150 μl of 20 mM Hepes, pH 7.9, containing 20% v/v glycerol, and 0.1 M KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride and then centrifuged at 4 °C in a microcentrifuge. The supernatant was collected, aliquoted into small volumes, and stored at −80 °C.

Western blot analysis. Western blot analyses were performed following standard procedures as described below. Nuclear proteins derived from each cell sample were fractionated by SDS–PAGE and transferred to a PVDF membrane (Bio-Rad Laboratories), and incubated overnight at 4 °C with a rabbit anti-PDX-1 antibody (1:2000 dilution) or a rabbit anti-MafA antibody (1:1000) (Bethyl). The membrane was then incubated at room temperature for 60 min in PBS with a 1:2000 dilution of anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (Zymed Laboratories). The antibody complex was visualized by incubation with chemiluminescence reagent (PerkinElmer Life Sciences).

Gel-mobility shift analysis. Nuclear protein extract (5 μg) was preincubated in mobility shift buffer (10 mM Hepes (pH 7.4), 100 mM NaCl, 2 mM DTT, 10% (v/v) glycerol, and 1 μg of poly (dI–dC)) for 15 min at 4 °C (total volume, 50 μl). The binding reaction was initiated by addition of a double-stranded ^{32}P-labeled probe (1 ng, 1×10^5 cpm) containing C1 ($^{-126}$TGGAAACTGCAGCTTCAGCCCCTCTG^{-101}) or A3 ($^{-214}$CCTC TTAAGACTCTAATTACCCT^{-192}) element sequences from the rat insulin II gene, followed by incubation for 30 min at 4 °C. The complexes were resolved by electrophoresis through a 6% nondenaturing polyacrylamide gel using high ionic strength polyacrylamide gel electrophoresis (PAGE) conditions.

Results

Rho expression in the pancreas of non-diabetic C57BL6 and diabetic C57BL/KsJ-db/db mice

It has been reported that Rho has a wide tissue distribution. First, to examine Rho expression in the pancreas, immunostaining for RhoA was performed using paraffin-embedded pancreas sections of non-diabetic C57BL6 and diabetic C57BL/KsJ-db/db mice at 16 weeks of age. As shown in Fig. 1A, RhoA was detected in pancreatic islets of C57BL6 mice. Furthermore, a larger amount of RhoA expression was observed in pancreatic islets of diabetic C57BL/KsJ-db/db mice. Next, to examine whether RhoA is expressed in β-cells, we performed double immunostaining for RhoA and PDX-1 using anti-RhoA and anti-PDX-1 antibodies, because it is known that PDX-1 is expressed only in β-cells in the adult pancreas. As shown in Fig. 1B, RhoA was co-localized with PDX-1, indicating that RhoA is indeed expressed in β-cells. Furthermore, an increase in RhoA expression was observed in PDX-1-expressing cells, indicating that RhoA expression is increased in β-cells under diabetic conditions.

Marked increase of insulin promoter activity and mRNA level in a β-cell-derived cell line by Rho-kinase inhibitors

To examine the effect of suppression of the Rho/Rho-kinase pathway on insulin mRNA levels, HIT-T15 cells, a β-cell-derived cell line, were treated with the Rho-kinase inhibitor, Y27632 (Calbiochem), for 48 h, followed by Northern blot analyses. As shown in Fig. 2A, insulin mRNA levels were markedly increased by Y27632 in a dose-dependent manner. To further examine the effect of suppression of the Rho/Rho-kinase pathway on insulin mRNA levels, HIT-T15 cells were treated with another

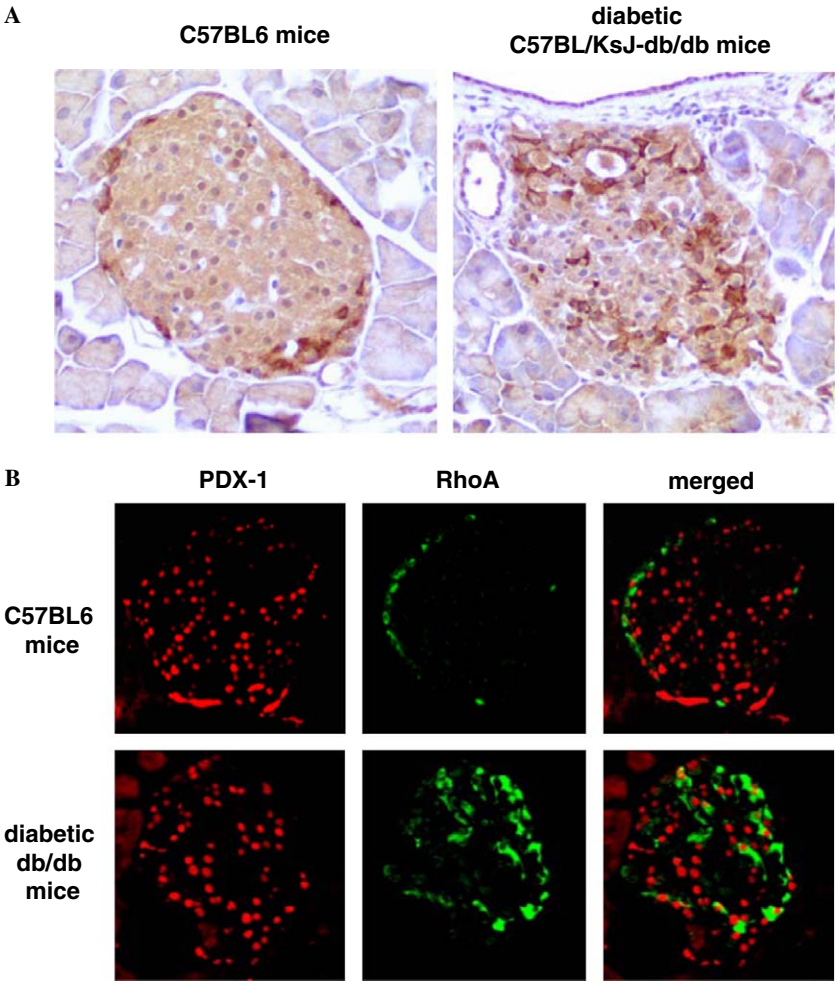

Fig. 1. Increase of RhoA expression in β-cells of diabetic C57BL/KsJ-db/db mice. (A) Immunostaining for RhoA was performed using paraffin-embedded pancreas sections of non-diabetic C57BL6 and diabetic C57BL/KsJ-db/db mice at 16 weeks of age. RhoA is expressed in islets of both non-diabetic and diabetic db/db mice, and a larger amount of RhoA expression is observed in islets of diabetic db/db mice. (B) Co-immunostaining for RhoA and PDX-1 was performed using paraffin-embedded pancreas sections of C57BL6 and diabetic C57BL/KsJ-db/db mice at 12 weeks of age. RhoA is co-localized with PDX-1, indicating that RhoA is expressed in β-cells of both non-diabetic and diabetic db/db mice. In addition, a larger amount of RhoA expression is observed in β-cells of diabetic db/db mice.

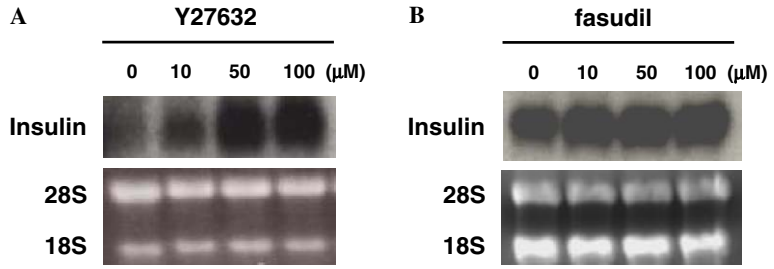

Fig. 2. Marked increase in insulin mRNA level by Rho-kinase inhibitors. A β-cell-derived cell line, HIT-T15 cells, was treated with the Rho-kinase inhibitors Y27632 (A) and fasudil (B) for 48 h, followed by Northern blot analyses using a mouse insulin II probe.

Rho-kinase inhibitor, fasudil (Asahi Kasei, Tokyo, Japan), for 48 h. As shown in Fig. 2B, insulin mRNA levels were also markedly increased by fasudil in a dose-dependent manner, as observed with Y27632. These results indicate that suppression of the Rho/Rho-kinase pathway leads to augmentation of insulin mRNA levels. Next, to examine the effect of suppression of the Rho/Rho-kinase pathway on insulin promoter activity, a human insulin promoter-luciferase plasmid was transfected into HIT-T15 cells, and Y27632 was added to the medium. The luciferase assay was performed 48 h after the transfection. As shown in Fig. 3A, insulin promoter activity was also markedly increased by Y27632 in a dose-dependent manner, which was associated with an increase in insulin mRNA level. These results suggest that suppression of the Rho/Rho-kinase pathway markedly increases insulin promoter activity, which leads to a significant increase in insulin mRNA level.

It is well known that PDX-1 and NeuroD are important transcription factors for the insulin gene and that insulin enhancer elements, A3-box (PDX-1 binding site) and E1-box (NeuroD binding site), play an important role in regulating cell-specific expression of the insulin gene. To examine whether activation of the A3-box and/or E1-box region is involved in the increase of insulin promoter activity by Y27632, an A3-box or E1-box-mutated insulin promoter-luciferase plasmid (rat insulin II promoter) was transfected into HIT-T15 cells, and Y27632 was added to the medium. The luciferase assay was performed 48 h after the transfection. As shown in Fig. 3B, A3-box or E1-box-mutated insulin promoter activity was also increased by Y27632 as observed with the wild type insulin promoter. These results suggest that the increase of insulin promoter activity by suppression of the Rho/Rho-kinase pathway is independent of A3-box or E1-box activation.

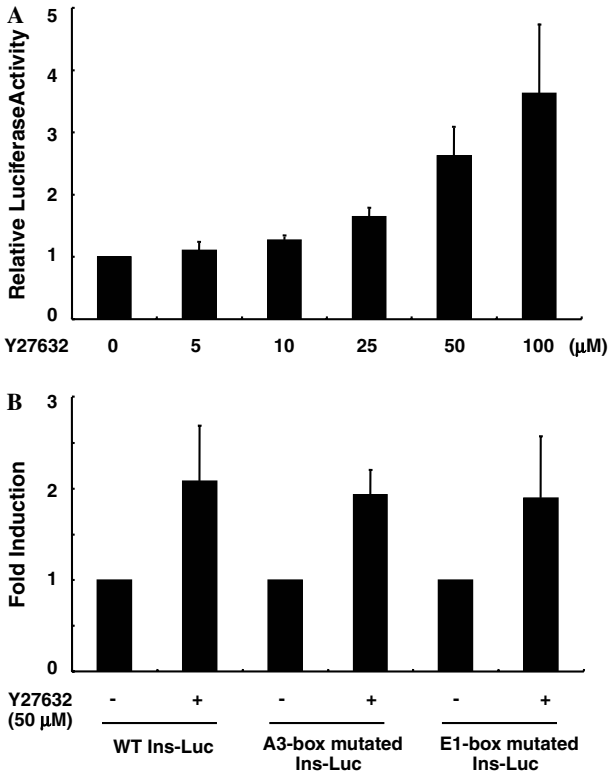

Fig. 3. Marked increase of insulin promoter activity by Rho-kinase inhibitors. (A) The wild type insulin promoter-luciferase plasmid was transfected into HIT-T15 cells, and Y27632 was added to the medium. The luciferase assay was performed 48 h after transfection. Data are shown as relative luciferase activities with the insulin promoter activity without treatment being arbitrarily set at 1. (B) A3-box or E1-box-mutated insulin promoter-luciferase plasmid was transfected into HIT-T15 cells, and Y27632 was added to the medium. The luciferase assay was performed 48 h after transfection. Data are shown as relative luciferase activities with the insulin promoter activity without treatment being arbitrarily set at 1. All data are shown as means ± SD of at least four individual experiments.

Rho-kinase inhibitor does not affect PDX-1 and MafA expression or their DNA binding activities to insulin gene promoter region

In the diabetic state, chronic hyperglycemia decreases insulin gene expression and secretion, which is often accompanied by a reduction of PDX-1 and MafA expression and their DNA binding activities to the insulin gene promoter region. These pancreatic transcription factors are very important for insulin gene transcription. Therefore, to examine the molecular mechanism as to how suppression of the Rho/Rho-kinase pathway leads to a marked increase in insulin gene transcription, we examined the effect of a Rho-kinase inhibitor on PDX-1 and MafA expression and their DNA binding activities to the insulin gene promoter region. To examine the effect of suppression of the Rho/Rho-kinase pathway on PDX-1 and MafA protein levels, HIT-T15 cells were treated with Y27632 for 48 h

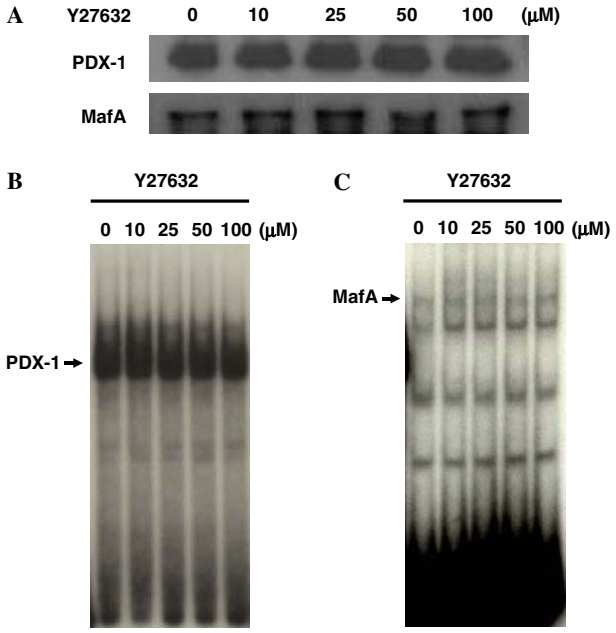

Fig. 4. Treatment of HIT-T15 cells with a Rho-kinase inhibitor does not affect PDX-1 and MafA expression levels and DNA binding activities to the insulin gene promoter region. (A) HIT-T15 cells were treated with Y27632 for 48 h, followed by nuclear protein extraction. PDX-1 and MafA expression levels in nuclei were subsequently evaluated by Western blot analyses. (B,C) HIT-T15 cells were treated with Y27632 for 48 h, followed by nuclear protein extraction. Subsequently, PDX-1 (B) and MafA (C) DNA binding activities to the insulin gene promoter region were evaluated by the gel-mobility shift assay. Similar results were obtained in two independent experiments.

and Western blot analyses were performed using nuclear extracts. As shown in Fig. 4A, PDX-1 and MafA protein levels were not affected by Y27632. We also examined the effect of Y27632 on mRNA levels of another important transcription factor, NeuroD, by RT-PCR, but there was no change in NeuroD mRNA levels (data not shown). We also examined the effect of suppression of the Rho/Rho-kinase pathway on the DNA binding activity of PDX-1 and MafA to the insulin gene promoter region. HIT-T15 cells were treated with Y27632 for 48 h and gel-mobility shift analyses were performed using nuclear extracts. As shown in Fig. 4B and C, PDX-1 and MafA DNA binding activities were not affected by Y27632. These results suggest that the increase in insulin gene transcription by suppression of the Rho/Rho-kinase pathway is independent of PDX-1 and/or MafA expression and their DNA binding activities to the insulin gene promoter region.

Discussion

In this study, we examined how Rho/Rho-kinase is involved in β-cell function and found that RhoA was expressed in pancreatic β-cells and that RhoA expression was increased in β-cells of diabetic db/db mice compared to non-diabetic mice. Furthermore, interestingly, treatment with Y27632 markedly increased insulin promoter activity and insulin mRNA level. These results suggest that Rho/Rho-kinase is involved in the β-cell dysfunction found in type 2 diabetes and that suppression of the Rho/Rho-kinase pathway may be a useful tool to augment insulin gene transcription.

In addition, to examine the molecular mechanism responsible for the marked increase of insulin promoter activity and mRNA level, we examined the effect of Y27632 on PDX-1 and MafA expression and their DNA binding activities to the insulin gene promoter region. However, PDX-1 and MafA expression and activity were not influenced by Y27632. Thus, although the molecular mechanism by which suppression of the Rho/Rho-kinase pathway increases insulin promoter activity remains unknown, there are a number of possibilities. For example, expression of several transcription factors such as c-Jun and/or c-Myc might be involved in the increase in insulin gene transcription by suppression of the Rho/Rho-kinase pathway. It was shown that RhoA activates the *c-jun* promoter and induces *c-jun* expression [17], and that c-Jun suppresses insulin gene transcription in β-cells [18,19]. Therefore, suppression of the Rho/Rho-kinase pathway might increase insulin promoter activity by reducing c-Jun expression. In addition, it was shown that c-Myc is a target of the Rho/Rho-kinase pathway [20] and that c-Myc also suppresses insulin gene transcription [21,22]. Therefore, suppression of the Rho/Rho-kinase pathway might increase insulin promoter activity by reducing c-Myc expression. Another possibility is that some other transcription factors which are important for maintaining insulin promoter activity are influenced by Rho-kinase inhibition, which leads to the increase in insulin gene transcription. To further examine the detailed molecular mechanism involved in the increase in insulin promoter activity by suppression of the Rho/Rho-kinase pathway, it will be useful to examine which part of the insulin promoter region is required for its activation by the Rho-kinase inhibitor. This point can be addressed by using various deletion constructs of the insulin promoter region.

In conclusion, we have demonstrated that RhoA expression is increased in β-cells under diabetic conditions and that suppression of the Rho/Rho-kinase pathway markedly increases insulin promoter activity and its mRNA level. These results imply that Rho/Rho-kinase activation is involved in the suppression of insulin biosynthesis and β-cell dysfunction found in type 2 diabetes, and that suppression of the Rho/Rho-kinase pathway could be a useful tool to augment insulin gene transcription.

Acknowledgments

We thank Dr. Christopher V.E. Wright (Vanderbilt University School of Medicine) for kindly providing the guinea pig anti-PDX-1 primary antibody and Dr. Helena Akiko Popiel for valuable comments on the manuscript. We also thank Ms. Yuko Sasaki for her technical assis-

tance and Ms. Chikayo Yokogawa for her secretarial assistance.

References

[1] A. Hall, Rho GTPases and the actin cytoskeleton, Science 279 (1998) 509–514.
[2] K. Riento, A.J. Ridley, ROCKS: multifunctional kinases in cell behaviour, Nat. Rev. Mol. Cell Biol. 4 (2003) 446–456.
[3] M. Uehata, T. Ishizaki, H. Satoh, T. Ono, T. Kawahara, T. Morishita, H. Tamakawa, K. Yamagami, J. Inui, M. Maekawa, S. Narumiya, Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension, Nature 389 (1997) 990–994.
[4] Y. Mukai, H. Shimokawa, T. Matoba, T. Kandabashi, S. Satoh, J. Hiroki, K. Kaibuchi, A. Takeshita, Involvement of Rho-kinase in hypertensive vascular disease: a novel therapeutic target in hypertension, FASEB J. 15 (2001) 1062–1064.
[5] S. Farah, Y. Agazie, N. Ohan, J.K. Ngsee, X.J. Liu, A Rho-associated protein kinase, ROKα, binds insulin receptor substrate-1 and modulates insulin signaling, J. Biol. Chem. 273 (1998) 4740–4746.
[6] N. Begum, O.A. Sandu, M. Ito, S.M. Lohmann, A. Smolenski, Active Rho kinase (ROK-α) associates with insulin receptor substrate-1 and inhibits insulin signaling in vascular smooth muscle cells, J. Biol. Chem. 277 (2002) 6214–6222.
[7] R. Sordella, M. Classon, K.Q. Hu, S.F. Mathson, M.R. Brouns, B. Fine, L. Zhang, H. Takami, Y. Yamada, J. Settleman, Modulation of CREB activity by the Rho GTPase regulates cell and organism size during mouse embryonic development, Dev. Cell 2 (2002) 553–565.
[8] N. Furukawa, P. Ongusaha, W. Jahng, K. Araki, C. Choi, H. Kim, Y. Lee, K. Kaibuchi, B. Kahn, H. Masuzaki, Role of Rho-kinase in regulation of insulin action and glucose homeostasis, Cell Metab. 2 (2005) 119–129.
[9] T. Kanda, S. Wakino, K. Homma, K. Yoshioka, S. Tatematsu, K. Hasegawa, I. Takamatsu, N. Sugano, K. Hayashi, T. Saruta, Rho-kinase as a molecular target for insulin resistance and hypertension, FASEB J. 20 (2006) 169–171.
[10] H. Ohlsson, K. Karlsson, T. Edlund, IPF1, a homeodomain-containing-transactivator of the insulin gene, EMBO J. 12 (1993) 4251–4259.
[11] C.P. Miller, R.E. McGehee, J.F. Habener, IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene, EMBO J. 13 (1994) 1145–1156.
[12] J. Leonard, B. Peers, T. Johnson, K. Ferreri, S. Lee, M.R. Montminy, Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells, Mol. Endocrinol. 7 (1993) 1275–1283.
[13] T.A. Matsuoka, L. Zhao, I. Artner, H.W. Jarrett, D. Friedman, A. Means, R. Stein, Members of the large Maf transcription family regulate insulin gene transcription in islet β cells, Mol. Cell. Biol. 23 (2003) 6049–6062.
[14] M. Olbrot, J. Rud, L.G. Moss, A. Sharma, Identification of β-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA, Proc. Natl. Acad. Sci. USA 99 (2002) 6737–6742.
[15] K. Kataoka, S.I. Han, S. Shioda, M. Hirai, M. Nishizawa, H. Handa, MafA is a glucose-regulated and pancreatic β-cell-specific transcriptional activator for the insulin gene, J. Biol. Chem. 277 (2002) 49903–49910.
[16] A. Sharma, R. Stein, Glucose-induced transcription of the insulin gene is mediated by factors required for B-cell-type-specific expression, Mol. Cell. Biol. 14 (1994) 871–879.
[17] M.J. Marinissen, M. Chiariello, J.S. Gutkind, Regulation of gene expression by the small GTPase Rho through the ERK (p38γ) MAP kinase pathway, Genes Dev. 15 (2001) 535–553.
[18] N. Inagaki, T. Maekawa, T. Sudo, S. Ishii, Y. Seino, H. Imura, c-Jun represses the human insulin promoter activity that depends on multiple cAMP response elements, Proc. Natl. Acad. Sci. USA 89 (1992) 1045–1049.
[19] G.L. Robinson, E. Henderson, M.E. Massari, C. Murre, R. Stein, c-Jun inhibit insulin control element-mediated transcription by affecting the transactivation potential of the E2A gene products, Mol. Cell. Biol. 15 (1995) 1398–1404.
[20] Y. Mizukami, K. Fujiki, E.M. Duerr, M. Gala, W.S. Jo, X. Zhang, D.C. Chung, Hypoxic regulation of vascular endothelial growth factor through the induction of phosphatidylinositol 3-kinase/Rho/Rock and c-Myc, J. Biol. Chem. 281 (2006) 13957–13963.
[21] H. Kaneto, A. Sharma, K. Suzuma, D.R. Laybutt, G. Xu, S. Bonner-Weir, G.C. Weir, Induction of c-Myc expression suppresses insulin gene transcription by inhibiting NeuroD/BETA2-mediated transcriptional activation, J. Biol. Chem. 27 (2002) 12998–13006.
[22] D.R. Laybutt, G.C. Weir, H. Kaneto, J. Lebet, R.D. Palmiter, A. Sharma, S. Bonner-Weir, Overexpression of c-Myc in β-cells of transgenic mice causes proliferation and apoptosis, downregulation of insulin gene expression, and diabetes, Diabetes 51 (2002) 1793–1804.