Plasma hyperosmolality stimulates leptin secretion acutely by a vasopressin-adrenal mechanism

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Am J Physiol Endocrinol Metab 287: E263–E268, 2004. First published April 6, 2004; 10.1152/ajpendo.00514.2003.—Glucose administration to rodents acutely stimulates leptin secretion. To investigate the mechanism, rats were infused intravenously with various concentrations of glucose, and plasma leptin concentrations were measured with time. The osmolality of the infusates was equalized with various concentrations of carbohydrates that are not metabolized. Hyperosmolar glucose stimulates leptin secretion in a dose-dependent manner, with peak plasma leptin concentrations occurring ~3 h after the end of the glucose infusion. Hypertonic infusions of galactose, mannitol, and sodium chloride independently stimulate leptin secretion with approximately one-half the strength of equivalent osmolar concentrations of glucose. Peak plasma leptin concentrations occur ~4 h after the end of the hypertonic solution infusion. Hypertonic solutions of mannitol do not stimulate leptin secretion in vasopressin-deficient or in adrenalectomized animals. In conclusion, intravenous infusions of hypertonic glucose and hypertonic mannitol independently stimulate leptin secretion. Hyperosmolality stimulates leptin secretion by a vasopressin-adrenal mechanism.

Leptin is a hormone secreted by adipocytes that has pleiotropic functions, including roles in body weight homeostasis, satiety, metabolism, reproduction, and angiogenesis (for review, see Ref. 1). The mechanisms of leptin secretion from adipocytes have been intensively studied. A number of factors, hormones, and conditions regulate leptin secretion. Fasting plasma leptin concentrations are mostly determined by body fat content; numerous investigations have demonstrated that both leptin mRNA and plasma leptin concentrations obtained from animals and humans after an overnight fast are directly proportional to measurements of body fat (6, 18, 19, 27). In addition, leptin secretion is regulated by body fat-independent mechanisms. In rodents and humans, plasma leptin concentration has a diurnal variation that most likely is entrained to meals (4, 23). In the rodent, enteral feeding (26) or the intravenous infusion of total parenteral nutrition or glucose alone (15, 16) causes a rise in plasma leptin concentration ~3 h after the calorie bolus. The rise in insulin and the transport and metabolism of energy-producing substrates in adipocytes appear to stimulate postprandial leptin secretion (17, 20).

We previously showed that leptin secretion from cultured adipocytes was proportional to the uptake and metabolism of glucose and to the intra-adipocyte concentrations of ATP (14). We wanted to determine whether the previous studies in cultured adipocytes could be extended to studies in whole animals. We hypothesized that the intravenous infusion of increasing concentrations of glucose would increase delivery and metabolism of glucose in adipose tissue and acutely increase leptin secretion and plasma leptin concentrations. For these experiments, we thought it necessary to control for the osmolality of the infusate. The controls for plasma osmolality may be particularly relevant, because plasma osmolality rises postprandially and has previously been proposed as a mechanism for satiety (10). We have found that glucose and hyperosmolality independently stimulate leptin secretion in vivo. Plasma hyperosmolality likely increases leptin secretion by central nervous system mechanisms, namely, through the vasopressin-corticosterone axis.

METHODS

Animals and diets. All animals were humanely treated, and the experimental protocols were reviewed and accepted by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Fischer 344 (F344), adrenalectomized F344, Long-Evans, and Long-Evans Brattleboro rats were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed in individual cages at 22°C. Lighting was controlled on a natural dark-light cycle (lights out at 1800; lights on at 0600). The animals were allowed free access to water and were fed a regular chow diet (Research Diets, New Brunswick, NJ) consisting of 16.4% protein, 70.8% carbohydrate, 4.6% fat and measuring 3.9 kcal/g. At the time of catheter insertions, all animals weighed 300–400 g.

Acute metabolic response to glucose and other osmoles. To measure the metabolic and leptin responses to the intravenous infusion of glucose, mannitol, galactose, or sodium chloride (NaCl), animals were anesthetized with isoflurane, and a catheter was inserted into the external jugular vein, as previously described (11). The infusion cannulas were tunneled subcutaneously to the back of the neck. The catheter was infused with heparinized saline to prevent clot formation at the intravenous tip of the catheter, and the external end of the catheter was plugged. The animals were allowed to recover for 3 days. On the 3rd day, the animals were fasted overnight. The next morning at approximately the same time of day, 0.5 ml of blood was withdrawn through the catheter, and then various concentrations of glucose, mannitol, galactose (between 0 and 40%), or sodium chloride (between 0.45 and 3%) were infused through the catheter over a 5-min period. Total volume of the infusate was 6 ml. After various times, as indicated in Figs. 1–8, 0.5 ml of whole blood was withdrawn through the catheter.

Plasma measurements. Osmolarity, albumin, leptin, vasopressin, and corticosterone concentrations were measured in the plasma. Blood was collected in heparinized microtubes and centrifuged at 4°C, 10,000 g for 5 min, and the plasma was separated and immediately stored at −70°C. The plasma was thawed at room temperature before...
the measurements listed below were performed. Plasma levels of leptin in F344 animals were measured by a rat radioimmunoassay kit (Linco Research, St. Charles, MO). The limit of sensitivity for this leptin assay was between 0.5 and 50 ng/ml. The interassay variation for the leptin assay at 1.7 ng/ml [Quality Control I (QCI)] was <6%. The interassay variation for the leptin assay at 6 ng/ml (QCCI) was <10%. Plasma leptin concentrations in Long-Evans animals were measured by a leptin mouse/rat RIA kit as described by the suppliers (American Laboratories Products, Windham, NH). The sensitivity and range of this assay were 6 pg/ml and 12.5–800 pg/ml, respectively. Plasma vasopressin concentrations were measured by Correlate-EIA, an enzyme immunoassay kit, as described by the suppliers (Assay Designs, Ann Arbor, MI), and the murine plasma corticosterone concentrations were measured by an RIA kit with a double antibody (ICN Diagnostics). The clinical laboratory at the McGuire Veterans Administration Medical Center assessed plasma albumin concentrations (Vitos ALB Slides) and osmolality (Fiske Associates, Norwood, MA).

Intracellular leptin concentration. Adipose tissue from the epididymal fat depot was weighed, and an equal volume of lysis buffer (1% Triton X-100, 0.3 M NaCl, 1 mM EDTA, and 0.05 M Tris, pH 7.4, with complete protease inhibitor cocktail; one tablet/10 ml; Boehringer Mannheim, Indianapolis, IN) was added. We then homogenized the tissue suspension with a polytron (PowerGen Model 125, Fisher Scientific). The tissue suspension was centrifuged, and cell debris was removed. The leptin concentration in the cell lysate was measured with the Rat Leptin RIA kit, as described above.

RESULTS

Fischer 344 animals were infused intravenously with increasing concentrations of glucose (from 0 to 40%). Each solution contained varying concentrations of galactose, a carbohydrate that is not metabolized, to equalize the osmolality of the infusates. Various times after the intravenous infusion, plasma leptin concentrations were measured. In preliminary experiments, intravenous glucose infusion increased serum glucose concentrations approximately fivefold within 30 min. By 60 min, the plasma glucose concentrations returned to baseline. Intravenous glucose infusion increased plasma insulin by approximately fourfold at 30 and 60 min. Plasma insulin concentrations returned to baseline by 120 min. Infusion of a nonmetabolizable carbohydrate did not increase plasma glucose or insulin concentrations. Plasma leptin concentrations 30 min after the infusions were not statistically different from plasma leptin concentrations before glucose or galactose infusions. As shown in Fig. 1, the intravenous infusion of glucose caused a dose-responsive increase in plasma leptin concentrations. Infusion of 10 and 40% glucose increased plasma leptin concentrations ~2 and 2.5-fold, respectively (Fig. 1, inset). The peak plasma leptin concentration occurred ~3 h after the glucose infusion. Unexpectedly, galactose, in the absence of glucose, also stimulated leptin secretion. An intravenous infusion of 40% galactose increased plasma leptin concentration about twofold. After galactose infusions, plasma leptin concentrations peaked at ~4 h, 1 h after the leptin peak observed with glucose infusions. The area under the leptin curve when animals were infused 40% glucose was 2.2-fold greater than that when animals were infused 40% galactose.

The intravenous infusions of other hyperosmolar solutions stimulated leptin secretion. Mannitol and hypertonic saline increased plasma leptin concentrations in a time- and dose-dependent manner. Plasma leptin concentrations peaked 4 h after the infusion of the hypertonic solutions. As shown in Fig. 2, infusion of 5, 10, and 20% mannitol increased plasma leptin concentrations by ~1.3-, 1.8-, and 3.3-fold, respectively. Infusion of normal saline (0.9%) did not increase plasma leptin concentrations. However, infusion of hypertonic saline (3%) increased plasma leptin concentrations by approximately twofold (Fig. 2).

Plasma hyperosmolality will induce an osmotic diuresis, which is the renal excretion of excess osmoles and free water. After the infusion of hypertonic solutions, the plasma concentrations of intravascular proteins, such as leptin or albumin, may increase with time, as the intravascular space loses free water. However, as shown in Fig. 3, plasma albumin concentrations do not rise after the intravenous infusions of hypertonic mannitol. In fact, plasma albumin concentrations decrease after the infusion of this relatively large intravenous volume. Therefore, hemocoagulation of proteins is an unlikely mechanism for the increase in plasma leptin concentrations after the infusion of hypertonic solutions.

The intravenous infusion of glucose or hyperosmolality may stimulate the synthesis of leptin or the secretion of stored leptin from adipose tissue. We therefore measured the intracellular concentrations of leptin in epididymal adipose tissue 3 h after the intravenous infusion of normal saline or equimolar concentrations of glucose or mannitol. As shown in Fig. 4, intravenous glucose and mannitol infusions increased intra-adipocyte leptin concentrations by ~2- and 1.75-fold, respectively.

The effect of infusion of hypertonic solutions on plasma osmolality and vasopressin concentrations is shown in Fig. 5. Preinfusion plasma osmolality was ~290 mosmol/kgH2O. Af-
ter infusion, the plasma osmolality increased rapidly and peaked about 5 min after the intravenous bolus of 20% mannitol, 20% glucose, and 3% NaCl. The plasma osmolality then gradually declined to prebolus osmolality values by 180 min. Hypotonic and isotonic solutions had minimal effects on plasma osmolality. Plasma vasopressin concentrations did not change before or after the intravenous infusion of iso- or hypotonic solutions (see Fig. 5). Plasma vasopressin concentrations rose precipitously and peaked 5 min after the intravenous infusion of 20% mannitol. The vasopressin concentration returned toward baseline values ~60 min after the intravenous bolus.

To test the hypothesis that vasopressin increases leptin secretion after the infusion of hypertonic solutions, we administered vasopressin intravenously or intraperitoneally and measured plasma leptin concentrations with time. Exogenous vasopressin had no effect on plasma leptin concentrations (data not shown). Furthermore, vasopressin did not stimulate leptin secretion in cultured adipocytes (data not shown). Therefore, vasopressin does not directly stimulate leptin secretion from peripheral adipose tissue. Because vasopressin crosses the blood-brain barrier poorly, the above experiments do not rule out the possibility that vasopressin acts within the central nervous system to stimulate leptin secretion. To test this possibility, we infused glucose (20%) or mannitol (20%) intravenously to rats with vasopressin deficiency (Brattleboro) and to rats with vasopressin sufficiency (control). Both groups of rats were from the same strain (Long-Evans). The Long-Evans strain has much less fat than the Fischer strain, and the fasting leptin concentrations were lower. As shown in Fig. 6A,
plasma leptin concentrations in control animals increased after the intravenous infusion of both hypertonic glucose and mannitol. However, in the vasopressin-deficient Brattleboro rat, plasma leptin concentrations increased after the intravenous infusion of glucose but did not increase after the intravenous infusion of mannitol (Fig. 6B).

Previous studies have demonstrated that vasopressin can stimulate the release of glucocorticoids. Because glucocorticoids are leptin secretagogues, hypertonicity may stimulate leptin secretion through a vasopressin-adrenal pathway. We measured, therefore, a time course of plasma corticosterone after the intravenous infusion of normal saline and hypertonic solutions. As shown in Fig. 7, plasma corticosterone concentrations at 30 min are approximately threefold greater in animals infused with hypertonic glucose and mannitol than in animals infused with normal saline. Plasma corticosterone concentrations decline after 60 and 120 min in animals infused with hypertonic glucose and mannitol, respectively. At 120 and 180 min, plasma corticosterone concentrations are more than threefold greater in animals infused with mannitol than in animals infused with glucose.

To further test the hypothesis that corticosterone mediates hypertonicity-induced leptin secretion, adrenalectomized F344 rats were infused with either hypertonic glucose or mannitol. As shown in Fig. 8, hypertonic glucose increased plasma leptin concentrations by ~30% over fasting plasma leptin concentrations. By contrast, hypertonic mannitol (20%) did not stimulate leptin secretion in adrenalectomized animals.

**DISCUSSION**

Chronic and acute mechanisms regulate leptin secretion in rodents. Fasting plasma leptin concentrations vary directly with indexes of body fat. However, plasma leptin concentrations can change acutely with no detectable variations in body fat. In the present study, we demonstrate that plasma leptin concentrations vary with the amount of intravenously infused glucose and peak ~3 h after the glucose infusion (Fig. 1). We
(16) and others (26) have previously shown that hyperosmolar glucose stimulates leptin secretion; these previous studies, however, measured plasma leptin concentrations after the intravenous infusion of only one glucose concentration and did not control for osmolality. In this study, we included controls for osmolality of the intravenous solution being infused. To our surprise, we found that hypertonic solutions containing galactose (Fig. 1), mannitol (Fig. 2), or NaCl (Fig. 2) independently stimulate leptin secretion. The leptin area under the curve after an intravenous infusion of a nonmetabolizable substrate is approximately one-half that after an intravenous infusion of glucose. Furthermore, the peak plasma leptin concentration after the intravenous infusion of mannitol or galactose occurs ~1 h after the peak plasma leptin concentration following the intravenous infusion of glucose (Fig. 1).

Stimulation of the vasopressin-adrenal axis is a likely mechanism for hypertonicity-induced leptin secretion. Infusions of hypertonic solutions acutely raise plasma osmolality (Fig. 5), vasopressin (Fig. 5), and corticosterone concentrations (Fig. 7). Furthermore, plasma leptin concentrations do not increase after the intravenous infusion of hypertonic mannitol in rats that lack vasopressin (Fig. 6) or corticosterone (Fig. 8). Therefore, we propose the following mechanism for hypertonicity-induced leptin secretion. Within minutes after the intravenous infusion of hypertonic solutions, plasma osmolality rises and stimulates the release of vasopressin from the neurohypophysis. Vasopressin is found in ~50% of neurons that contain corticotropin-releasing hormone in supraoptic and paraventricular nuclei; these hormones are cosecreted into the hypophysial-portal circulation, bind to the anterior pituitary, and synergistically stimulate the production and release of corticotropin (5). Within a couple of hours of the hypertonic stimulus, circulating corticotropin enhances corticosterone release from the adrenal glands (Fig. 7). Corticosterone then binds to glucocorticoid receptors within adipocytes and stimulates the gene expression, synthesis, and secretion of leptin (3, 12, 25). In this study, hypertonic mannitol increases intra-adipocyte leptin concentrations (Fig. 4); peak plasma concentrations of leptin occur 2–3 h from peak plasma corticosterone concentrations (see Figs. 1 and 7), a time period consistent with the binding of corticosterone to glucocorticoid receptors and the initiation of transcription, translation, packaging, and secretion of leptin from adipocytes.

The magnitude and the peak plasma leptin concentrations differ in response to hypertonic glucose and mannitol. This finding suggests that hypertonic glucose and mannitol stimulate leptin secretion by different mechanisms. We propose that hypertonic glucose stimulates leptin secretion by vasopressin-/corticosterone-independent and -dependent mechanisms. Plasma leptin concentrations increase within hours after the intravenous infusion of glucose in animals that lack vasopressin (Fig. 6) and corticosterone (Fig. 8). Some investigators (3, 21) have suggested that insulin stimulates the exocytosis and secretion of leptin that has been stored within cytoplasmic vesicles in much the same way that glucose stimulates the release of insulin from pancreatic islets. This hypothesis is largely based on studies that demonstrate enhanced insulin-mediated leptin secretion within 30 min, a time frame too short for induction of transcription, translation, trafficking, and secretion of leptin (2). Furthermore, other investigators have shown that cultured adipocytes can secrete leptin even when pretreated with protein synthesis inhibitors (3, 21). Our present studies and studies in cultured adipocytes (17) fail to support this mechanism. We and others (22, 28) have shown that glucose (and/or the associated rise in insulin) increases leptin gene transcription and protein synthesis (Fig. 4). We have not demonstrated that glucose or insulin stimulates leptin secretion before 30 min. Rather, we find in whole animals (the present study) and in cultured adipocytes (17) that glucose or insulin increases serum or medium leptin concentrations well after 30 min. Furthermore, we have demonstrated that cultured adipocytes, which cannot synthesize proteins, cannot secrete leptin (17). In the latter studies, we measured leptin secretion in adipocytes that were continuously incubated, rather than briefly incubated (3, 21), with protein synthesis inhibitors. We propose that glucose and/or insulin stimulates the synthesis and the constitutive secretion of enhanced intra-adipocyte concentrations of leptin.

Hypertonic glucose likely stimulates leptin secretion by a vasopressin-corticosterone-dependent mechanism. Corticosterone concentrations, however, increase less after hypertonic glucose than after hypertonic mannitol (Fig. 7) despite similar effects on serum osmolarity (Fig. 5). We do not know the reason for the difference in the corticosterone response between the two hypertonic solutions. We speculate that the difference in metabolism of glucose and mannitol contributes to the variability in the corticosterone response. Perhaps cells metabolize glucose, and not mannitol, before the glucose can interact with receptors that respond to osmolality. The 1-h difference in the time to maximal leptin response between glucose and the nonmetabolizable carbohydrates (Fig. 1) likely reflects differences in the corticosterone time course. The corticosterone time course shifts to the right by ~1 h after the mannitol infusion compared with the glucose infusion (Fig. 7). In future studies, we can estimate the contribution of the corticosterone-dependent pathway by measuring glucose-mediated leptin secretion before and after adrenalectomy. We predict that the corticosterone-dependent leptin secretion with hypertonic glucose will be much less than that with hypertonic mannitol.

We propose that the fasting serum leptin concentrations and the leptin response to glucose were less robust in Brattleboro and adrenalectomized animals than in F344 animals because of several reasons. Infusion of hypertonic glucose stimulates leptin release by both glucose and hypertonicity mechanisms. In vasopressin- or corticosterone-deficient animals, the hypertonicity-induced component of leptin secretion is eliminated. Second, the Long-Evans strain of rat has less body fat and is likely more leptin sensitive than the relatively obese, leptin-resistant F344 animal (13). Baseline and stimulated plasma leptin concentrations are increased in rats with more adiposity and leptin resistance. Finally, corticosterone-deficient animals have a relative paucity of body fat and, therefore, relatively lower fasting and stimulated leptin concentrations.

We did not measure satiety in animals infused with hypertonic glucose or mannitol. Previous investigations have implicated serum osmolality as one variable governing food intake regulation. Kakolewsky and Deaux (9, 10) initially proposed that the serum osmolality determines meal initiation and cessation. These investigators demonstrated that, in water-deprived animals reexposed to water, the latency to eat is in-
versely related to the amount of water consumed or to the osmolality of an ingested solution. Postgastric mechanisms of satiety may be sensitive to the osmolality of intestinal contents. Duodenal administration of isosmotic nutrient and nonnutrient solutions that are hypertonic reduces food intake similarly in intact rats (24) and pigs (8). We propose that osmolality-induced leptin secretion may be contributing to the anoxia observed with water restriction or hypertonic food intake. We predict that food intake may not change in Brattleboro or adrenalectomized animals that are water restricted or administered isosmotic nutrients.

GRANTS

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REFERENCES