BDNF Action in the Brain Attenuates Diabetic Hyperglycemia via Insulin-Independent Inhibition of Hepatic Glucose Production

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Recent evidence suggests that central leptin administration fully normalizes hyperglycemia in a rodent model of uncontrolled insulin-deficient diabetes by reducing hepatic glucose production (HGP) and by increasing glucose uptake. The current studies were undertaken to determine whether brain-derived neurotrophic factor (BDNF) action in the brain lowers blood glucose in uncontrolled insulin-deficient diabetes and to investigate the mechanisms mediating this effect. Adult male rats implanted with cannulas to either the lateral cerebral ventricle or the ventromedial hypothalamic nucleus (VMN) received either vehicle or streptozotocin to induce uncontrolled insulin-deficient diabetes. Three days later, animals received daily intracerebroventricular or intra-VMN injections of either BDNF or its vehicle. We found that repeated daily intracerebroventricular administration of BDNF attenuated diabetic hyperglycemia independent of changes in food intake. Instead, using tracer dilution techniques during a basal clamp, we found that BDNF lowered blood glucose levels by potently suppressing HGP, without affecting tissue glucose uptake, an effect associated with normalization of both plasma glucagon levels and hepatic expression of gluconeogenic genes. Moreover, BDNF microinjection directly into the VMN also lowered fasting blood glucose levels in uncontrolled insulin-deficient diabetes, but this effect was modest compared with intracerebroventricular administration. We conclude that central nervous system BDNF attenuates diabetic hyperglycemia via an insulin-independent mechanism. This action of BDNF likely involves the VMN and is associated with inhibition of glucagon secretion and a decrease in the rate of HGP. ***Diabetes*** **62:1512–1518, 2013**

Growing evidence suggests that the brain has a surprisingly potent capacity to normalize blood glucose levels in animals with uncontrolled insulin-deficient diabetes. Even at low doses, leptin infusion directly into the brain completely normalizes diabetic hyperglycemia despite persistent, severe insulin deficiency (1–4). Thus, glucose-lowering in this setting appears to involve mechanisms that are largely independent of either insulin secretion or insulin sensitivity, and identifying neurocircuits that underlie these effects has emerged as an important scientific priority.

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Neurons in the ventromedial hypothalamic nucleus (VMN) that express brain-derived neurotrophic factor (BDNF) are of interest with respect to brain mechanisms of glucose-lowering in diabetes. In addition to its well-established roles in the survival, differentiation, and plasticity of neurons (5), BDNF also is implicated in the regulation of energy homeostasis and glucose homeostasis through its effects in the central nervous system. Consistent with this, either systemic or central BDNF administration dose-dependently reduces food intake and body weight (6) in rodent models of both impaired (diet-induced obesity) and deficient (*db/db* mice) leptin signaling (7,8). Moreover, BDNF neurons in the VMN are activated by leptin (9) and, like leptin, the glucose-lowering effects of BDNF are mediated, at least in part, via a mechanism independent of reduced food intake (10). Conversely, reduced hypothalamic BDNF signaling causes a hyperphagic, obese, diabetic phenotype in rodents (11) and, similarly, humans with BDNF haploinsufficiency (12) or de novo missense in the high-affinity receptor for BDNF, tropomyosin-related kinase B, are characterized by hyperphagia, obesity, and associated metabolic disturbances (13).

Thus, BDNF is implicated in the central control of both energy balance and glucose metabolism, but the mechanisms underlying these effects remain poorly understood. Therefore, the current studies were undertaken to determine whether the central action of BDNF to lower blood glucose is detectable in animals with uncontrolled insulin-deficient diabetes, is dependent on insulin, or is mediated via effects on hepatic glucose production (HGP), tissue glucose uptake, or both.

RESEARCH DESIGN AND METHODS

Animals. Adult male Wistar rats (Harlan) were individually housed with ad libitum access to water and chow (PMI Nutrition), unless otherwise stated, in a temperature-controlled room with a 12:12-h light–dark cycle under specific pathogen-free conditions. All procedures were performed in accordance with National Institutes of Health Guidelines for Care and Use of Animals and were approved by the Animal Care Committee at the University of Washington.

Surgery. Animals underwent implantation of either a single cannula to the lateral ventricle (Alzet) as previously described (1) or a bilateral cannula to the VMN (Plastics One) at the following stereotaxic coordinates: 2.8 mm posterior to bregma, ±0.6 mm lateral, and 8.5 mm below the skull surface (14). Intracerebroventricular or bilateral intraparenchymal injections were administered using a microinjector needle that extended 1 mm beyond the tip of the cannula over the course of 60 s in a final volume of up to 5 μL or 0.5 μL, respectively.

Experimental protocol.

Effect of intracerebroventricular BDNF on food intake and blood glucose levels in streptozotocin-induced diabetes. Adult male Wistar rats bearing lateral ventricle cannulas either were made diabetic with two consecutive daily subcutaneous injections of streptozotocin (STZ; 40 mg/kg body weight) or received vehicle (NaCit, pH 4.5) and remained nondiabetic, as previously described (1,15). Three days later, animals with STZ-induced diabetes received daily intracerebroventricular injections of either vehicle or

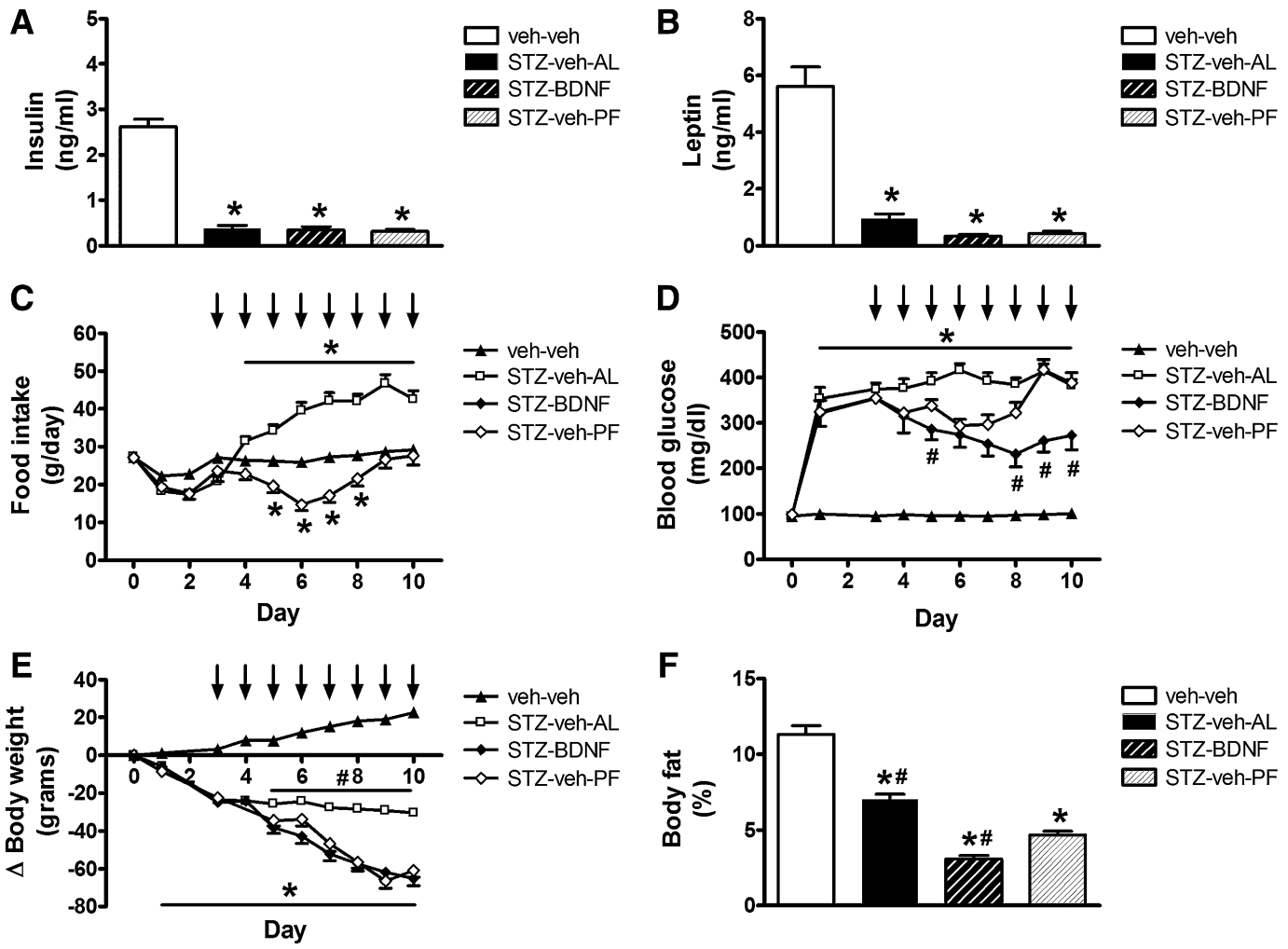

FIG. 1. Effect of intracerebroventricular BDNF on food intake, blood glucose levels, and body weight in uncontrolled insulin-deficient diabetes. Plasma insulin (*A*), plasma leptin (*B*), mean daily food intake (*C*), fed blood glucose levels (*D*), body weight change (*E*), and percent body fat (*F*) in nondiabetic controls (veh-veh) or in STZ-induced diabetic animals receiving intracerebroventricular vehicle and fed ad libitum (STZ-veh-AL) or pair-fed (STZ-veh-PF) or intracerebroventricular BDNF (STZ-BDNF) (n = 8–10 per group). Arrows indicate the days of intracerebroventricular injections. Data represent mean ± SEM. *$P < 0.05$ vs. veh-veh; #$P < 0.05$ vs. STZ-veh-PF.

BDNF (Peprotech) at a dose (5 μg) known to induce anorexia (6,10). To control for the effect of BDNF to reduce food intake, an additional group STZ-vehicle–treated animals was pair-fed to the intake of the STZ-BDNF group, as previously described (1,16). Food intake, body weight, and blood glucose levels were measured daily.

Effect of intracerebroventricular BDNF on HGP and glucose uptake in STZ-induced diabetes. Adult male Wistar rats bearing catheters to the right jugular vein and left carotid artery (Harlan) were studied using the same protocol as described. Ten days after administration of STZ or vehicle, tracer dilution techniques were used to determine the effect of intracerebroventricular BDNF on HGP and tissue glucose uptake. Measures of glucose appearance using [3-^{3}H] glucose and tissue glucose uptake using 2[^{14}C]-deoxyglucose (2-DG) were obtained as previously described (1).

Effect of VMN BDNF on food intake and blood glucose levels in STZ-induced diabetes. Animals followed the protocol described, except that they underwent implantation of a bilateral cannula directed to the VMN. Three days after STZ-induced diabetes, animals received daily intraparenchymal microinjections of either BDNF (0.5 μg each side) or its vehicle to the VMN.

Immunohistochemical verification of cannula and injection site. The cannula placement and the injection site were verified postmortem using histological techniques (17), and the spread of injection was verified after bilateral microinjection of a Cy3-labeled recombinant peptide (leptin, 0.5 μL per side; Phoenix Pharmaceuticals) to the VMN as previously described (17). Representative photomicrographs of cannula placement and the injection site are shown in Fig. 4*A*. Only data from rats that were positively verified were included in the analysis.

Blood collection and tissue processing. At study completion, liver, skeletal muscle, and brown adipose tissue (BAT) samples were harvested, frozen on dry ice, and stored at −80°C. Blood samples for plasma hormonal measures were collected in appropriately treated tubes (1) and centrifuged, and the plasma was removed, aliquoted, and stored at −20°C for subsequent assay. Plasma insulin and leptin levels were determined by ELISA (Crystal Chem). Corticosterone levels were determined using an enzyme immunoassay (DSLabs). Thyroid levels (T4) were determined using an ELISA (Calbiotech). Total ketone bodies were determined using a colorimetric kit (Wako Chemicals). Glucagon levels were determined using a RIA kit (Linco Research).

RT-PCR. Total RNA was extracted from liver and BAT using TRIzol B according to the instructions of the manufacturer (MRC). RNA was quantitated by spectrophotometry at 260 nm (Nanodrop 1000) and reverse-transcribed with AMV reverse transcriptase (Promega), and real-time PCR was performed on an ABI Prism 7900 HT (Applied Biosystems) as described previously (1,16).

Body composition analysis. Determinations of lean mass and fat mass were made using quantitative magnetic resonance spectroscopy (Echo Medical Systems) using the Nutrition Obesity Research Center Energy Balance and Glucose Metabolism Core Laboratory at the University of Washington.

Statistical analyses. All results are expressed as mean ± SEM. Comparisons between multiple groups were made using a two-way ANOVA with a least significant difference post hoc test for comparisons between groups. For two-group comparisons, a two-sample, unpaired Student t test was used. Statistical analyses were performed using Statistica (version 7.1; StatSoft). $P < 0.05$ was considered significant.

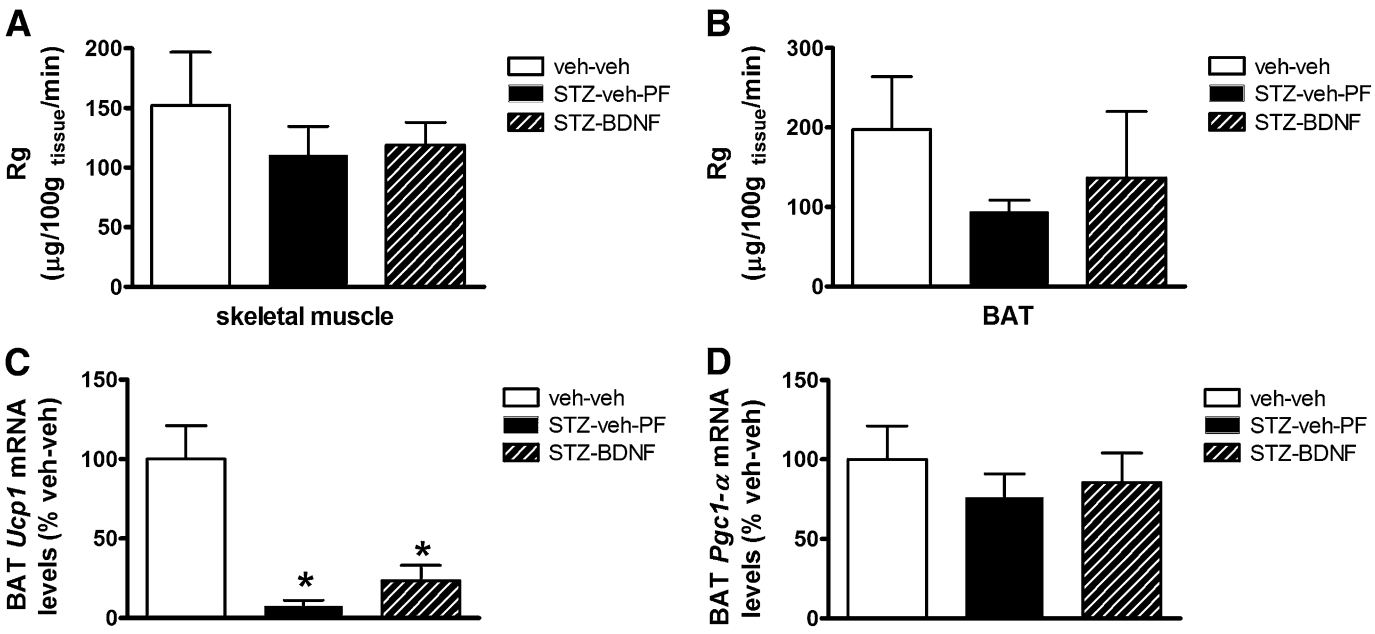

FIG. 2. Central nervous system BDNF infusion fails to increase tissue glucose uptake in uncontrolled insulin-deficient diabetes. Tissue glucose uptake (Rg) determined from 2[^{14}C]-deoxyglucose studies in tibialis anterior muscle (*A*) and BAT (*B*) and BAT expression of uncoupling protein-1 (Ucp1) (*C*) and peroxisome proliferator–activated receptor γ-coactivator-1 α (Pgc-1α) (*D*) in nondiabetic controls or in STZ-induced diabetic animals receiving intracerebroventricular vehicle and pair-fed or intracerebroventricular BDNF (n = 5–6 per group). Data represent mean ± SEM. *P < 0.05 vs. veh-veh.

RESULTS

Effect of intracerebroventricular BDNF on food intake and blood glucose levels in STZ-induced diabetes. As expected, plasma insulin and leptin levels were markedly reduced in all animals with STZ-induced diabetes relative to nondiabetic controls (Fig. 1*A* and *B*), but the characteristic increase in food intake (15) was prevented by intracerebroventricular BDNF (Fig. 1*C*). Relative to blood glucose levels in nondiabetic controls, the marked hyperglycemia characteristic of STZ-induced diabetes was attenuated, but not fully normalized, among diabetic animals receiving daily intracerebroventricular BDNF injection. However, this glucose-lowering effect of BDNF cannot be fully explained by reduced food intake because hyperglycemia was not attenuated in pair-fed controls that received intracerebroventricular vehicle rather than BDNF (Fig. 1*D*).

As expected, animals with STZ-induced diabetes lost body weight relative to nondiabetic controls, despite dramatically increased food intake, presumably because of increased glycosuria (Fig. 1*E*). Although animals with STZ-induced diabetes that received intracerebroventricular BDNF lost more body weight than those receiving vehicle, this effect is largely explained by the associated reduction of food intake (Fig. 1*E*). In BDNF-treated rats with STZ-induced diabetes, however, this loss of body weight was characterized by a greater loss of body fat than that observed in vehicle-treated rats with STZ-induced diabetes fed ad libitum or pair-fed (Fig. 1*F*).

Effect of intracerebroventricular BDNF on HGP and glucose uptake in STZ-induced diabetes. Because of the confounding effect of glycosuria in uncontrolled insulin-deficient diabetes, we measured tissue glucose uptake using labeled 2-deoxyglucose rather than tracer-based estimates of glucose disappearance. In contrast to the effect of BDNF to lower elevated blood glucose levels in rats with STZ-induced diabetes, glucose uptake in skeletal muscle and BAT was not increased in animals with STZ-induced diabetes receiving intracerebroventricular BDNF compared with intracerebroventricular vehicle (Fig. 2*A* and *B*). Moreover, the effect of uncontrolled insulin-deficient diabetes to dramatically reduce activity of BAT, as judged by *Ucp1* mRNA levels, was unaffected by central BDNF treatment (Fig. 2*C*). These findings suggest that intracerebroventricular BDNF is unlikely to ameliorate diabetic hyperglycemia via a mechanism involving increased tissue glucose uptake.

As expected, the rate of endogenous glucose appearance was significantly increased in pair-fed STZ-diabetic vehicle-treated animals relative to nondiabetic controls (Fig. 3*B*), supporting the concept that increased rates of HGP contribute to diabetic hyperglycemia in uncontrolled insulin-deficient diabetes. Importantly, this increased rate of glucose appearance was normalized to nondiabetic control values in rats with STZ-induced diabetes that received intracerebroventricular BDNF (Fig. 3*B*). In addition, hepatic expression of genes encoding the key gluconeogenic enzymes glucose-6-phosphatase (*G6Pase*) and phosphoenolpyruvate carboxykinase (*Pepck*) was elevated in animals with STZ-induced diabetes that received intracerebroventricular vehicle, and this effect was reversed in animals receiving intracerebroventricular BDNF (Fig. 3*C* and *D*). Thus, the glucose-lowering effect of intracerebroventricular BDNF in uncontrolled insulin-deficient diabetes is likely mediated, at least in part, via reduced HGP and involves inhibition of gluconeogenic gene expression.

Effect of intracerebroventricular BDNF on plasma parameters in STZ-induced diabetes. Uncontrolled insulin-deficient diabetes is characterized by hyperglucagonemia and hypercorticosteronemia, responses that increase HGP and that are normalized when intracerebroventricular leptin is administered at doses that normalize hyperglycemia (1). As expected, plasma glucagon levels were elevated in pair-fed

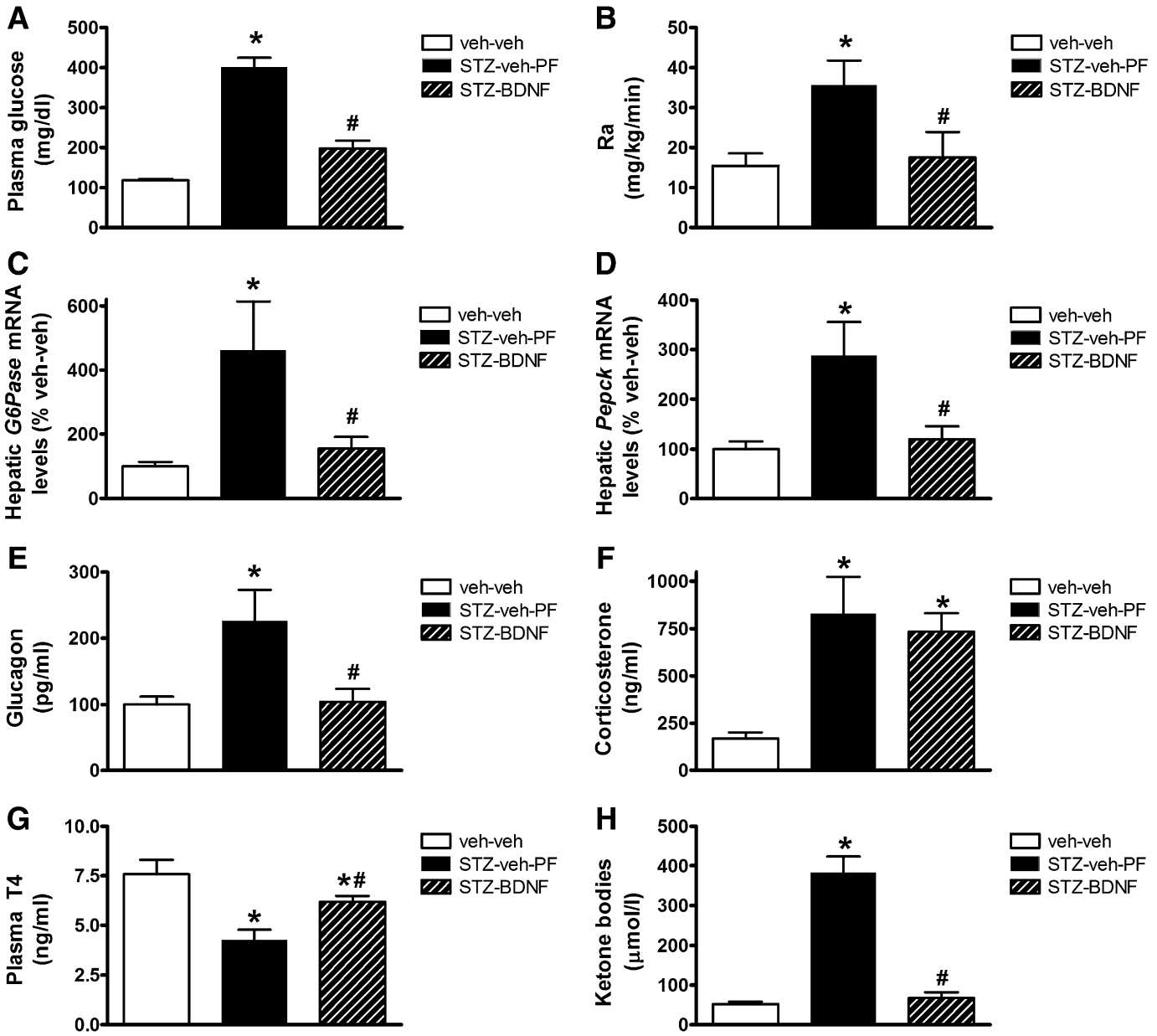

FIG. 3. Central nervous system BDNF infusion suppresses HGP and normalizes elevated expression levels of hepatic gluconeogenic genes and plasma glucagon levels in uncontrolled insulin-deficient diabetes. The 3-h fasted plasma glucose levels (*A*), rate of glucose appearance (Ra) as determined from [3-^{3}H] glucose tracer studies (*B*), and hepatic expression of glucose-6-phosphatase (*G6Pase*) (*C*) and phosphoenolpyruvate carboxykinase (*Pepck*) (*D*) using real-time PCR and plasma glucagon (*E*), corticosterone (*F*), T4 levels (*G*), and total ketone bodies (*H*) in animals with STZ-induced diabetes receiving intracerebroventricular vehicle and pair-fed or intracerebroventricular BDNF relative to nondiabetic controls (n = 5–6 per group). Data represent mean ± SEM. *P < 0.05 vs. veh-veh; #P < 0.05 vs. pair-fed STZ-diabetic vehicle-treated.

STZ-diabetic vehicle-treated rats relative to nondiabetic rats, and this effect was normalized with BDNF (Fig. 3*E*). In contrast, whereas rats with STZ-induced diabetes also displayed the expected increase of plasma corticosterone levels, treatment with BDNF was without effect (Fig. 3*F*). Moreover, we replicated the effect of STZ-induced diabetes to lower circulating T4 levels (Fig. 3*G*). However, whereas T4 levels were increased by intracerebroventricular BDNF, the effect was small such that T4 levels remained well below control levels. Plasma levels of ketone bodies also were elevated in pair-fed STZ-diabetic vehicle-treated animals, and these were normalized with intracerebroventricular BDNF administration (Fig. 3*H*).

The VMN as a target for BDNF action on food intake and blood glucose levels in STZ-induced diabetes. Because BDNF is abundantly expressed in the VMN (5), animals with STZ-induced diabetes received daily bilateral microinjections of BDNF to this brain area. We found that whereas blood glucose levels in the fed state were reduced in STZ-induced diabetic animals that received BDNF directly to the VMN relative to vehicle-treated controls only on day 11 (Fig. 4*C*), 4-h fasted blood glucose levels were consistently reduced (Fig. 4*D*). Thus, BDNF microinjection into the VMN lowered fasting blood glucose levels in uncontrolled insulin-deficient diabetes, but this effect was modest compared with intracerebroventricular administration.

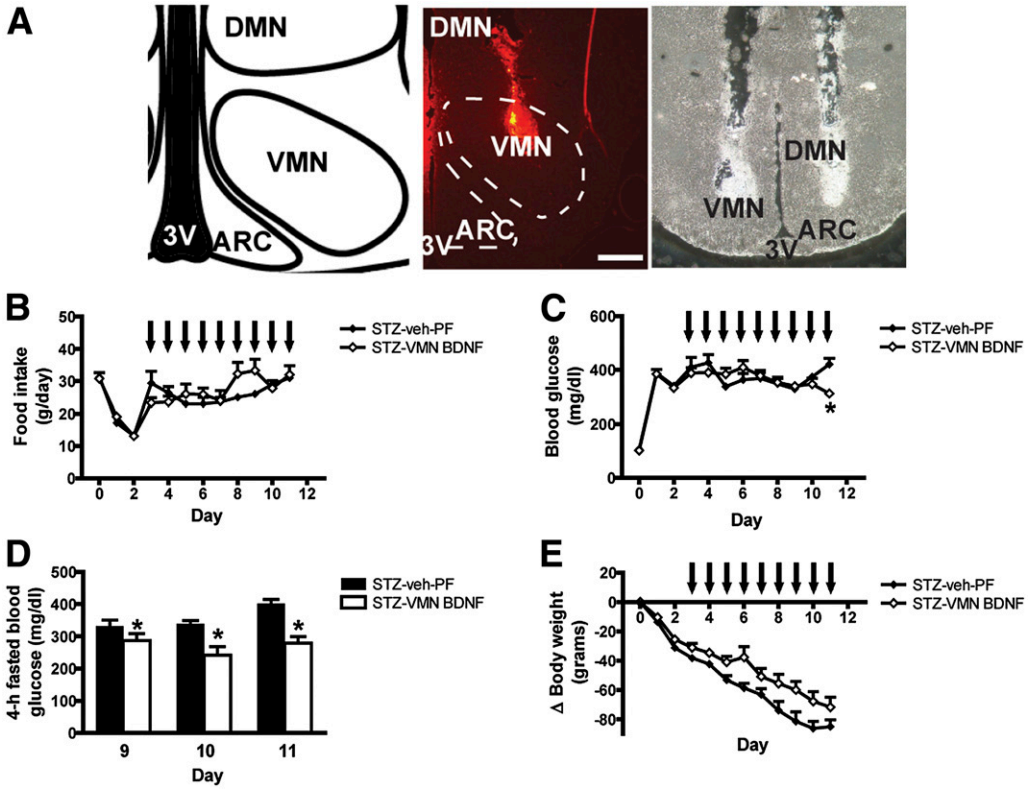

FIG. 4. Effect of VMN BDNF on food intake and blood glucose levels in uncontrolled insulin-deficient diabetes. Verification of cannula placement and spread of injection after microinjection into the VMN. *A*: A fluorescent micrograph of a representative injection site of a cy3-labeled peptide within the VMN using a ×4 objective lens and a representative example of cannula placement from coronal sections at the level of the VMN using a ×2 objective lens. For two animals, cy3-labeled leptin overlapped with the borders of other hypothalamic areas or lined the walls of the third ventricle and were excluded from the analysis. Mean daily food intake (*B*), fed blood glucose levels (*C*), 4-h fasted blood glucose levels (*D*), and body weight change (*E*) in animals with STZ-induced diabetes receiving vehicle and pair-fed (STZ-veh-PF, closed diamonds) or BDNF directed to the VMN (STZ-VMN BDNF, open diamonds). *n* = 7–10 per group. Arrows indicate the days of bilateral VMN injections. Data represent mean ± SEM. **P* < 0.05 vs. STZ-veh-PF. ARC, arcuate nucleus; DMN, dorsomedial nucleus; 3V, third ventricle. (A high-quality color representation of this figure is available in the online issue.)

DISCUSSION

Previous studies have demonstrated that central BDNF administration improves glucose metabolism in rodent models of type 2 diabetes via effects that cannot be explained by reduced food intake (5,7,8,10). Although the mechanism whereby BDNF action in the brain improves glucose metabolism is unknown, much of the published literature has focused on its ability to increase insulin sensitivity (10,18). Thus, the question of whether intracerebroventricular injection of BDNF can lower blood glucose in a setting in which diabetes results exclusively from severe insulin deficiency (and, hence, must involve an insulin-independent mechanism) has yet to be investigated. Here, we report for the first time that chronic central administration of BDNF attenuates diabetic hyperglycemia despite persistent, severe insulin deficiency. Moreover, this effect occurs independently of changes of food intake, likely involves an action of BDNF in the VMN, and the underlying mechanism is mediated by a potent suppression of HGP that is accompanied by normalization of increased levels of both plasma glucagon and expression of gluconeogenic genes in the liver without effects on tissue glucose uptake.

Glucagon increases plasma glucose levels by stimulating HGP, and several lines of evidence suggest that hyperglucagonemia contributes to elevated blood glucose levels in uncontrolled insulin-deficient diabetes (19). Similar to recent evidence that leptin action in the brain potently suppresses HGP and reduces plasma glucagon levels in uncontrolled insulin-deficient diabetes (1), we found that central nervous system BDNF signaling also reverses the effect of STZ-induced diabetes to increase glucagon secretion. Because glucagon action on the liver induces expression of gluconeogenic genes and increases HGP, our data suggest that suppression of hyperglucagonemia contributes to the glucose-lowering effect of intracerebroventricular BDNF. However, because physiological replacement of leptin levels in uncontrolled insulin-deficient diabetes normalizes plasma glucagon levels without ameliorating diabetic hyperglycemia (16), mechanisms additional to normalizing glucagon levels likely contribute to BDNF-mediated suppression of HGP.

Interestingly, whereas elevated levels of both plasma glucagon and HGP were normalized with central BDNF treatment, diabetic hyperglycemia was not fully normalized (~50%), suggesting that other factors are not normalized by BDNF signaling. Consistent with this, central infusion of BDNF did not stimulate tissue glucose uptake in uncontrolled insulin-deficient diabetes. This observation is in contrast to a previous report that found that systemic BDNF treatment to *db/db* mice increased tissue glucose uptake, albeit to a small extent, relative to tissue glucose uptake in vehicle-treated wild-type littermates (20). The discrepancy between these findings is not likely explained by a direct action of BDNF on peripheral tissues because BDNF fails to increase insulin-stimulated glucose uptake

in cultured L6 muscle cells and 3T3-L1 adipocytes (18). Rather, this finding suggests that BDNF-induced glucose uptake is mediated via increased insulin sensitivity and therefore requires intact insulin signaling. Because uncontrolled insulin-deficient diabetes is characterized by severe insulin deficiency, and because this was not altered by intracerebroventricular BDNF, an increase of tissue insulin sensitivity, which may have occurred, is unlikely to lower blood glucose because ambient levels of insulin are too low to increase tissue glucose uptake, regardless of insulin sensitivity.

Although the neuronal mechanism whereby BDNF mediates these affects remains to be elucidated, our data point to a local action in the VMN. First, BDNF is abundantly expressed in this brain area (5), and the VMN plays an important role in the control of glucagon secretion (21) and in sensing and mounting responses to hypoglycemia (22). We found that whereas BDNF action in the VMN lowers fasting blood glucose levels in uncontrolled insulin-deficient diabetes independent of changes of food intake, this effect cannot fully account for the glucose-lowering effects of intracerebroventricular BDNF, suggesting that BDNF acts in additional brain areas as well. Because BDNF is expressed in additional hypothalamic and extrahypothalamic areas involved in the regulation of metabolism (5), future studies are warranted to examine the sites and downstream mechanism of BDNF action, (i.e., tropomyosin-related kinase B).

Because BDNF is a key regulator of neural growth and synaptic plasticity (23), it is possible that the glucose-lowering effects of BDNF involve synaptic changes in hypothalamic areas that regulate glucose homeostasis. Consistent with this hypothesis, recent evidence implicates the long form of *Bdnf* mRNA in the control of synaptic plasticity in mice, whereas the short form is not (24). Furthermore, BDNF translated from the long form, but not the short form, of *Bdnf* mRNA is essential for the regulation of energy homeostasis and responses to leptin (9).

It is also worth noting that BDNF action in the brain mimics many, but not all, of the beneficial metabolic effects of leptin in uncontrolled insulin-deficient diabetes. Thus, whereas intracerebroventricular BDNF lowers blood glucose levels and mimics the effect of leptin to suppress HGP and hyperglucagonemia in uncontrolled insulin-deficient diabetes (1), it does not mimic the ability of leptin to normalize blood glucose levels, presumably because, unlike leptin, BDNF does not increase tissue glucose utilization. Because leptin activates BDNF neurons (9), these data raise the possibility that BDNF is in the pathway linking leptin action to the control of HGP, but not tissue glucose uptake. Similarly, leptin (1,16), but not BDNF, normalizes the increase of corticosterone levels characteristic of uncontrolled insulin-deficient diabetes, suggesting that leptin regulation of the hypothalamic-pituitary-adrenal axis (25) occurs independent of BDNF signaling.

In summary, these data suggest that in diabetic animals, increased BDNF signaling in the brain normalizes HGP via a mechanism that is largely insulin-independent and substantially attenuates diabetic hyperglycemia. Moreover, the persistent, moderate hyperglycemia during central BDNF administration to animals with uncontrolled insulin-deficient diabetes likely reflects the lack of any increase of tissue glucose uptake.

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T.H.M. researched data, contributed to discussion, and reviewed and edited the manuscript. B.E.W., J.P.T., and S.J.G. contributed to discussion and reviewed and edited the manuscript. M.E.M. researched data and reviewed and edited the manuscript. J.D.F. researched data. G.J.T. and M.W.S. contributed to discussion and reviewed and edited the manuscript. G.J.M. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. G.J.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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