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Improvement of Hyperglycemia and Sexual Dysfunction in Diabetic Female Rats by an Artificial Endocrine Pancreas Developed from Mouse β Cells

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Abstract: We investigated the effects of a bioartificial endocrine pancreas (Bio-AEP) produced by mouse β cells on sexual dysfunction of streptozotocin (STZ)-induced diabetic female rats. Female rats were administered STZ (60 mg/kg BW, i.v.) at the age of 10 weeks and transplanted with a Bio-AEP including mouse β cells at the age of 14 weeks (STZ+Bio-AEP group). Lordosis and proceptive sexual behavior of female rats were observed. The results showed that after the Bio-AEP transplant blood glucose recovered from 380–450 mg/dl induced by streptozotocin to 140–230 mg/dl and suppressed lordosis and proceptive behavior also recovered. These results suggest that it is possible to reverse sexual dysfunction by continuous administration of mouse insulin.

Key words: bioartificial endocrine pancreas, diabetic rats, streptozotocin

Diabetes is one of the causes of sexual dysfunction, inducing neuroendocrine dysfunction [11, 13, 19, 21] and apoplexy [2] based on hyperglycemia and insulin insufficiency. Rats are usually used as a diabetic animal models as diabetes can be induced by streptozotocin (STZ) for observations of sexual behavior [18, 21, 22, 23]. Both men and women can contract type 1 and 2 diabetes. Especially, diabetes during pregnancy is common in primiparous women and it is significantly associated with a number of risks of pregnancy and birth, including hypertension [20], intra-amniotic infection [1], and an increased rate of Caesarean section [1, 12]. In addition, the children born to mothers with diabetes in pregnancy are more likely to be premature [12] and develop metabolic abnormalities in later life [17]. Therefore, we focused on sexual behavior testing in females in this study.

Ohgawara *et al*. (1995) created a diffusion chamber as a bioartificial endocrine pancreas (Bio-AEP) with β cells for xenotransplantation [15]. The β cells in the Bio-AEP were maintained for a long time because Bio-AEP included β cells with an agarose-PVMA-collagen matrix [15]. Ohgawara *et al*. (1996) reported that the Bio-AEP suppressed hyperglycemia in STZ-induced diabetic rats [16]. We became interested in these reports on the Bio-AEP, since we thought it might improve

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sexual dysfunction induced by neuroendocrine dysfunction [11, 13, 19, 21] based on hyperglycemia and insulin insufficiency. In addition, the Bio-AEP may make possible transplantation of β cells from different species because the Bio-AEP shows promise for long-term xenotransplantation without immunosuppression [7, 8]. Therefore, the object of our study was to confirm whether or not mouse β cells could improve hyperglycemia and sexual dysfunction caused by diabetes mellitus.

Subject: Fifteen Wistar strain female rats obtained from the Imamichi Institute for Animal Reproduction (Kasumigaura, Ibaraki, Japan) were used in this study. The animals were kept in a room at a temperature of 22–27 degrees Celsius under a light-schedule of 14 h on and 10 h off (lights off at 19:00). They were provided with pelletized diet MB-1 (Funabashi Farms Co., Ltd., Chiba, Japan) and water *ad libitum*. On reaching 8 weeks of age, all female rats were ovariectomized under ether anesthesia and divided into three groups. The first group of five female rats was administered STZ (60 mg/kg BW, i.v.) at the age of 10 weeks and transplanted with Bio-AEP including mouse β cells at the age of 14 weeks (STZ+Bio-AEP group). A second group of five female rats administered STZ (60 mg/kg BW, i.v.) at the age of 10 weeks was transplanted with a diffusion chamber without mouse β cells at the age of 14 weeks as a sham operation (STZ group). The remaining five female rats were administered vehicle without STZ and transplanted with a diffusion chamber without mouse β cells (vehicle group).

Drugs: Streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline acidified to pH 4.5 with citrate and immediately administered intravenously in a volume of 0.1 ml/100 g body weight. Vehicle control females were given the same volumes of acidified saline alone.

Preparation of Bio-AEP: MIN6 cells [14] were passed serially in a 25-cm^2 Corning tissue-culture plastic flask in Dulbecco's modified Eagle's medium (DMEM; Gibco Oriental, Tokyo, Japan) containing 25 mmol/l glucose, supplemented with 12% heat incubated fetal bovine serum (FBS) and penicillin-streptomycin (Gibco). The cells were then incubated in humidified 5% CO_2 and air at 37 degrees Celsius. For embedded-culture of MIN6 cells and transplantation of Bio-AEP, 2×10^7 MIN6 cells were suspended in heat-inactivated FBS and added to agarose-PVMA-collagen gel [14]. Then, 0.5 ml of the mixture was placed in a multiwell plate (24 wells; Corning Glass Works, Corning, NY, USA), and heated to 30 degrees Celsius to allow polymerization of the agarose-PVMA-collagen. The incubation was continued at 37 degrees Celsius under an atmosphere of 5% CO_2 and air. Under these conditions, the agarose-PVMA-collagen mixture polymerized rapidly, trapping the MIN6 cells in the agarose-PVMA-collagen matrix (mixed matrix). The MIN6 cells were suspended in the mixed matrix with 10 nmol/l nicotinamide and 10% FBS (the final concentration of the cells was 4×10^6/ml of the mixed matrix). Insulin secretion from the embedded MIN6 cells was confirmed by ELISA (Mesacup Insulin Test, MBL Co., Nagoya, Japan). The embedded MIN6 cells that showed more than 300 μU/ml were transferred into diffusion chambers as Bio-AEP (Fig. 1[A]). The Bio-AEPs were transplanted into the abdominal cavity of STZ-induced diabetic females (Fig. 1[B]).

Sexual behavior testing: The females were subcutaneously treated with 2 μg estradiol benzoate (EB, dissolved in 0.1 ml sesame oil) daily for 3 days and 0.5 mg progesterone 4–6 h before the behavioral test on the fourth day. In the behavioral test, each female was placed in a semi-circular observation cage (radius 40, high 50 cm), with one sexually active male of the same strain. When the tests were initiated at 19:00 in the dark period, dim red light was provided. The female was observed until she received a total of 10 mounts. The presence of lordosis was recorded after each mount and the results were expressed in terms of the lordosis-to-mount (L/M) ratio and used as an index of sexual receptivity. In addition, the incidences of proceptive behaviors such as ear-wiggling, hopping and darting were also recorded.

Experimental procedure: The experimental design is shown in Fig. 1 [C].

Two weeks after ovariectomy, females received a single injection of vehicle or a dose of STZ, 60 mg/kg body weight. Diabetes was verified by blood glucose concentrations (>180 mg/dl). Body weights and blood glucose of rats were measured every week, the day before and the day after STZ injection. Blood samples were taken from orbital sinus using a heparinized capillary tube. Blood glucose concentrations were measured using an automatic glucose meter (Arkray, Kyoto, Ja-

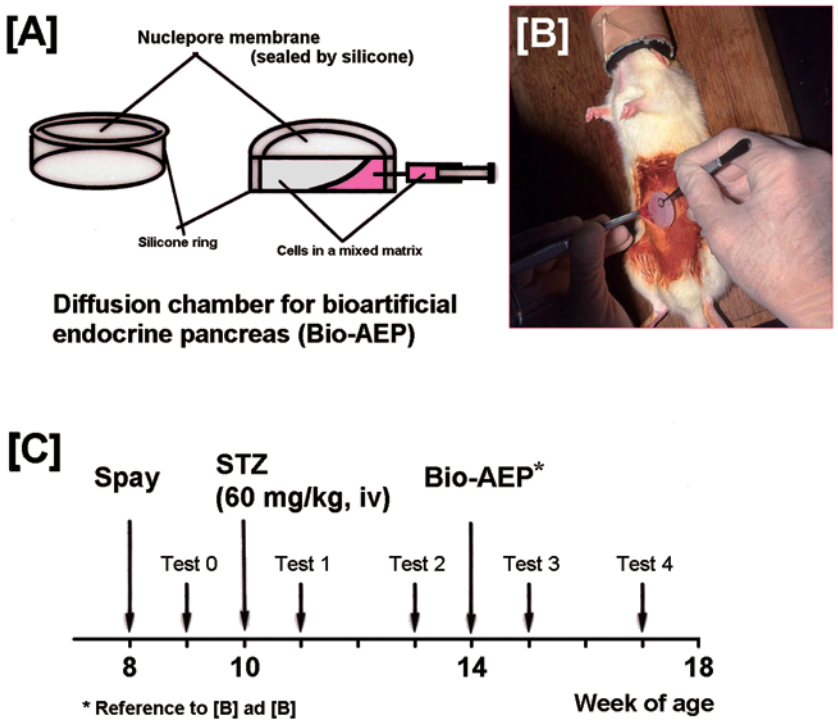

Fig. 1. Experimental design and operation of the diffusion chamber for the bioartificial endocrine pancreas (Bio-AEP). [A]: Time schedule for spaying, STZ administration and transplantation of Bio-AEP. [B]: Preparation of Bio-AEP with mouse β cells. [C]: Intraperitoneal transplantation of Bio-AEP in a rat anesthetized with 1–3% isoflurane.

pan). Four weeks after STZ injection, Bio-AEP was transplanted into diabetic females. As shown in Fig. 1 [C], sexual behavior testings were carried out in Tests 0–4. All procedures were in accordance with the NIH Guidelines of the Animal Care and Use Committee of Nippon Veterinary and Life Science University.

Values of body weight, blood glucose concentration and lordosis were expressed as mean ± SE. Differences of body weight, blood glucose concentrations, and L/M ratio among vehicle, SZT, and STZ+Bio-AEP group were analyzed by 1-way analysis of variance and Tukey's test. Incidences of proceptive behavior were analyzed by Fisher's exact probability test. Values were considered significantly different at $P<0.05$.

The effects of STZ administration and Bio-AEP transplantation on body weights and blood glucose concentrations are shown in Fig. 2.

There were no differences in body weights and blood glucose concentrations among the three groups, the STZ, STZ+Bio-AEP, and vehicle groups. Body weights after STZ administration and Bio-AEP transplantation in both the STZ and STZ+Bio-AEP groups did not increase when compared with the vehicle group. However, blood glucose concentrations that were significantly increased by STZ administration were suppressed by Bio-AEP transplantation. After Bio-AEP transplantation, the blood glucose concentrations of the STZ+Bio-AEP group did not recover to the level of the vehicle group, but the blood glucose concentrations of the STZ+Bio-AEP group decreased more than those of the STZ group ($P<0.05$).

The effects of STZ administration and Bio-AEP transplantation on the lordosis to male mount (L/M) ratio are shown in Fig. 3. In Test 0 as a pretreatment, there were no differences in the L/M ratio among the three groups. In Test 2, the L/M ratio after STZ administration in both the STZ and STZ+Bio-AEP groups decreased compared with that of the vehicle group ($P<0.05$). In Test 3 and Test 4, the L/M ratio after transplantation of β cells in

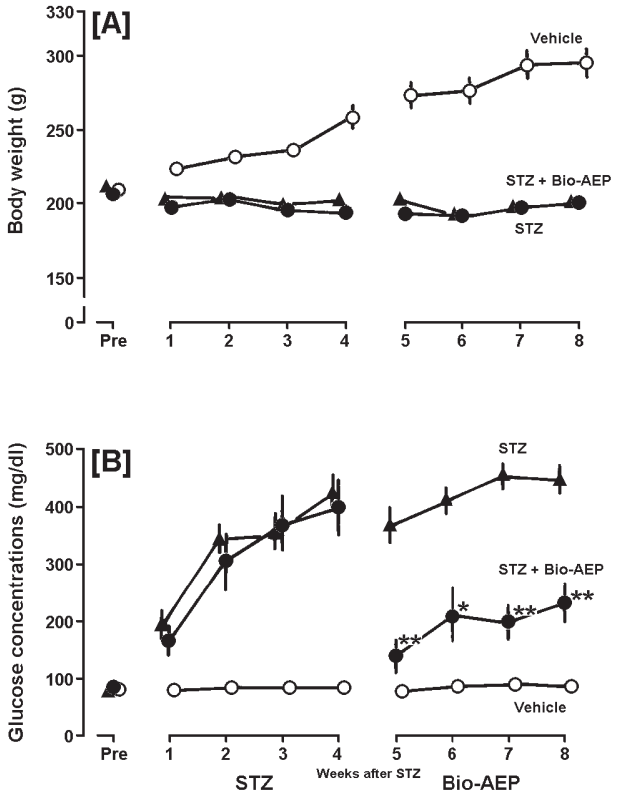

Fig. 2. Changes of body weight [A] and blood glucose concentration [B] with STZ administration and transplantation of Bio-AEP. Values are expressed as mean ± SE: STZ group (n=5), STZ+Bio-AEP group (n=5), and vehicle group (n=5). **P*<0.05 and ***P*<0.01 indicate that values of the STZ+Bio-AEP group were significantly different from those of the STZ group.

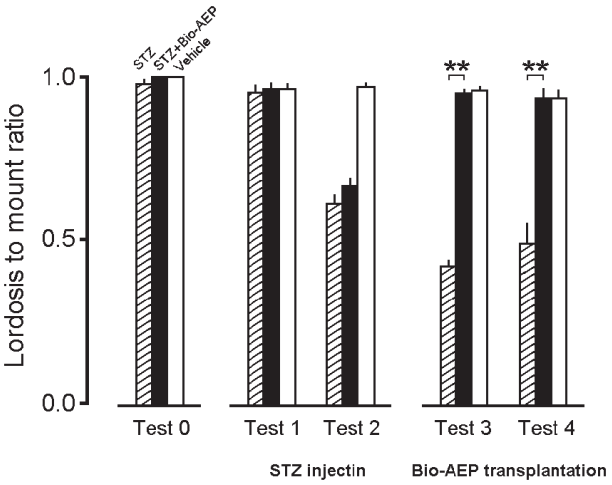

Fig. 3. Changes in the lordosis to mount ratio with STZ administration and transplantation of Bio-AEP. Values are expressed as mean ± SE: STZ group (n=5), STZ+Bio-AEP group (n=5), and vehicle group (n=5). **P*<0.05 and ***P*<0.01 indicate that values of the STZ+Bio-AEP group were significantly different from those of the STZ group.

the STZ+Bio-AEP group recovered compared with that of the STZ group ($P<0.05$). In addition, in Test 3 and Test 4, the L/M ratio of the STZ+Bio-AEP group was similar to that of the vehicle group. Incidences of proceptive behavior including both ear wiggling and darting in the vehicle group, STZ group, and STZ+Bio-AEP group were 100% (5/5), 20% (1/5), and 100% (5/5) respectively. The incidence of proceptive behavior in the STZ+Bio-AEP group was higher than that in the STZ group ($P<0.05$).

In this study, Bio-AEP with mouse β cells improved hyperglycemia and revitalized the sexual behavior of STZ-induced diabetic female rats. The cause of sexual dysfunction induced by diabetes is neuroendocrine dysfunction [11, 13, 19, 21]. Therefore, these results suggest that the recovery of lordosis after using Bio-AEP in STZ-induced diabetic female rats was based on the recovery of neuroendocrine function. We used mouse β cells to facilitate Bio-AEP transplantation after inducing diabetes with STZ in female rats at the start of this study. Insulin shows glucose uptake action even when insulin from different species is administered. Since human insulin is often used for the insulin tolerance test in mice [4–6], we assumed that mouse insulin would have an effect on STZ-induced diabetic female rats. In this study, it was possible to transplant mouse β cells into rats using Bio-AEP. This result was in agreement with those reported in previous studies [7, 8]. Edamura *et al*. (2004) reported pancreatectomized dogs could be transplanted with porcine pancreatic endocrine cells in a Bio-AEP [3]. Our results showed that it is possible to reverse the diabetic suppression of sexual behavior of female rats, that are not immunodeficient, by continuous administration of mouse insulin. A study on the differentiation of ES and iPS cells into β cells has been reported [24]. However, applications to humans is still difficult. Even if techniques using iPS cells could be applied to humans, treatments using them would take too long to be of benefit to patients with acute hyperglycemia and with fulminant type 1 diabetes, which can rapidly become fatal

[9, 10]. Because differentiation of iPS cells to β cells is difficult, Bio-AEP will be necessary in the future for the transplantation of β cells from different species and unrelated persons.

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