Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.3, No.6, 291-296, 2016

Original Article

Streptozotocin-induced diabetic state triggers glucose-dependent insulinotropic polypeptide (GIP) expression in the rat liver

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(Received December 13, 2016; Accepted December 20, 2016)

ABSTRACT — Streptozotocin (STZ), a toxic glucose analogue for pancreatic β cells, has been demonstrated as a treatment option for insulinoma. Glucose-dependent insulinotropic polypeptide (GIP), an incretin hormone, is secreted by K cells in the duodenum in response to food intake and acts on pancreatic β cells, leading to increased secretion of insulin. We previously reported that GIP gene expression in the liver is modified in a diabetic setting. However, the role of GIP in the liver has not been fully elucidated; we aimed to discover its effects on type 1 diabetes by focusing on GIP protein expression in a type 1 diabetes rat model following STZ administration. In this study, we assessed whether glucose and lipid metabolism affected GIP expression in the liver following STZ-induced diabetes. Diabetes was induced by intraperitoneal injection with 70 mg/kg STZ. We expected that blood glucose levels would be higher because of STZ treatment as a result of reduced insulin secretion in pancreatic β cells. Interestingly, GIP was expressed only in the liver of STZ-treated rats; however, blood glucose levels were not elevated. On the other hand, blood triglyceride and cholesterol levels were higher in STZ-treated rats with hepatic GIP protein expression than in control rats. These findings indicated that GIP protein expression in the liver possibly has hypoglycemic action, which may ameliorate the damage of pancreatic β cells induced by STZ treatment, rather than affect glycolipid metabolism.

Key words: Streptozotocin, Pancreatic β cell toxicity, Hepatic GIP protein, Hypoglycemic action, Diabetes

INTRODUCTION

The effectiveness of streptozotocin (STZ), a toxic glucose analogue for pancreatic β cells (Szkudelski, 2001), as a treatment option for insulinoma has been demonstrated through clinical applications (Kouvaraki *et al*., 2004). Diabetes treatment and the mechanism of diabetic complications are heavy topics of research, and some of these investigations have been conducted using a type 1 diabetes rat model via STZ administration (Lenzen, 2008; Kohda *et al*., 2008, 2009, 2012).

We demonstrated that expression of various proteins associated with progression and development of diabetic complications is accelerated (Kohda *et al*., 2008, 2009, 2010; Tanaka *et al*., 2010). Furthermore, we evaluated the transcriptomic profiles of the livers of obese diabetic rats using microarrays, which suggested transcriptomic modification of genes involved in glucose metabolism, lipid metabolism, vascular physiology, and carcinogenesis in the liver (Kohda *et al*., 2012). *Gip* encodes the gastric inhibitory polypeptide, also known as the glucose-dependent insulinotropic polypeptide (GIP). We particularly discovered the transcriptional modulation of GIP in the liver (Kohda *et al*., 2012). GIP is a gut-derived incretin hormone that is secreted in response to nutrient ingestion (Gautier *et al*., 2005; Baggio and Drucker, 2007). Increased GIP signaling has been suggested to regulate fat metabolism within adipocytes (Yip and Wolfe, 2000; Getty-Kaushik *et al*., 2006; Kim *et al*., 2007). Hence, GIP is described as the obesity hormone and may contribute to the pathogenesis of type 2 diabetes (Marks *et al*., 2006). However, GIP potentiates glucose-dependent insulin secretion and enhances pancreatic β-cell survival through regulation of β-cell proliferation and apoptosis (Kim *et al.*, 2005). Incretin hormone, such as glucagon-like peptide-1 and GIP, receptor signaling exerts physio-

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logically relevant actions critical for glucose homeostasis and represents a pharmacologically attractive target for the development of therapeutic agents for diabetes (Gault *et al.*, 2003).

GIP is secreted by K cells in the duodenum in response to food intake and acts on pancreatic β cells, leading to increased secretion of insulin (Gautier *et al*., 2005; Baggio and Drucker, 2007). Type 1 diabetes is an affliction wherein insulin secretion is either reduced or exhausted, depending on the surviving pancreatic β cells. We reported the amplification of GIP gene expression in the livers of obese type 2 diabetic rats (Kohda *et al*., 2012). In the present study, we primarily focused on the expression of GIP in the livers of type 1 diabetic rat model and assessed its effects on the diabetic state. Herein, we report its effects on type 1 diabetes via GIP protein liver expression in the STZ-induced type 1 diabetic rat model.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Wako Pure Chemical Industries, Japan. For blood glucose testing, a glucose pilot meter and blood glucose test strips were used (Aventir Biotech, Carlsbad, CA, USA). The antibodies used were anti-GIP and horseradish peroxidase (HRP)-conjugated anti-goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Saline solution was supplied by Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Mammalian tissue lysis and extraction reagent was purchased from Sigma (St. Louis, MO, USA). Protease inhibitor cocktail and blocking solution were supplied by Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity available from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals

Male Sprague Dawley (SD) rats (6 weeks of age) were used in all experiments. They were the progeny of rats obtained from the Japan SLC, Inc. (Shizuoka, Japan) and were maintained in the central animal facility of our university. The animals were provided with a commercial diet and water *ad libitum* under temperature-, humidity-, and lighting- controlled conditions (22 ± 2°C, 55 ± 5%, and a 12:12-hr light-dark cycle, respectively). Experimental protocols and animal care methods were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

Experimental design

SD rats had standard pellet food and tap water *ad libitum*. Diabetes was induced by an intraperitoneal injection of 70 mg/kg STZ in saline solution; on day 7 of STZ administration, blood was drawn from the tail vein, and blood glucose levels were measured using a blood glucose test strip (Aventir Biotech). Only rats with high blood glucose levels (> 250 mg/dL) 7 days after STZ administration were used as STZ diabetic rats with hyperglycemia. On the other hand, rats with low blood glucose levels 7 days after STZ administration were used as STZ-treated rats with non-hyperglycemia. Rats treated with a single intraperitoneal injection of vehicle saline solution were used as controls.

Measurement of blood pressure and blood parameters

Systolic blood pressure and heart rate were measured using a tail-cuff system (BP98A, Softron) in conscious animals. Casual blood glucose levels were measured using a blood glucose test strip (Aventir Biotech). Following these tests, all animals were sacrificed 34 days after STZ or vehicle injection, blood was drawn from the ventral aorta into tubes containing heparin, and livers were extracted. Plasma was immediately separated by centrifugation. Cholesterol and triglycerides levels in supernatants were measured using the total cholesterol and triglycerides test (Wako), respectively.

Preparation of the protein extraction solution from rat livers

Immediately after livers were removed and weights were obtained, liver tissue was immediately immersed and rinsed in ice-cold saline. They were then minced, and liver (100 mg) tissue was homogenized at 4°C in 900 μL tissue lysis and extraction reagent with a protease inhibitor cocktail. Homogenates were centrifuged at 15,000 rpm for 15 min, and supernatants were used for western blot analysis to examine the expression of GIP protein.

Western blot analysis

Protein samples were separated by 16% polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked with blocking buffer for 1 hr at room temperature. The membranes were then incubated with the specific primary anti-GIP antibody in signal enhancer solution A (Nacalai Tesque) overnight at 4°C. Membranes were washed three times in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Tween 20 (TBST) to remove unbound antibody. They were then incubated with an HRP-conjugated secondary antibody in signal enhancer solution B

(Nacalai Tesque) for 1 hr at room temperature. Chemiluminescence for GIP protein expression was detected by the Ez-Capture MG machine (ATTO Corp., Tokyo, Japan) using an enhanced chemiluminescence reagent (Nacalai Tesque).

Statistical analysis

Data are expressed as mean ± S.E. Statistical analysis of the data from multiple groups were performed by ANOVA followed by Scheffe's F-tests. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of streptozotocin administration on blood glucose levels in SD rats

We expected that blood glucose levels of rats would be higher because insulin secretion in pancreatic β cells may have been reduced or exhausted due to STZ treatment. However, some rats did not have high blood glucose levels (> 250 mg/dL) upon STZ treatment (Fig. 1). In this study, 7 days after STZ and vehicle injection, rats were divided into the following groups: group 1, controls; group 2, STZ diabetic rats with hyperglycemia; and group 3, STZ-treated rats with non-hyperglycemia.

Effect of streptozotocin on cardiac function, body weight, and organ weight levels

The Table 1 shows mean heart rate, systolic blood pressure (BP), body weight, liver weight, and epididymal fat weight 34 days after STZ injection. Fluctuations in heart rate were not observed after STZ treatment. Systolic BP in STZ-treated rats with non-hyperglycemia was significantly less than in the control rat group. No difference in body weight was observed between the three groups at day 34 after STZ treatment. Livers in STZ diabetic rats with hyperglycemia weighed less compared to the rest of the groups. Livers and epididymal fat in STZ-treated rats with non-hyperglycemia weighed similarly to those in the control group.

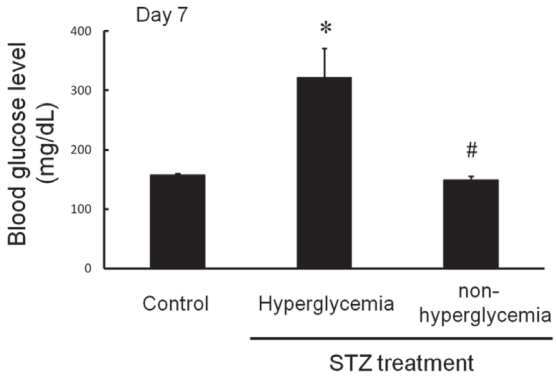

Fig. 1. Blood glucose levels in rats 7 days after intraperitoneal injection of streptozotocin (STZ, 70 mg/kg, i.p.). Glycemic parameter levels were determined from fed blood samples. Each value represents the mean ± S.E. n = 3-4 per group. *p < 0.05, compared with the control. #p < 0.01, compared with the "STZ treatment hyperglycemia group."

Effect of streptozotocin on biochemical measurement levels

Blood glucose levels increased in group 2, STZ diabetic rats with hyperglycemia (Fig. 2A). However, blood glucose levels were not altered by the STZ treatment on day 34 in group 3, STZ-treated rats with non-hyperglycemia (Fig. 2A). On the other hand, plasma triglycerides and total cholesterol levels increased in both groups 2 and 3 (Figs. 2B and 2C).

GIP protein liver expression upon exposure to STZ

There is a suggested relationship between GIP and pancreatic β cells. We have previously reported that the GIP gene is expressed in livers exposed to a diabetic state. STZ treatment with non-hyperglycemia, in which blood glucose levels were not elevated, caused marked abundance of the GIP protein (Fig. 3). There was no significant GIP protein expression in control group and STZ treatment hyperglycemia group (Fig. 3).

Table 1. Heart rate, systolic blood pressure (BP), body weight, liver weight, and epididymal fat weight in rats 34 days after injection of streptozotocin (STZ, 70 mg/kg, i.p.).

Group	Heart rate (bpm)	Systolic BP (mmHg)	Body weight (g)	Liver weight (g)	Epididymal fat (g)
1. Control	347 ± 32.4	130 ± 4.6	363 ± 10	15.7 ± 0.37	2.6 ± 0.15
2. STZ hyperglycemia	340 ± 2.9	137 ± 9.0	295 ± 42	11.8 ± 1.10*	1.4 ± 0.62
3. STZ non-hyperglycemia	339 ± 14.2	118 ± 1.3*	375 ± 11	15.0 ± 0.60	3.0 ± 0.33

Each value represents the mean ± S.E. n = 3-4 per group. *p < 0.05, compared with the control.

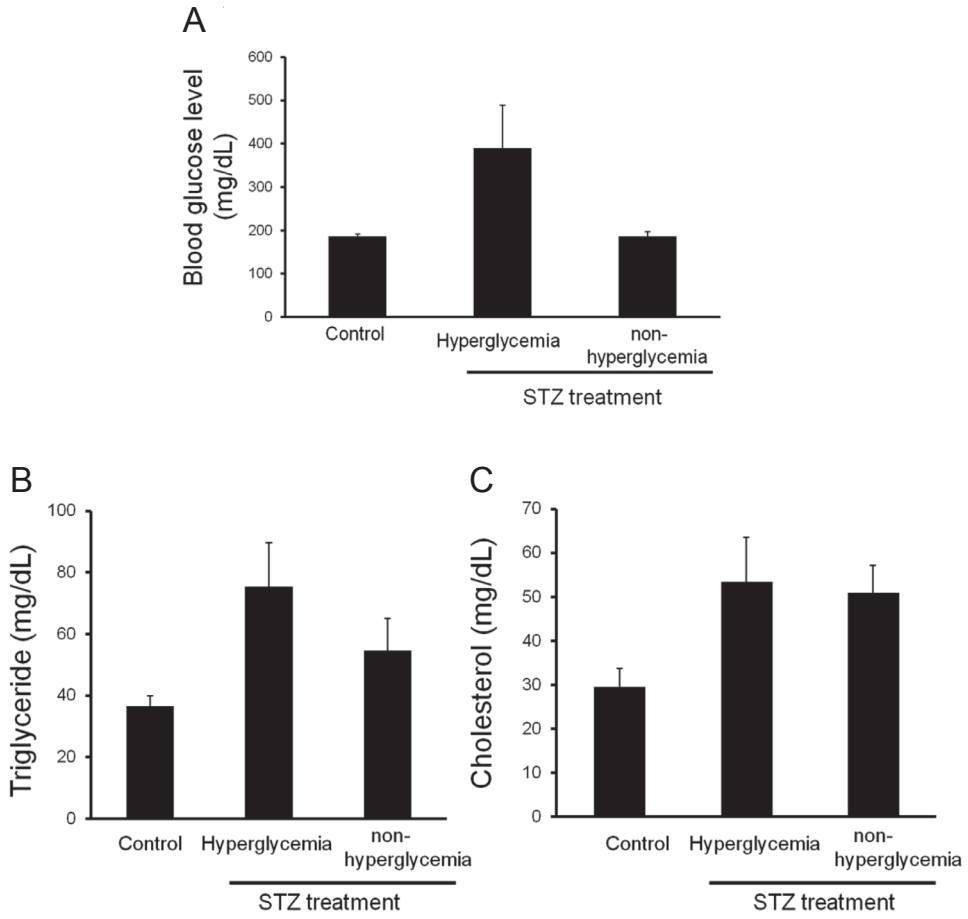

Fig. 2. Blood glucose (A), plasma triglyceride (B), and plasma cholesterol (C) levels in rats 34 days after administration of streptozotocin (STZ, 70 mg/kg, i.p.). In this experiment, 7 days after the STZ or vehicle treatment, rats were placed in one of three groups as follows: group 1, Controls; group 2, STZ diabetic rats with hyperglycemia; and group 3, STZ-treated rats with non-hyperglycemia. Metabolic parameters were determined from fed blood samples. Each value represents the mean ± S.E. n = 3-4 per group.

DISCUSSION

Interestingly, GIP was expressed only in the livers of STZ-treated rats with non-hyperglycemic conditions. The triglyceride and cholesterol levels of STZ-treated rats with hepatic GIP expression increased compared with control rats. It has been previously reported that transgenic GIP over expression has the potential to improved glucose homeostasis (Kim *et al*., 2012). We suggested that GIP protein expression in the liver exerts hypoglycemic action, thus, affecting glucose, rather than glycolipid, metabolism. GIP may contribute to the pathogenesis of obese type 2 diabetes (Marks *et al*., 2006). In this study, fluctuations in body weight, liver weight, and epididymal fat weight were not observed in rats with hepatic GIP protein expression compared with control rats. Additionally, GIP protein expression in the rat liver on STZ treatment non-hyperglycemic group, affected systolic blood pressure levels, which support the notion that incretin therapy improve cardiovascular damage and cardiac functions (Lee *et al*., 2013; Wang *et al*., 2013). Dipeptidyl peptidase-4 inhibitors are therapeutic agents for diabetes that control blood glucose by preventing the degradation of the incretin hormones such as glucagon-like peptide-1 and GIP (Cernea, 2011; Irwin and Flatt, 2015).

The supposed mechanism of action of GIP is dependent on blood glucose levels (Gautier *et al*., 2005; Baggio and Drucker, 2007). This dependency allows GIP to promote insulin secretion and induce an increase in β-cell volume via modulating apoptosis and formation and pro-

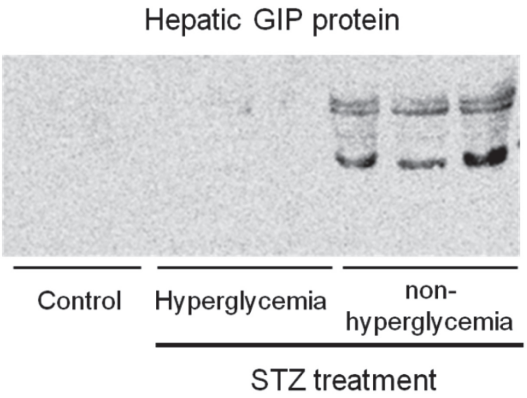

Fig. 3. Expression of the glucose-dependent insulinotropic polypeptide (GIP) protein in the liver exposed to streptozotocin (STZ) treatment. In this experiment, 7 days after STZ or vehicle treatment, rats were divided into the following groups: group 1, Controls; group 2, STZ diabetic rats with hyperglycemia; and group 3, STZ-treated rats with non-hyperglycemia. GIP protein levels were detected by western blotting using specific antibody.

liferation of β cells (Kim *et al.*, 2005). Expression of GIP in pancreatic β cells has been reported by Fukami *et al*. (2013), but the effect of hepatic GIP protein dynamics in diabetes was unclear. In this study, it should be considered that insulin concentration and the condition of pancreatic β cells after STZ treatment might affect the landscape of GIP-diabetes dynamics. The exact details of GIP's mechanism of action remain unknown and warrant future studies. However, we suggest that these hypoglycemic actions may occur by ameliorating the STZ-induced damage to pancreatic β cells, and, thus may be a preconditioning effect induced by mild STZ treatment. Whether hepatic GIP protein expression is a protective cellular reaction in response to STZ-induced transient acute damage to pancreatic β cells will be a topic of future investigations.

Type 1 diabetes is generally considered as an autoimmune disease that destroys the pancreatic β cells that make insulin. Type 1 diabetes patients need to take insulin in order to control blood glucose levels and reduce diabetic complications. Glycemic control can reduce the risk for metabolic disorders. Considering the possible role of hepatic GIP in achieving satisfactory glycemic control, normalization of insulin secretion capacity, and improvement in pancreatic β cell function in type 1 diabetes patients, we believe that detailed investigation of the relationship between GIP expression in the liver and insulin secretion is necessary.

Conflict of interest---- The authors declare that there is no conflict of interest.

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