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WTC rat has unique characteristics such as resistant to streptozotocin

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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.

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 °C until use. Insulin concentrations were measured by a rat insulin ELIZA kit (Shibayagi Co., Japan).

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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

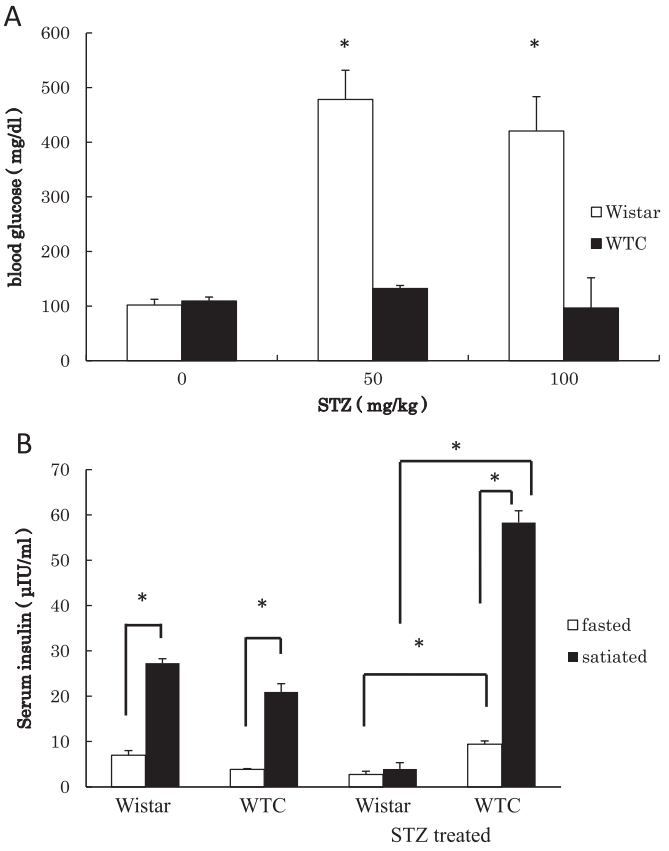

Fig. 1. Effects of STZ on glycemia and insulin secretion in Wistar rats and WTC rats. (A) Open bars show Wistar rat (n=6). Closed ones show WTC rat (n=6). *:$p < 0.05$ VS 0 mg/kg. (B) Serum insulin levels were measured with and without STZ treatment in Wistar rats and WTC rats during fasted (open bars)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

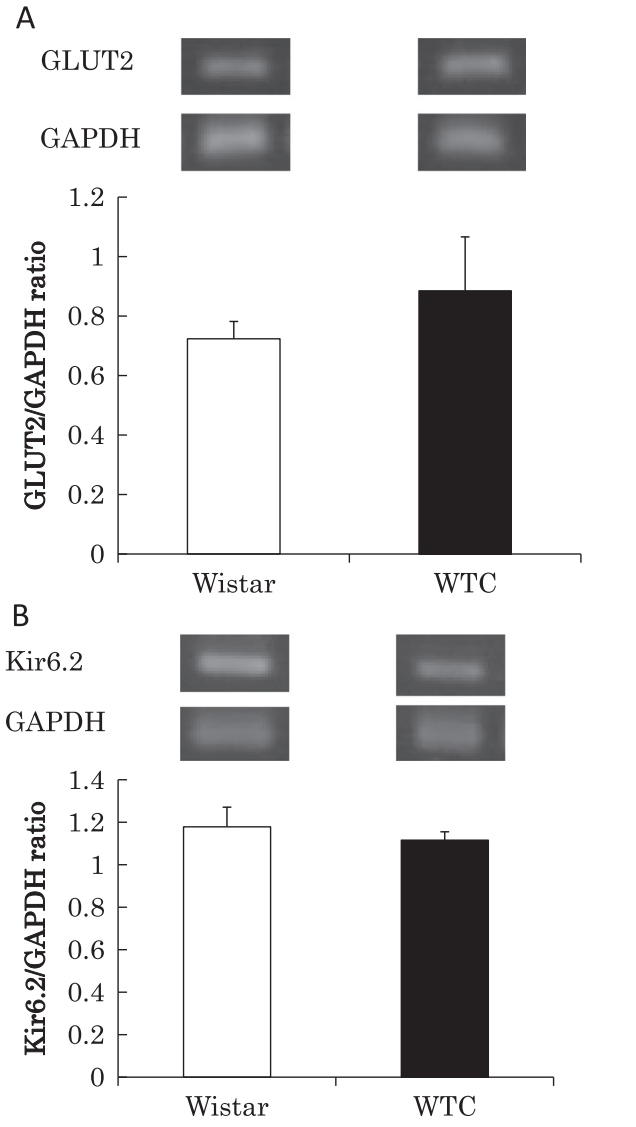

Fig. 2. The gene expressions of possible mechanisms relevance to uptake of STZ or enhanced secretion of insulin for the STZ resistance in WTC rats. (A) GLUT2 and (B) Kir6.2 expressions in Wistar rats (n=6) and WTC rats (n=6) were detected by RT-PCR. GAPDH was used as house keeping gene.

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