Leptin Activates a Novel CNS Mechanism for Insulin-Independent Normalization of Severe Diabetic Hyperglycemia

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The brain has emerged as a target for the insulin-sensitizing effects of several hormonal and nutrient-related signals. The current studies were undertaken to investigate mechanisms whereby leptin lowers circulating blood glucose levels independently of insulin. After extending previous evidence that leptin infusion directly into the lateral cerebral ventricle ameliorates hyperglycemia in rats with streptozotocin-induced uncontrolled diabetes mellitus, we showed that the underlying mechanism is independent of changes of food intake, urinary glucose excretion, or recovery of pancreatic β-cells. Instead, leptin action in the brain potently suppresses hepatic glucose production while increasing tissue glucose uptake despite persistent, severe insulin deficiency. This leptin action is distinct from its previously reported effect to increase insulin sensitivity in the liver and offers compelling evidence that the brain has the capacity to normalize diabetic hyperglycemia in the presence of sufficient amounts of central nervous system leptin. **(*Endocrinology* 152: 394–404, 2011)**

In studies dating to the 19th century, Claude Bernard observed that puncturing the floor of the fourth ventricle induces diabetes ("piqure diabetique") (1) in rabbits, suggesting that the brain plays an important role in the control of blood glucose. After the discovery of insulin (2) and the subsequent demonstration that insulin action in peripheral tissues, such as liver, muscle, and adipose tissue, accounts for most aspects of insulin-mediated glucose metabolism, interest shifted away from the brain as a regulator of glucose homeostasis. Although insulin remains the cornerstone of therapy for type 1 diabetes in humans, recent evidence suggests that pharmacological administration of leptin systemically (3, 4) can also normalize blood glucose levels in rodent models of uncontrolled diabetes mellitus (uDM). Moreover, the central nervous system (CNS) is implicated in this effect, because leptin administration directly into the brain normalizes blood glucose in rats with uDM (5–9) at doses that are ineffective when administered peripherally. Although accumulating evidence suggests that leptin (10–13), insulin (14, 15), and nutrient-related signals (16–18) in the CNS improve hepatic insulin sensitivity, how CNS leptin signaling normalizes diabetic hyperglycemia in uDM, and whether this involves leptin-dependent effects on hepatic glucose production (HGP) or tissue glucose uptake (Rg), is unknown.

Here, we report that the antidiabetic effect of CNS leptin action in rats with uDM is not due to reduced food intake, increased urinary glucose loss, or recovery of pancreatic β-cells. Instead, we show that CNS leptin administration induces both potent suppression of HGP and increased Rg via insulin-independent mechanisms. Although the action of leptin in the CNS was also associated

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Abbreviations: BAT, Brown adipose tissue; CNS, central nervous system; 2-DG, 2[^{14}C]-deoxyglucose; G6Pase, glucose-6-phosphatase; HGP, hepatic glucose production; icv, intracerebroventricular; Igfbp, Igf binding protein; NEFA, nonesterified free fatty acid; Pepck, phosphoenolpyruvate kinase; PF, pair fed; Ra, glucose rates of appearance; Rd, glucose rates of disposal; Rg, tissue glucose uptake; SNS, sympathetic nervous system; STZ, streptozotocin; uDM, uncontrolled diabetes mellitus; veh, vehicle.

with normalization of elevated plasma glucagon levels, consistent with an earlier report (4), our data suggest that factors, in addition to glucagon, contribute to the glucose-lowering effect of CNS leptin action. These data establish that the brain has an ability to normalize diabetic hyperglycemia, and we emphasize that this effect is distinct from leptin's previously reported action to increase hepatic insulin sensitivity (10–13).

Materials and Methods

Animals

Adult male Wistar rats (Harlan Laboratories, Inc., Indianapolis, IN) were housed individually under specific-pathogen-free conditions, in a temperature-controlled room with a 12-h light, 12-h dark cycle and *ad libitum* access to water and chow (Purina 5053, Purina Mills, St. Louis, MO) unless otherwise stated. All procedures were performed in accordance with National Institutes of Health Guidelines for the Care and Use of Animals and were approved by the Animal Care Committee at the University of Washington.

Effect of CNS leptin infusion on blood glucose levels in streptozotocin (STZ)-induced diabetes

Animals were implanted with a cannula to the lateral ventricle (Alzet; DURECT Corp., Cupertino, CA) under isoflurane anesthesia at stereotaxic coordinates: 1.5 mm lateral, 0.8 mm posterior to bregma, and 3.5 mm below the skull surface (19). At least 1 wk after cannula implantation, animals received either two consecutive daily sc injections of STZ (40 mg/kg · body weight) to induce uDM or veh (vehicle) [NaCit (pH 4.5)]. On the same day, under isoflurane anesthesia, animals were implanted with an osmotic minipump sc that was connected to the lateral ventricle to enable direct infusion of either veh [PBS (pH 7.9)] or leptin (1 μg/d; Dr. Parlow; National Hormone Peptide Program, Torrance, CA) to the brain for up to 11 d. This dose of leptin was selected based on the data generated from a leptin dose-response study. To control for the effects of leptin to reduce food intake, a third group of STZ-veh-treated animals was pair fed (PF) to the intake of STZ-leptin-treated animals as previously described (20). An additional control group was added, in which STZ-veh-treated animals were treated with leptin (1 μg/d) systemically, to determine whether the central effect of leptin was due to leakage into the periphery. Thus, a total of five groups of rats were studied: 1) veh-veh, 2) STZ-veh, 3) STZ-leptin, 4) STZ-veh-PF, and 5) STZ-leptin-sc (n = 7–9 per group). Food intake, body weight, water intake, and blood glucose levels were measured daily midlight cycle for a total of 11 d.

The experimental paradigm described above was repeated in separate groups of animals, with the exception that an indwelling catheter was inserted into the left carotid artery, as previously described (13), to permit blood sampling from conscious, unstressed rats for determination of plasma glucagon, corticosterone, and catecholamine levels.

Measures of glucose tolerance

Intraperitoneal glucose-tolerance tests (30% D-glucose; 2 g/kg) were conducted in 4-h fasted animals on d 9 after STZ administration. Blood glucose levels were measured at t = 0, 30, 60, 90, and 120 min, and a blood sample was obtained at t = 0 and 30 min for determination of plasma insulin concentrations.

Effect of CNS leptin infusion on HGP and glucose uptake in STZ-diabetic rats

Eight days after the administration of STZ, we used tracer dilution techniques to determine whether CNS leptin treatment reduces HGP, increases glucose uptake, or both in STZ-induced diabetic rats. Animals were fasted for 4 h, and a blood sample was obtained at t = 0 min for determination of fasting plasma glucose, insulin, leptin, glucagon, and corticosterone levels in appropriately treated tubes. A 24 μCi prime of [3-^{3}H] glucose was given at t = 0 min for 3 min followed by a continuous 0.2 μCi/min infusion for 90 min. A bolus of 50 μCi 2[^{14}C]-deoxyglucose (2-DG) was given at t = 48 min. Blood samples were taken at 10-min intervals from 60 to 90 min and processed to determine plasma [3-^{3}H]glucose and 2-DG. At t = 90 min, animals were anesthetized with ketamine:xylazine and the liver, red gastrocnemius muscle, brown adipose tissue (BAT), heart, and brain were rapidly excised and stored at −80 C until analyzed. To more directly test whether the ability of intracerebroventricular (icv) leptin to enhance glucose utilization occurs through an insulin-independent mechanism, nondiabetic and STZ-treated animals that received icv leptin were prepared as described above. At t = 0 min, animals were administered a 24 μCi prime of [3-^{3}H]glucose for 3 min followed by a continuous 0.2 μCi/min infusion for 90 min. At t = 90 min, a primed continuous variable infusion of 50% dextrose was administered and periodically adjusted to clamp the blood glucose concentration at approximately 210 mg/dl in veh-veh- and STZ-leptin-treated animals. A bolus of 50 μCi 2[^{14}C]DG was given at t = 178 min. Blood samples were taken at 10-min intervals from 180 to 210 min and processed to determine plasma [3-^{3}H]glucose, 2[^{14}C]DG and insulin levels. At t = 210 min, animals were anesthetized with ketamine:xylazine, tissues rapidly excised and stored at −80 C for later analysis.

Blood and urinary collection and assay

Plasma for [3-^{3}H]glucose determinations were deproteinized with $Ba(OH)_2$ and $ZnSO_4$, then dried overnight at 60 C. An aliquot of urine was collected into chilled tubes from animals that were housed briefly in metabolic cages in the fed state during the midlight cycle, 10 d after STZ administration, and were subsequently stored at −20 C. Plasma glucose and urinary glucose levels were measured using a GM9D glucose direct analyzer (Analox Instruments, London, UK). Daily blood glucose levels were measured using a hand-held glucometer (Accu-Chek, Indianapolis, IN) on blood obtained from tail capillary samples. Blood samples were collected for measurement of insulin, leptin, nonesterified free fatty acids (NEFAs), and ketone bodies from the tail vein in chilled EDTA-treated tubes. Blood for catecholamine and glucagon assays was collected from arterial blood samples collected on EGTA/glutathione tubes and tubes containing 10 μl of 1 M benzamidine (Sigma, St. Louis, MO) and 1 U heparin, respectively, whereas blood for corticosterone was collected in EDTA-treated tubes. Whole blood was centrifuged at 1500 rpm for 20 min, and plasma was removed, aliquoted, and stored at −20 C. Plasma immunoreactive insulin and leptin levels were determined by ELISA (Crystal Chem, Inc., Downers Grove, IL), NEFAs using a colorimetric assay kit (Wako Chemicals,

Richmond, VA) and total ketone bodies using a colorimetric kit (Wako Chemicals). Catecholamine levels were measured using a sensitive and specific radioenzymatic assay (21), glucagon was assayed by a glucagon RIA kit (Linco Research, St. Charles, MO), whereas plasma corticosterone levels were measured using enzyme immunoassay (Diagnostics Systems Laboratories, Webster, TX).

Reverse transcription-polymerase chain reaction

Total RNA was extracted from liver and pancreas using TRIzol B according to manufacturers' instructions (MRC, Cincinnati, OH). RNA was quantitated by spectrophotometry at 260 nm (Nanodrop 1000; Thermo Scientific, Rockford, IL) and reverse-transcribed with avian myeloblastosis virus reverse transcriptase (1 μg; Promega, Madison, WI). Levels of mRNA for glucose-6-phosphatase (*G6Pase*), phosphoenolpyruvate kinase (*Pepck*), Igf binding protein (*Igfbp*)-2 and 18S RNA (internal control) were measured by semiquantitative real-time PCR on an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA). The primer sequences for *18S RNA*, G6Pase, Pepck, and Igfbp-2 were designed using Primer Express (version 2.0.0; Applied Biosystems) and are as follows: 18S RNA forward, CGGACAGGATTGACAGATTG and reverse, CAAATCGCTCCACCAACTAA; *G6Pase* forward, TCAACCTCGTCTTCAAGTGGATT and reverse, CTGCTTTATTATAGGCACGGAGCT; *Pepck* forward, GGCGGAGCATATGCTG-ATCC and reverse, CCACAGGCACTAGGGAAGGC; and *Igfbp-2* forward, TCTACAACGAGCAGCAGG and reverse, ATCCTCCACACCCATCAC. PCR data were analyzed using the Sequence Detection System software (SDS version 2.2; Applied Biosystems). Expression levels of each gene were normalized to a house-keeping gene (*18S RNA*) and expressed as a percentage of veh-veh controls. Nontemplate controls were incorporated into each PCR run.

Body composition analysis

Liver tissue triglyceride content and measures of body lean and fat mass were determined using quantitative magnetic resonance spectroscopy (Echo Medical Systems, Houston, TX). Liver glycogen levels were determined using a colorimetric assay (Biovision, Mountain View, CA). Both liver and glycogen were standardized to grams wet weight.

Glucose rates of appearance (Ra), disposal (Rd), and Rg

After deproteinization and drying, plasma [3-^{3}H]glucose and 2[^{14}C]DG radioactivity was determined by liquid scintillation counting (Packard TRI-CARB 2900TR) (22) with Ultima Gold (Packard) as scintillant (22). Ra, Rd, and endogenous Ra were determined as described previously (13). For 2[^{14}C]DG measures, tissue samples were homogenized in 0.5% perchloric acid and neutralized with KOH. One sample was measured directly to count 2[^{14}C]DGP and 2[^{14}C]DG, whereas a second sample was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ to measure only 2[^{14}C]DG. Tissue glucose clearance was calculated by dividing the 2[^{14}C]DGP dpm for each tissue by the plasma area under the curve 2[^{14}C]DG as determined by the trapezoid method. Multiplying tissue glucose clearance by the average plasma glucose concentration gives the rate of Rg (23). In all experiments, the accumulation of 2[^{14}C]DGP was normalized to tissue weight.

Statistical analysis

All results are expressed as mean ± SEM. Statistical analyses were performed using Statistica (version 7.1; StatSoft, Inc., Tulsa, OK). A one-way ANOVA with a least significant difference *post hoc* test was used to compare mean values between multiple groups and a two-sample unpaired Student's *t* test was used for two-group comparisons. In all instances, probability values of less than 0.05 were considered significant.

Results

CNS leptin infusion normalizes blood glucose levels in STZ-induced diabetes

Consistent with previous data (24, 25), plasma levels of both insulin and leptin (Fig. 1, A and B) were significantly reduced within 48 h in all animals that received STZ and remained very low relative to nondiabetic controls, confirming previous reports that uDM is a model of leptin and insulin deficiency (24). As expected (5, 6), food intake was significantly increased by d 5 after STZ administration in diabetic animals receiving icv veh, and this effect was completely blocked by icv leptin (Fig. 1C). To control for differences in food intake, we included a group of STZ-diabetic rats that were PF (STZ-veh-PF) to the mean daily intake of the STZ-leptin group.

Although blood glucose levels were markedly increased in diabetic animals receiving icv veh throughout the study, blood glucose levels normalized to nondiabetic levels by d 7 in STZ-diabetic rats receiving icv leptin (Fig. 1D). This effect was due neither to a leptin-mediated reversal of diabetic hyperphagia, because hyperglycemia was not attenuated in STZ-veh-PF animals, nor to a peripheral effect of leptin, because sc infusion of leptin at the same dose did not affect blood glucose in STZ-diabetic rats (Fig. 1D). After the induction of diabetes in STZ-treated rats receiving icv veh, body weight continuously decreased despite pronounced hyperphagia, because the excess calories cannot be stored as fat and are ultimately lost through the urine. Interestingly, leptin-treated STZ-diabetic rats exhibited similar weight loss to STZ-diabetic rats receiving icv veh but exhibited a greater loss of fat mass (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). These data suggest that icv leptin treatment reduces food intake and stimulates energy expenditure, but these animals do not lose more weight compared with STZ-diabetic animals receiving veh, presumably because they lose less calories through the urine.

Potential mechanism(s) mediating the glucose-lowering action of CNS leptin

One potential mechanism that could contribute to the glucose-lowering effect of leptin is an increase in urinary glucose loss. However, urinary glucose was undetectable

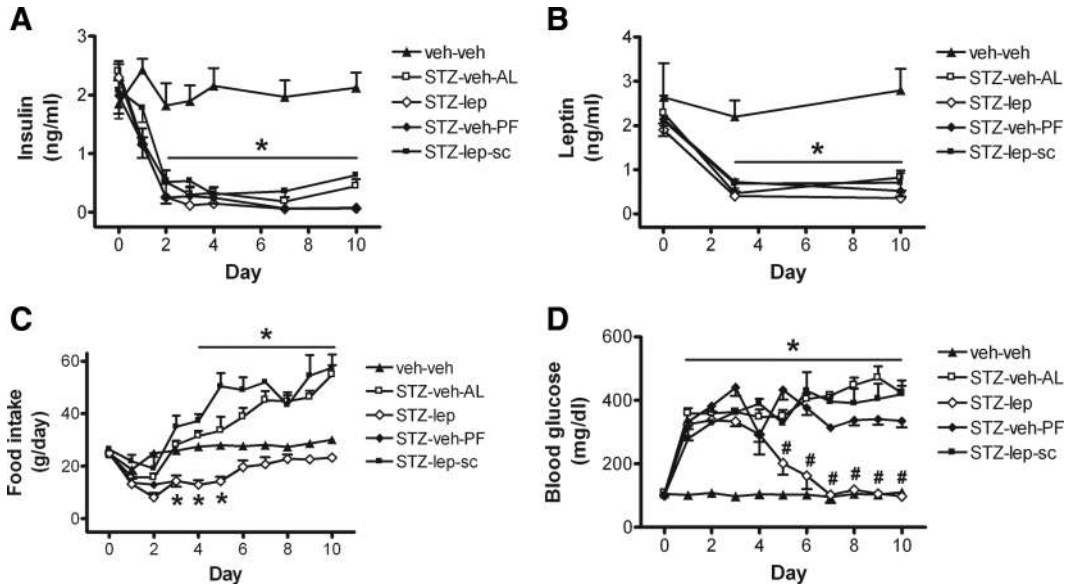

FIG. 1. CNS leptin normalizes blood glucose levels in uDM. Plasma insulin (A), plasma leptin (B), mean daily food intake (C), and blood glucose levels (D) in nondiabetic controls (veh-veh) or in STZ-induced diabetic animals receiving either icv veh and fed *ad libitum* (STZ-veh-AL) or PF (STZ-veh-PF), or leptin (1 μg/d) delivered either sc (STZ-leptin-sc) or directly into the brain (STZ-leptin) (n = 7–9 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh; #, $P < 0.05$ *vs.* STZ-veh-PF. lep, Leptin.

in diabetic animals that received icv leptin similar to nondiabetic controls, whereas STZ-veh-treated animals displayed the expected, marked glycosuria (Fig. 2A). Similarly, water intake was markedly increased in both groups of STZ-veh-treated animals, owing to the dehydrating effect of pronounced glycosuria in uDM (26), whereas water intake in diabetic animals receiving icv leptin was equivalent to that of nondiabetic controls (Fig. 2B). Thus, the effect of leptin to normalize blood glucose levels in uDM is not mediated by increased urinary glucose loss and in fact occurs despite the complete reversal of glycosuria.

Another potential mechanism by which leptin action in the CNS normalizes diabetic hyperglycemia is by restoring pancreatic β-cells. However, arguing against this hypothesis, basal plasma insulin levels remained very low in the fed state in STZ-treated animals, irrespective of icv treatment status, and pancreatic preproinsulin mRNA levels were severely reduced (by >95%) in all animals that received STZ (data not shown). Last, 30 min after a glucose challenge (2 g/kg ip), plasma insulin levels increased as expected in nondiabetic animals but remained extremely low in both veh and leptin-treated STZ-diabetic rats (Fig. 2C). Thus, the severe insulin deficiency (under both basal and glucose-stimulated conditions) caused by STZ-induced β-cell destruction was not ameliorated by icv leptin. However, despite the absence of any increase in circulating insulin, STZ-leptin-treated animals exhibited near normal glucose tolerance compared with nondiabetic controls, implying the existence of an insulin-independent mechanism for rapid glucose clearance that is activated by CNS leptin action (Fig. 2D).

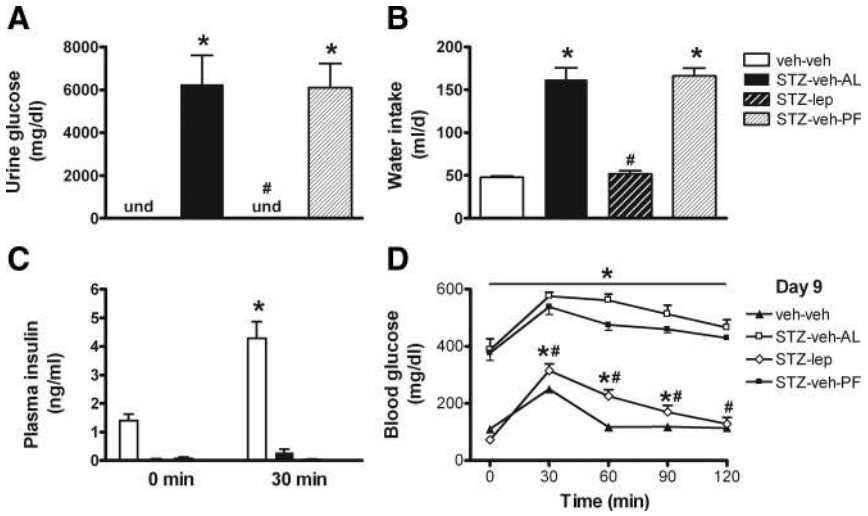

FIG. 2. Glucose-lowering effect of CNS leptin is not explained by glycosuria or recovery of β-cells. Urinary glucose (A) and daily water intake (B) and plasma insulin (C) and blood glucose profiles (D) during an ip glucose tolerance test (2 g/kg · body weight), in nondiabetic controls or in STZ-induced diabetic animals receiving either icv veh and fed *ad libitum* or PF, or icv leptin (n = 7–9 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh; #, $P < 0.05$ *vs.* STZ-veh-PF. lep, Leptin.

Effect of icv leptin infusion on HGP and glucose uptake in STZ-induced diabetes

We next used tracer dilution techniques to determine whether icv leptin normalized Ra or Rd (or both) in rats

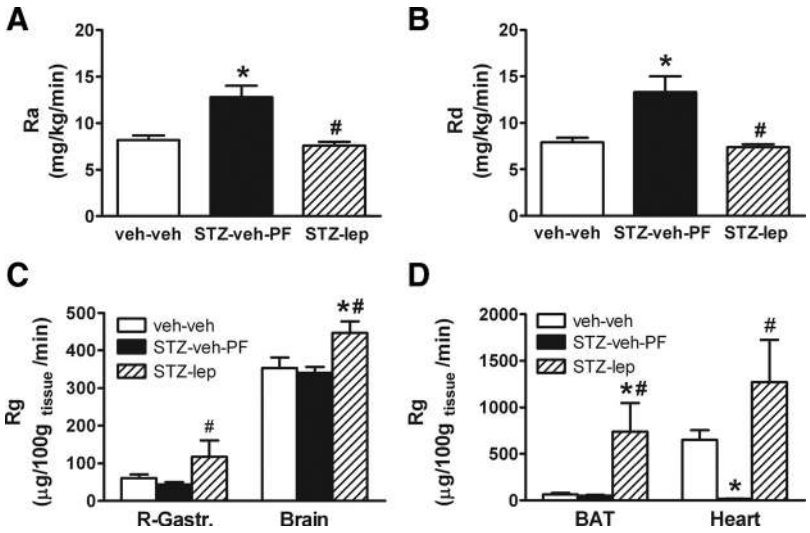

FIG. 3. CNS leptin suppresses HGP and increases Rg in uDM. Ra (A) and Rd (B) determined from [3-^{3}H]glucose tracer studies and Rg determined from 2[^{14}C]DG studies in red gastrocnemius muscle and brain (C), BAT and heart (D) in nondiabetic controls or in STZ-induced diabetic animals receiving icv veh and PF or icv leptin (n = 5–6 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh; #, $P < 0.05$ *vs.* STZ-veh-PF. lep, Leptin.

with uDM. Consistent with the key role played by increased HGP in the hyperglycemia of uDM (27), basal Ra measured by tracer dilution were markedly elevated in STZ-veh-PF animals compared with nondiabetic controls (Fig. 3A). Central leptin administration dramatically reduced Ra to nondiabetic control values (Fig. 3A), strongly suggesting that normalization of diabetic hyperglycemia resulted, at least in part, from reduced HGP. Because this suppression of HGP occurs despite near undetectable plasma insulin levels, it differs from the previously documented effect of CNS leptin to increase hepatic insulin sensitivity (10–13) and implies the existence of an insulin-independent mechanism.

Because animals with uDM excrete large amounts of glucose in the urine, tracer-based estimates of the Rd are artificially increased in STZ-diabetic animals receiving veh, because Rd values encompass both whole-body glucose utilization and urinary glucose loss (Fig. 3B). In contrast, Rd values in STZ-diabetic animals that receive icv leptin are not confounded by this problem, because the animals are neither hyperglycemic nor glycosuric. We found that basal Rd values were similar between STZ-diabetic animals receiving leptin and nondiabetic controls but were unable to compare these values with veh-treated STZ-diabetic rats due to the confounding effects of glycosuria on Rd. We therefore measured Rg using labeled 2-DG and found that glucose uptake was significantly increased in red gastrocnemius muscle and brain in STZ-diabetic animals that received icv leptin relative to veh (Fig. 3C). In BAT, glucose uptake was significantly increased in STZ-diabetic rats receiving leptin compared with both those receiving veh and nondiabetic controls, whereas in heart, glucose uptake was significantly reduced in STZ-diabetic rats receiving veh compared with nondiabetic controls, and this effect was reversed with leptin treatment (Fig. 3D).

To investigate the role of the liver in the antidiabetic effects of leptin, we examined hepatic gene expression using real-time PCR as well as measures of liver triglyceride and glycogen content. As expected, hepatic expression of the key gluconeogenic genes *G6Pase* and *Pepck* were markedly increased in the liver of STZ-veh-treated animals relative to nondiabetic controls (Fig. 4, A and B). Because these enzymes are rate-limiting for gluconeogenesis, their increased expression likely contributes to elevated rates of HGP in this setting. By comparison, hepatic expression of these genes was significantly reduced in STZ-diabetic rats receiving icv leptin, suggesting that leptin action in the brain reduces HGP in rats with uDM by inhibiting gluconeogenic gene expression, independent of insulin signaling (Fig. 4, A and B).

Igfbp-2 was recently identified as a leptin-regulated hepatic gene implicated in the action of leptin to suppress HGP (28). Surprisingly, we found that hepatic expression of *Igfbp2* mRNA was increased by mote than 25-fold in leptin-deficient STZ-diabetic rats compared with nondiabetic controls. Even though hepatic *Igfbp2* mRNA levels were further increased by leptin, this effect seems unlikely to explain the effect of leptin to normalize HGP ($P < 0.05$) (Fig. 4C). Although hepatic triglyceride content was also reduced in STZ-leptin-treated animals compared with other groups, this effect was relatively small (Fig. 4D), whereas liver glycogen levels were dramatically reduced in STZ-treated animals receiving icv leptin compared with the other groups (Fig. 4E), a finding that may underlie the effect of icv leptin to reduce HGP.

To directly test the hypothesis that icv leptin suppresses HGP and stimulates glucose utilization in animals with uDM through an insulin-independent mechanism, we subjected both leptin-treated STZ-diabetic animals and veh-treated nondiabetic controls to a hyperglycemic clamp (Fig. 5). In nondiabetic animals, plasma insulin and C-peptide levels were significantly increased during the hyperglycemic clamp ($P < 0.05$) (Fig. 5, A and B), resulting in complete suppression of HGP and enhancement of glucose utilization by more than 250% (Fig. 5, E–H). Similarly, despite persistent, severe insulin deficiency, HGP was suppressed and glucose utilization was increased in

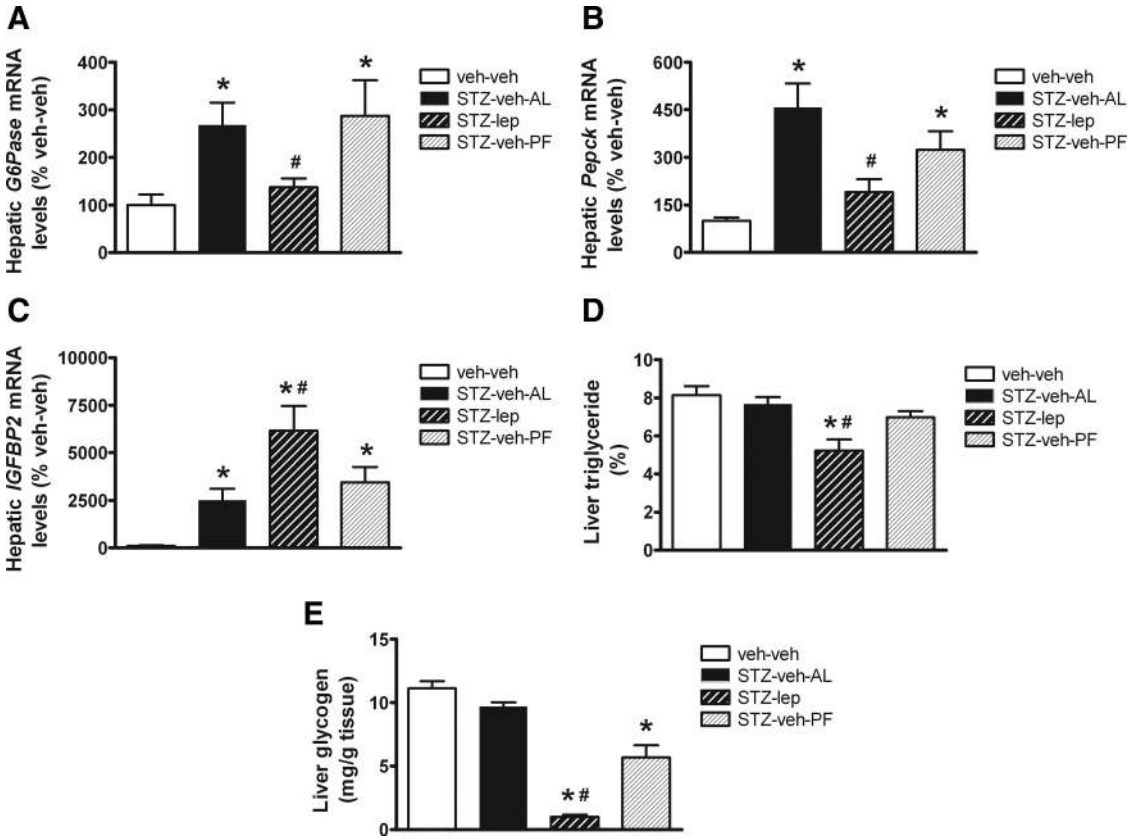

FIG. 4. CNS leptin infusion normalizes hepatic gluconeogenic gene expression in uDM. Hepatic expression of *G6Pase* (A) and *Pepck* (B) using real-time PCR, *Igfbp-2* (C), liver triglyceride (D), and liver glycogen content (E) in nondiabetic controls or STZ-induced diabetic animals receiving either icv veh and fed *ad libitum* or PF or icv leptin (n = 7–9 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh; #, $P < 0.05$ *vs.* STZ-veh-PF. lep, Leptin.

STZ-diabetic rats receiving icv leptin, although less than that observed in nondiabetic animals (Fig. 5, E–H). Furthermore, the effect of hyperglycemia to increase Rg in skeletal muscle and heart was similar between STZ-leptin and nondiabetic controls, and icv leptin treatment further increased glucose uptake into BAT (Fig. 5, I and J).

CNS leptin infusion normalizes plasma glucagon and corticosterone levels in STZ-induced diabetes

Elevated plasma glucagon and corticosterone levels are implicated in the pathogenesis of hyperglycemia in uDM (29–31). As was observed in response to pharmacological hyperleptinemia in an earlier report (4), the elevation in plasma glucagon levels observed in STZ-treated animals was prevented by central leptin administration (Fig. 6A). Plasma corticosterone levels were similarly elevated in animals with STZ diabetes, a response also prevented by icv leptin but not PF (Fig. 6B). These findings raise the possibility that in uDM, reduced leptin action in the brain stimulates the secretion of counterregulatory hormones and that the antidiabetic effect of icv leptin is mediated in part via normalization of these hormone levels. However, we recently reported that in rats with STZ diabetes, physiological leptin replacement is sufficient to normalize circulating glucagon and corticosterone levels, yet does not reverse hyperglycemia (20). Although changes in plasma norepinephrine and epinephrine levels are unlikely to explain leptin's effects (Fig. 6, A and D), plasma levels of NEFAs and ketone bodies were also elevated in STZ-veh animals and normalized with icv leptin administration but only after blood glucose levels had returned to normal (Fig. 6, E and F).

Discussion

Although effective management of uDM generally requires insulin therapy, recent studies (3–9) have shown that hyperglycemia in rat and mouse models can be ameliorated by induction of pharmacological hyperleptinemia. In the current work, we sought to determine how CNS leptin action can mediate this effect and whether the underlying mechanism is insulin-independent. We found that continuous infusion of leptin directly into the brain normalizes blood glucose levels in STZ-induced diabetic rats via a mechanism that cannot be explained by reduced food intake, increased urinary glucose losses, or recovery of pancreatic β-cells and does not involve a peripheral

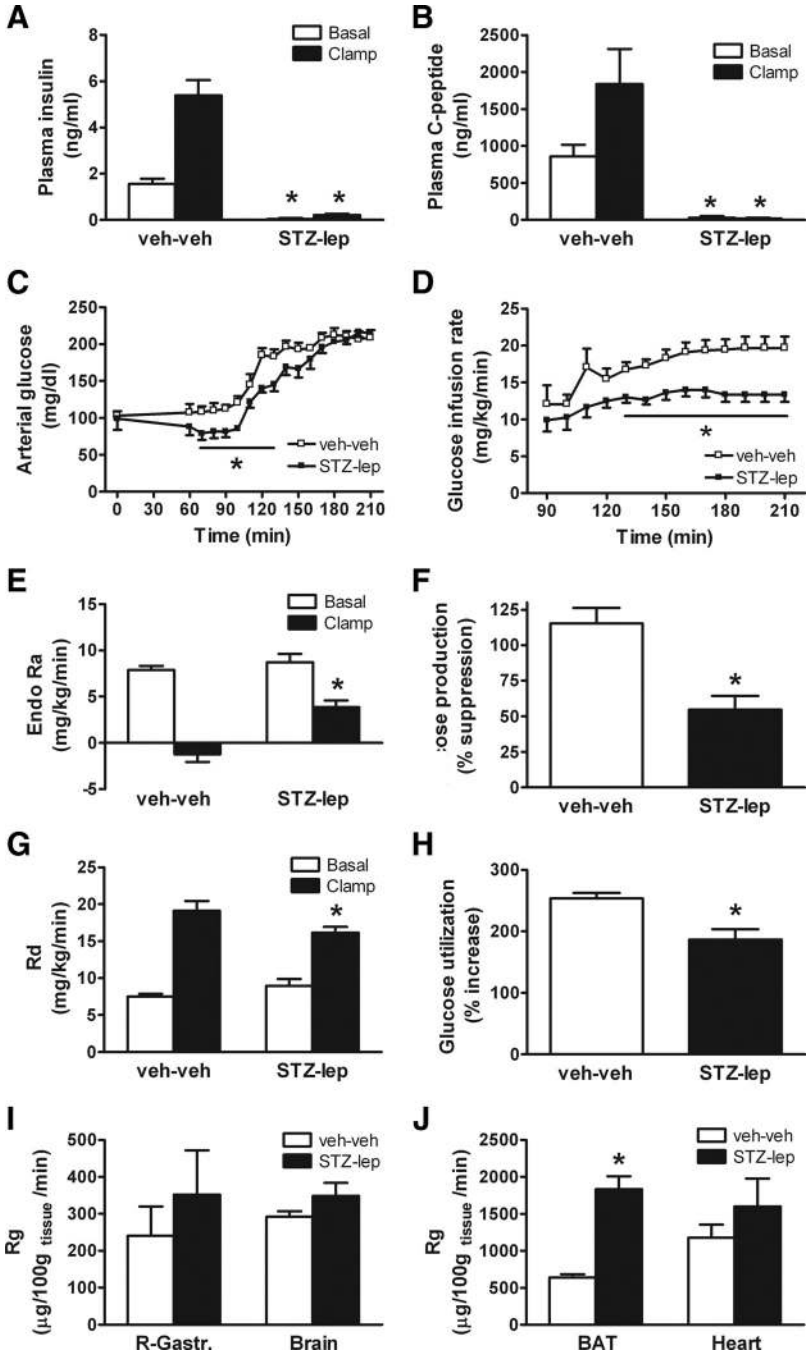

FIG. 5. CNS leptin suppresses HGP and increases glucose utilization in uDM during a hyperglycemic clamp. Basal and glucose-stimulated plasma insulin (A) and C-peptide levels (B) and arterial glucose levels (C) and glucose infusion rate (D) required to maintain blood glucose levels at approximately 210 mg/dl during the hyperglycemic clamp, endogenous (Endo) Ra (E), percentage suppression of HGP (F), Rd (G), percentage increase in glucose utilization (H), and Rg in red gastrocnemius muscle and brain (I) BAT and heart (J) in STZ-induced diabetic animals receiving icv leptin or in nondiabetic controls receiving icv veh (n = 5 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh. lep, Leptin.

action of leptin. Rather, we demonstrate that leptin acts in the CNS to potently inhibit glucose production and simultaneously increase tissue glucose utilization in diabetic animals. Because each of these effects occurs despite persistent, severe insulin deficiency, even during a hyperglycemic clamp, our findings constitute direct support for the existence of a novel brain mechanism for insulin-independent normalization of blood glucose in animals with uDM. This leptin effect is distinct from its previously reported action to increase hepatic insulin sensitivity (10–13) and suggests that through its central action, leptin has important potential as an effective, insulin-independent adjunct to the treatment of type 1 diabetes in humans.

Because the effect of leptin to ameliorate diabetic hyperglycemia could not be explained by reduced food intake, increased urinary glucose excretion, or a recovery of pancreatic β-cells, we investigated whether it involves suppression of HGP, increased glucose utilization, or both. Insulin-mediated inhibition of HGP is essential for normal glucose homeostasis as evidenced by the contribution of unrestrained HGP (through increases of both glycogenolysis and gluconeogenesis) to severe hyperglycemia in uDM (32). Although we and others have previously observed the ability of CNS leptin to increase insulin-induced suppression of HGP during a euglycemic-insulin clamp (10–13), our findings demonstrate that this can occur in insulin-deficient rats, constituting direct evidence of a brain mechanism for reducing HGP independent of insulin (33).

Several lines of evidence suggest that like leptin, insulin action in the brain of nondiabetic animals lowers ambient glycemia by increasing hepatic insulin sensitivity (14, 15). Moreover, hypothalamic effects of both insulin and leptin are dependent on neuronal phosphatidylinositol-3-OH kinase signaling, suggesting a shared neuronal mechanism mediating effects of both hormones on glucose metabolism (34, 35). However, unlike leptin, icv infusion of insulin at a dose that attenuates diabetic hyperphagia fails to normalize blood glucose levels in STZ-diabetic rats (36), suggesting that the neurocircuitry that mediates leptin's glucose-lowering effects is distinct from those CNS pathways activated by insulin. This concept is supported by recent evidence that although both hormones exert phosphatidylinositol-3-OH kinase-dependent effects on hypothalamic POMC cells, these effects are in fact mediated on distinct proopiomelanocortin neuronal subsets (37, 38).

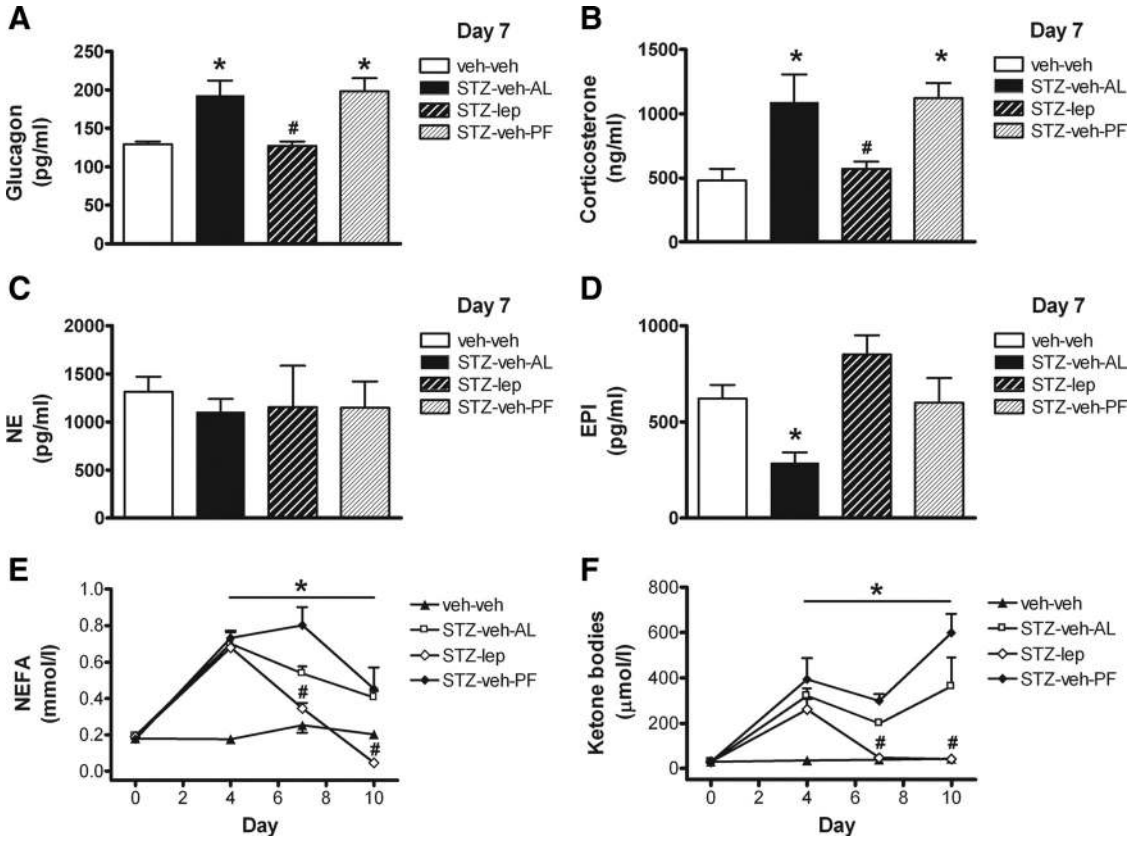

FIG. 6. CNS leptin normalizes plasma glucagon and corticosterone levels in uDM. Plasma glucagon (A), plasma corticosterone (B), norepinephrine (NE) (C), epinephrine (EPI) (D), NEFAs (E), and total ketone bodies (acetoacetate and β-hydroxybutyrate) (F) in STZ-induced diabetic animals receiving either icv veh and fed *ad libitum* or PF, icv leptin or icv veh in nondiabetic controls (n = 7–9 per group). Data represent mean ± SEM; *, $P < 0.05$ *vs.* veh-veh; #, $P < 0.05$ *vs.* STZ-veh-PF. lep, Leptin.

One mechanism that could contribute to suppression of HGP in diabetic rats receiving icv leptin is inhibition of excess glucagon secretion. Hyperglucagonemia contributes to diabetic hyperglycemia (29, 30) in part by driving hepatic expression of the key gluconeogenic enzymes *Pepck* and *G6Pase*. A recent study demonstrated that adenovirally induced hyperleptinemia ameliorates hyperglycemia in STZ diabetes, and the authors hypothesized that suppression of hyperglucagonemia plays a causal role in leptin-mediated inhibition of hepatic gluconeogenesis in uDM (4). Our finding that icv leptin reversed the effect of STZ diabetes to increase both glucagon secretion and the expression of these gluconeogenic genes (6) is therefore consistent with the hypothesis that reversal of hyperglucagonemia contributed to the resolution of hyperglycemia. Furthermore, our finding that the effect of STZ diabetes to raise plasma glucagon levels is prevented by icv leptin, combined with the marked reduction of circulating leptin levels in this setting, supports the hypothesis that increased glucagon secretion in uDM arises as a consequence of deficient leptin signaling in the brain. This hypothesis is compatible with evidence that neurons situated in the ventromedial hypothalamic nucleus are important in the control of glucagon secretion (39–41) and are leptin-sensitive, expressing high concentrations of leptin receptors relative to most other brain areas (42, 43). However, whether reduced glucagon levels contribute to, or are a result of, leptin-mediated resolution of hyperglycemia is unknown. We recently observed that in rats with STZ diabetes, physiological leptin replacement is sufficient to normalize plasma glucagon levels but has little effect on diabetic hyperglycemia (20). Combined with our finding that icv leptin also increases glucose uptake in rats with uDM, an effect not readily explained by changes of plasma glucagon (which does not affect glucose utilization), we conclude that mechanisms in addition to normalization of glucagon levels likely contribute to leptin's antidiabetic effects.

Similar to glucagon, circulating levels of corticosterone were elevated in STZ-diabetic rats (44), and this effect was prevented by icv leptin treatment. Increased glucocorticoid secretion is thought to contribute to hyperglycemia in uDM, because adrenalectomy attenuates the rise in blood glucose in STZ-diabetic rats, and this is partially reversed with corticosteroid replacement (45). Glucocorticoids can exacerbate hyperglycemia by increasing hepatic gluconeogenesis (by inducing expression of *G6Pase* and *Pepck*) (46) and glycogen synthesis (47) and inhibiting peripheral

glucose uptake in muscle and adipose tissue (48). Leptin-mediated normalization of glucocorticoid levels may therefore have contributed to reversal of diabetic hyperglycemia. Although the mechanisms underlying leptin regulation of the hypothalamic-pituitary-adrenal axis remain incompletely understood, conditions associated with leptin deficiency, such as fasting and uDM, clearly raise corticosterone levels via a mechanism that can be reversed with leptin treatment (44, 45, 49). In uDM, therefore, reduced leptin signaling in the brain may constitute a stimulus to the hypothalamic-pituitary-adrenal axis, raising plasma corticosterone levels. This effect, in concert with insulin deficiency and glucagon excess, may help to drive hyperglycemia.

A recent study suggested that peripheral leptin administration up-regulates hepatic expression of *Igfbp2* and that overexpression of Igfbp-2 ameliorates hyperglycemia in insulin-resistant and in STZ-induced diabetic mice (28). However, we observed that in diabetic animals, hepatic expression of *Igfbp2* was elevated more than 25-fold over nondiabetic controls, despite markedly increased HGP and reduced plasma leptin levels. Given the already elevated levels of *Igfbp2* in uDM, a further increase in hepatic *Igfbp2* by leptin seems unlikely to inhibit HPG. This assessment is consistent with previous reports that hepatic Igfbp-2 expression is tonically inhibited by insulin and hence dramatically up-regulated by insulin deficiency (50–52).

Using tracer dilution techniques, we found that Rd was similar between STZ-diabetic rats receiving icv leptin and nondiabetic controls. Although it could be argued that the action of CNS leptin to normalize Ra could in and of itself normalize Rd (by reducing hyperglycemia), this possibility is inconsistent with our finding of increased glucose uptake in muscle, brain, BAT, and heart in STZ-diabetic animals receiving icv leptin compared with icv veh. Moreover, during the hyperglycemic clamp, whole-body Rd were substantially increased in STZ-diabetic animals receiving icv leptin despite the absence of any glucose-stimulated increase of circulating insulin, and this effect was accompanied by a further increase in glucose uptake in BAT.

Based on these data, we infer that icv leptin normalizes blood glucose levels in STZ-diabetic rats at least in part via an insulin-independent mechanism that increases glucose utilization in tissues such as BAT and skeletal muscle. We also suspect that autonomic responses play a role, because leptin activation of BAT in nondiabetic animals clearly involves increased sympathetic nervous system (SNS) outflow that in turn induces uncoupling protein-1 (53, 54). We therefore hypothesize that a similar mechanism contributes to the effect of leptin to increase glucose uptake in BAT in uDM. Consistent with this hypothesis, leptin administration directly into the brain of nondiabetic rats stimulates glucose uptake in BAT, as well as skeletal muscle and heart, by increasing SNS outflow (55, 56). However, neither chemical denervation of the SNS (8) nor combined α- and b-adrenergic receptor blockade attenuated the effect of leptin to restore euglycemia in rats with uDM, nor was activation of the β3-adrenergic receptors required or sufficient to mediate the effects of leptin on blood glucose levels (7), although Rg was not measured in these studies.

In summary, these findings suggest that in response to leptin stimulation, the brain has the inherent ability to normalize blood glucose levels in uDM via a novel, insulin-independent mechanism characterized by reduced rates of glucose production and increased rates of glucose uptake. These observations are distinct from any previously described CNS leptin action and have important implications for understanding the role of the brain in the pathogenesis of diabetic hyperglycemia and its potential as a target in the treatment of this condition.

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