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Brain-Derived Neurotrophic Factor Reduces Blood Glucose Level in Obese Diabetic Mice but Not in Normal Mice

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family. However, it is not yet known if BDNF works on the endocrine system itself. Here we report that BDNF improves hyperglycemia in obese diabetic animals. BDNF reduced the blood glucose level in obese *db*/*db* diabetic mice in which the effect of BDNF was age-dependent and high under the condition of hyperinsulinemia, while BDNF showed no effect on non-diabetic *db*/*m* mice. These results suggest that BDNF ameliorates insulin resistance by enhancing insulin action in peripheral tissues. Furthermore, BDNF was found to reduce the plasma insulin level in *db*/*db* mice. Among the neurotrophin family, NT-3 also reduced the blood glucose level in *db*/*db* mice. These results provide a novel insight that neurotrophin functions on the endocrine system as well as the nervous system.

Much attention has been drawn to the interaction among the nervous, immune, and endocrine systems. This is because it has been becoming more obvious that this so-called "triangle" of body system is more interrelated than previously thought. Brain-derived neurotrophic factor (BDNF) is a member of a neurotrophin family that includes nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) (1). BDNF promotes neurite outgrowth and provides a trophic support for certain neurons during development and in the adulthood (1). BDNF has been demonstrated to be effective on the animal models with nervous disorders such as diabetic neuropathy in addition to motor neuron and peripheral nerve disorders (2-4). Recently, however, the functional NGF receptor has been clarified to be present on non-neuronal cells as well as neuronal cells. It has been pointed out that NGF might be involved in the maintenance of a balanced interaction between the nervous, immune and endocrine systems (5). In contrast to NGF, the activity of BDNF on non-neuronal cells has not yet been understood.

EXPERIMENTAL PROCEDURES

Animals. Female *db*/*db* mice were purchased from Clea Japan Inc. Animals were maintained on a 12-h day/night schedule with continuous *ad libitum* access to water. All animal experiments were conducted according to the Guidelines of Experimental Animal Care issued by the Japanese Prime Minister's office.

Measurement of blood glucose level. Human recombinant BDNF (N-terminal methionine-free, Regeneron Pharmaceuticals) was administered subcutaneously at a dosage of 20 mg/kg to female *db*/*db* mouse. PBS was used as a vehicle. Pair-feeding was performed by providing the same amount of food to each pair-fed *db*/*db* mouse as the food consumed by BDNF-treated mice during the previous 24-h period. Blood samples were collected from tail blood vessel before BDNF administration and glucose levels were analyzed with blood glucose analyzers, Tide (Byer/Sankyo, Japan) or Antsense II (Daikin, Japan) according to the manufacturer's protocol.

Oral glucose tolerance test. Glucose was orally administered at a dosage of 3g/kg to *db*/*db* mice which were administered with BDNF or PBS for 12 days and then fasted for 20 hours before the test. Animals were kept fasted and the blood glucose was analyzed in a similar manner to the described above.

Measurement of insulin level. For the analysis of insulin, plasma was prepared by the addition of heparin and the subsequent centrifugation at 16,000 rpm for 15 min. Insulin concentration was measured by ELISA method according to the manufacture's protocol (Morinaga Biochemistry Research Center, Japan).

RESULTS AND DISCUSSION

db/*db* mice, originated from C57BL/KsJ, are well known to be a type II diabetic model animal because these mice begin showing obesity at 3 to 4-week-old, and induce hyperglycemia and hyperinsulinemia (6, 7). We used female *db*/*db* mice (7 to 10-week-old if not specified) to study the effect of BDNF on glucose metab-

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olism. Since BDNF reduces food intake and decreases a weight gain in rodents (8), the effect of BDNF administration on glucose metabolism was studied in comparison with pair-fed control animal. *db*/*db* mice were subcutaneously administered daily with 20mg/kg BDNF for 8 weeks. The blood glucose level of *db*/*db* mice 8 weeks post-administration with BDNF was statistically significantly low ($P<0.01$ by Student's *t*-test), 33 and 46 % of that of mice treated with phosphate-buffered saline (PBS) in *ad libitum*- and pair-fed condition, respectively (Fig. 1A). At that time the average food intake of BDNF group and pair-fed PBS group was approximately 60% compared to *ad libitum* PBS group, their body weights being 90% of the latter. Next, in order to address the question when this phenomenon is observed at the earliest, *db*/*db* mice were treated for 12 days. One week after administration, at the earliest, the blood glucose level of BDNF-treated mice was controlled to be approximately 170 mg/dl, significantly lower than that of pair-fed control mice treated with PBS (approximately 300 mg/dl)(Fig. 1B). Thus, the effect of BDNF on blood glucose was clearly demonstrated, being not simply caused by the reduced food uptake in obese *db*/*db* mice. Similar results were obtained using male *db*/*db* mice. After the consecutive daily administration for 12 days, an oral glucose tolerance test was performed on the both groups of the *db*/*db* mice which were fasted for overnight before the test. The blood glucose levels of BDNF-treated mice was shifted to be significantly lower than that of pair-fed control mice, and the cumulative glucose amount (AUC: Area under curve) in blood during 2 hours after glucose load (3 g/kg) in BDNF-treated mice was 65 % of that of the pair-fed mice. These results indicate that BDNF treatment reduces the increased blood glucose level and ameliorates the impaired glucose tolerance of obese diabetic *db*/*db* mice.

In addition to the effect of BDNF on blood glucose level (Fig. 2A), plasma insulin level of *db*/*db* mice treated with 20 mg/kg BDNF for 2 weeks was demonstrated to be significantly reduced by about 50% to the normal level, compared with that of PBS-treated control mice in *ad libitum*-fed condition (Fig. 2B). In contrast, no reduction of blood glucose (Fig. 2A) was seen in lean control *db*/*m* mice treated with BDNF. Very interestingly, plasma insulin level in lean control mice decreased by the treatment with BDNF (Fig. 2B). This suggests that BDNF increases insulin sensitivity of peripheral tissues so that low level of insulin is enough to keep the blood glucose level normal in lean control mice. Plasma insulin levels of *db*/*db* mice are known to be age-dependent. In their second month of age, *db*/*db* mice become hyperglycemic. In parallel, insulin levels increase to 10 times normal values when the mice are 3-month-old. When the mice are 3 to 6-month-old, hyperinsulinemia diminishes and the mice manifest symptoms of insulin deficiency (6). We investigated the effect of BDNF on blood glucose levels at various ages of *db*/*db* mice. As shown in Fig. 3, the response of blood glucose reduction caused by BDNF in *db*/*db* mice was intensively big and rapid when the treatment started at

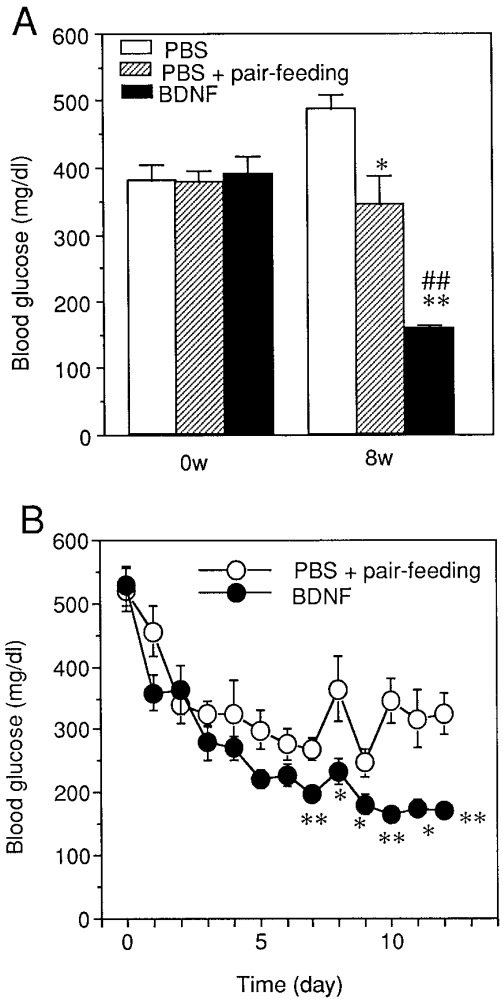

FIG. 1. Effects of chronic administration with BDNF on blood glucose. (A) PBS or BDNF at a dosage of 20 mg/kg was subcutaneously administered 5 times a week for 8 weeks to respective 8 to 10 female *db*/*db* mice (10-week-old). Pair feeding was performed as described in the methods. Student's *t*-test was used for statistical analysis. Blood glucose level of the BDNF-treated group at 8 weeks was significantly different from the PBS-treated group with *ad libitum*-feeding (**$P<0.01$) and the PBS-treated group with pair-feeding ($^{\#\#}P<0.01$). Blood glucose level of pair-fed mice was significantly different (*$P<0.05$) from *ad libitum*-fed mice in PBS-treated groups. (B) BDNF at a dosage of 20 mg/kg or PBS was administered subcutaneously 7 times a week for 12 days to female *db*/*db* mice (10-week-old). Seven and eight mice were used for BDNF-treated and PBS-treated groups, respectively. Pair-feeding was performed as described in the methods. Blood glucose concentration was measured. Bars indicate s.e.m. Blood glucose level of the BDNF-treated group was significantly different from the PBS-treated group (*$P<0.05$ and **$P<0.01$, Student's *t*-test).

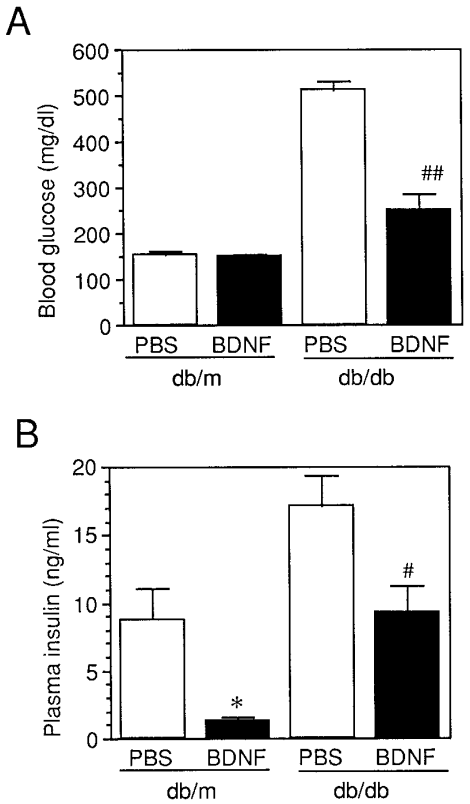

FIG. 2. Blood glucose and plasma insulin levels in obese diabetic *db*/*db* mice and lean control *db*/*m* mice following BDNF administration. BDNF was administered subcutaneously 7 times a week for 2 weeks at a dosage of 20 mg/kg to *db*/*db* mice (female, 7-week-old) and *db*/*m* mice (female, 7-week-old). Nine and ten mice were used for BDNF-treated and PBS-treated groups, respectively. Mice were allowed *ad libitum* access to food. Twenty-four hours after the final administration, a blood sample was collected. Blood glucose and plasma insulin levels of *db*/*db* mice in the BDNF-treated group were significantly different ($^{\#}P<0.05$ and $^{\#\#}P<0.01$, Student's *t*-test or Welch's *t*-test) from PBS-treated *db*/*db* mice. Plasma insulin level of *db*/*m* mice in the BDNF-treated group was significantly different ($^{*}P<0.05$, Welch's *t*-test) from PBS-treated *db*/*m* mice. (A) Blood glucose. (B) Plasma insulin.

7-week-old, moderate at 11-week-old and less at 23-week-old in our experiments. No reduction of blood glucose caused by BDNF was observed again in 11-week-old lean *db*/*m* mice. These results imply that BDNF requires relatively high level of insulin to reduce blood glucose level in *db*/*db* mice.

In an effort to further evaluate the role of BDNF in glucose metabolism, the effects of other factors in a neurotrophin family have been studied. As shown in Fig. 4A, a single subcutaneous administration of NGF at a dosage of 20 mg/kg did not show any effect on the blood glucose level. In contrast, a single administration with the same amount of NT-3 more extensively reduced the glucose level than did BDNF in *db*/*db* mice, but within 24 hours restored it to almost the same level as that of 0 hour. The effect of BDNF on glucose level was different from those of ciliary neurotrophic factor (CNTF) or Insulin-like growth factor (IGF-I). CNTF has been reported to reduce blood glucose level not only in diabetic mice (9) but also in normal mice (10). In contrast, blood glucose level remained normal even after the administration at a dosage of 20 and 200 mg/kg BDNF in the male normal C57BL/6NCrj mice under the fasted condition (Fig. 4B) as well as in fasted normal rats (data not shown), while IGF-1 (20 mg/kg) markedly reduced the blood glucose level (Fig. 4B). Thus, it was shown that BDNF did not cause hypoglycemia in normal animals. BDNF did not show effects on blood glucose level in streptozotocin-induced type I diabetic rats, the pancreatic-cells of which were damaged by streptozotocin and could not secrete insulin (data not shown). These observations indicate that BDNF does not show a direct insulin-like hormonal action to peripheral tissues such as skeletal muscle, adipose and liver. Our preliminary results showed that BDNF administration also reduced blood glucose level in obese and hyperinsulinemic *ob*/*ob* mice. Together with that BDNF decreased the blood glucose level of obese diabetic *db*/*db* mice at 7 to 11-week-old when they showed hyperinsulinemia and also reduced plasma insulin level without any change of blood glucose level in non-diabetic *db*/*m* mice, these findings lead us to speculate that BDNF might enhance and/or modulate insulin actions in peripheral tissues and namely

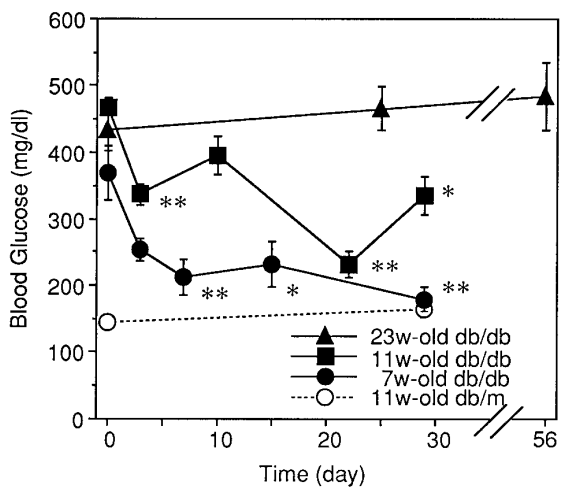

FIG. 3. Age-dependency of BDNF action on blood glucose in *db*/*db* mice. BDNF was administered subcutaneously 5 times a week for 24 to 56 days at a dosage of 20 mg/kg to young and old female *db*/*db* mice or lean control *db*/*m* mice under *ad libitum* condition; bars indicate s.e.m. Difference from initial blood glucose level was analyzed by Student's *t*-test ($^{*}P<0.05$ and $^{**}P<0.01$) adjusted by Bonferroni procedure. Eight 7-week-old *db*/*db*, ten 11-week-old *db*/*db*, seven 11-week-old *db*/*m*, and six 23-week-old *db*/*db* mice were used for this study. PBS treatment did not reduce the blood glucose levels of *db*/*db* mice (data not shown).

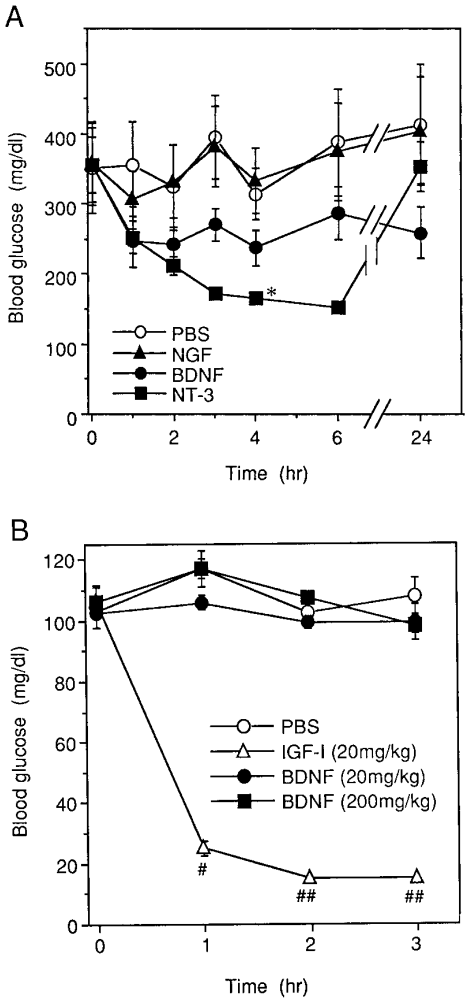

FIG. 4. Effects of neurotrophins on blood glucose. (A) Effects of neurotrophins in *db*/*db* mice were examined under *ad libitum* fed condition for 24 hours. Neurotrophic factors of NGF (mouse 2.5S NGF: Serotech), BDNF, and NT-3 (human recombinant NT-3: PreproTech) were administered subcutaneously at a single injection of 20 mg/kg and the time course of the blood glucose level was analyzed. Three to four *db*/*db* mice were used for respective groups. Blood glucose level of NT-3-treated mice was significantly different (*$P<0.05$, Dunnet test) from PBS-treated mice. Vertical bars indicate s.e.m. (B) Effects of BDNF or IGF-I (Pharmacia & Upjohn) in male normal C57BL/6NCrj mice. BDNF or IGF-I was administered subcutaneously at a single injection of 20 mg/kg (also 200 mg/kg for BDNF) to 5 male normal mice respectively after 20 hours fasting. Mice were further kept under fasted condition and blood glucose level was analyzed for the subsequent 3 hours. Blood glucose levels of three mice (#) or all mice (##) were below the detection limit (20 mg/dl). Vertical bars indicate s.e.m.

reduce insulin resistance in *db*/*db* mice of type II diabetic model. However, it remains to be elucidated whether BDNF ameliorates impaired glucose tolerance by directly calling off insulin resistance rather than simply controlling blood glucose level.

BDNF and NT-3 are well known to act on neuronal cells through high affinity receptor, trkB and trkC, respectively (1), in association with p75, the low affinity receptor (11). However, information on the function of these receptors in non-neuronal tissues which might be involved in insulin resistance is limited. p75 is expressed in rat pancreas, liver and muscle (12, 13). The truncated but not full-length form of trkB is expressed in rat muscle (13) and adipose tissue (our preliminary data). There remains possibility that BDNF might act through these tissues, in which BDNF bound to trkB alone and/or trkB in association with p75 induces signals to increase glucose incorporation and consumption. The effect of BDNF on these cells remains to be clarified. Alternative interesting hypothesis is that BDNF and NT-3 might act on hypothalamo-pituitary axis, leading the blood glucose reduction. NGF, BDNF and NT-3 were reported to penetrate blood-brain barriers to reach hypothalamus by a systemic administration (14). p75, trkB and trkC, but not trkA are expressed in hypothalamus (15, 16) and pituitary (17, 18). Thus, our finding that BDNF and NT-3, but not NGF reduced blood glucose level coincides with the presence of their cognate receptors in hypothalamus and pituitary. Our result would support the physiological role in neuroendocrine system of BDNF in hypothalamo-pituitary axis.

In conclusion, we report here that BDNF and NT-3 regulate glucose metabolism. These results demonstrate that BDNF and NT-3 show some physiological role on not only the nervous system but also the endocrine system.

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