Bilateral Midbrain Transection Induced Hyperphagia Accelerates The Development of Diabetes in Spontaneously Diabetic Torii (SDT) Rats

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Abstract: The Spontaneously Diabetic Torii (SDT) rat is a model of severe type 2 diabetes and its complications. Although the diabetic features of the SDT rat make it a useful research tool for studying diabetes, the usefulness of this tool is limited by the slow onset of the disease. To solve this problem, we performed bilateral midbrain transection on SDT rats and evaluated whether hyperphagia accelerates the onset of diabetes. By severing fibers ascending from the nucleus tractus solitarius to the limbic system through the ventral and dorsal tegmental nuclei, food consumption was significantly increased and the onset of diabetes was accelerated. The cumulative incidence of diabetes was 88.9% at 7 weeks after surgery in midbrain-transsected SDT rats (14 weeks of age) and 20.0% in sham operated rats. Increased food consumption was correlated to body weight, plasma glucose level, plasma triglyceride level, and plasma insulin level. In conclusion, the overeating caused by blocking anorexigenic signals in the brain significantly accelerates the onset of diabetes in SDT rats. The early development of type 2 diabetes may accelerate microvascular complications and is considered useful in the study of the disease in SDT rats.

Keywords: Bilateral midbrain transection, Diabetes, Hyperphagia, SDT rats

INTRODUCTION

Diabetes is one of the most common metabolic diseases. According to the International Diabetes Federation (IDF), 463 million adults are living with this disease and the number of adult patients is expected to increase to 700 million by 2045 [1]. In addition, more than 50% of diabetics develop microvascular complications, such as diabetic retinopathy, diabetic nephropathy, or diabetic peripheral neuropathy, all of which lower the patients’ quality of life. Approximately 4.2 million adults died from diabetes and its complications in 2019 [1].

Today, many drugs lower blood glucose levels, but still, their efficacies are not sufficient enough to prevent the development of microvascular complications. Thus, new drugs targeting the underlying mechanism of diabetic complications are needed. To develop new drugs, a good animal model that functionally and pathologically mimics human diabetes is essential. In addition, the proportion of non-obese patients with type 2 diabetes is higher in Asian countries than in Western countries [2]. Therefore, an animal models of non-obese type 2 diabetes have been developed following the need for testing of various drugs [3]. The Spontaneously Diabetic Torii (SDT) rat is a model of non-obese type 2 diabetes and is reported to develop significant hyperglycemia with severe diabetic complications in eyes, kidneys, and peripheral nerves [4,5]. These SDT rat features are very useful for investigation purposes; however, their slow development (manifesting at approximately 17-25 weeks of age [4,6] sometimes limited the usefulness of this animal model. Because excess intake of a high-calorie diet or overeating is known to accelerate the onset of diabetes in animal models, such as Otsuka-Long-Evans-Tokushima Fatty (OLETF) rats [7], we performed bilateral midbrain transection on SDT rats before diabetes developed and evaluated whether overeating accelerates the onset of diabetes in this animal model.

METHODS

Animals

Seven-week-old male SDT rats (CLEA Japan, Tokyo, Japan) were used in the study. Fifteen SDT rats were divided into two groups: a bilateral midbrain transection group containing 10 rats and a sham operation group containing 5 rats. All animal protocols complied with guidelines of the Animal Care Committee of Central Pharmaceutical Research Institute at Japan Tobacco Inc. Animals were housed in the controlled environment of an animal room (temperature 23 ± 3°C, humidity 55 ± 15%, 12-hour lighting cycle) and allowed free access to diet (CRF-1; Oriental Yeast, Tokyo, Japan) and water.

Bilateral Midbrain Transection

Bilateral midbrain transection was performed on SDT rats as previously reported [8,9]. Briefly, the head
was fixed in a stereotaxic instrument in a 2.4-mm nose-down position under appropriate isoflurane anesthesia. A 1.5-mm-wide razor was carefully inserted into the brain bilaterally in a coronal plane, 1 mm anterior to the lambdoidal suture, 0.5 mm bilaterally from the midline and 7.7 mm ventral to the dura. The lesion location details are presented by Crawley et al. [8]. Sham operated animals were also anesthetized to open up the head, but the incision was sutured without transection. One out of the ten surgically operated animals was excluded from the experiment because of the development of a behavioral abnormality; therefore, there were nine evaluable animals in the bilateral midbrain transection group. After the surgery, rats were bred under the same conditions as before surgery and daily food intakes were monitored for 4 weeks.

**Blood Biochemical Parameters**

Blood biochemical parameters were measured before and 1, 2, 3, 4, and 7 weeks after surgery. Blood was collected from the tail vea, and plasma samples were prepared by centrifuging the blood. Plasma glucose and triglyceride (TG) levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic biochemical analyzer (Hitachi 7180; Hitachi High-Tech Corporation, Tokyo, Japan). Plasma insulin level was measured with a rat-insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan).

**Statistical analysis**

All results are expressed as mean ± SEM. Statistical differences between midbrain-transected rats and sham-transected rats were analyzed using the F-test, followed by the Student’s t-test for equal variances or Aspin-Welch t test for unequal variances. The Pearson’s chi-square test was performed to evaluate correlations between food consumption and blood parameters or body weight. Log-rank test was performed to test for the differences in the cumulative incidence of diabetes. Diabetes was considered to have developed in animals with plasma blood glucose level over 250 mg/dL. Statistical analysis was performed using Statlight 2000 statistical software (Yukms Co., Ltd., Kawasaki, Japan) or GraphPad Prism 6.07 (GraphPad Software, La Jolla, CA, USA) and differences were accepted as significant at \( p < 0.05 \) (2-sided).

**RESULTS AND DISCUSSIONS**

Bilateral midbrain transection was performed on 7-week-old SDT rats before the development of diabetes. According to previous reports, SDT rats at this age show impaired glucose tolerance (IGT), but not hyperglycemia [10]. Although severe diabetes and its microvascular complications spontaneously develop in this animal model, the diabetes takes too long to develop, making this animal model cumbersome to use as a research tool. To solve this problem, Masuyama et al. inserted the fa allele of obese Zucker fatty (ZF) rats into the SDT rat genome and established the SDT fatty rat. Hyperphagia, obesity, and early onset of severe diabetes with microvascular complications [11,12], as well as osteoporosis [13], non-alcoholic steatohepatitis (NASH) [14], sarcopenia [15], and central nerve diseases [16] develop in the SDT fatty rat, making it a multifaceted research tool for studying metabolic disorders and related diseases. On the other hand, some characteristics of the SDT fatty rat are different from the original SDT rat. The most striking difference is in their eyes. The SDT rat undergoes severe retinal changes, such as folding around the optic disk and detachment of the neurosensory retina from the retinal pigment epithelium due to the formation of a neovascular fibrous membrane. In some severe cases, massive hemorrhage associated with fibrous proliferation around the iris was observed [4,17,18]. However, as far as we know, no such report has noted this in the SDT fatty rat. This difference is probably caused by unexpected changes in traits that are linked to SDT fatty rat production. To avoid such unwanted changes in characteristics, we subjected SDT rats to another treatment. Previously, we fed SDT rats high-fat diet (HFD) because we hypothesized that such a diet would accelerate the onset of diabetes in this model; however, the HFD caused hypersecretion of insulin from pancreatic β-cells in SDT rats [19]. Because the HFD accelerates diminishment in the number of pancreatic β-cells and leads to hyperglycemia and hypoinsulinemia in obese Zucker diabetic fatty (ZDF) rats [20], we suppose that the vulnerability of pancreatic β-cells to the effects of the HFD is different between strains. Sucrose/fat-enriched diet also failed to worsen diabetes in SDT rats; elevation of blood ketone body seems to improve glucose metabolism [21].

Therefore, in this study, we performed bilateral midbrain transection on SDT rats to accelerate the development of diabetes. Cholecystokinin (CCK) is an anorexigenic peptide produced by endocrine I cells mainly distributed in the duodenum and upper small intestine. The attenuation of the anorexigenic effect of CCK by vagotomy indicates its mediation by the gastric vagal fibers [22]. In addition, by severing fibers ascending from the nucleus tractus solitarius to the limbic system through the ventral and dorsal tegmental nuclei that transmit the CCK signals, the dorsal midbrain transection technique prevents CCK signal transmission thereby blocking the anorexigenic effect of CCK and causing hyperphagia [8].

With this surgery, SDT rats clearly developed diabetes at an earlier age compared to sham operated rats and sham transected rats.
As shown in Figure 1A, food consumption was significantly increased after surgery. The incidence of diabetes in male SDT rats has been reported. Shinohara et al. noted that the cumulative incidence of diabetes was 52.4% at age 25 weeks and 100% at 40 weeks [4]. In another report, SDT rats spontaneously developed diabetes after 22 weeks of age [23], and the mean age at diabetes onset was 25.4 weeks [10]. In the present study, SDT rats with bilateral midbrain transection developed diabetes at 10 weeks of age, i.e., three weeks after surgery. Diabetes developed in seven out of nine animals (77.8%) at 10 weeks of age and eight animals (88.9%) at 11 weeks of age. On the other hand, none of the sham treated SDT rats were hyperglycemic at 11 weeks of age. Diabetes developed in only one out of five (20.0%) animals in the sham treated group, even at 14 weeks of age. The cumulative incidence of diabetes is presented in Figure 1B.

Hyperphagia was found even at five days postoperatively (data not shown). The body weight of SDT rats was significantly increased two weeks after the operation (Figure 2A). Postoperative overweight was observed until four weeks after surgery. After the onset of diabetes, the body weight gain was attenuated and finally reversed in sham-operated animals. Plasma glucose level was dramatically increased three weeks after midbrain transection (Figure 2B). At 14 weeks of age (7 weeks post-operation), the plasma glucose level in midbrain transection animals reached 638.7 ± 167.8 mg/dL (sham animals: 209.6 ± 32.2 mg/dL). Similarly, glycated hemoglobin (HbA1c) was 7.25 ± 1.39% (midbrain transection) and 3.77 ± 0.17% (sham). Plasma insulin level in sham SDT rats increased gradually with age. Midbrain transected SDT rats showed a transient increase in plasma insulin level and thereafter, insulin level decreased but the decrease was not statistically significant (Figure 2C). Although we have not evaluated the histopathological changes in pancreas in this study, previous reports suggest that decreased number and mass of pancreatic β-cells leads to hypoinsulinemia and subsequent hyperglycemia in SDT rats [10,24]. Plasma TG level also increased concomitantly with hyperglycemia in SDT rats after midbrain transection (Figure 2D). Increased food consumption was significantly correlated to body weight, plasma glucose level, and plasma TG level (Figure 3). Plasma insulin level also tended to correlate with food consumption, but the correlation was not statistically significant. These findings also support that hyperphagia accelerates the development of diabetes in SDT rats. By using bilateral midbrain transection technique, we succeeded in accelerating the development of diabetes in SDT rats. This classic method increases the convenience and usefulness of SDT rats as models of type 2 diabetes and microvascular complications such as diabetic retinopathy, diabetic nephropathy, and diabetic peripheral neuropathy.
Figure 2. Blood biochemical parameters of SDT rats with or without bilateral midbrain transection. (A) Body weight, (B) plasma glucose level, (C) plasma insulin level, and (D) plasma triglyceride (TG) level. Closed circle: bilateral midbrain transection group; open circle: sham group. Each value represents the mean ± SEM (n=5 or 9). *p<0.05 vs. sham group (Student’s t-test), ##p<0.01 vs. sham group (Aspin-Welch t test).

Figure 3. Correlation between food consumption and other parameters in SDT rats with and without midbrain transection. (A) Correlation between food consumption and body weight, (B) plasma glucose level, (C) plasma insulin level, and (D) plasma triglyceride (TG) level. Pearson’s correlation coefficient and p-value are indicated in each graph.
CONCLUSIONS

In this study, we demonstrated that overeating caused by blocking an anorexigenic signal in the brain significantly accelerates the onset of diabetes in SDT rats received bilateral midbrain transection. We regard the early development of type 2 diabetes and the attendant microvascular complications as useful for the study of these diseases in SDT rats.

CONFLICT OF INTEREST

Tomohiko Sasase, Makoto Ito, and Yukihito Ishii are employees of Japan Tobacco Inc. The authors declare that there are no conflicts of interest regarding this article.

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