Brain-Derived Neurotrophic Factor Regulates Glucose Metabolism by Modulating Energy Balance in Diabetic Mice

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We previously reported that brain-derived neurotrophic factor (BDNF) regulates both food intake and blood glucose metabolism in rodent obese diabetic models such as C57BL/KsJ-lepr^{db}/lepr^{db} (*db*/*db*) mice. To elucidate the effect of BDNF on glucose metabolism, we designed a novel pellet pair-feeding apparatus to eliminate the effect of appetite alteration on glucose metabolism. The apparatus was used to synchronize food intake precisely between BDNF-treated and vehicle-treated *db*/*db* mice. It was shown using this pellet pair-feeding apparatus that BDNF administered daily (20 mg · kg^{-1} · day^{-1}) to *db*/*db* mice significantly lowered blood glucose compared with pellet pair-fed *db*/*db* mice. To evaluate the effect of BDNF on insulin action, we used streptozotocin-induced type 1 diabetic mice. In this case, BDNF did not lower blood glucose concentration but rather enhanced the hypoglycemic action of insulin. In hyperglycemic *db*/*db* mice, pancreatic insulin content was reduced and glucagon content was increased compared with normoglycemic *db*/*m* mice. BDNF administered to *db*/*db* mice significantly restored both pancreatic insulin and glucagon content. Histological observations of aldehyde-fuchsin staining and immunostaining with anti-insulin indicated that insulin-positive pancreatic β-cells were extensively regranulated by BDNF administration. We also studied the effect of BDNF on KK mice, normoglycemic animals with impaired glucose tolerance. In these mice, BDNF administration improved insulin resistance in the oral glucose tolerance test. To elucidate how blood glucose was metabolized in BDNF-treated animals, we investigated the effect of BDNF on the energy metabolism of *db*/*db* mice. Body temperature and oxygen consumption of the pellet pair-fed vehicle-treated mice were remarkably lower than the ad libitum–fed vehicle-treated mice. Daily BDNF administration for 3 weeks completely ameliorated both of the reductions. Finally, to clarify its action mechanism, the effect of intracerebroventricular administration of BDNF on *db*/*db* mice was examined. Here, a small dose of BDNF was found to be effective in lowering blood glucose concentration. This indicates that BDNF regulates glucose metabolism by acting directly on the brain. *Diabetes* 49:436–444, 2000

Neurotrophins are important regulators in the embryogenesis, development, and functioning of nervous systems (1,2). At present, four neurotrophins are known: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/NT-5 (3–6) in mammals. BDNF, discovered long after NGF, enhances the survival and differentiation of several classes of neurons in the central and peripheral nervous systems, including motoneurons and sensory neurons. BDNF and its receptor, TrkB, are widely expressed in a variety of neurons through the embryonic, postnatal, and adult stages (7–10). These results and the many activities of BDNF described in in vitro cell cultures and lesioned animal studies (11,12) indicate that BDNF is likely to have multiple functions.

In addition to the efficacy of BDNF in neurological disorders, we previously found that BDNF reduces food intake and blood glucose concentration in rodent obese diabetic models, such as C57BL/KsJ-*db*/*db* mice (13,14). To eliminate the effect of reduced food intake on the regulation of glucose metabolism, we evaluated the hypoglycemic effect of BDNF in *db*/*db* mice using the conventional pair-feeding protocol in which the amount of food provided to each pair-fed mouse was the same as the average amount of food eaten by the BDNF-treated mice during the preceding 24-h period (13,14). However, because such hyperphagic diabetic mice given a vehicle ate all of the food over a period of several hours, leaving them in a fasting condition until the next feeding, this protocol was inappropriate to study the effect of antidiabetic agents on glucose metabolism by mechanisms other than appetite alteration.

In this study, to overcome these drawbacks, we designed a novel pellet pair-feeding apparatus and evaluated the effect of BDNF on glucose metabolism by precisely synchronizing food intake between BDNF-treated *db*/*db* mice and vehicle-treated control *db*/*db* mice. The hypoglycemic effect of BDNF was found to be stronger in younger *db*/*db* mice than in older mice (13). Because *db*/*db* mice are characterized by hyperglycemia and hyperinsulinemia in the early stage followed by reduced insulin levels in the late stage (15–19), it was felt that BDNF should facilitate insulin action and lower blood glucose concentration in obese diabetic mice. To eval-

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BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay; NGF, nerve growth factor; NT, neurotrophin; PBS, phosphate-buffered saline; PPAR-γ, peroxisome proliferator-activated receptor-γ; STZ, streptozotocin.

uate this expected effect of BDNF on insulin action, we conducted a detailed investigation of the relationship between BDNF and insulin using streptozotocin-induced type 1 diabetic mice. In addition, we studied the insulin content and histochemical changes in the pancreas from BDNF-treated *db*/*db* mice. To evaluate the antidiabetic effect of BDNF on another animal model, we demonstrated the efficacy of BDNF on impaired glucose tolerance of KK mice, normoglycemic obese animals (20). We then tried to ascertain how blood glucose is metabolized in BDNF-treated animals. Because BDNF increased neither body weight nor fat weight in *db*/*db* mice, it seemed likely that BDNF enhanced the energy expenditure in these animals. To test this hypothesis, we measured body temperature and oxygen consumption of *db*/*db* mice to determine the role of BDNF in the regulation of energy metabolism.

Lastly, to clarify the action mechanism of BDNF, we evaluated the effect of intracerebroventricular administration of BDNF on *db*/*db* mice. Below, we discuss the antidiabetic effects of BDNF in diabetic models.

RESEARCH DESIGN AND METHODS

Animals. Male C57BL/KsJ-*db*/*db* mice were obtained from Clea Japan (Tokyo). Mice were singly housed and were used for the experiment at 11–12 weeks of age. Age-matched male heterozygous C57BL/KsJ-*db*/*m* mice were used as a nondiabetic control. Male C57BL/6Ncrj mice were obtained from Charles River Japan (Yokohama, Japan) and were treated with streptozotocin (STZ) at 8 weeks of age. Male KK mice were obtained from Clea and used for the experiment at 12 weeks of age. Animals were given food and water ad libitum, except during the pair-feeding experiment. All animal experiments were conducted according to the guidelines of the Sumitomo Pharmaceuticals Committee on Animal Research.

Synchronized pair-feeding apparatus. A synchronized pellet pair-feeding apparatus was newly developed (Sumitomo Pharmaceuticals, Osaka, Japan, and Osaka Micro Systems, Settsu, Japan) to carry out experiments under precise pair-feeding conditions. The apparatus is composed of a controller, counter printer, and eight pairs (master and slave) of cage units (Fig. 1*A*). There are a pellet feeder and pellet detector in each cage unit; each of the eight cage unit pairs is independently controlled. The supply of pellets to the master cage is not limited, but the supply of pellets to the slave cage is limited to the number of pellets consumed by the mouse in the master cage. The number of pellets consumed in each cage unit is recorded per unit time.

Administration of BDNF. Human recombinant BDNF (NH_2-terminal methionine-free; Regeneron Pharmaceuticals, Tarrytown, NY) was administered subcutaneously. Phosphate-buffered saline (PBS) or 0.01% Tween 80 and 1% Mannitol in PBS was used as a vehicle. For intracerebroventricular administration, *db*/*db* mice were anesthetized with diethyl ether, the bregma was identified through the skin, and 15 μg/mouse of BDNF (~3 μl/shot) was injected into the third cerebral ventricle every other day (total five times).

Induction of diabetes by STZ and experimental protocols. Diabetes was induced in C57BL/6Ncrj mice by two consecutive daily intraperitoneal injections of STZ (200 mg/kg) (Sigma, St. Louis, MO) dissolved in citrate buffer (pH 5.5). On the third day after the last STZ injection, the diabetic mice were divided into four groups. Treatment for each group was as follows: group 1, PBS and saline; group 2, BDNF and saline; group 3, PBS and insulin (0.5 U/kg) (Novo Nordisk, Copenhagen); and group 4, BDNF and insulin. BDNF or PBS was administered 1 h before insulin or saline administration (subcutaneously). It was necessary to maintain fasting in all groups after BDNF or PBS administration, since BDNF treatment reduced food intake in STZ-induced diabetic animals (data not shown).

Oral glucose tolerance test. After a period of overnight fasting, 3g/kg D-glucose was administered orally to KK mice. Blood glucose concentrations were measured at 0 min (before) and 15, 45, 95, and 135 min after glucose administration. Plasma insulin concentrations were also measured at 0 min (before) and 15, 45, and 135 min after glucose administration.

Measurement of blood glucose and percent HbA_{1c}. Blood samples were collected from the tail vein, and blood glucose was measured by the Glucose C-Test Wako (Mutarotase-glucose oxidase method; Wako Chemical, Osaka, Japan) or with a blood glucose analyzer (Antsense II; Daikin, Osaka, Japan). Percent HbA_{1c} was measured by DCA2000 (Bayer-Sankyo, Tokyo) according to the manufacturer's protocol (21).

Histological analysis and measurement of insulin and glucagon. Blood samples were collected from tail vein, and plasma insulin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Levis-insulin-mouse; Shibayagi, Gunma, Japan). At the end of the treatment, the whole pancreas was resected from each mouse and divided into splenic and duodenal regions. Splenic regions were weighed, minced, and homogenized in acid-ethanol solution (75% ethanol, 23.5% distilled water, 1.5% concentrated HCl). After overnight incubation at 4°C, the suspensions were centrifuged, and the supernatants were collected and assayed for insulin content (see above). Pancreatic glucagon content was measured by radioimmunoassay (Linco Research, St. Louis, MO). Duodenal regions were fixed in Bouin's solution, embedded in paraffin, sectioned (3 μm), and stained using Gomori's aldehyde-fuchsin technique (22) or the immunohistochemical method (anti-insulin, anti-glucagon, or anti-somatostatin).

Body temperature and themographic imaging analysis. Body temperature was measured using an electron thermistor (Model BAT-12; Physitemp, Clifton, NJ), equipped with rectal probe (RET-3; Physitemp). Skin temperature was imaged by thermography (TVS-8000MkII; Abionics, Tokyo).

Oxygen consumption. Oxygen consumption was measured with an O_2/CO_2 metabolism measuring system (Model MK-5000; Muromachikikai, Tokyo). Each mouse was placed in a sealed chamber (560 ml volume) with an air flow of 0.18 l/min for ~3 h at 25°C. Air was taken every 3 min, and the consumed oxygen concentration was converted to milliliters per minute by multiplying it by the flow rate.

Statistical analysis. All data were presented as means ± SD. Differences between individual groups were analyzed by the Student's *t* test, Tukey-Kramer test, William's test, or Dunnett's test. The statistical calculations were performed using SAS software (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

RESULTS

Control of food intake with a synchronized pellet pair-feeding apparatus. We have previously shown that BDNF reduces both blood glucose concentration and food intake in *db*/*db* diabetic mice (13,14). To elucidate the actual role of BDNF on glucose metabolism, it is very important to know whether or not the hypoglycemic effect of BDNF is due solely to appetite reduction. Therefore, in this study, we have developed a new synchronized pellet pair-feeding apparatus to control food intake precisely between BDNF-treated *db*/*db* mice and vehicle-treated *db*/*db* mice. The mice receiving BDNF were housed singly in each of the master cages, and the corresponding pair-fed mice receiving vehicle were housed singly in the paired slave cages (Fig. 1*A*). The daily profile of food intake by *db*/*db* mice housed in the apparatus is shown in Fig. 1*B*. Both the number of pellets eaten by each mouse per hour and the time course of the food intake during 24 h were very well synchronized between individual pairs of BDNF-treated mice (master cage) and vehicle-treated mice (slave cage).

Antidiabetic effect of BDNF on *db*/*db* mice housed in the synchronized pellet pair-feeding apparatus. Using the synchronized pellet pair-feeding apparatus, we studied the effect of BDNF on blood glucose concentration in *db*/*db* mice during daily administration of 20 mg/kg BDNF. As shown in Fig. 2*A*, the food intake of vehicle-treated *db*/*db* mice in the slave cages was synchronized precisely with the BDNF-treated animals in the master cages. Even under these precise pellet pair-feeding conditions, the blood glucose concentration of the BDNF-treated mice was reduced gradually and was found to be significantly lower than the pair-fed control mice after 8 days of BDNF administration (Fig. 2*B*). Furthermore, repetitive administration of BDNF did not induce hypoglycemia. Body weights decreased almost identically in both the BDNF-treated and pair-fed vehicle-treated groups (Fig. 2*C*).

Effect of BDNF on glycemic control of STZ-induced type 1 diabetic mice. We have previously reported that the efficacy of BDNF in lowering blood glucose concentration was stronger in younger more hyperinsulinemic *db*/*db* mice than in older less hyperinsulinemic mice (13). In our previous study, we showed that BDNF had no effect on blood glucose

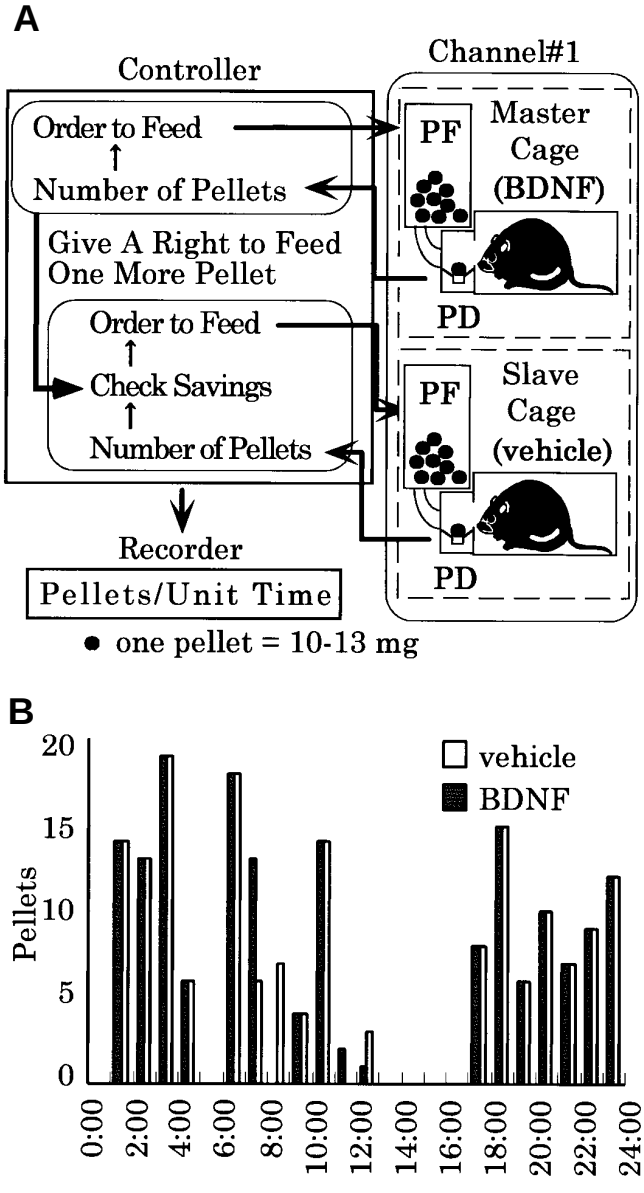

FIG. 1. *A*: Components of novel pellet pair-feeding apparatus. A set of master and slave cages is shown. PF, pellet feeder; PD, pellet detector. *B*: Feeding profile of a 20 mg/kg BDNF-treated *db*/*db* mouse and the pellet pair-fed control during 24 h. Total numbers of pellets eaten by each mouse per hour are shown.

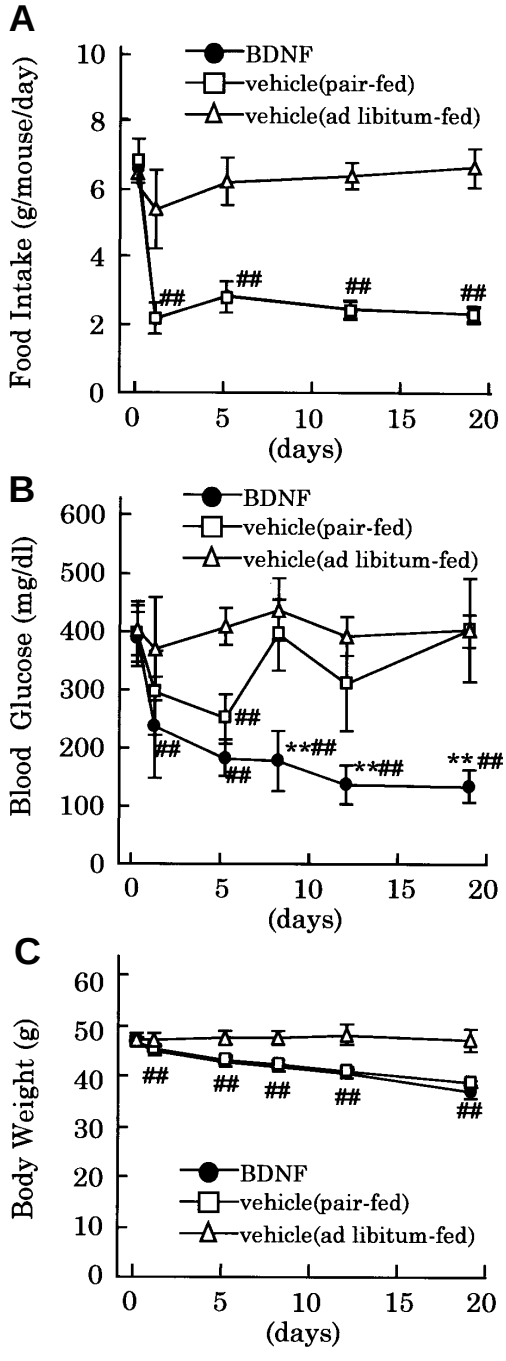

FIG. 2. Effect of daily administration of BDNF on food intake (*A*), blood glucose concentrations (*B*), and body weights (*C*) in *db*/*db* mice. There was 20 mg/kg BDNF or vehicle administered daily to *db*/*db* mice housed in the pellet pair-feeding apparatus for 3 weeks. Data are means ± SD ($n = 7$). $^{**}P < 0.01$ vs. pair-fed group; $\#\#P < 0.01$ vs. ad libitum–fed group by Tukey-Kramer test.

in the STZ-induced model (13). To confirm the effect of BDNF on insulin action, we evaluated the efficacy of BDNF in STZ mice with and without insulin co-administration. As shown in Fig. 3, monotherapy with BDNF did not reduce blood glucose concentration in the experiment. However, when administered concomitantly with insulin, BDNF showed a significant hypoglycemic effect compared with vehicle-treated STZ mice. Moreover, the blood glucose concentration of the STZ mice that received a combination of BDNF and insulin was significantly lower than insulin-treated mice at 1 and 2 h after insulin administration. Blood glucose concentrations of insulin-treated mice, irrespective of BDNF treatment, returned to pretreatment levels within 3 h after insulin treatment. These data indicate that BDNF enhances the hypoglycemic action of insulin.

Antidiabetic effect of BDNF on KK mice. Our findings in this study showed that BDNF is effective on obese hyperglycemic diabetic *db*/*db* mice and that combination therapy with insulin is effective on lean STZ-induced mice. We then decided to study the effect of BDNF on KK mice, normoglycemic animals with impaired glucose tolerance. After daily subcutaneous administration of 5 or 20 mg/kg BDNF for 3 weeks, blood glucose concentrations of KK mice given

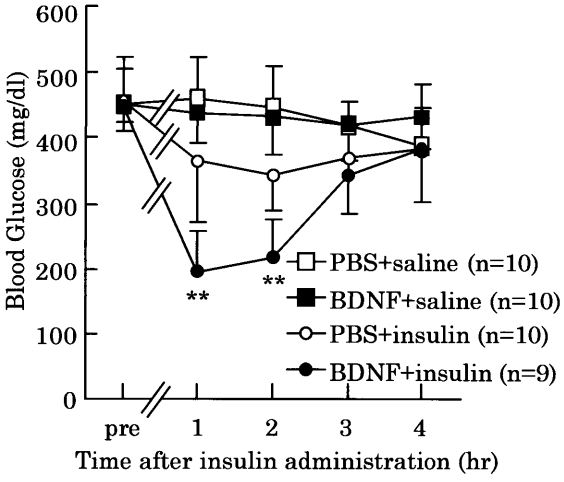

FIG. 3. Effect of BDNF on glycemic control of STZ-induced diabetic mice. There was 20 mg/kg BDNF or PBS administered 1 h before insulin (0.5 U/kg) or saline administration (subcutaneously). Fasting was maintained in all groups after BDNF or PBS administration. Data are means ± SD. $^{**}P < 0.01$ vs. PBS + insulin by Tukey-Kramer test.

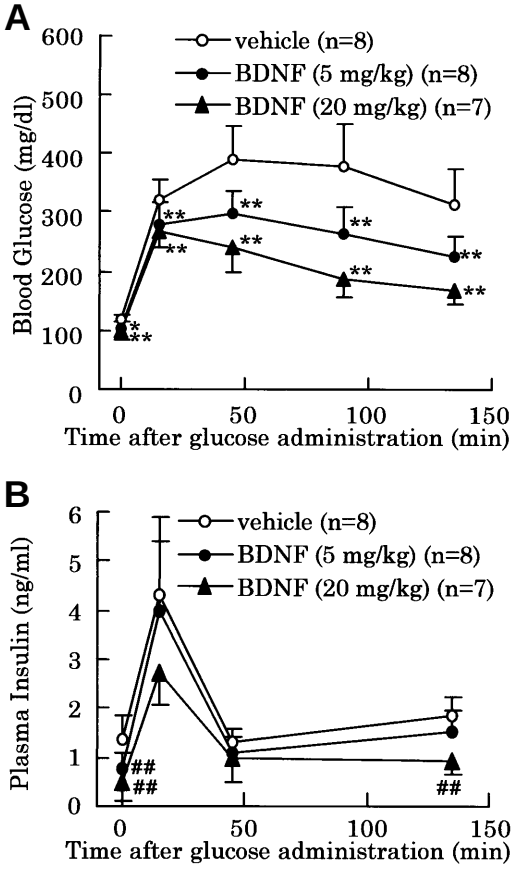

FIG. 4. Effect of BDNF on an oral glucose tolerance test in KK mice. There was 5 or 20 mg/kg BDNF administered daily to KK mice for 3 weeks. At the end of treatment, mice were fasted overnight and 3 g/kg of glucose was administered orally. Blood glucose concentrations (*A*) were measured at 0 min (before) and 15, 45, 95, and 135 min after glucose administration. Plasma insulin concentrations (*B*) were also measured at 0 min (before) and 15, 45, and 135 min after glucose administration. Data are presented as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle by William's test; $^{\#\#}P < 0.01$ vs. vehicle by Dunnett's test.

BDNF were significantly lowered during the oral glucose tolerance test compared with KK mice given vehicle (Fig. 4*A*). This decrease, taken together with the lower plasma insulin concentrations in BDNF-treated KK mice (Fig. 4*B*), indicates that BDNF improves impaired glucose tolerance in KK mice. Although blood glucose concentrations of KK mice given BDNF were lowered to some extent by the reduced food intake, glucose concentrations and HbA_{1c} levels were within normal levels (Table1).

Effect of BDNF treatment on pancreas. The effect of BDNF on blood glucose concentration prompted us to investigate its effect on the pancreas. Insulin content in the pancreas of *db*/*db* mice was determined by ELISA after daily administration of 20 mg/kg BDNF for 3 weeks. The pancreatic insulin content of ad libitum–fed vehicle-treated *db*/*db* mice was reduced to ~30% of the age-matched normoglycemic *db*/*m* mice, as shown in Table 2. In contrast, the pancreatic insulin content of BDNF-treated *db*/*db* mice with reduced percent HbA_{1c} levels was ~10-fold higher than vehicle-treated mice. The pancreatic glucagon content of *db*/*db* mice given vehicle was about threefold higher than that of the *db*/*m* mice, but was significantly lower for the *db*/*db* mice given BDNF.

Aldehyde-fuchsin staining of pancreas from these *db*/*db* mice is shown in Fig. 5*A*. Compared with *db*/*m* mice, the vehicle-treated mice showed extensive degranulation and an indistinct boundary between the endocrine and exocrine regions. In contrast, pancreas from BDNF-treated *db*/*db* mice showed many more positive granules and a distinguishable endocrine/exocrine boundary region. Immunohistochemical staining of pancreatic islets with anti-insulin antibody revealed faintly stained cells in vehicle-treated mice, whereas many strongly stained cells were observed in BDNF-treated mice (Fig. 5*B*). These data correlated well with the pancreatic insulin content obtained by ELISA (Table 2).

Immunohistochemical staining with anti-glucagon antibody was also used to identify pancreatic α-cells in the *db*/*db* mice (Fig. 5*C*). Staining with anti-glucagon antibody was obscure in most, if not all, of the islets from mice given vehicle with only a very few glucagon-positive cells found in the islets. In contrast, glucagon-positive cells were located peripherally in the islets from BDNF-treated mice, indicating a normalized cell distribution. Similar results were obtained with immunostaining for somatostatin (Fig. 5*D*).

BDNF prevented the reduction of body temperature in the food-restricted *db*/*db* mice. Our results showed that under precise pellet pair-feeding conditions, the blood glucose concentration of BDNF-treated mice was significantly lower than the vehicle-treated mice (Fig. 2*B*). There was no significant difference between fat weights (subcutaneous and intraperitoneal) of *db*/*db* mice given BDNF and pellet pair-fed *db*/*db* mice given vehicle (data not shown), nor was there any significant difference in body weights (Fig. 2*C*). To elucidate how blood glucose was metabolized, body temperature was measured as one of the indices of energy expenditure. Leptin-resistant *db*/*db* mice show a reduced energy metabolism and lower body temperature, similarly to leptin-deficient *ob*/*ob* mice (23–26). Furthermore, they are thought to be unable to activate thermogenesis and maintain body temperature when their food intake is restricted. In the control

TABLE 1
Effects of BDNF on blood glucose concentration, HbA_{1c}, and food intake in KK mice

BDNF dose	n	Blood glucose (mg/dl)	HbA_{1c} (%)	Food intake (g · mouse^{-1} · day^{-1})
Vehicle	8			
Before		172.8 ± 12.1	3.1 ± 0.3	5.4 ± 0.3
After		192.6 ± 38.8	4.1 ± 0.5	5.1 ± 0.2
5 mg/kg	8			
Before		177.0 ± 10.1	3.4 ± 0.4	5.6 ± 0.2
After		157.7 ± 9.5†	3.7 ± 0.3*	4.5 ± 0.2†
20 mg/kg	7			
Before		184.0 ± 12.6	3.0 ± 0.4	5.5 ± 0.2
After		149.7 ± 9.6†	3.5 ± 0.2†	4.1 ± 0.2†

Data are means ± SD. There was 5 or 20 mg/kg of BDNF administered daily to KK mice for 3 weeks. Food intake was determined by recording the amount of food remaining in food dishes. *P < 0.05, †P < 0.01 vs. vehicle by William's test.

TABLE 2
Effects of BDNF on pancreas of *db/db* mice

	HbA_{1c} (%)	Pancreatic insulin (ng/mg tissue)	Pancreatic glucagon (ng/mg tissue)
db/m	3.4 ± 0.5	187.7 ± 25.3	1.9 ± 0.4
db/db: vehicle	8.2 ± 0.8†	64.9 ± 21.3*	6.9 ± 1.6†
db/db: BDNF	4.8 ± 0.7‡	672.3 ± 130.0†‡	4.1 ± 0.4†‡

Data are means ± SD (n = 8). There was 20 mg/kg of BDNF administered daily to *db/db* mice for 3 weeks. HbA_{1c} level was measured at the end of treatment. Splenic regions of pancreas were extracted by acid-ethanol solution. Insulin and glucagon contents were measured by ELISA and radioimmunoassay, respectively. *P < 0.05, †P < 0.01 vs. *db/m* mice, ‡P < 0.01 vs. vehicle-treated *db/db* mice by Tukey-Kramer test.

db/db mice given vehicle, food intake was suppressed by pair-feeding with mice given BDNF (Fig. 2*A*). Dramatic reductions in body temperature were also observed compared with the ad libitum–fed *db/db* mice (Fig. 6*A*). These reductions indicate that control of body temperature is impaired in such hyperphagic obese *db/db* mice. However, after daily administration of BDNF for 3 weeks, the reduction of body temperature in the *db/db* mice was significantly ameliorated and normalized to those of the ad libitum–fed *db/db* mice (Fig. 6*A*).

Skin temperature was analyzed by thermography to determine which body areas of the *db/db* mice showed higher temperatures. The skin temperatures of the pellet pair-fed *db/db* mice were lowered over the entire body compared with the ad libitum–fed *db/db* mice (Fig. 6*B*). On the other hand, skin temperatures of the *db/db* mice given BDNF were higher than those of the pair-fed *db/db* mice (Fig. 6*B*). Relatively strong signals were observed, especially on the back skin around the scapula, suggesting that BDNF treatment stimulated thermogenesis in brown adipose tissue. To analyze the effects of BDNF on energy expenditure, we measured oxygen consumption in *db/db* mice. In the pair-fed *db/db* mice given vehicle, oxygen consumption was lower than that in the ad libitum–fed *db/db* mice. In contrast, the BDNF-treated *db/db* mice showed a significantly higher oxygen consumption rate than the pair-fed animals (Fig. 6*C*). Consistent with these findings, the CO_2 release rate of the BDNF-treated *db/db* mice was higher than that of the pair-fed animals. In addition, there was no difference between locomotor activity in the BDNF-treated *db/db* mice and the pair-fed animals (data not shown).

Effects of intracerebroventricular administration of BDNF on glucose metabolism. To clarify the action mechanism of BDNF, we studied the effect of intracerebroventricular administration of BDNF on *db/db* mice. Although intracerebroventricular administration of BDNF decreased food intake, our pellet pair-feeding apparatus synchronized food intake very well between BDNF-treated and vehicle-treated *db/db* mice (Fig. 7*A*). Under such strict pellet pair-feeding conditions, intracerebroventricular administration of 15 µg/mouse BDNF (five times every other day) significantly lowered blood glucose concentrations in *db/db* mice (Fig. 7*B*). As shown in Fig. 7*B*, blood glucose concentrations in the BDNF-treated mice remained significantly lower than those in the vehicle-treated mice for an additional 25 days after completion of treatment. This result indicates that intracerebroventricular administration of BDNF shares the same anorectic and hypoglycemic actions seen in peripheral administration.

DISCUSSION

We have previously shown that BDNF reduces food intake together with blood glucose in obese diabetic *db/db* mice (13,14). Our interest was then focused on determining whether BDNF actually regulates glucose metabolism by a mechanism other than appetite alteration. In our initial attempt, we used a conventional pair-feeding protocol and demonstrated the hypoglycemic effect of BDNF on *db/db* mice. However, because these diabetic mice are very hyperphagic, pair-fed control *db/db* mice under such a protocol usually eat all of the food within several hours, leaving them in a fasting condition for almost half a day. Accordingly, patterns of food intake during the day are quite different between BDNF-treated and pair-fed vehicle-treated mice. Worse yet, blood glucose concentrations in the pair-fed mice, if measured before the next feeding, must be reduced because of the long fasting period. Thus, this is an inappropriate control group to study the effects of antidiabetic agents on glucose metabolism by mechanisms other than appetite regulation.

In this study, we designed a novel pellet pair-feeding apparatus and succeeded in precisely synchronizing food intake between BDNF-treated *db/db* mice and vehicle-treated control mice. We then clearly demonstrated that BDNF given to *db/db* mice brings on a significant hypoglycemic effect compared with pellet pair-fed mice. This finding demonstrates that BDNF plays a regulatory role in glucose metabolism independently of appetite alteration in obese diabetic mice. In these experiments, where both the amount of food and the feeding pattern were precisely matched using our apparatus, reductions in body weights and fat weights of both the BDNF-treated mice and pellet pair-fed mice were found to be nearly the same. BDNF neither made these mice too thin nor aggravated their obesity. In this study, the pellet pair-fed *db/db* mice had significantly lower body temperature and oxygen consumption rate than the ad libitum–fed *db/db* mice. This indicates that *db/db* mice required excessive food intake

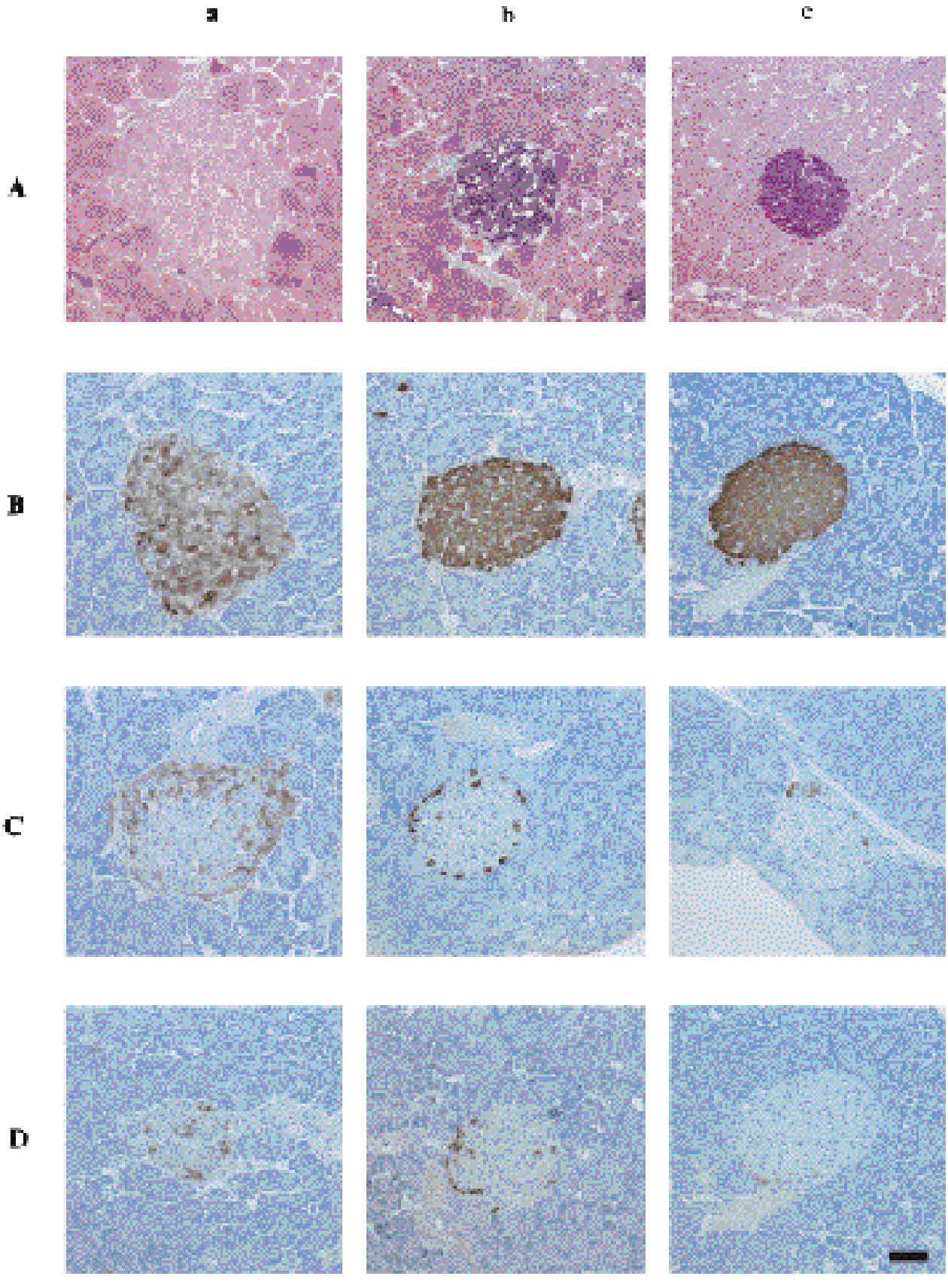

FIG. 5. Histological analyses of pancreatic islets from vehicle-treated db/db mice (a), BDNF-treated db/db mice (b), and age-matched lean db/m mice (c). There was 20 mg/kg BDNF or vehicle administered daily to db/db mice for 3 weeks. At the end of the treatment, the whole pancreas was resected and fixed in Bouin's solution. Aldehyde-fuchsin (A), anti-insulin (B), anti-glucagon (C), and anti-somatostatin (D) stainings were performed. Size bar = 200 µm.

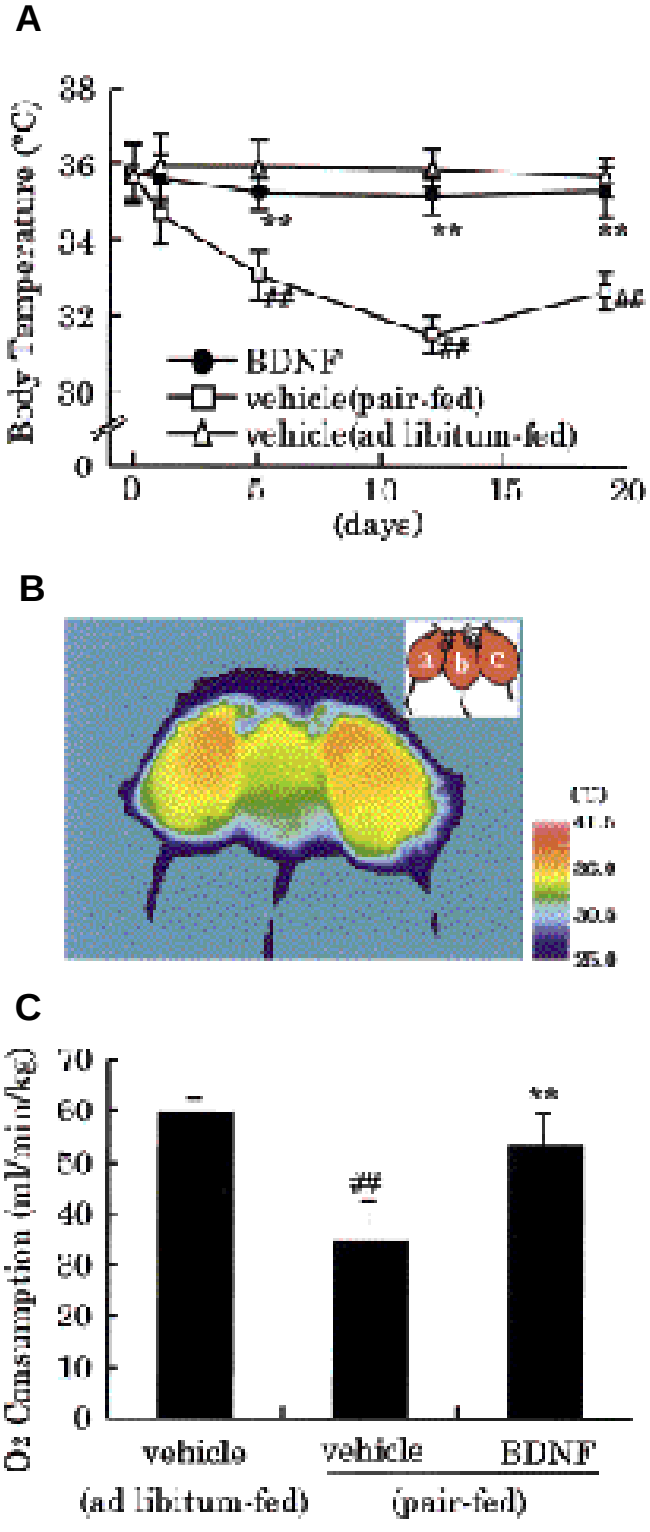

FIG. 6. Effect of BDNF on energy metabolism in *db*/*db* mice. BDNF (20 mg/kg) or vehicle was administered daily for 3 weeks to *db*/*db* mice housed in a synchronized pellet pair-feeding apparatus. Body temperature was measured at indicated day (*A*). BDNF-treated mice (*a*), vehicle-treated mice pair-fed to BDNF-treated mice (*b*), and vehicle-treated mice fed ad libitum (*c*) underwent thermographic imaging analysis at 2 h after the last injection (*B*). Oxygen consumption was measured at 15–17 days (*C*). Data are means ± SD (n = 7). $^{**}P < 0.01$ vs. pair-fed group; $^{\#\#}P < 0.01$ vs. ad libitum–fed group by Tukey-Kramer test.

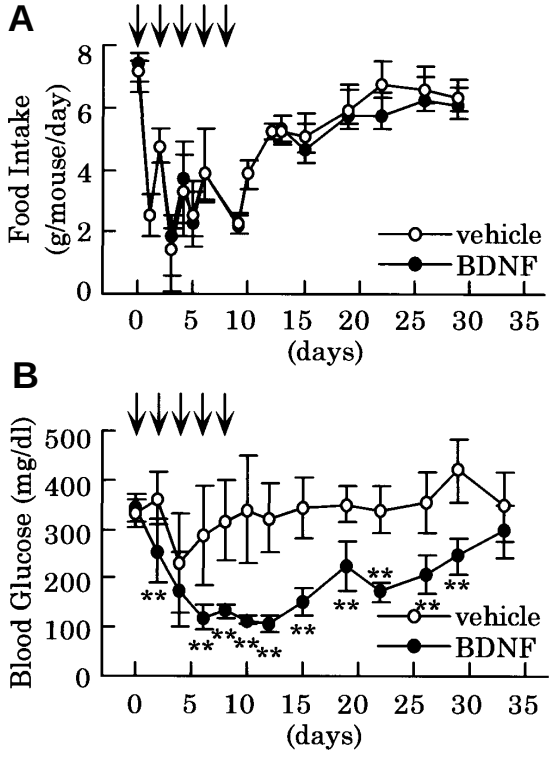

FIG. 7. Effect of intracerebroventricular administration of BDNF on food intake (*A*) and blood glucose concentrations (*B*) in *db*/*db* mice. BDNF (15 µg/shot) or vehicle was administered five times every other day. Mice were housed in the pellet pair-feeding apparatus until day 13 and then were fed ad libitum. Arrows indicate the day of BDNF injection. Data are means ± SD (n = 7). $^{**}P < 0.01$ vs. vehicle by Student's t test.

to maintain body temperature because their energy expenditure was reduced. We found that in spite of the reduced food intake, *db*/*db* mice given BDNF maintained nearly the same body temperature and metabolic rate as ad libitum–fed animals. Taken together with the hypoglycemic effect of BDNF, these results strongly suggest that BDNF enhances the expenditure of excess energy and normalizes diabetic metabolism in *db*/*db* mice.

This action of BDNF may share some features with the anorectic protein leptin, which has been reported to regulate appetite and energy expenditure. It has been suggested that leptin administered by the peripheral route produces these effects through its action on the hypothalamus (27–29), the regulatory center of the autonomic nervous system controlling both food intake and energy expenditure (30). The present results with intracerebroventricular administration indicate that BDNF acts on the brain and produces its hypoglycemic and anorectic effects via the central nervous system. Intracerebroventricular administration of BDNF in doses as small as 15 µg/mouse (~300 µg/kg) proved to be effective in lowering blood glucose concentrations. Furthermore, intracerebroventricular administration of only 1.5 µg/mouse (~30 µg/kg) BDNF was also found effective (data not shown). Such doses are roughly 1/100 and 1/1,000 of those used in subcutaneous administration. In addition, these doses of BDNF were not effective when delivered peripherally (M.O., Y.I., T.No., T.Na., C.N., M.T., H.N., unpublished observations). Therefore, it is reasonable to assume that when BDNF is

administered subcutaneously, a small portion of the dose enters the brain and is directly responsible for lowering blood glucose concentrations. Because the BDNF receptor, TrkB, is expressed also in the hypothalamus (10), BDNF may regulate glucose metabolism by modulating the autonomic function in this region.

We have previously reported that the hypoglycemic action of BDNF was stronger in younger more hyperinsulinemic *db*/*db* mice than older less hyperinsulinemic mice (13). BDNF was thus seen to exert its hypoglycemic action most efficiently in *db*/*db* mice with sufficient plasma insulin levels. These data indicate that BDNF facilitates insulin action and lowers blood glucose concentrations in obese diabetic mice. In the current study of the effect of BDNF on insulin action, we found that BDNF itself does not lower the blood glucose concentration but enhances insulin action in STZ mice. Moreover, only a single administration of BDNF was effective in enhancing insulin action in STZ mice. This is the first evidence to show that BDNF acts quickly to enhance insulin action. In recent years, it has been reported that thiazolidinediones, newly discovered antidiabetic agents, increase insulin sensitivity in insulin-dependent glucose-utilizing tissues (31,32) and, with repetitive administration, improve insulin resistance in obese diabetic animals and humans (33,34). It has also been reported that thiazolidinediones themselves have no effect on blood glucose concentration in the STZ-induced diabetic model (35) but when administrated with a minimal dosage of insulin, improve insulin resistance (36).

In addition to *db*/*db* mice, we have found that BDNF exhibits its hypoglycemic action in other obese diabetic animals, such as *ob*/*ob* mice, KK*Ay* mice, and Zucker diabetic fatty rats (data not shown). Therefore, BDNF is effective in several obese hyperglycemic models, irrespective of genetic mutation sites, mutation types, or functional deficiencies. In combination therapy with insulin, BDNF is even effective on genetically normal but chemically induced hyperglycemic diabetic STZ-induced mice. These observations indicate that BDNF regulates glucose metabolism in a variety of hyperglycemic diabetic animals. Furthermore, in this study, we demonstrated that BDNF ameliorates impaired glucose tolerance of a normoglycemic obese animal—the KK mouse. This means that BDNF improves insulin resistance in these animals without severe glucose toxicity.

Thiazolidinediones are thought to enhance the insulin sensitivity in the peripheral tissues by activating peroxisome proliferator-activated receptor-γ (PPAR-γ), a subtype of the nuclear receptor superfamily expressed in adipose tissue (37). On the other hand, BDNF neither activates PPAR-γ–dependent transcription nor enhances 2-deoxyglucose transport in cultured adipocytes and muscle cells (A.T., T.Na., Y.I., J. Ichihara, M.T., H.N., unpublished observations). Thus, it is not likely that BDNF acts directly on peripheral tissues to enhance insulin action. Because BDNF is a neurotrophic factor that acts on both the central and peripheral nervous systems, it is natural to speculate that BDNF enhances insulin action indirectly via regulation of such neural pathways. Our results showed that intracerebroventricular administration of BDNF has the same hypoglycemic action as peripheral administration. These data apparently reinforce the action mechanism by which BDNF acts directly on the brain to regulate glucose metabolism, resulting in enhancement of insulin action in peripheral tissues.

We have also demonstrated that BDNF increases insulin content compared with pretreatment levels and restores granulated β-cells in *db*/*db* mice pancreatic islets. This demonstration supports our previous results that BDNF lowers blood glucose levels of *db*/*db* mice, as determined by an oral glucose tolerance test (13,14). It is not likely that the effect of BDNF on the pancreas is due to lowered food intake, since there was no difference in pancreatic insulin content between the pellet pair-fed and ad libitum–fed controls. Because BDNF enhances insulin sensitivity and ameliorates insulin resistance, *db*/*db* mice given BDNF require less insulin secretion from β-cells to achieve prolonged near-normoglycemia and increase both pancreatic insulin content and granulation of β-cells. However, another possibility that cannot be excluded is that BDNF directly affects the pancreas and normalizes the function of islets. This view is supported by a report that pancreatic α-cells express the BDNF receptor, TrkB (38), which in turn suggests a potential paracrine interaction between α- and β-cells in the islets. Intensive insulin therapy that manages blood glucose well and prevents progressive onsets of diabetic complications is expected to ameliorate pancreatic function in type 2 diabetic patients (39,40). Therefore, it should be emphasized that BDNF, in place of insulin, could be especially useful in restoring the pancreatic function of type 2 diabetic patients.

In summary, we report here that BDNF normalizes glucose metabolism and energy balance in type 2 diabetic mice and enhances the hypoglycemic effect of insulin in type 1 diabetic mice. Our findings confirm that this antidiabetic effect was not due to appetite alteration. Furthermore, it appears that BDNF ameliorates pancreatic dysfunction as well as insulin resistance. These antidiabetic effects are also observed in the intracerebroventricular administration of BDNF. Our results thus indicate that BDNF possesses a potentially useful profile as an antidiabetic agent.

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