Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

WTC rat has unique characteristics such as resistant to streptozotocin

Yoshiaki Nagaki, Koichi Ito, Masayoshi Kuwahara *

Department of Veterinary Pathophysiology and Animal Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

ARTICLE INFO

ABSTRACT

Because we found that WTC rats might be resistant to streptozotocin (STZ), we have elucidated the mechanisms of resistant to the diabetogenic effects of STZ in the WTC rats. Dose response to STZ was evaluated with glucose levels. No significant changes in glucose level to STZ administration were observed in WTC rats. Insulin secretion by suppling glucose was preserved in WTC rats even after STZ administration. Although there was no significant difference in gene expression of both GLUT2 and Kir6. 2, which were involved in STZ resistance, between WTC rats and Wistar rats, the expression of metallothionein 2a in pancreas and liver to STZ administration of WTC rats was significantly higher than that of Wistar rats. Moreover, alloxan did not induce diabetes in WTC rats as same as STZ. These results suggest that WTC rats might have powerful antioxidant property to protect β cells in pancreas. Because the STZ-resistant property is very close characteristics to human beings, WTC rats will become a useful animal model in diabetic researches.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Type I diabetes is a chronic metabolic disorder characterized by a loss of pancreatic islet β cell mass, decreased serum insulin, and hyperglycemia. Although the pathogenic mechanisms of this disease have not been fully characterized, genetic, environmental, and autoimmune factors have been postulated. Streptozotocin (STZ)-induced diabetes in mice and rats has been used widely as an animal model to study type I diabetes [1,2], because STZ causes β cell necrosis and insulin-dependent diabetes mellitus in many species [3]. Although a number of mammalian species are sensitive to STZ, rabbits are highly resistant with little metabolic or histologic evidence of β cell damage [4]. Even in rodents, mice are much less sensitive to the diabetogenic effects of STZ than rats. Moreover, it seems that human islets are likely to be relatively resistant to STZ from considerable evidences *in vitro* and *in vivo* studies [5]. WTC rats (control rats for tremor rats derived from Kyoto: Wistar rats), a congenic strain derived from an inbreeding line of tremor rats and bred as an inbred strain without the *tm* gene. The WTC shows neither neuropathological alteration nor abnormal phenotypes from defects in the CNS [6]. Because, unexpectedly, we found that the WTC rats might be highly resistant to STZ, we have elucidated the mechanisms of resistant to the diabetogenic effects of STZ in the WTC rats.

2. Materials and methods

2.1. Animals and induction of diabetes

All experimental procedures conformed to the animal use guidelines of the Committee for Ethics on Animal Experiments of The University of Tokyo. WTC (the National BioResource Project for the Rat in Japan, Kyoto University) and Wistar rats (Japan SLC, Inc.) were maintained under a controlled conditions at 23 °C with a 12-h light/dark cycle, and given free access to food and water. In 12-week-old male WTC rats and Wistar rats, the STZ (50 and 100 mg/kg) or alloxan (150 mg/kg) were administrated to render diabetic. Glycemia was measured using blood sample obtained from a tail vein 4 days post-injection of these drugs with One Touch Ultra (Johnson and Johnson, Japan) [7].

2.2. Measurement of insulin

For plasma insulin concentration measurement, animals were separated to two groups with and without after 4 days 50 mg/kg STZ injection. Each group was further separated to two groups: one group was fasted for overnight, another group was left for 60 min after orally given 2 g/kg glucose after fasted. Then, blood samples were drawn from the inferior vena cava under urethane (1 g/kg i.p.) anesthesia and centrifuged for 2 min, and the plasma was stored at $-80\ ^{\circ}$C until use. Insulin concentrations were measured by a rat insulin ELIZA kit (Shibayagi Co., Japan).

* Correspondence to: Department of Veterinary Pathophysiology and Animal Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

Table 1
The primers for RT-PCR.

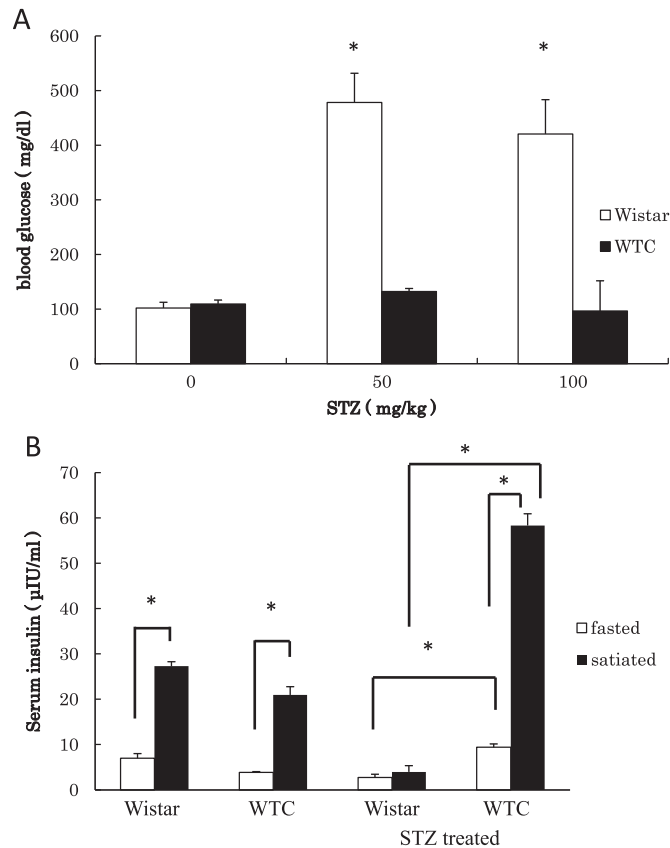| gene	Sense primer	Antisense primer
GLUT2	CAATTTCATCATCGCCCTCT	TGCAGCAATTTCGTCAAAAG
Kir6.2	CGCATGGTGACAGAGGAATG	GTGGAGAGGCACAACTTCGC
Mt1a	GGACCCCAACTGCTCCTG	CGAGGCACCTTTGCAGACAC
Mt2a	CAGCGATCTCTCGTTGATCTCC	CTTGTCCGAAGCCTCTTTGC
GAPDH	CTCATGACCACAGTCCATGC	TTCAGCTCTGGGATGACCTT

Fig. 1. Effects of STZ on glycemia and insulin secretion in Wistar rats and WTC rats. (A) Open bars show Wistar rat (n=6). Closed ones show WTC rat (n=6). *:p < 0.05 VS 0 mg/kg. (B) Serum insulin levels were measured with and without STZ treatment in Wistar rats and WTC rats during fasted (open bars) or satiated (closed bars). *: p < 0.05.

2.3. Relative quantity of mRNA

The relative quantity of mRNA was measured for GLUT2 and Kir6.2, which were involved in insulin secretion and STZ-resistance [8–10], and metallothionein [11] using reverse transcription-polymerase chain reaction (RT-PCR) using TaKaRa PCR Amplification kit (TaKaRa BIO INC.). The target genes' primers for RT-PCR are shown in Table 1 [11–14]. Tissue samples for these experiments were collected from pancreas, skeletal muscles and liver. The expression was calculated with Image J and the raw data was normalized with the internal control GAPDH.

2.4. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed by unpaired t-test. Values were considered statistically significant at p < 0.05.
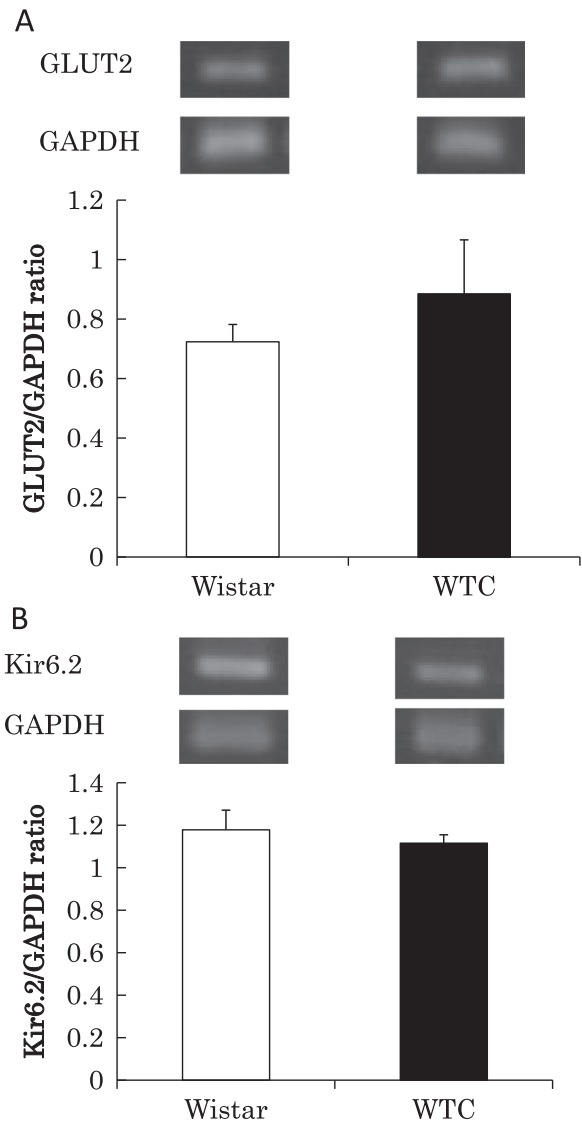

Fig. 2. The gene expressions of possible mechanisms relevance to uptake of STZ or enhanced secretion of insulin for the STZ resistance in WTC rats. (A) GLUT2 and (B) Kir6.2 expressions in Wistar rats (n=6) and WTC rats (n=6) were detected by RT-PCR. GAPDH was used as house keeping gene.

3. Results

3.1. STZ resistance in WTC rats

Wistar rats were uniformly diabetic at doses of either 50 or 100 mg/kg STZ. However, no significant changes were observed in WTC rats (Fig. 1A). Insulin secretion by suppling glucose was significantly increased to almost the same level in Wistar rats and WTC rats. Although STZ injection did not affect insulin secretion both fasting and suppling glucose in Wistar rats, insulin secretion in WTC rats was significantly increased in both conditions (Fig. 1B). From these data, we considered that the WTC rats have unique STZ-resistant characteristics.

3.2. GLUT2, Kir6.2 and metallothionein 2a mRNA expression

Because GLUT2 and Kir6.2 are involved in resistance against STZ [8–10], we examined the gene expression of GLUT2 and Kir6.2 at pancreas by RT-PCR to evaluate STZ-resistance in WTC rats. There was no significant difference in gene expression of both GLUT2 and Kir6.2 between WTC rats and Wistar rats (Fig. 2A and
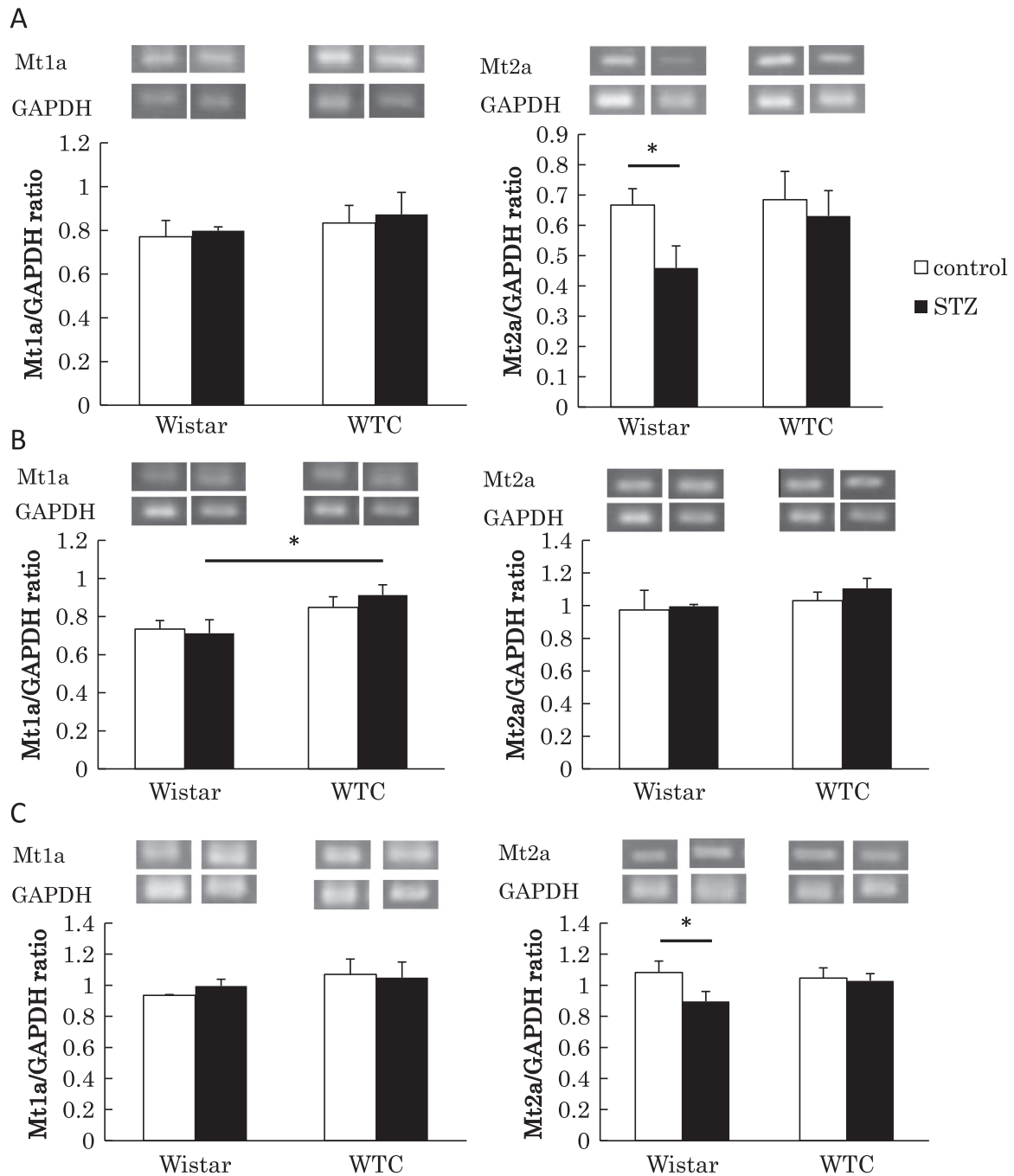
Fig. 3. The gene expressions of antioxidant property in some organs. Metallothionein (MT) 1A and 2A in (A) pancreas, (B) skeletal muscle and (C) liver were detected by RT-PCR. Open bars show their expression before STZ injection (n=4). Closed ones show the expression after STZ injection (n=4). GAPDH was used as house keeping gene. *: p < 0.05.

B). While STZ leads β cell death by promoting production of oxidant, we further examined the gene expression of metallothionein which was reported to have not only antioxidant but also anti diabetic function [15]. The expression of metallothionein 2a in pancreas and liver of Wistar rats was significantly decreased by STZ injection, but WTC rats did not show any changes (Fig. 3A–C). Therefore, WTC rats might have powerful antioxidant property to protect β cells in pancreas.

3.3. Effect of alloxan on diabetes in WTC rats

To confirm this property of WTC rats, alloxan which is another diabetogenic agent via production of reactive oxygen species was tested [15]. As shown in Fig. 4, alloxan could not induce diabetes in WTC rats as same as STZ.

4. Discussion

In this study, we found that WTC rats were highly resistant to STZ, and clearly showed STZ-resistant characteristics in these rats. No significant changes in glucose level to STZ administration were observed in WTC rats. Insulin secretion by suppling glucose was preserved in WTC rats even after STZ administration. Although there was no significant difference in gene expression of both GLUT2 and Kir6.2 between WTC rats and Wistar rats, the
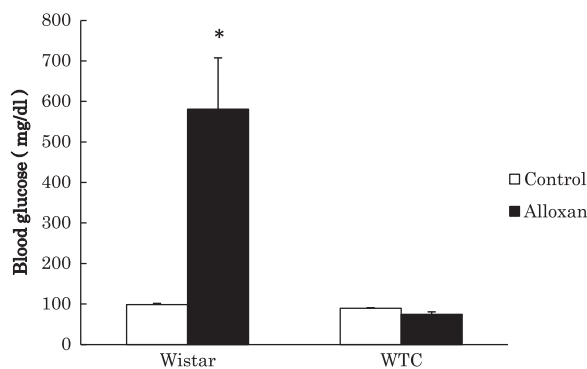
Fig. 4. Effect of alloxan on glycemia in Wistar rat and WTC rat. Open bars show Wistar rat (n=6). Closed ones show WTC rat (n=4). *: p < 0.05 VS control.

expression of metallothionein 2a in pancreas and liver to STZ administration of WTC rats was significantly higher than that of Wistar rats. Moreover, alloxan did not induce diabetes in WTC rats as same as STZ. These results suggest that WTC rats might have powerful antioxidant property to protect β cells in pancreas. Because the STZ-resistant property is very close characteristics to human beings, WTC rats will become a useful animal model in diabetic researches.

At first we thought that possible mechanisms for the marked STZ resistance in WTC rats could be impaired uptake of STZ or enhanced secretion of insulin by pancreatic β cells. Expression in the plasma membrane of β cells of the glucose transporter isoform GLUT2 [17,18], which has a high Km for glucose (17 mM) [19,20] may be required for the normal functioning of the β cell glucose sensor [17–21]. Thus a decreased expression of GLUT2 is associated with the β cell dysfunction of rodent models of insulin dependent diabetes mellitus and non-insulin-dependent diabetes mellitus and decreased GLUT2 levels may impair normal glucose uptake and metabolism thereby preventing glucose sensing [22]. Therefore, we evaluated the gene expression of G to know how STZ resistance came out in WTC rat. But there were no significant differences of this gene between Wistar rat and WTC rat.

Closure of ATP-sensitive K^+ channels (K_{ATP} channels) in response to metabolically generated ATP or binding of sulfonylurea drugs stimulates insulin release from pancreatic β cells. The physiological importance of K_{ATP} channels in insulin secretion was established > 20 years ago [23]. Heterozygous gain-of-function mutations in the KCJN11 gene encoding the Kir6.2 subunit of this channel are found in ~47% of patients diagnosed with permanent diabetes at < 6 months of age. At substimulatory glucose concentrations, K^+ efflux through open K_{ATP} channels maintains the β cell membrane at a hyperpolarized potential of around −70 mV, which keeps voltage-gated Ca^{2+} channels closed [24]. Elevation of the blood glucose concentration increases glucose uptake and metabolism by the β cell, producing changes in cytosolic nucleotide concentrations that cause K_{ATP} channel closure. This leads to a membrane depolarization that opens voltage-gated Ca^{2+} channels initiating β cell electrical activity and Ca^{2+} influx, and the subsequent rise in $[Ca^{2+}]_i$ triggers exocytosis of insulin granules. Therefore, we also evaluated the gene expression of Kir6.2. However, we could not find any difference in the expression of this gene between Wistar rats and WTC rats.

On the other hand, because STZ leads β cell death by producing oxidative stress, we investigated the gene expression of metallothionein which has not only anti-oxidative but also anti-diabetic function [25]. Because liver and skeletal muscles are glucometabolic tissues, we investigated the gene expression in not only pancreas but also liver and skeletal muscles. In fact, the antioxidant capacity in Wistar rats was weakened by STZ injection, but not in WTC rats.

Thus, it seems that the STZ-resistant mechanism in WTC rats may be its own possessed antioxidant ability. Earlier studies have shown that metallothionein has antioxidant effect by absorbing heavy metal, such as cadmium and so on, which makes from hydrogen peroxide to hydroxyl radical [26]. However, while it was shown that STZ-induced diabetes related to nitric oxide, the details of the reactive oxygen species (ROS) that STZ produces were still unknown. STZ and alloxan, cytotoxic glucose analogs, are the most prominent diabetogenic chemicals in diabetes research, although their cytotoxicity is achieved via different pathways [27]. The chemical properties of alloxan is generation of ROS, while DNA alkylation is thought main mechanisms of β cell death with STZ. Even though STZ is also generator of ROS including superoxide and hydroxyl radicals. Therefore, we investigated the response to alloxan, which induced diabetes by producing hydroxyl radical [16], to make it clear whether the course where hydrogen peroxide changes to a hydroxyl radical participates in STZ resistance in WTC rats. Alloxan also did not induce diabetes in WTC rats. These results suggest that the strength of antioxidant ability of production and resolution of hydrogen peroxide affects STZ resistance in WTC rats.

WTC rats are sometimes used as a control of WTC-deafness KYOTO (WTC-*dfk*) rats [28]. WTC-*dfk* rats have a spontaneous mutation of KCNQ1 gene which produces a slowly activating delayed rectifier potassium current. Mutations of the human KCNQ1 gene are associated with the congenital long-QT syndrome and increases the risk of sudden death from cardiac arrhythmias. Moreover, a multistage genome-wide association study has shown that variants in KCNQ1 are associated with susceptibility to diabetes [29]. WTC and WTC-*dfk* strains are coisogenic and have an identical genetic background except for the *dfk* deletion. Therefore, WTC-*dfk* rats may also have the STZ-resistant characteristics same as WTC rats. Of course further studies will be needed, both of these strains of rats might be useful for diabetes research in the future.

Moreover, there are a lot of animal models both type I and type II diabetes including chemically as well as genetically induced models in diabetes research [30]. In these models, there are many studies about diabetes using STZ-induced diabetic rats. Human islets are likely to be relatively resistant to STZ, but, typically, rats are high sensitive to STZ. And so, it is more difficult to discuss the relations between the results of the studies about diabetes using STZ-induced diabetic rats and the studies of treatments of human diabetes. So, WTC rats are expected to give us useful information about research on discoveries of the treatment of human diabetes.

In conclusion, WTC rats are STZ resistant for its own strong antioxidant ability and WTC rats are expected to be useful to develop studies about diabetes.

Conflict of interest

None.

Acknowledgments

References

[1] Z.Z. Chaudhry, D.L. Morris, D.R. Moss, E.K. Sims, Y. Chiong, T. Kono, C. Evans-Molina, Streptozotocin is equally diabetogenic whether administered to fed or fasted mice, Lab. Anim. 47 (2013) 257–265.
[2] M.C. Deeds, J.M. Anderson, A.S. Armstrong, D.A. Gastineau, H.J. Hiddinga,

A. Jahangir, N.L. Eberhardt, Y.C. Kudva, Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models, Lab. Anim. 45 (2011) 131–140.

[3] N. Sakata, G. Yoshimatsu, H. Tsuchiya, S. Egawa, M. Unno, Animal models of diabetes mellitus for islet transplantation, Exp. Diabetes Res. 2012 (2012) 256707.

[4] B. Kushner, M. Lazar, M. Furman, T.W. Lieberman, I.H. Leopold, Resistance of rabbits and guinea pigs to the diabetogenic effect of streptozotocin, Diabetes 18 (1969) 542–544.

[5] H. Yang, J.R. Wright Jr, Human beta cells are exceedingly resistant to streptozotocin in vivo, Endocrinology 143 (2002) 2491–2495.

[6] Y. Shirasaka, M. Ito, H. Mikawa, T. Serikawa, J. Yamada, Alterations of benzodiazepine receptor binding in tremor rats with absence-like seizures, J. Neurochem. (1992) 2292–2295.

[7] A. Velangi, G. Fernandes, T.M. Wolever, Evaluation of a glucose meter for determining the glycemic responses of foods, Clin. Chim. Acta 356 (2005) 191–198.

[8] M. Hosokawa, W. Dolci, B. Thorens, Differential sensitivity of GLUT1- and GLUT2-expressing beta cells to streptozotocin, Biochem. Biophys. Res. Commun. 289 (2001) 1114–1117.

[9] T. Miki, K. Nagashima, F. Tashiro, K. Kotake, H. Yoshitomi, A. Tamamoto, T. Gonoi, T. Iwanaga, J. Miyazaki, S. Seino, Defective insulin secretion and enhanced insulin action in KATP channel-deficient mice, Proc. Natl. Acad. Sci. USA 95 (1998) 10402–10406.

[10] J. Xu, L. Zhang, A. Chou, T. Allaby, G. Belanger, J. Radziuk, B.J. Jasmin, T. Miki, S. Seino, J.M. Renaud, KATP channel-deficient pancreatic beta-cells are streptozotocin resistant because of lower GLUT2 activity, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E326–E335.

[11] J. Duprez, L.P. Roma, A.F. Close, J.C. Jonas, Protective antioxidant and anti-apoptotic effects of ZnCl2 in rat pancreatic islets cultured in low and high glucose concentrations, PLoS One 7 (2012) e46831.

[12] L. Gao, G. Wang, H. Liu, C. Yan, Effect of combined treatment with clozapine and metformin on fasting blood glucose, insulin level and expression of the glucose transporter-2 (GLUT2) in sprague-dawley rats, Shanghai Arch. Psychiatry 25 (2013) 149–156.

[13] Y. Huang, Y. Zhang, Z. Shao, K.A. O'Hara, M.A. Kopilas, L. Yu, T. Netticadan, H. D. Anderson, Suppression of endothelin-1-induced cardiac myocyte hypertrophy by PPAR agonists: role of diacylglycerol kinase zeta, Cardiovasc. Res. 90 (2011) 267–275.

[14] M. Zhou, H.J. He, M. Hirano, M. Sekiguchi, O. Tanaka, K. Kawahara, H. Abe, Localization of ATP-sensitive K$^+$ channel subunits in rat submandibular gland, J. Histochem. Cytochem. 58 (2010) 499–507.

[15] M. Sato, T. Kawakami, M. Kondoh, M. Takiguchi, Y. Kadota, S. Himeno, S. Suzuki, Development of high-fat-diet-induced obesity in female metallothionein-null mice, FASEB J. 24 (2010) 2375–2384.

[16] M. Katoh, K. Sakurai, Y. Fujimoto, Alloxan radical-induced generation of reactive oxygen species in the reaction system of alloxan with ascorbate, Yakugaku Zasshi 122 (2002) 831–839.

[17] B. Thorens, H.K. Sarkar, H.R. Kaback, H.F. Lodish, Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells, Cell 55 (1988) 281–290.

[18] L. Orci, B. Thorens, M. Ravazzola, H.F. Lodish, Localization of the pancreatic beta cell glucose transporter to specific membrane domains, Science 245 (1989) 295–297.

[19] J.H. Johnson, C.B. Newgard, J.L. Milburn, H.F. Lodish, B. Thorens, The high Km glucose transporter of islets of Langerhans is structurally identical and functionally similar to the high Km transporter of liver, J. Biol. Chem. 265 (1990) 6548–6551.

[20] B. Thorens, Molecular and cellular physiology of GLUT2, a high-Km facilitated diffusion glucose transporter, Int. Rev. Cytol. 137A (1992) 209–238.

[21] B. Thorens, M.J. Charron, H.F. Lodish, Molecularphysiology of glucose transporters, Diabetes Care 13 (1990) 209–218.

[22] R.H. Unger, Diabetic hyperglycemia: link to impaired glucose transport in pancreatic beta cells, Science 251 (1991) 1200–1205.

[23] F.M. Ashcroft, D.E. Harrison, S.J. Ashcroft, Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells, Nature 312 (1984) 446–448.

[24] F.M. Ashcroft, P. Rorsman, Electrophysiology of the pancreatic β-cell, Prog. Biophys. Mol. Biol. 54 (1989) 87–143.

[25] A. El-Mahmoudy, Y. Shimizu, T. Shiina, H. Matsuyama, M. El-Sayed, T. Takewaki, Successful abrogation by thymoquinone against induction of diabetes mellitus with streptozotocin via nitric oxide inhibitory mechanism, Int. Immunopharmacol. 5 (2005) 195–207.

[26] K.S. Min, Physiological significance of metallothionein in oxidative stress, Yakugaku Zasshi 127 (2007) 695–702.

[27] S. Lenzen, The mechanisms of alloxan- and streptozotocin-induced diabetes, Diabetologia (2008) 216–226.

[28] H. Gohma, T. Kuramoto, M. Kawamura, R. Okajima, N. Tanimoto, et al., WTC deafness Kyoto (dfk): a rat model for extensive investigations of Kcnq1 functions, Physiol. Genom. 24 (2006) 198–206.

[29] H. Unoki, A. Takahashi, T. Kawaguchi, K. Hara, M. Horikoshi, G. Andersen, et al., SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations, Nat. Genet. 40 (2008) 1092–1097.

[30] A.J.F. King, The use of animal models in diabetes research, Br. J. Pharm. (2012) 877–894.