Intermittent Administration of Brain-Derived Neurotrophic Factor Ameliorates Glucose Metabolism in Obese Diabetic Mice

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We have previously shown that brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, interacts with the endocrine system in obese diabetic mice, and systemic peripheral administration of BDNF regulates glucose metabolism in this model. Results from the present study show that the hypoglycemic effect induced by 2 weeks' daily administration of BDNF (20 mg/kg/d) to *db/db* mice lasts for several weeks after treatment cessation, irrespective of food reduction. On the other hand, the antidiabetic agent, metformin had no lasting effect. This duration of the BDNF hypoglycemic action prompted us to examine the efficacy of BDNF intermittent administration on glucose metabolism. BDNF administered once or twice per week (70 mg/kg/wk) to *db/db* mice for 3 weeks significantly reduced blood glucose concentrations and hemoglobin A_{1c} (HbA_{1c}) as compared with ad libitum-fed phosphate-buffered saline (PBS)-treated and pair-fed PBS-treated groups. This suggests that BDNF not only temporarily reduced blood glucose concentrations but also ameliorated systemic glucose balance in this obese diabetic mouse model during the experimental period. Our results indicate that BDNF could be a novel hypoglycemic agent with an exceptional ability to normalize glucose metabolism even with treatment as infrequently as once per week.

BRAIN-DERIVED neurotrophic factor (BDNF) is one of the neurotrophins, a family that also includes nerve growth factor, neurotrophin-3, and neurotrophin-4/5. BDNF promotes neurite outgrowth and provides trophic support for certain neurons during development and in adulthood.[1] In addition to motor neuron and peripheral nerve disorders, there is extensive evidence indicating that BDNF is effective in animal models of nervous disorders such as diabetic neuropathy.[2-5] Recently, we reported that systemic administration of BDNF reduced nonfasting blood glucose concentrations in *db/db* mice,[6] a model for obesity and non–insulin-dependent diabetes mellitus (NIDDM).[7,8] BDNF did not affect blood glucose levels in nondiabetic mice or insulin-dependent diabetic rats. Although BDNF also decreased food intake and body weight in hyperphagic and obese *db/db* mice, pair-feeding experiments confirmed that the hypoglycemic action of BDNF was not caused by a reduction of food intake.

Here, we continue along the lines of our previous study to demonstrate the potency of BDNF as a novel hypoglycemic agent to treat NIDDM. We compared the effects of BDNF and another antidiabetic agent, metformin, during and after the respective treatments in diabetic mice. Surprisingly, the hypoglycemic actions of BDNF lasted for several weeks after treatment cessation. We thus decided to investigate whether intermittent administration of BDNF could be effective in the treatment of NIDDM.

MATERIALS AND METHODS

Animals

Female C57BL/KsJ-*db/db* mice were obtained from Clea Japan (Tokyo, Japan). Animals were used for experiments at 12 weeks of age and housed in group cages with ad libitum access to water under a 12-hour day/night schedule. All animal experiments were performed according to the guidelines of the Sumitomo Pharmaceuticals Committee on Animal Research.

Administration of BDNF or Metformin

Human recombinant BDNF (*N*-terminal methionine-free; Regeneron Pharmaceuticals, Tarrytown, NY) was administered subcutaneously to *db/db* mice at a dosage of 1 to 70 mg/kg. Phosphate-buffered saline (PBS) was used as a vehicle. Metformin (Sigma, St Louis, MO) was administered by chow feeding (1% metformin hydrochloride by weight). The daily doses of metformin were calculated from the food intake and body weight of each mouse.

Pair-Feeding

The amount of chow provided to each pair-fed mouse was the same as the average amount of chow consumed per animal in the BDNF treatment group during the preceding 24-hour period. Food intake was determined by recording the amount of chow remaining in the food dishes.

Measurement of Blood Glucose and Hemoglobin A_{1c}

Blood samples were collected from a tail vein before BDNF administration. Glucose concentrations and hemoglobin A_{1c} (HbA_{1c}) were analyzed with a blood glucose analyzer (Antsense II; Bayer-Sankyo, Tokyo, Japan) and a HbA_{1c} analyzer (DCA 2000; Bayer-Sankyo) according to the manufacturers' protocol.

Statistics

Results are the mean ± SEM or SD for each parameter. Statistical analysis was performed using either Dunnett's test or the Tukey-Kramer test.

RESULTS

Dose-Dependent Effect of BDNF

To study the dose-dependency of the BDNF action on the blood glucose concentration, we administered BDNF in doses of 1 to 20 mg/kg to *db/db* mice daily for 5 days (Fig 1). BDNF decreased blood glucose in a dose-dependent manner. Administration of greater than 10 mg/kg BDNF significantly reduced the blood glucose concentration. BDNF also produced dose-

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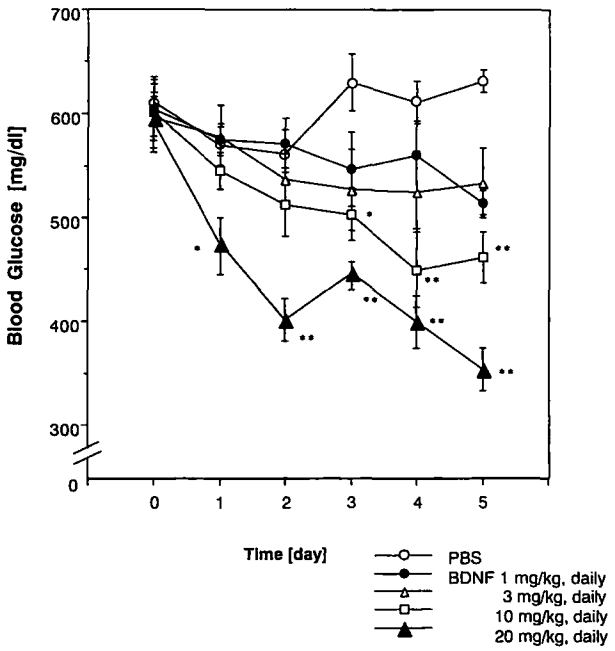

Fig 1. Dose-dependent effects of BDNF on blood glucose concentrations with daily administration. PBS or BDNF at a dose of 1-20 mg/kg was subcutaneously administered to female *db/db* mice (12 weeks old) daily for 5 days and blood glucose concentrations were measured. All mice were allowed ad libitum access to food. Data are the mean ± SEM for 8 or 9 mice per experimental group. *$P < .05$ or **$P < .01$ for BDNF-treated *v* PBS-treated (Dunnett's test).

dependent decreases in body weight and food intake (data not shown).

Duration of BDNF Action

Next, we compared the efficacy of BDNF and the antidiabetic agent metformin over a 2-week treatment period (Fig 2). During treatment, blood glucose declined more slowly in BDNF-treated *db/db* mice versus metformin-treated mice. However, in the second week of treatment, both groups had equivalent blood glucose concentrations. Both BDNF and metformin reduced food intake, but the reduction was more pronounced in the BDNF group during the second week of treatment. BDNF reduced body weight, but metformin showed no such effect in this study.

The recovery of blood glucose concentrations was also investigated during an extended period after cessation of BDNF and metformin treatment (Fig 2). On the next day after stopping treatment, blood glucose concentrations and food intake in *db/db* mice given metformin quickly returned to nearly the same levels as in mice given PBS. In contrast, blood glucose remained lower in the BDNF group versus the control animals given PBS for approximately 4 weeks after treatment cessation. Food intake in the BDNF group started to increase on the day after treatment was stopped and gradually returned, although not as rapidly as metformin, to the control (PBS) level in 2 weeks. These results indicate that the duration of the BDNF action to keep blood glucose concentrations low was not simply caused by altered food intake in *db/db* mice.

Intermittent Administration of BDNF

The long-lasting effects of BDNF on glucose metabolism indicate the possibility that treatment less frequently than daily would also be effective in *db/db* mice. Therefore, we examined the efficacy of BDNF administered once or twice per week over a 3-week period. Since our results indicated that daily administration of more than 10 mg/kg/d BDNF reduces blood glucose

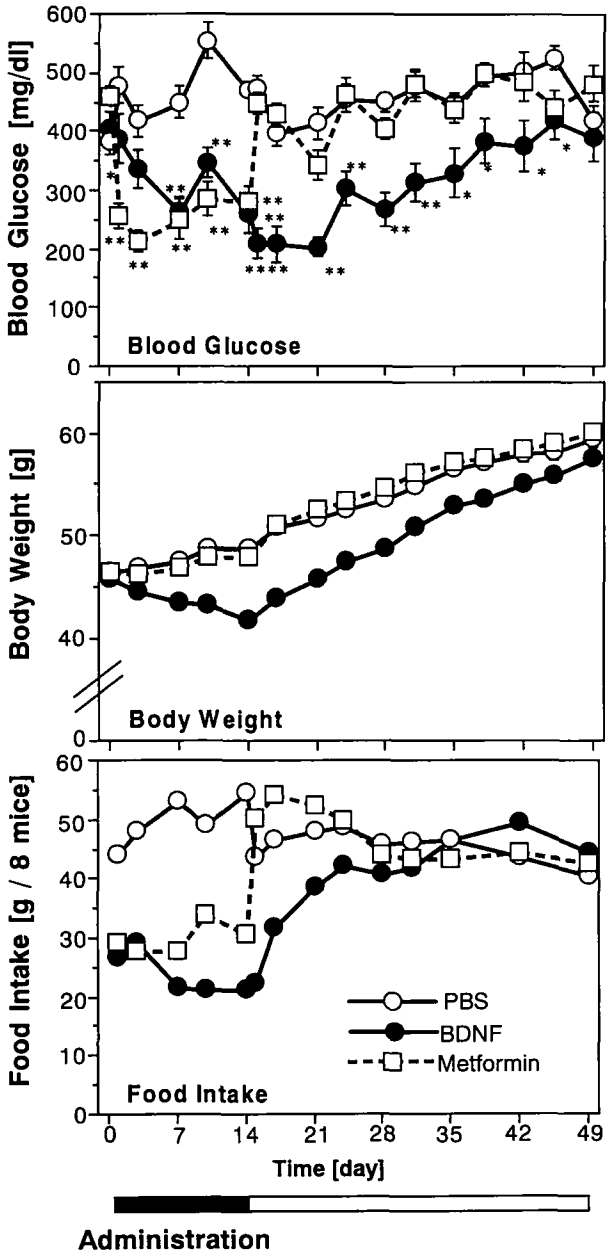

Fig 2. Sustained effect of BDNF on blood glucose concentrations. BDNF was administered subcutaneously at a dosage of 20 mg/kg/d and metformin was administered by chow feeding (1% metformin hydrochloride by weight) for 2 weeks to female *db/db* mice (12 weeks old). All mice were allowed ad libitum access to food, and blood glucose, body weight, and food intake were monitored. Data are the mean ± SEM for 8 mice per experimental group. *$P < .05$ or **$P < .01$ for BDNF-treated *v* PBS-treated (Dunnett's test).

concentrations significantly (Fig 1), it was estimated that approximately 70 mg/kg/wk BDNF would be necessary to ameliorate blood glucose concentrations in *db/db* mice. The effects of 2 different intermittent administration schedules were then investigated: 70 mg/kg BDNF once per week (1/w) and 35 mg/kg BDNF twice per week (2/w). In this experiment, PBS-treated mice were either fed ad libitum or kept under pair-feeding conditions.

Blood glucose was significantly lower during days 7 to 21 in both the 2/w BDNF-treated group and 1/w BDNF-treated group versus the ad libitum-fed PBS group (Fig 3). Blood glucose remained lower in the group pair-fed to the 2/w BDNF-treated group versus the group pair-fed to the 1/w BDNF-treated group. This indicates that a twice-weekly administration of BDNF induced a larger reduction in food intake and tended to maintain lower blood glucose concentrations than the same amount of BDNF administered once per week. Except for a transient decrease at day 1, blood glucose concentrations were significantly lower in the 2/w BDNF-treated and 1/w BDNF-treated groups versus the respective pair-fed PBS-treated groups during days 11 to 21.

We also measured HbA_{1c} as an index of blood glucose management during the experiments[9] (Fig 4). After 3 weeks of BDNF treatment, HbA_{1c} was significantly lower in the 2/w BDNF-treated and 1/w BDNF-treated groups versus the ad libitum-fed PBS-treated control groups. The reduced food intake of BDNF-treated *db/db* mice had no effect on HbA_{1c} levels during the 3-week experimental period. HbA_{1c} levels were significantly lower in the 2/w BDNF-treated group and 1/w BDNF-treated group versus each pair-feeding control.

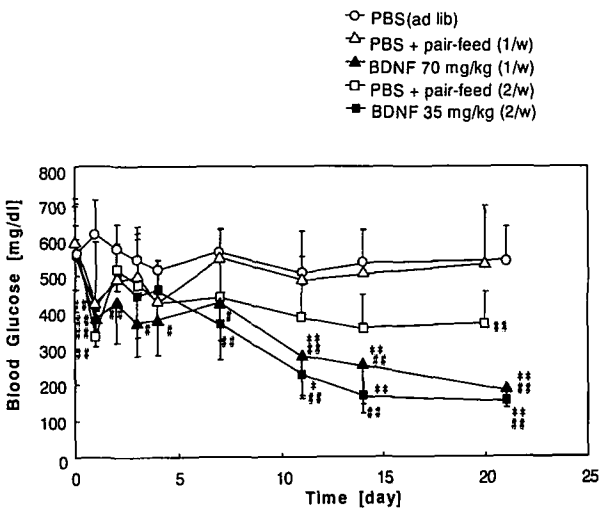

Fig 3. Effect of intermittent administration of BDNF on blood glucose concentrations in *db/db* mice. BDNF was administered subcutaneously once or twice per week for 3 weeks at a dosage of 70 mg/kg/wk to female *db/db* mice (12 weeks old) with ad libitum access to food. PBS-treated mice were either fed ad libitum or kept under pair-feeding conditions. The amount of chow provided to each pair-fed mouse was the same as the average amount of chow consumed per animal in the BDNF treatment group during the preceding 24-hour period. Blood glucose data are the mean ± SD for 7 or 8 mice per experimental group. #$P < .05$ or ##$P < .01$ for BDNF-treated *v* ad libitum PBS-treated. *$P < .05$ or **$P < .01$ for BDNF-treated *v* pair-fed PBS-treated (Tukey-Kramer test).

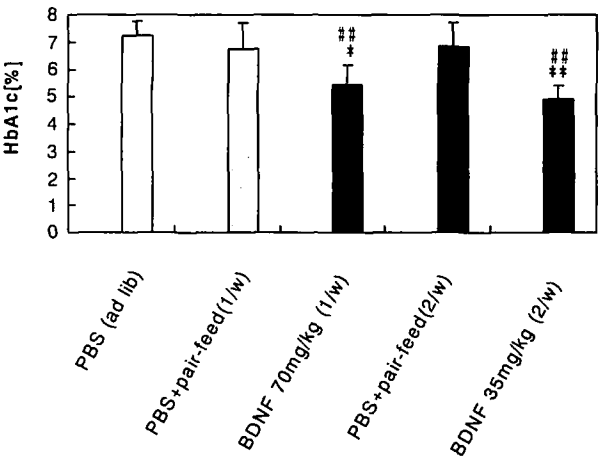

Fig 4. Effects of BDNF intermittent administration on HbA_{1c} levels. BDNF was administered subcutaneously once or twice per week for 3 weeks at a dosage of 70 mg/kg/wk to female *db/db* mice (12 weeks old) with ad libitum access to food. PBS-treated mice were either fed ad libitum or kept under pair-feeding conditions. The amount of chow provided to each pair-fed mouse was the same as the average amount of chow consumed per animal in the BDNF treatment group during the preceding 24-hour period. After 3 weeks of BDNF treatment, blood samples were collected from a tail vein. Data are the mean ± SD for 7 or 8 mice per experimental group. ##$P < .01$ for BDNF-treated *v* ad libitum PBS-treated. *$P < .05$ or **$P < .01$ for BDNF-treated *v* pair-fed PBS-treated (Tukey-Kramer test).

DISCUSSION

In this study, daily administration of BDNF reduced blood glucose concentrations in obese diabetic *db/db* mice. Surprisingly, BDNF was also shown to have a unique long-lasting hypoglycemic effect that persisted for several weeks after treatment cessation. There was some reduction in food intake due to BDNF treatment, but food intake returned to the level of the PBS-treated group approximately 1 week after BDNF treatment was stopped. The change in blood glucose was not associated with food intake, and reduced glucose levels persisted for an additional 4 weeks (Fig 2). This indicates that the long-lasting hypoglycemic effect is not simply due to the reduction of food intake and confirms the previous finding.[10]

Metformin is an oral hypoglycemic agent widely used in the management of NIDDM. Although its exact mechanism of action is poorly understood, it has been reported that metformin suppresses hepatic gluconeogenesis in the liver[11] and increases peripheral insulin sensitivity.[12] As shown in the present study, the efficacy of metformin to reduce the blood glucose concentration and food intake in *db/db* mice was observed only during repeated administration of the medication. The reduced blood glucose levels and food intake increased again after treatment was stopped. It is known that long-term management of hyperglycemia with antidiabetics improves glucose tolerance in both diabetic models and humans.[13,14] However, short-term treatment for 2 weeks with metformin in this study did not improve the ability of *db/db* mice to maintain lower blood glucose levels after treatment cessation. Metformin is reported to undergo rapid renal excretion, with a mean plasma elimination half-life of approximately 1.5 to 5 hours in rats after oral administration.[15] Taken together, our current results indicate

that lower blood glucose concentrations can be maintained in diabetic mice with metformin only if the agent is present in the blood.

In contrast, the hypoglycemic effect of BDNF lasted for several weeks after treatment cessation. The most likely explanation for such a long-lasting hypoglycemic action is that BDNF has a long plasma half-life which helps it to generate a prolonged biological effect. However, the mean plasma elimination half-life after subcutaneous administration is only approximately 3.5 hours in rats (data not shown), and plasma BDNF was not detectable 10 days after BDNF injection.[10] Therefore, it is unlikely that BDNF remained in the circulation for several weeks. This suggests that BDNF not only temporarily reduced blood glucose concentrations through its interaction with peripheral tissue but also normalized the total glucose metabolism in *db/db* mice. It can be further speculated that this normalizing effect of BDNF, once established, can last for weeks. The molecular mechanism responsible for this unique effect needs to be clarified in further studies. After cessation of treatment, the effects of BDNF on food intake and body weight did not last as long as the hypoglycemic effect. Therefore, it is interesting to speculate that BDNF may primarily regulate energy metabolism and appetite, and such actions may lead to secondary amelioration of insulin resistance and glucose toxicity in obese diabetic animals. Leptin, which is produced and secreted from adipose tissue,[16,17] is well known to regulate both food intake and energy metabolism[18-20] through its action on the central nervous system. Even when administered via a peripheral route, leptin also has been suggested to enter the hypothalamic region,[21-23] the regulatory center of the autonomic nervous system, and to control both food intake and energy expenditure.[24] An alternative indication of its possible mode of action is found in a report that intracerebroventricular infusion of BDNF induces weight loss in normal rats.[25] Since the receptor for BDNF, trkB, is also expressed in the hypothalamic region,[26] BDNF may therefore ameliorate the glucose metabolism of diabetic animals via its effect on the hypothalamus, like leptin.

The efficacy of intermittent administration of BDNF in regulating blood glucose concentrations in *db/db* mice supports the idea of an extended period of action for BDNF. Once- or twice-weekly administration of BDNF (70 mg/kg/wk) was shown to reduce blood glucose concentrations significantly as compared with the pair-fed control condition. The efficacy of BDNF was also confirmed by our finding that HbA_{1c} was lower in *db/db* mice given BDNF intermittently versus ad libitum-fed mice or pair-fed mice given PBS. HbA_{1c} levels of pair-fed mice were not significantly affected as compared with ad libitum-fed mice in this study. Less frequent dosing of BDNF via the subcutaneous route would be clinically acceptable in terms of patient compliance. This suggests that the need for frequent administration would be eliminated with BDNF, making this a uniquely advantageous agent for the treatment of diabetes.

In conclusion, we have demonstrated that intermittent administration of BDNF is sufficient to modulate systemic glucose metabolism in obese diabetic mice. These findings indicate that BDNF may be developed into a unique hypoglycemic agent for the treatment of diabetes at a fundamental level with good patient compliance.

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