Intracerebroventricular Leptin Regulates Hepatic but Not Peripheral Glucose Fluxes

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LiSen Liu, George B. Karkanias, Jose’ C. Morales, Meredith Hawkins, Nir Barzilai, Jiali Wang, and Luciano Rossetti‡

From the Diabetes Research and Training Center and Division of Endocrinology, Albert Einstein College of Medicine, Bronx, New York 10461

Acute intravenous infusions of leptin markedly altered hepatic glucose fluxes (Rossetti, L., Massillon, D., Barzilai, N., Yuguin, P., Chen, W., Hawkins, M., Wu, J., and Wang, J. (1997) J. Biol. Chem. 272, 27758–27763). Here we examine whether intracerebroventricular (ICV) leptin administration regulates peripheral and hepatic insulin action. Recombinant mouse leptin (n = 14; 0.02 or 1 μg/kg/h) or vehicle (n = 9) were administered ICV for 6 h to conscious rats, and insulin action was determined by insulin (3 milliunits/kg-min) clamp and tracer dilution techniques. During physiologic hyperinsulinemia (−65 microunits/ml), the rates of glucose uptake (Rd) were lower in leptin- versus vehicle-infused rats. ICV leptin resulted in a 2–3-fold increase in hepatic phosphoenolpyruvate carboxykinase mRNA levels. Glycolysis and PEP-glucogenoses were similar in rats receiving low- and high-dose leptin versus vehicle. ICV leptin resulted in a 2–3-fold increase in hepatic phosphoenolpyruvate carboxykinase mRNA levels. Glycolysis and PEP-glucogenoses contributed similarly to endogenous glucose production (GP) in the vehicle-infused group. However, glucogenoses accounted for ~80% of GP in both groups receiving ICV leptin, while hepatic glycolysis was markedly suppressed (0.7 ± 0.3 and 1.2 ± 0.3 versus 2.2 ± 0.4 mg/kg/min, in rats receiving low- and high-dose leptin versus vehicle, respectively; p < 0.01). In summary, short-term ICV leptin administration: 1) failed to affect peripheral insulin action, but 2) induced a striking re-distribution of intrahepatic glucose fluxes. The latter effect largely reproduced that of leptin given systemically at much higher doses. Thus, the regulation of hepatic glucose fluxes by leptin is largely mediated via its central receptors.

Leptin, the protein encoded by the ob gene, is an anorectic hormone secreted by adipose cells (1–6). The effects of leptin on food intake are reproduced by its injection directly in the central nervous system, thus suggesting a prominent role of the hypothalamic receptors in mediating its actions (7–12). In the long term, circulating leptin levels correlate with adiposity (3, 8, 10, 13, 14) and leptin has been suggested to function as a “biochemical messenger” between fat depots and the hypothalamus (7, 10, 11, 14, 15).

Nutritional and hormonal factors can also regulate ob gene expression in adipose cells and leptin levels in plasma (3, 13, 14, 16, 17). Most important, it has become increasingly evident that leptin plays in turn an important role in the regulation of carbohydrate and lipid metabolism (9, 14, 18–24). We have previously demonstrated that marked acute elevations in the plasma leptin concentrations modulate the hepatic gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and the rate of glucogenoses (22). It is presently unknown whether the latter metabolic effects of leptin are, at least in part, mediated through its action on hypothalamic receptors.

Therefore the primary aim of this study was to examine the metabolic impact of intracerebroventricular (ICV) infusions of leptin on peripheral and hepatic glucose metabolism under basal conditions and in response to physiologic hyperinsulinemia. Furthermore, as recent studies have suggested that a highly selective β3-adrenergic receptor agonist (CL 316,243) exerts potent “leptin-like” effects in the hypothalamic regulation of food intake (5), its metabolic effects were also examined.

EXPERIMENTAL PROCEDURES

Experimental Animals—Thirty-four male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were studied. Rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)–dark (6 p.m. to 6 a.m.) cycle. One week before the in vivo study, a group of rats (n = 23; at ~10 weeks of age) were equipped with chronic catheters placed in the third cerebral ventricle, the left carotid artery, and the right internal jugular vein. Rats were anesthetized with intraperitoneal ketamine (Ketaset, 87 mg/kg) and xylazine (Rompun, 11 mg/kg) and fixed in a stereotaxic apparatus with ear bars and a nose piece set at +5.0 mm. A 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was chronically implanted into the third ventricle using the following coordinates from bregma: anterior–posterior, +0.2 mm; dorsal–ventral, −9.0 mm; medial–lateral, 0.0 mm. This paper is available on line at http://www.jbc.org

1 The abbreviations used are: PEPCK, phosphoenolpyruvate carboxykinase; ICV, intracerebroventricular; FFA, free fatty acid; RTPCR, reverse transcriptase-polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GIB, glucose infusion rate; GP, glucose production.
Frozen brains were mounted and placed in a cryostat, and sectioned in the coronal plane. Every fifth 40-μm frontal section was examined and the neuroanatomical location of the cannulae tips was verified.

ICV Studies—Rats were divided in three experimental groups. One consisted of 9 rats receiving an ICV infusion of vehicle for 6 h (leptin 0) and the other 2 groups contained ICV infusions of recombinant mouse leptin (gift of Dr. M. McCaleb, Amgen, Inc., Thousand Oaks, CA; >95% pure by SDS-polyacrylamide gel electrophoresis) at the rate of 20 ng/kg/h and 1 μg/kg/h for 6 h (leptin 30 ng, and leptin 1.5 μg). All rats received the euclidean clamp protocol described below during the last 2 h of the 6-h vehicle or leptin infusions. Each group of rats was divided into three experimental groups. One consisted of 5 rats receiving an intra-arterial infusion of vehicle for 6 h (CON). A second group of 3 rats received a systemic infusion of recombinant mouse leptin at the rate of 30 μg/kg/h for 6 h (LEP). A third group of 3 rats received a systemic infusion of the selective β3-adrenoceptor agonist CL 316,243 (gift of Dr. K. Steyer, Wyeth-Ayerst Research, Princeton, NJ) at a dose of 125 μg/kg/h for 6 h (β3). All rats received the euclidean clamp protocol described below during the last 2 h of the 6-h vehicle, leptin, or CL 316,243 infusions.

Euglycemic Hyperinsulinemic Clamp Studies—Studies were performed in unrestrained rats using the insulin clamp technique (25–27), in combination with high performance liquid chromatography-purified [3-3H]glucose and [U-14C]lactate infusions, as described previously (27, 28). The protocol followed during the insulin clamp study was similar to that previously described (22, 25–27). Briefly, a primed-continuous infusion of regular insulin (3 milliunits/kg/min) was administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentration at 7–8 mm. In order to control for possible effects of leptin on the endocrine pancreas (20), somatostatin (1.5 μg/kg/min) was also infused to inhibit endogenous insulin secretion in both groups. Plasma samples for determination of [3H]glucose and [3H]water specific activities were obtained at 10-min intervals during the basal and clamp periods. Steady state conditions for the plasma glucose consumption pathway (gluconeogenesis) were achieved within 90 min in both the basal and clamp periods of the studies. Plasma samples for determination of plasma insulin and FFA concentrations were obtained at 30-min intervals during the study. The total volume of blood withdrawn was ~3–3.5 ml/study; to prevent volume depletion and anemia, a solution (1:1 v/v) of 4.0 ml of fresh blood (obtained by heart puncture from littersmates of the test animals) and heparin (100 I.U./ml) was infused at a rate of 20 μl/min. All determinations were also performed on portal vein blood obtained at the end of the experiment.

At the end of the in vivo studies, rats were anesthetized (pentobarbital 60 mg/kg body weight, intravenously), the abdomen was quickly opened, portal vein blood was obtained, and liver was freeze-clamped in situ with aluminum tongs pre-cooled in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the tissues was less than 45 s. All tissue samples were stored at ~80 °C for subsequent analysis. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Reverse Transcription and Amplification of Complementary DNA—Hepatic glucose-6-phosphatase (G6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA abundance were assessed by semiquantitative RT-PCR. Total RNA isolation was performed using TRIzol reagent. Briefly, 40 mg of liver tissues were homogenized in 1 ml of TRIzol reagents. Chloroform was added at 1.5:1 of the initial volume of TRIzol. Sample was incubated at room temperature for 15 min and then centrifugated at 12,000 × g for 15 min at 4 °C. After centrifugation RNA was removed as the aqueous phase and transferred to a new tube, mixed with 0.5 ml of cold isopropyl alcohol, placed at 4 °C for 20 min, and then centrifuged at 12,000 × g for 10 min at 4 °C. The RNA pellet was washed with 75% ethanol, dried by air, and dissolved in 0.1% diethyl pyrocarbonate water. The purify and amounts of the RNA obtained were checked by measuring optical density at 260 and 280 nm. cDNA synthesis: total RNAs were reverse transcribed using a commercial kit (Life Technologies, Inc.). Two micrograms of total RNA was incubated at 65 °C for 5 min with 2 μl of oligo(dT)12-18 (0.5 μg/μl) and water to a final volume of 10 μl and subsequently kept on ice. Eight microliters of 5 × synthesis buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl2, 4.0 μl of 10 mM dNTP mixture, 4.0 μl of 0.5 mM dGTP, 1.5 mM dATP, and 1.5 mM dCTP) and 1 μl of 30 μM each primer (the sequences for PEPCK, sense: 5′-TTGTCTTGGACTTCCTGC-3′, and antisense: 5′-ACCGCCTTGGCCTGATCTG-3′; for G6-Pase, sense: 5′-AGTGGACGCCAAAGTTAGATCC-3′, and antisense: 5′-TGCTTGGGTCTGATGCTGCTG-3′), 1.5 unit of TaqDNA polymerase and 1 μl of RT product. Amplification reaction: initial denaturation at 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min 30 s, and then a final incubation at 72 °C for 10 min. In addition, the GAPDH gene was used as an external control for identification of the amount of mRNA. Quantitation of RT-PCR products: following amplification, a 10-μl aliquot of PCR product was electrophoresed through a 2.0% agarose gel containing 0.5 μg/ml ethidium bromide. DNA was visualized on a UV transilluminator. After photographing, the picture was scanned with Scan Jet 4C and quantitated with Bioimaging analyzer.

Analytical Procedures—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Inc., Palo Alto, CA). Plasma insulin, glucagon, and leptin (rat Leptin RIA kit, Linco Research Inc., St. Charles, MO) concentrations were measured by radioimmunoassay. The plasma concentration of free fatty acids was determined by an enzymatic method with an automated kit according to the manufacturer’s specifications (Waco Pure Chemical Industries, Osaka, Japan). Plasma [3H]glucose radioactivity was measured in duplicates in the supernatants of BarOHa and ZnSO4 precipitates (Somogyi procedure) of plasma samples (20 μl) after evaporation to dryness to eliminate tritiated water. The rates of glycolysis were estimated as described previously (26). Briefly, plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritated water or [3-3H]glucose. Urdine diphosphoglucose (UDP-Glc) and phosphoenolpyruvate (PEP) concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (27–30).

Calculations—Under steady-state conditions for plasma glucose concentrations, the rate of glucose disappearance (Rg) equals the rate of glucose appearance (Rg). The latter was calculated as the ratio of the rate of infusion of [3-3H]glucose (dpm/min) and the steady-state plasma glucose concentration. When exogenous glucose was given, the rate of endogenous glucose production was calculated as the difference between Rg and the infusion rate of glucose. The rates of glycolysis were estimated as described previously (26). Briefly, plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritated water or [3-3H]glucose. Although tritium may also be released during fructose-6-phosphate cycling and/or pentose phosphate cycling, these pathways account for only a small percentage of glucose turnover. Glycogen synthesis was estimated by subtracting the glycolytic rate from the Rg. The percent of the hepatic glucose-6-phosphate pool directly derived from plasma glucose (direct pathway) was calculated as the ratio of [3H]UDP-Glc and plasma [3H]glucose specific activities. The percent of the hepatic glucose-6-phosphate pool derived from PEP-glucogenesis (indirect pathway) was calculated as the ratio of the specific activities of [3-3C]UDP-Glc and 2 × [14C]PEP following in vivo labeling with [U-14C]lactate (27, 28). Comparisons between groups were made by analysis of variance and all values are presented as the mean ± S.E.

RESULTS

General Characteristics of the Experimental Animals Receiving the ICV Infusions (Table I)—To examine the effect of the acute ICV administration of leptin on peripheral and hepatic...
insulin action, 14 rats received a primed-constant infusion of recombinant mouse leptin (total of 30 ng or 1.5 \( \mu \)g in 6 h) and were compared with 9 control rats receiving vehicle infusion. Only rats displaying a complete recovery from the surgery were studied. There were no differences in the mean body weights and average food intake among the three groups of rats. Similarly, following ~6 h fast (postabsorptive state), the plasma insulin, glucose, and FFA concentrations and the rate of endogenous glucose production were similar in the rats assigned to the three experimental groups.

**Euglycemic Hyperinsulinemic Clamp Studies (Table II)**—In order to investigate whether ICV leptin modulated the metabolic effects of insulin in vivo, a physiologic increase in the plasma insulin concentrations was generated during the last 120 min of the 6-h ICV infusions and the plasma glucose concentrations were maintained at ~8 mM by a variable glucose infusion (Table II). The plasma FFA concentration and the rates of endogenous glucose production were similarly suppressed during the hyperinsulinemic clamp in the three groups (Table II). The rates of glucose infusion (GIR) required to maintain the plasma glucose concentration at the target level during the hyperinsulinemic clamp studies were also similar in the rats receiving vehicle and leptin (Table II).

**Effect of ICV Leptin on Insulin-mediated Glucose Disposal, Glycolysis, and Glycogen Synthesis (Fig. 1)**—The effect of a similar increase in the circulating insulin concentrations on the rates of tissue glucose uptake (\( R_{glc} \)), glycolysis, and glycogen synthesis are displayed in Fig. 1. All measurements were performed during the final 30 min of the clamp study, a time when steady-state conditions were achieved for plasma glucose and insulin concentrations, glucose specific activity, and rates of glucose infusion. The rates of whole body glucose disappearance (21.7 ± 0.6, 20.1 ± 0.6, and 23.1 ± 0.7 mg/kg/min in rats receiving ICV vehicle, 30 ng of leptin, and 1.5 \( \mu \)g of leptin, respectively, Fig. 1A) were similar in the three groups. Thus, ICV leptin did not modify the effect of physiologic increments in the plasma insulin concentration on whole body glucose disposal. We next examined whether ICV leptin exerts any effect on the partitioning of glucose disposal into glycogen synthesis and glycolysis. As shown in Fig. 1B the rates of glycogen synthesis and glycolysis during the hyperinsulinemic clamp studies were also similar in the two groups receiving ICV leptin compared with the control group.

**Effect of ICV Leptin on the Hepatic Gene Expression of Glc-6-Pase and PEPCK (Fig. 2)**—The relative abundance of Glc-6-Pase and PEPCK mRNA in the liver was determined by semiquantitative RT-PCR and compared with the expression of a reference gene, GAPDH. The calibration curve used for quantification in the RT-PCR assays for Glc-6-Pase, PEPCK, and GAPDH mRNA were rectilinear with a slope not significantly different from 1 (data not shown). Multiple densitometric scanning of PCR products (example is shown in Fig. 2B) shows that the rats receiving ICV leptin for 6 h manifested a 2–3-fold increase in PEPCK mRNA concentrations compared with vehicle-infused rats. Thus, ICV leptin appeared to antagonize the inhibitory effect of physiological hyperinsulinemia on PEPCK gene expression in vivo. At the highest dose (1.5 \( \mu \)g) ICV leptin also increased the hepatic abundance of Glc-6-Pase mRNA. Thus, ICV leptin even at extremely low rates of infusion (30 ng) was able to reproduce the effects of the systemic administration of a much larger leptin dose (~500 \( \mu \)g) on PEPCK gene expression (22). We next examined whether this molecular effect of ICV leptin also lead to a redistribution of hepatic glucose fluxes.
between gluconeogenesis and glycogenolysis.

**Effect of ICV Leptin on Hepatic Glucose Fluxes (Table III and Fig. 3)**—A marked increase in the contribution of gluconeogenesis to GP was demonstrated in rats receiving ICV leptin with PEP-gluconeogenesis almost entirely accounting for GP. In a net sense, the hepatic glucose 6-phosphate pool receives three major inputs: 1) plasma glucose directly transported and phosphorylated to form glucose 6-phosphate (direct pathway); 2) glucosyl units derived from glycogen; and 3) glucosyl units neo-formed from 3-carbon precursors (indirect or gluconeogenic pathway). The relative contribution of plasma glucose and gluconeogenesis to the hepatic glucose 6-phosphate pool is estimated here by tracer methodology while the contribution of glycogenolysis is calculated as the difference between GP and gluconeogenesis. We examined whether ICV leptin modified the relative contributions of plasma glucose, gluconeogenesis, and glycogenolysis to the hepatic glucose 6-phosphate pool.

Table III displays the [3H]UDP-glucose and the [3H]glucose specific activities which are used to calculate the contribution of plasma glucose (Direct in Table III) to the hepatic glucose 6-phosphate pool. The ratio of the specific activities of 3H-labeled hepatic UDP-glucose and portal vein plasma glucose provided an estimate of the contribution of the direct pathway. As shown in Table III, the contributions of plasma-derived glucose to the hepatic UDP hexose pool measured at the end of the clamp studies were similar in the three groups. Table III also displays the [14C]UDP-glucose and the [14C]PEP specific activities which are used to calculate the contribution of gluconeogenesis (Indirect in Table III) to the hepatic glucose 6-phosphate pool. The ratio of the specific activities of [14C]UDP-glucose and [14C]PEP provided an estimate of the contribution of gluconeogenesis to the hepatic glucose 6-phosphate pool.

The following abbreviations were used: UDPGlc, uridine diphosphate glucose; Glc, plasma glucose; PEP, phosphoenolpyruvate; DIRECT, percent of the hepatic Glc-6-P pool derived from plasma glucose, calculated as the ratio of the specific activities of [3H]UDPGlc (Glc) and [3H]-Glc; INDIRECT, percent of the hepatic Glc-6-P pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [14C]UDPGlc (Glc) and 2 x [14C]PEP.

**TABLE III**

| Leptin (μg) | [3H]Glc | [3H]UDP-Glc | Direct |
|-------------|---------|-------------|--------|
| 0           | 35.2 ± 2.0 | 10.5 ± 1.1  | 29 ± 2 |
| 0.03        | 44.9 ± 2.5 | 14.9 ± 1.1  | 33 ± 2 |
| 1.5         | 27.9 ± 2.3 | 8.0 ± 0.9   | 28 ± 2 |

Leptin (μg) | [14C]PEP | [14C]UDP-Glc | Indirect |
|-------------|---------|-------------|---------|
| 0           | 23.3 ± 0.7 | 19.8 ± 1.1  | 36 ± 3  |
| 0.03        | 14.9 ± 0.9 | 19.2 ± 3.1  | 58 ± 5* |
| 1.5         | 15.9 ± 1.7 | 15.7 ± 2.1  | 53 ± 7* |

*p < 0.01 versus 0.

**Fig. 2**. Effect of intracerebroventricular leptin on the relative expression of PEPCK, glucose-6-phosphatase (Glc-6-Pase), and GAPDH mRNA in liver. The upper panel displays the mean levels of PEPCK, Glc-6-Pase, and GAPDH (G3PDH) mRNA (as a percent of control levels) in rats receiving intracerebroventricular infusions of vehicle (0) or leptin (0.03, 1.5 μg). Semiquantitative RT-PCR was performed in liver freeze-clamped in situ at the completion of all in vivo studies. The lower panel depicts two representative experiments from each of the three experimental groups. ICV leptin resulted in a significant increase in PEPCK and Glc-6-Pase mRNA.

**Fig. 3**. Effect of intracerebroventricular leptin on the relative contribution of gluconeogenesis and glycogenolysis to GP during the hyperinsulinemic clamp studies. A, percent contribution of gluconeogenesis and glycogenolysis to GP in rats receiving ICV infusions of vehicle (0) or leptin (0.03, 1.5 μg). B, rates of gluconeogenesis (■) and glycogenolysis (●) in rats receiving intracerebroventricular infusions of vehicle (0) or leptin (0.03, 1.5 μg) are displayed in this panel. Intracerebroventricular infusions of leptin resulted in a marked shift in the pathways of formation of hepatic glucose 6-phosphate with ~80% of GP accounted for by PEP-gluconeogenesis in rats receiving either dose of leptin.
activities which are used to calculate the contribution of PEP-glucogenesis (indirect in Table III) to the hepatic glucose 6-phosphate pool. PEP-glucogenesis accounted for 36% of the hepatic UDP-glucose pool in the vehicle-infused group. This contribution was markedly increased to ~55% in rats receiving ICV leptin. These data allowed us to estimate the in vivo fluxes through glucogenesis and glycogenolysis and their contribution to GP in rats receiving ICV vehicle or leptin. Fig. 3 depicts the dramatic effects of ICV leptin on the intrahepatic partitioning of glucose fluxes. Glucogenesis and glycogenolysis similarly contributed to GP (~50% of GP or ~2.2 mg/kg/min) in the control group (Leptin 0 in Fig. 3, A and B). However, following ICV leptin administration glucogenesis markedly increased and accounted for ~80% of GP, while glycogenolysis was markedly suppressed.

These effects of ICV leptin on hepatic PEPCK gene expression and hepatic glucose fluxes closely resemble those previously demonstrated with high rates of systemic leptin infusion (22). This may indicate that centrally administered leptin activates a neuronal pathway which in turn mediates its actions on the liver. We next wished to explore the hypothesis that increased adrenergic outflow may directly or indirectly mediate the effects of leptin on hepatic glucose fluxes.

**General Characteristics of the Experimental Animals Receiving Systemic Infusions (Table IV)—**To examine the effects of a highly selective β3-adrenoreceptor agonist and of a physiologic increase in plasma leptin levels on peripheral and hepatic insulin action, rats received either a primed-constant infusion of recombinant mouse leptin (0.5 μg/kg/min for 6 h) or CL 316,243 (125 μg/kg/h) for 6 h and were compared with 5 control rats receiving systemic vehicle infusions. There were no differences in the mean body weights and average food intake among the three groups of rats. Similarly, following ~6 h fast (post-absorptive state), the plasma insulin, glucose, and FFA concentrations and the rate of endogenous glucose production were similar in the rats assigned to the three experimental groups.

**Euglycemic Hyperinsulinemic Clamp Studies (Table V)—**Hyperinsulinemic (5 milliunits/kg/min) clamp studies were performed during the last 2 h of the vehicle, leptin, or CL 316,243 infusions and the plasma glucose concentrations were maintained at ~7.4 mM by a variable glucose infusion (Table V). The plasma FFA concentration and the rates of endogenous glucose production were similarly suppressed during the hyperinsulinemic clamp in the three groups (Table V). The rates of GIR required to maintain the plasma glucose concentration at the target level during the hyperinsulinemic clamp studies were also similar in the rats receiving vehicle, leptin, and CL 316,243 (Table V). The rates of whole body glucose disappearance (17.1 ± 1.5, 15.0 ± 0.4, and 15.6 ± 0.6 mg/kg/min), glycogen synthesis (4.9 ± 1.0, 4.1 ± 1.2, and 4.3 ± 1.0 mg/kg/min) and glycolysis (12.1 ± 1.4, 11.0 ± 1.3, and 11.4 ± 1.4 mg/kg/min in rats receiving vehicle, leptin, and CL 316,243, respectively) during the hyperinsulinemic clamp studies were similar in the three groups.

**Hepatic Gene Expression of Glc-6-Pase and GAPDH (G3PDH) mRNA in Liver.** The upper panel displays the mean levels of PEPCK, Glc-6-Pase, and GAPDH (G3PDH) mRNA (as a percent of control levels) in rats receiving an infusion of either vehicle (0) or CL 316,243 (β3). Semiquantitative RT-PCR was performed in liver freeze-clamped in situ at the completion of all in vivo studies. The lower panel depicts three representative experiments from each of the two experimental groups. CL 316,243 resulted in a significant increase in PEPCK and Glc-6-Pase mRNA in liver.

| Table IV | Baseline characteristics of rats receiving systemic infusions of vehicle (CON), leptin (LEP), or β3-agonist (β3) |
|----------|----------------------------------------------------------|
| Group    | CON | LEP | β3 |
| n        | 5   | 3   | 3  |
| Body weight (g) | 278 ± 12 | 267 ± 6 | 274 ± 14 |
| Food intake (g)  | 20 ± 2 | 21 ± 3 | 22 ± 1 |
| Plasma glucose (mM) | 7.6 ± 0.3 | 7.5 ± 0.3 | 7.7 ± 0.2 |
| Plasma insulin (microunits/ml) | 31 ± 4 | 30 ± 5 | 29 ± 3 |
| Plasma FFA (meq/liter) | 0.82 ± 0.08 | 0.88 ± 0.08 | 0.79 ± 0.09 |
| Basal GP (mg/kg/min)  | 14.0 ± 0.9 | 14.8 ± 0.3 | 14.5 ± 0.3 |

| Table V | Steady-state plasma glucose, insulin, and FFA concentrations and average rates of GIR and endogenous GP during the hyperinsulinemic clamp studies in rats receiving systemic infusions of vehicle (CON), leptin (LEP), or β3-agonist (β3) |
|----------|----------------------------------------------------------|
| Group    | CON | LEP | β3 |
| Glucose (mM) | 7.3 ± 0.2 | 7.4 ± 0.3 | 7.4 ± 0.2 |
| Insulin (microunits/ml) | 0.39 ± 0.09 | 0.42 ± 0.07 | 0.39 ± 0.03 |
| GIR (mg/kg/min) | 10.2 ± 1.7 | 9.1 ± 1.9 | 9.4 ± 1.3 |
| GP (mg/kg/min) | 7.4 ± 0.7 | 6.9 ± 0.5 | 7.0 ± 0.5 |

Fig. 4. Effect of the systemic infusion of a β3-adrenoreceptor agonist on the relative expression of PEPCK, Glc-6-Pase, and GAPDH (G3PDH) mRNA in liver.
tration of leptin on PEPCK gene expression.

Hepatic Glucose Fluxes (Table VI and Fig. 6)—Fig. 6 depicts the effects of systemic leptin or β3-adrenoreceptor agonist on the intrahepatic partitioning of glucose fluxes. A marked increase in the contribution of gluconeogenesis to GP was demonstrated in rats receiving either leptin or CL 314,243 with PEP-gluconeogenesis almost entirely accounting for GP. Table VI displays the [3H]UDP-glucose and the [3H]glucose specific activities which are used to calculate the contribution of plasma glucose (Direct in Table VI) to the hepatic glucose 6-phosphate pool. The ratio of the specific activities of [3H]-labeled hepatic UDP-glucose and portal vein plasma glucose provided an estimate of the contribution of the direct pathway. The contribution of the direct pathway to the hepatic UDP hexose pool measured at the end of the clamp studies were similar in the three groups. Table VI also displays the [14C]UDP-glucose and the [14C]PEP specific activities which are used to calculate the contribution of PEP-gluconeogenesis (Indirect in Table VI) to the hepatic glucose 6-phosphate pool. The indirect pathway accounted for 37% of the hepatic UDP-glucose pool in the vehicle-infused group. This contribution was markedly increased to 53% in rats receiving systemic leptin and to 60% in rats receiving the β3-adrenoreceptor agonist. These data allowed us to estimate the in vivo fluxes through gluconeogenesis and glycolysis and their contribution to GP. Following leptin or β3-adrenoreceptor agonist administration, gluconeogenesis markedly increased and accounted for ~80% of GP, while glycolysis was markedly suppressed.

DISCUSSION

This study demonstrates that an acute ICV infusion of recombinant leptin antagonizes the action of insulin on the gene expression of PEPCK in the liver and results in marked changes in the intrahepatic partitioning of glucose fluxes with increased gluconeogenesis and decreased glycogenolysis (Fig. 7). It is likely that these metabolic effects of leptin participate in the regulation of hepatic glucose metabolism under physiologic conditions. In fact, the total amount of leptin required to provoke these metabolic effects is well within the range of ICV leptin required for anorectic actions in rats (8, 15). These data also indicate that the hepatic effects of circulating leptin are largely mediated via ob receptors within the central nervous system. In fact, in the present study 30 ng of ICV leptin entirely reproduced the effects of 50 µg of leptin infused systemically on both PEPCK gene expression and rate of gluconeogenesis.

Leptin plays an important role in the regulation of energy stores and in the choice of fuels to be utilized under various nutritional conditions (14, 21, 23). The latter metabolic effects of leptin are likely to neutralize or diminish the compensatory mechanisms which normally favor regaining normal body weight after fasting or food restriction (15, 31, 32). We have proposed that since the net flux through glycolysis/gluconeogenesis determines the hepatic concentration of malonyl-CoA (22, 33–35), the effects of leptin on PEPCK and gluconeogenesis are likely to limit the hepatic formation of triglycerides by favoring FFA entry in the mitochondria and their β-oxidation (22, 23, 33–36). These biochemical actions occur in response to minimal amounts of ICV leptin and are therefore likely to participate in its “lipostatic” action. While the liver is the principal site for de novo lipogenesis in humans (35, 36), leptin has also been shown to promote lipid oxidation and decrease tissue triglycerides at other tissue sites (14, 17, 18, 21, 23, 37). These effects of leptin on carbohydrate and lipid metabolism, while are likely to be mediated by diverse molecular and biochemical events in various tissues, may all contribute to the dramatic and beneficial effects of long-term leptin administration on hepatic and peripheral insulin action (6, 9, 24, 38). However, it should be pointed out that in the present study we did not demonstrate a significant increase in either basal or insulin-stimulated rates of glucose disposal following acute ICV leptin administration. Conversely, a recent report in mice demonstrated parallel increases in both glucose production and disposal 5 h after ICV leptin administration (39). Since plasma glucose and plasma insulin did not change significantly, the authors suggested that leptin activated effenter signals to the central nervous system to the liver and peripheral tissues. The apparent inconsistency between these results and ours may be explained on the basis of differences between species and experimental protocols, e.g., pancreatic and insulin clamp was performed in this study. However, it is also possible that a stimulatory effect of ICV leptin on peripheral glucose uptake requires high peak levels of leptin in a cerebral ventricle. In fact, in the present study, in an attempt to approximate physiological conditions, we used a constant infusion of leptin rather than a bolus injection and our overall doses were quite low. Regardless of effects (or lack thereof) on peripheral glucose uptake, it is clear that this protocol was sufficient to unveil potent effects of ICV leptin on hepatic PEPCK gene expression and glucose fluxes. It is well established that PEPCK activity is primarily regulated at the level of gene transcription (40, 41).

**TABLE VI**

Specific activities of hepatic substrates used to calculate the direct pathway and the “indirect pathway” at the end of the [3-3H]glucose-[U-14C]lactate infusions in rats receiving systemic infusions of vehicle (CON), leptin (LEP), or β3-agonist (β3). The following abbreviations were used: UDPGlc, uridinediphosphoglucose; Glc, plasma glucose; PEP, phosphoenolpyruvate; DIRECT, percent of the hepatic Glc-6-P pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [3H]UDP-glucose (Glc) and [3H]glucose (GP); INDIRECT, percent of the hepatic Glc-6-P pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [14C]UDP-glucose (Glc) and 2 × [14C]PEP.

| Group   | [3H]Glc dpm/nmol | [3H]UDPglc dpm/nmol | Direct % | [14C]PEP dpm/nmol | [14C]UDPglc dpm/nmol | Indirect % |
|---------|------------------|---------------------|----------|------------------|---------------------|------------|
| CON     | 42.4 ± 0.4       | 11.6 ± 0.8          | 29 ± 4   | 24.4 ± 0.9       | 12.3 ± 0.5          | 27 ± 1     |
| LEP     | 47.4 ± 1.4       | 17.6 ± 2.0          | 32 ± 2   | 17.7 ± 2.9       | 21.7 ± 2.4          | 53 ± 3a    |
| β3      | 35.1 ± 7.4       | 8.7 ± 2.1           | 26 ± 1   | 22.2 ± 4.7       | 23.4 ± 5.1          | 60 ± 5a    |

*p < 0.01 versus CON.
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FIG. 6. Effects of physiologic hyperleptinemia and of the systemic infusion of a $\beta_3$-adrenoreceptor on the relative contribution of gluconeogenesis and glycogenolysis to GP during hyperinsulinemic clamp studies. A, percent contribution of gluconeogenesis (■) and glycogenolysis (■) to GP in rats receiving systemic infusions of vehicle (CON), leptin (LEP), or CL 316,243 ($\beta_3$). B, rates of gluconeogenesis and in rats receiving systemic infusions of vehicle, leptin, or CL 316,243 are displayed in this panel. Systemic infusions of either leptin (0.5 $\mu$g/kg/min) or the $\beta_3$-adrenoreceptor agonist CL 316,243 resulted in a marked shift in the pathways of formation of hepatic glucose 6-phosphate with ~80% of GP accounted for by PEP-gluconeogenesis in the treated rats versus 38% in the vehicle control.

The correspondence here between in vivo fluxes through PEPCK (PEP-gluconeogenesis) and its mRNA levels is consistent with this notion.

The effects of leptin on the hepatic gene expression of PEPCK and Glc-6-Pase occurred in the presence of fixed and moderate hyperinsulinemia. Thus, it may be argued that leptin antagonized the suppressive action of insulin on these promoters perhaps by interfering with hepatic insulin signaling. Alternatively, leptin may antagonize the effects of insulin on PEPCK and Glc-6-Pase indirectly via increased sympathetic tone and cAMP levels. Since leptin has been shown to exert a marked shift in the pathways of formation of hepatic glucose 6-phosphate with a marked increase in the contribution of PEP-gluconeogenesis (~80%) to GP paralleled by a marked decrease in glycogenolysis.

by which acute stimulation of $\beta_3$-adrenoreceptors modulates hypothalamic activity remains to be delineated, our results taken together with those of Mantzoros et al. (5) suggest that an hypothalamic pathway which is regulated by leptin and $\beta_3$-adrenergic receptors may play a role in both regulation of appetite and hepatic metabolism. Alternatively, ICV leptin and systemic CL 314,243 infusions may regulate the production of an unknown protein or substrate in adipose tissue which in turn mediates their hepatic effects. Further studies will be required to explore these possibilities.

In conclusion, we have provided evidence for potent effects of intracerebroventricular leptin administration on hepatic PEPCK gene expression and gluconeogenesis. This suggests the operation of an efferent pathway relating areas of the hypothalamus involved in the regulation of satiety with hepatic glucose and lipid metabolism. Elucidation of the mechanism(s) by which activation of the leptin and $\beta_3$-adrenoreceptor systems regulate hepatic metabolic events may have major implications for our understanding of the role of the central nervous system in metabolic homeostasis.

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