Hypothalamic Responses to Long-chain Fatty Acids Are Nutritionally Regulated*

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Central administration of the long-chain fatty acid oleic acid inhibits food intake and glucose production in rats. Here we examined whether short term changes in nutrient availability can modulate these metabolic and behavioral effects of oleic acid. Rats were divided in three groups receiving a highly palatable energy-dense diet at increasing daily caloric levels (below, similar, or above the average of rats fed standard chow). Following 3 days on the assigned diet regimen, rats were tested for acute biological responses to the infusion of oleic acid in the third cerebral ventricle. Three days of overfeeding virtually obliterated the metabolic and anorectic effects of the central administration of oleic acid. Furthermore, the infusion of oleic acid in the third cerebral ventricle failed to decrease the expression of neuropeptide Y in the hypothalamus and of glucose-6-phosphatase in the liver following short term overfeeding. The lack of hypothalamic responses to oleic acid following short term overfeeding is likely to contribute to the rapid onset of weight gain and hepatic insulin resistance in this animal model.

Obesity and type 2 diabetes mellitus (DM2)1 share several metabolic features, which include insulin resistance (1–3). The incidence of obesity and DM2 has risen significantly in developed and developing countries. For example, in the United States alone there has been a significant increase in the prevalence of obesity among both children and adults over the last 10 years (4, 5). Consumption of high calorie diets and sedentary lifestyles play major roles in this trend (2, 4, 6). Similarly, exposure to palatable diets with high caloric density (high in fat) induces a variable degree of weight gain and insulin resistance in mice (7, 8), rats (9–11), pigs (12), dogs (13), and monkeys (14).

Evolutionary pressures may have favored the selection of genes, which maximize energy storage when food availability is high (15–18). We and others have proposed that a rapid increase in caloric intake initiates a "tug of war" between peripheral “anabolic signals” (19) and hypothalamic “catabolic signals” (20–26). The effects of hormones, such as leptin (27–31) and insulin (24, 25, 32, 33), and perhaps nutrients, such as fatty acids (21, 23, 26), within the hypothalamus initiate a negative feedback, which includes restraint on food intake, stimulation of energy expenditure, and decreased output of nutrients from endogenous sources (mainly from the liver). Animals and humans may be susceptible to weight gain and altered metabolic regulation when this negative feedback is disrupted. The rapid onset of leptin resistance in rodents models of voluntary overfeeding provides initial support for this theory (34, 35).

Here we test the hypothesis that short term increase in caloric intake rapidly induces resistance to the central effects of the long-chain fatty acid, oleic acid (OA). Thus, we examined whether changes in nutritional status lead to alterations in the central effects of OA on feeding behavior and glucose production.

EXPERIMENTAL PROCEDURES

Animals and Experimental Design—Ten-week-old male Sprague-Dawley or Zucker Fatty rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were housed in individual cages and subjected to a standard light dark cycle (0600–1800/1800–0600). At 14 days before the in vivo studies, all rats received implantations of ICV catheters by stereotaxic surgery as described previously (36). Rats were allowed to recover completely before initiation of all in vivo studies. Animals were fed a standard chow (SC, catalog number 5001, Purina Mills Ltd., with 59% calories provided by carbohydrate, 28% by protein, and 12% by fat, 3.3 kcal/g), a high sucrose (HS; animals were given free access to a 20% sucrose solution in addition to their standard chow diet), or a highly palatable high fat diet (HF, catalog number 9389; Purina Mills Ltd., with 45% calories provided by carbohydrate, 22% by protein and 33% by fat, 5.32 kcal/g) that was generated by supplementing SC diet with 10% lard. The lard contained 2% myristic acid, 24% palmitic acid, 13% stearic acid, 46% oleic acid, and 12% linoleic acid. The total composition of fats in the HF diet was 5.2% saturated, 6.2% monounsaturated, and 2.7% polyunsaturated fat by weight. The SC diet contained 1.15, and 1.6% of saturated, monounsaturated, and polyunsaturated fats by weight, respectively. Five groups of animals were included in the feeding and metabolic studies (described below) after 3 days on the following diet regimens (Table I): 1) SC ad libitum (SC; ~80 kcal/day); 2) HS ad libitum (HS; ~95 kcal/day); 3) HF ad libitum (HF140; ~140 kcal/day); 4) HF calorie-restricted (HF55; ~55 kcal/day); and 5) HF pair-fed to SC (HF80; ~80 kcal/day). In addition, we performed metabolic studies on Zucker Fatty rats (n = 10) fed an SC diet.

Central Delivery of OA—OA was complexed with the polymer hydroypropylβ-cyclodextrin (HPB, Cycloextrin Technologies Development Inc.). The latter has been shown to provide an excellent vehicle for the central delivery of fatty acids (23, 37, 38). OA was solubilized in 45% HPB to a final concentration of 17 mg/ml. The HPB-OA solution was diluted in artificial cerebrospinal fluid to the appropriate concentration used for each ICV injection (30 or 300 nmol/5 μl). HPB alone at a similar
Feeding Behavior Studies—This experimental protocol was designed to examine the acute effect of ICV OA on food intake in three experimental groups fed a standard chow (SC), a high sucrose diet (HS95), and a high fat diet (HF140). SC and HF140 animals were allowed to eat their diets ad libitum. HS animals were given free access to a 20% sucrose solution in addition to the standard chow diet for 3 days. Following 3 days of ad libitum feeding in all groups, on study day 0 (Fig. 1A), rats were given an ICV bolus injection of either 5 µl of OA (30 nmol) or vehicle at a rate of 1 µl/min using a gas-tight syringe (Hamilton Corp.) 1 h before the start of the dark cycle. Food intake was measured at the same time daily for 5 days post-injection.

Insulin Action Studies—For analysis of NPY expression, we studied two groups of rats, HF55 and HF140. After 3 days on the respective diet regimen, ICV vehicle (10% HPB in artificial cerebrospinal fluid) or ICV OA (30 nmol) was injected as a bolus into the third cerebral ventricle 1 h before the start of the dark cycle. Food was withdrawn, and hypothalami were harvested 16 h following injection.

Insulin Action Studies—The experimental protocol herein was designed to examine the effect of nutritional status on the ability of long-chain fatty acids in the hypothalamus to modulate carbohydrate metabolism. Sprague-Dawley rats (n = 43) and Zucker fatty rats (n = 10) were implanted with chronic catheters as described previously (36). After full recovery from the catheterization, Sprague-Dawley rats were randomized into three groups (HF55 = 16), HP60 (n = 9), or HF140 (n = 18) and were allowed to consume their allocated diet for 3 days. On the night prior to the in vivo study, all rats received 55 kcal to ensure a similar nutritional state at the start of the metabolic studies. In a separate experiment, Zucker fatty rats (n = 10) were fed a standard chow diet and allowed to fast for 5 h prior to the metabolic studies. All studies were performed in awake, unstressed, chronically catheterized rats. At t = 0 (Fig. 3A), a primed continuous infusion of ICV OA (total dose 30 or 300 nmol) or vehicle (HPB 10% in artificial cerebrospinal fluid) was initiated and maintained throughout the duration of the study. Plasma glucose was measured periodically initiating at the onset (t = 0) of the ICV infusion and lasting throughout the duration of the study. Since plasma samples for determination of insulin, leptin, and nonesterified fatty acid concentration were obtained at the onset (t = 0) and at 30-min intervals during the study. At t = 120, a primed continuous infusion of high pressure liquid chromatography-purified [3-3H]glucose (PerkinElmer Life Sciences; 40 µCi bolus, 0.4 µCi/min for duration of the study) was initiated and maintained for the last 4 h of the study. Samples for determination of [3-3H]glucose-specific activity were obtained at 10-min intervals throughout infusions. Finally, at t = 240, a pancreatic insulin clamp study was initiated and maintained for 2 h. During this procedure, a primed continuous infusion of regular insulin (1 milliunit/kg/min) was administered, and a variable infusion of 25% glucose solution was started at t = 240 and periodically adjusted to maintain plasma glucose concentrations of approximately 7–8 mM. The rate of insulin infusion was designed to replace the plasma insulin concentration at approximately the average basal levels in post-absorptive rats. In order to control for possible effects of the ICV injections on endocrine pancreas, somatostatin (3 µg/kg/min) was co-infused with insulin to inhibit endogenous insulin secretion. At the end of the in vivo studies, rats were anesthetized (pentobarbital 55 mg/kg body weight, intravenously), and tissue samples were freeze-clamped in situ with aluminum tongs pre-cooled in liquid nitrogen. All tissue samples were stored at −80 °C for subsequent analysis.

Analytical Procedures and Calculations—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Plasma insulin and leptin levels were determined by radioimmunoassay (rat leptin radioimmunoassay kit, Linco Research Inc., St. Charles, MO). Serum adiponectin was measured by radioimmunoassay (Linco Research, Inc.). Plasma nonesterified fatty acid concentrations were determined by an enzymatic method using an automated kit according to the manufacturer's specifications (Biodata, Chemical Industries Ltd., Tsukuba, Japan). Hepatic glucose production was measured by a two sequential chromatographic separation as described previously (35, 36). Calculations were performed as described previously (43).

Gene Expression Analysis—Total RNA was isolated from hypothalamus and liver with Trizol (Invitrogen). NPY, Glc-6-Pase, or PEPCK expression was measured by Northern blot analysis. Hypothalamic RNA was analyzed using probes for prepro-NPY and β-actin. To assess the effect of ICV OA on the expression of hepatic enzymes, total RNA was isolated from freeze-clamped liver tissues from rats subjected to insulin clamp studies. Glc-6-Pase and PEPCK cDNA were obtained as described previously (44–46). Probes were labeled with α-32PdCTP by using a random primer kit (Stratagene). Quantification was performed by scanning densitometry, normalizing for β-actin signal and 18 S ribosomal RNA to correct for loading variabilities. Agouti-related protein (AGRP) and pro-opiomelanocortin (POMC) gene expressions were measured by quantitative PCR. Single-stranded cDNA synthesis and real time PCRs with hypothalamic total RNA were performed as described before (25). The following primers were used for the quantitative PCR: agouti-related protein, forward, 5′-CCATGCTGACTGAAACGATC-3′; reverse, 5′-TCGGTCTAGTGGCGACATCAGA-3′; pro-opiomelanocortin, forward, 5′-CCAGGCCAAGGGAGATGAAC-3′; and reverse, 5′-TCAGTGGCCTTTCCTGTC-3′; and β-actin, forward, 5′-TGGAGACCTTCAACACCCCCACC-3′; and reverse, 5′-GAGTACTTGCCGTCAGAAGGAG-3′. The copy number of each transcript was measured against a copy number standard curve of cloned target templates. Expression of each transcript was normalized to the copy number for β-actin. Normalization with the glyceraldehyde phosphohydrogenase copy number yielded similar results (data not shown).

Comparisons between groups were made by analysis of variance, and all values are presented as the mean ± S.E. Specifically, for the feeding data presented in Fig. 1, the two curves for vehicle and oleic acid were first compared by each other by using analysis of variance for repeated measures. If statistical differences were revealed, the differences between each time point were estimated using Student's t test. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

RESULTS

To induce voluntary hyperphagia, male Sprague-Dawley rats were fed a highly palatable diet, ad libitum, for 3 days (Table I). Their food intake and adaptation to the increased caloric content of the diet was monitored daily. Despite hyper-leptinemia and hyperinsulinemia, animals in this group failed to adapt to the enhanced caloric content of the diet and increased their daily energy intake by −70–140 kcal/day. Because central rats underwet the same experimental procedures consumed −80 kcal/day when exposed to standard chow, we also compared the ad libitum fed rats to a group of pair-fed rats receiving 80 kcal/day and to a group of calorie-restricted rats receiving 55 kcal/day of the same highly palatable diet. This approach allowed us to investigate the central effects of OA at three levels of daily caloric intake reflecting moderate caloric restriction, pair-feeding, and overfeeding. It should be noted that data reported below for the pair-fed group are quite similar to those obtained in rats fed standard chow (23). Thus, this experimental approach allowed us to examine whether the central effects of OA on food intake and insulin action are modulated by short term changes in caloric intake.

Voluntary Overfeeding Rapidly Curtails the Effects of Central OA on Feeding Behavior and Hypothalamic NPY and AGRP Expression—We examined whether ICV OA modulates feeding behavior in animals following 3 days of voluntary overfeeding. Food intake was monitored daily in animals fed ad libitum either a high palatable diet (HF140) to induce hyperphagia or a standard chow (SC; caloric intake 78 ± 3 kcal/day) diet for 3 days prior to ICV injections. To examine the effects of macronutrient composition of the diet (e.g. high fat versus high carbohydrate) on OA sensitivity, we also studied an additional group of animals that were allowed free access to a 20% sucrose solution in addition to their standard chow (HS; caloric intake of 93 ± 20% compared with SC animals). After 3 days on their assigned regimen, all animals received a bolus of either OA (30 nmol) or vehicle (HPB) via an indwelling ICV catheter 1 h prior to the onset of the dark cycle. Food intake was monitored for a total of 3 days following ICV injections.
The effects of OA were compared with those elicited by vehicle alone within each experimental group. ICV OA resulted in a 48 and 52% decrease in food intake compared to baseline (Fig. 1, A and B) on the 1st and 2nd day following the ICV injection, respectively. However, after 3 days of moderate overfeeding (HS95), ICV OA decreased daily food intake by 21% on the 1st day and by 17% on the 2nd day (Fig. 1, D and E). Finally, following 3 days of marked voluntary hyperphagia (HF140), ICV OA decreased daily food intake by only 11% on the 1st day and by −2% on the 2nd day (Fig. 1, C and D). The modest decreases observed in the HS95 and HF140 groups were not statistically different from those observed after ICV injection of vehicle alone (Fig. 1, C and D). All groups returned to base-line food intake 3 days after the OA injection (Fig. 1, B–E).

We next examined a potential mechanism by which resistance to hypothalamic OA may develop. We reported previously that hypothalamic NPY mRNA was decreased by −50% after ICV OA compared with ICV vehicle in rats fed standard chow following prolonged (16 h) fasting (23). Here we asked whether changes in the nutritional status modulate the ability of ICV OA to restrain the hypothalamic expression of NPY. To this end, we assessed the abundance of NPY mRNA in the hypothalamus by Northern blot analysis following 3 days of ad libitum feeding (−140 kcal/day) on a highly palatable diet or restricted to 55 kcal/day on the same diet. Both experimental groups were given a single bolus injection of 30 nmol of OA or vehicle (HPB) followed by an overnight fast. Hypothalamic tissue was harvested the following morning, and Northern blot analysis was performed (Fig. 2A). Quantification by densitometry, utilizing β-actin as a reference transcript, demonstrated that the average NPY mRNA levels were similar in HF55 and HF140 (Fig. 2C). ICV OA suppressed NPY mRNA expression in the 55-kcal/day group by −60% (Fig. 2D). This decrease is similar to that reported previously (23) in rats fed standard chow at −70 kcal/day following prolonged fasting. However, ICV OA decreased hypothalamic NPY mRNA by only 28% in fasted animals following 3 days of voluntary overfeeding (Fig. 2, C and D). These data provide evidence that the hyperphagia displayed in animals given free access to a highly palatable diet is partly due to defective regulation of NPY expression in the hypothalamus. To investigate further the potential mechanisms responsible for the OA resistance, we next analyzed the effect of OA on the gene expression of the hypothalamic neuropeptides, agouti-related protein (AGRP) and pro-opiomelanocortin (POMC). Total hypothalami were obtained from ICV vehicle (n = 9) or ICV OA (n = 8) administered animals that were fed either 55 or 140 kcal/day for 3 days. Quantitative analysis of hypothalamic RNA demonstrated that OA suppressed AGRP expression by −75% in animals fed 55 kcal/day as compared with vehicle (Fig. 2D). Conversely, animals fed 140 kcal/day failed to suppress AGRP expression when treated with ICV OA. In fact, the expression of AGRP was −120% of its level in vehicle-infused animals (Fig. 2D). On the other hand, hypothalamic POMC expression was not altered following ICV OA treatment (Fig. 2E). Thus, 140 kcal/day animals displayed marked resistance to the ability of OA to alter AGRP expression in the hypothalamus, and this may partially account for the hyperphagia displayed by these animals.

Voluntary Overfeeding Rapidly Blunts the Effects of Central OA on Insulin Action and Hepatic Glucose Production—We next examined whether short term changes in nutritional status were sufficient to alter the effect of OA on in vivo insulin action (Fig. 3A). The effect of ICV OA on insulin action was assessed in conscious rats using a combination of ICV infusions and pancreatic-epinephrine clamp studies (Fig. 3B) The three experimental groups (HF55, HF80, and HF140) were randomized to receive either ICV OA or vehicle (HPB) treatment (Table I). The basal plasma FFA and glucose concentrations were similar in all groups (Table I). On the day prior to pancreatic-euglycemic clamp studies, all rats received the same amount of calories (55 kcal) to ensure similar post-absorptive states prior to metabolic measurements (Fig. 3A). In the presence of ICV vehicle and near basal levels of circulating insulin, marginal rates of glucose infusion (GIR) were required to maintain euglycemia in the three experimental groups (HF55–2.4, HF80–1.2, and HF140–1.4 mg/kg/min) (Fig. 3C). In contrast, in both the HF55 and HF80 groups, following the ICV infusion of OA (30 nmol), the GIR required to maintain euglycemia was markedly increased (9.55 and 4.64 mg/kg/min, respectively). However, ICV infusion of the same dose of OA failed to increase GIR in the ad libitum fed group. In fact, even the infusion of a 10-fold higher dose of OA (300 nmol) did not significantly increase GIR in this group (Fig. 3C). Because the stimulatory effect of ICV OA on GIR was significantly (−2-fold) higher in the HF55 than in the HF80 group, this increase appears to be highly dependent on the preceding nutritional status of the animal even within the normal to low range of caloric intake. Plasma FFA concentrations did not change during the clamp.
(sucrose), whereas another group was kept on standard chow. All groups were allowed to eat ad libitum, did not significantly modify eating behavior.

To provide base-line values. On day 0, OA (30 nmol) or vehicle (HPB) were injected as an ICV bolus. Daily food intake was monitored for 3 days following injection of ICV OA in animals fed standard chow; however, ICV OA failed to significantly alter feeding behavior in rats receiving high sucrose or high fat chow. Values are mean ± S.E. **, p < 0.001 versus vehicle; **, p < 0.0001 versus vehicle; #, p ≤ 0.05 versus standard chow group; $, ≤0.03 versus standard chow group.

period (Table I) indicating that the metabolic effects induced by ICV OA were initiated by its action within the central nervous system.

The increased requirement for exogenous glucose elicited by the ICV administration of OA in the presence of basal insulin levels could be due to stimulation of glucose uptake and/or to suppression of endogenous glucose production (GP). However, the rates of glucose disappearance (Fig. 3D, Rd) were similar in the three experimental groups, and most important, ICV OA did not significantly modify them. Thus, the increase in whole body insulin action induced by ICV OA in the HF55 and HF80 groups did not reflect an increase in peripheral glucose uptake (Fig. 3D). On the other hand, ICV OA (at 30 nmol) markedly inhibited GP in the HF55 and HF80 groups but not in the ad libitum fed rats (HF140). Indeed, a 10-fold higher central infusion of OA also failed to significantly decrease GP in the ad libitum fed group (Fig. 4A). Consistent with the effect observed on GIR, the inhibition of GP by ICV OA was more pronounced (71% decrease from basal) in the HF55 group than in the HF80 group (~45% decrease from basal) (Fig. 4B). Finally, the inhibition of GP induced by ICV OA entirely accounted for its stimulation of GIR. To examine further the impact of the nutritional status in modulating the effect of ICV OA on metabolic fluxes, we plotted the changes in GP induced by ICV OA or vehicle as a function of percent decreases in body weight (Fig. 4C) and daily food intake (Fig. 4D). The inhibitory effect of ICV OA was directly proportional to the decrease in daily food intake and weight gain. Most important, there was no significant correlation between changes in GP and GIR and changes in food intake/body weight in the groups receiving ICV vehicle (data not shown). A similar correlation with OA-induced inhibition of GP was also found by plotting only rats fed ad libitum the standard chow at various levels of caloric intake (data not shown). Thus, the degree of inhibition of GP in response to central administration of OA appears to be highly dependent on short term changes in caloric intake and/or body weight.

To examine whether central resistance to LCFA-CoAs is also a feature of genetic obesity, we next examined the effect of central OA on insulin action and glucose metabolism in obese Zucker Fatty rats (n = 10). We assessed insulin action by a combination of ICV infusions with pancreatic insulin (3 milliunits/kg/min) clamp studies. In the presence of similar plasma insulin concentrations, the rate of glucose infusion required to maintain the plasma glucose at basal levels was marginal and similar in ICV OA-treated (n = 4) and control rats (n = 6; 0.48 mg/kg/min versus 0.29 mg/kg/min, respectively). Basal rates of glucose production between the two groups were similar. ICV infusion of OA did not significantly alter the rate of glucose
production in either group (ICV OA 11.05 mg/kg/min versus ICV vehicle 11.22 mg/kg/min). Thus, ICV OA failed to modify hepatic insulin action in this obese animal model.

ICV OA Markedly Decreases the in Vivo Flux through Glc-6-Pase—GP represents the net contribution of glucosyl units derived from gluconeogenesis and glycolysis. However, a portion of glucose entering the liver via phosphorylation by glucokinase is also a substrate for de-phosphorylation via Glc-6-Pase. This futile cycle between glucokinase and Glc-6-Pase is commonly named glucose cycling and accounts for the difference between the total glucose output (flux through Glc-6-Pase) and GP (Fig. 5A).

To delineate further the mechanisms responsible for the effect of ICV OA infusion on hepatic glucose production, we estimated the in vivo flux through Glc-6-Pase and the relative contribution of glucose cycling to glucose output (Table II; Fig. 5, B and C). Fig. 5, B and C, depicts the effect of ICV OA and ICV vehicle on the rates of hepatic glucose fluxes during the pancreatic insulin clamp procedure. In the presence of similar plasma insulin concentrations, the rate of glucose production (Fig. 4A) was decreased by ICV OA in the HF55 and HF80 but not in the ad libitum fed rats (HF140). Table II displays the [3H]UDP-glucose, and the [3H]glucose-specific activities used to calculate the contribution of plasma glucose (% direct in Table II) to the hepatic glucose 6-phosphatase pool. These data allowed us to estimate the in vivo fluxes through Glc-6-Pase (Fig. 5B, total glucose output) and the rates of glucose cycling (Fig. 5C) in all groups. As shown in Fig. 5B, ICV OA markedly decreased the flux through Glc-6-Pase in parallel with the effect on GP in HF55 animals. Consistent with this marked decrease in overall glucose output, the rate of glucose cycling was also decreased in rats receiving ICV OA compared with ICV vehicle (Fig. 5C). However, the decline in the rate of glucose cycling was much less pronounced than the decline in Glc-6-Pase flux and indeed it did not achieve statistical significance. The latter finding taken together with the moderate increase in the % direct pathway (Table II) is consistent with a stimulatory effect of central OA on hepatic glucose phosphorylation. Thus, short term central infusion of OA leads to marked decrease in the net de-phosphorylation of glucose 6-phosphate to glucose, which is largely due to diminished in vivo flux through Glc-6-Pase coupled with a moderate stimulation of the in vivo flux through glucokinase. These effects of ICV OA are blunted after 3 days of voluntary overfeeding.

Effect of ICV OA on the Hepatic Expression of Glc-6-Pase and PEPCK—The potent effects of the central administration of OA on hepatic glucose fluxes prompted us to examine whether changes in the liver mRNA levels of key metabolic enzymes can partly account for these effects. Thus, we next measured the liver abundance of Glc-6-Pase and PEPCK mRNA as a function of 18 S ribosomal RNA (Fig. 5D) or β-actin mRNA (not shown) in tissue samples obtained after ICV infusion of either OA or vehicle in calorie-restricted and overfed rats. ICV OA markedly decreased glucose-6-phosphatase gene expression in calorie-restricted rats. Quantification of multiple blots by densitometry (Fig. 5, E and F), utilizing β-actin as a reference transcript, demonstrated that ICV OA suppressed Glc-6-Pase mRNA expression in the HF55 group by ~73% with no changes detected in the HF140 group. Conversely, ICV OA failed to significantly alter the liver expression of PEPCK in either group. The marked inhibitory effect of ICV OA on liver Glc-6-Pase expression is likely to contribute to its potent inhibition of the in vivo flux through glucose-6-phosphatase in calorie-restricted rats.
hypothalamic NPY expression is blunted following 3 days of voluntary overfeeding in rats. Similarly, exposure to a highly palatable diet leading to hyperphagia induces resistance to the anorectic and metabolic effects of leptin and insulin in the hypothalamus in susceptible animal models (6, 16–18). NPY, a potent orexigenic neuropeptide, is a downstream target of both anorectic and metabolic effects of leptin and insulin in the hypothalamus (26). In this regard, the well established biochemical link between cellular carbohydrate and lipid metabolism may play a particularly important role in modulating this nutrient signal (26).

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The development of obesity and DM2 is influenced by a complex interaction of environmental and genetic factors (47–49). We and others have advanced the notion that common central pathways are able to elicit a response to nutrient availability that includes changes in both feeding behavior and metabolic processes (20, 24, 25, 43, 50–53). In this regard, nutrient-dependent signals (e.g., leptin and insulin) convey the nutritional status of an organism to the hypothalamus, where messages are integrated and transduced into efferent signals that are aimed to limit the input of exogenous and endogenous nutrients into the circulation. Furthermore, it has been postulated recently that lipid metabolism within selective hypothalamic neurons is a primary biochemical sensor for nutrient availability, which in turn exerts a negative feedback on food intake (21, 23, 26) and endogenous GP (23, 26). In fact, central administration of fatty-acid synthase inhibitors (21), of carnitine palmitoyltransferase-1 (CPT-1) antagonists (26), or of the LCFA oleic acid (23) leads to decreased food intake (22, 24, 26) and inhibition of endogenous glucose production (24, 26). Based on these findings, we have postulated that a common effect of these central anorectic agents is to increase the cellular concentration of LCFA-CoAs within the arcuate nuclei of the hypothalamus (26). Although it remains to be determined whether circulating lipids can generate a similar increase in hypothalamic LCFA-CoAs, it is reasonable to postulate that such an effect may in turn take part in a negative feedback system that is intended to regulate the amount of nutrients in the circulation in response to changes in their availability (26).

Here we report that the ability of a central administration of the long-chain fatty acid oleic acid to inhibit food intake and hypothalamic NPY expression is blunted following 3 days of voluntary overfeeding in rats. Similarly, exposure to a highly palatable diet leading to hyperphagia induces resistance to the anorectic and metabolic effects of leptin and insulin in the hypothalamus in susceptible animal models (6, 16–18). NPY, a potent orexigenic neuropeptide, is a downstream target of both anorectic and metabolic effects of leptin and insulin in the hypothalamus (26). In this regard, the well established biochemical link between cellular carbohydrate and lipid metabolism may play a particularly important role in modulating this nutrient signal (26).

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FIG. 3. The effect of ICV OA on whole body insulin action is modulated by daily caloric intake. A, schematic representation of the experimental design for the pancreatic insulin clamp studies. Surgical implantation of ICV catheters was performed at least 2 weeks before diet interventions in order to allow time for adequate recovery. Similarly the implantation of intravascular catheters was completed 4 days before diet interventions. On day 0, rats were administered high fat chow at 55 or 80 kcal/day or ad libitum (~140 kcal/day) for 3 days. On the evening preceding the clamp procedures, all rats received a fixed portion of food (55 kcal) to ensure that they were in a comparable post-absorptive state at the start of the metabolic experiments. B, pancreatic insulin clamp procedure. ICV OA or vehicle infusions were initiated at the beginning of the study (t = 0) and lasted throughout the duration of the entire clamp procedure. Infusion of labeled glucose began at t = 120 and was continued for the last 4 h of the study. Finally, the infusions of somatostatin and insulin were initiated at t = 240 and lasted for the remaining 2 h. A 25% glucose solution was infused as needed to prevent any decline in the plasma glucose concentration. C, GIR during pancreatic insulin clamp studies. In the presence of ICV injection of HPB (■) and near basal insulin concentrations, GIR was negligible in all groups. However, during ICV injection of OA (□), exogenous glucose at the rate of ~4.5 and 9 mg/kg/min was required to prevent hypoglycemia in the groups receiving 55- and 80 kcal/day, respectively. In the group fed ad libitum (140), GIR was less than 2 mg/kg/min in the presence of ICV vehicle, low dose (30 nmol, □) and high dose (300 nmol, □) OA infusion. D, the rate of glucose disappearance (Rd) during the pancreatic insulin clamp period. The rate of glucose disappearance was not significantly affected by ICV OA in any of the experimental groups. **, p = 0.0001 versus vehicle infusion; *, p < 0.03 versus vehicle infusion.
may be able to respond to increased availability of either lipids or carbohydrates. It is therefore possible that the sustained increase in cellular LCFA-CoAs leads to adaptive changes in cellular lipid metabolism (e.g., inhibition of ACC leading to decreased formation of malonyl-CoA). In keeping with this postulate, we found that increasing the daily caloric intake via either a high fat or a high sucrose diet similarly blunted the effect of ICV OA on food intake, suggesting that changes in daily caloric intake rather than in macronutrient composition of the diet are likely to account for this effect. In this regard, it is intriguing that the anorectic effects of the fatty-acid synthase inhibitor C75 are preserved in diet-induced and genetic obesity in mice (54). By contrast, the poor response to ICV OA in overfed rats may be secondary to decreased esterification of LCFA due to decreased activity of acyl-CoA synthase or increased activity of acyl-thiosterases and/or accelerated metabolism of LCFA-CoAs due to increased activity of CPT-1 or depletion of malonyl-CoA. As in other models of diet-induced obesity (6, 16–18), the lack of response to an anorectic agent may indicate impaired action (of oleic acid) on feeding behavior and/or the inability to counteract the high palatability of the high fat and high sucrose diets.

Why does a short term increase in food intake lead to impaired hypothalamic response? Perhaps this is an attempt to promote efficient energy storage as an “adaptive” response to the increased availability of food. This mechanism may have developed as a result of evolutionary pressure in keeping with the thrifty genotype hypothesis by Neel (17). It is of interest that a similar paradoxical adaptation to overfeeding has also been demonstrated for a peripheral nutrient-sensing pathway whose stimulation appears to decrease mitochondrial function and energy expenditure in response to increased nutrient availability (22).

Recent studies on the metabolic effects of the central administration of insulin (24), leptin (36), melanocortins (55), and free fatty acids support the notion that these central hypothalamic pathways are also involved in the regulation of hepatic glucose output and insulin action. Because glucose production by the liver is the major source of endogenous fuel, we have postulated that central neural circuitries concomitantly modulate exogenous and endogenous sources of energy in keeping with a negative feedback system designed to monitor and regulate the input of nutrients in the circulation (23). Circulating fatty acids are mostly bound to albumin and cross the blood–brain barrier mainly by simple diffusion in the unbound form. Unbound fatty acids can also be derived via hydrolysis of lipoproteins by lipoprotein lipase within blood or at the cerebral capillary bed. Thus, chylomicrons are likely to be a major circulating source of brain fatty acids in the post-meal state, although a combination of unbound fatty acids and locally hydrolyzed lipoproteins contribute to the brain fatty acid pool in the fasting state. A small portion of fatty acid entry into the brain may also occur via direct uptake of lipoprotein particles mediated by lipoprotein receptors in the luminal surface of the cerebrovascular endothelium (56, 57). Overall, the access of circulating free fatty acids to the central nervous system is generally proportional to their plasma concentration (58, 59), and their concentration in cerebral spinal fluid is ~6% of plasma concentration in fasted anesthetized dog (60). Thus, whereas one cannot simply extrapolate the effects of ICV oleic...
acid to physiological conditions, our findings raise the possibility that nutritionally induced changes in the potent behavioral and metabolic effects of fatty acids within the hypothalamus can contribute to the regulation of both energy balance and insulin action. On the other hand, it has long been recognized that changes in caloric intake also have a dramatic impact on the actions of insulin on glucose metabolism (61). In this regard, hepatic insulin resistance develops within days and/or a few weeks following overfeeding in animals and humans (62).

Here we report a strong correlation between the inhibition of GP induced by ICV OA and the nutritional status of the animal (i.e., body weight/caloric intake). In the presence of basal insulin levels, stimulation of hypothalamic insulin signaling (24) or central administration of OA (23) leads to inhibition of endogenous glucose production. This “insulin-like” central effect of LCFA appears to be at odds with their well-established actions in the peripheral tissues (63–65). For instance, elevated circulating and hepatic FFAs levels reduce insulin suppression of endogenous glucose production (i.e., induces hepatic insulin resistance), whereas elevated levels of central fatty acids enhance the suppression of endogenous glucose production, even in the presence of basal insulin. Similarly, increased availability of fatty acids induces the expression of Glc-6-Pase in the liver (66), whereas the central administration of OA decreases the hepatic expression of the same enzyme. It is conceivable that central effects of fatty acids provide an important restraint on their peripheral action on hepatic glucose fluxes. Because the central administration of OA failed to inhibit GP in overfed rats, we postulate that the inability of OA to inhibit hepatic glucose production and Glc-6-Pase expression leaves the peripheral effects of fatty acids unopposed by their central effects. It is therefore conceivable that a lack of response to LCFA in the hypothalamus leads to increased rate of glucose output and may contribute to the hepatic insulin resistance observed in this model.

This hypothalamic nutrient sensing may also play a role in fuel partitioning. Under normal conditions, increased availability of fatty acids in the hypothalamus results in decreased output of glucose from the liver (23, 26) in order to promote the preferential utilization of lipid in muscle and other peripheral tissues. However, when the increased availability of fatty acids is sustained, it triggers the activation of “thrifty” metabolic mechanisms designed to promote the efficient flux of lipid into energy storage (triglyceride) sites. The increased production of

### Table II

| ICV | HF55 | HF140 |
|-----|------|-------|
| Vehicle | OA | Vehicle | OA |
| UDP-Glc | 6.8 ± 1.0 | 7.4 ± 2.0 | 9.7 ± 1.1 | 10.0 ± 1.4 |
| Liver SA (dpm/nmol) | 55.8 ± 5.4 | 41.2 ± 4.2 | 58.2 ± 3.7 | 54.5 ± 3.1 |
| [%] Direct | 12 ± 3 | 17 ± 4 | 17 ± 3 | 18 ± 2 |
glucose from the liver in overfed rats may serve this goal by providing alternative fuel for oxidation.

The downstream mechanism(s) by which OA modulates hepatic glucose fluxes has yet to be delineated. However, the marked decrease in both in vivo flux through glucose-6-phosphatase and in the hepatic expression of the glucose-6-phosphatase catalytic subunit induced by ICV OA is likely to play a key role. How does lipid sensing within the hypothalamus modulate hepatic glucose fluxes and gene expression? It is likely that rapid changes in autonomic nervous system outflow play a leading role.

The downstream mechanism(s) by which OA modulates hepatic glucose fluxes and gene expression is likely to play a leading role. ICV leptin administration increases autonomic outflow in various regional sites (36). It is well known that both sympathetic and parasympathetic systems provide direct innervation of the liver, pancreas, and adipose tissue (via the splanchnic nerve and vagus nerve, respectively). Indeed, ventromedial hypothalamic lesions lead to acute and chronic hyperinsulinemia, and this can be reversed by subdiaphragmatic vagotomy (68, 69). Electrical stimulation of the lateral hypothalamus, on the other hand, fails to increase insulin secretion or change plasma glucagon concentration (68–70). However, it should be pointed out that all present studies were performed in the presence of pancreatic clamp conditions. Thus, it is not likely that changes in the levels of these pancreatic hormones can account for the effects of ICV OA on hepatic glucose fluxes. Of note, electrical stimulation of the ventromedial hypothalami causes an increase in the activity of PEPCK and Glic-6-Pase, key gluconeogenic enzymes, and a marked suppression of pyruvate kinase, a key glycolytic enzyme in rat liver (69, 71). Stimulation of the lateral hypothalamus, on the other hand, leads to a decrease in PEPCK activity. Finally, various adipose depot sizes receive input from autonomic nervous system. The latter has been shown in turn to regulate the gene expression and secretion of fat-derived hormones and cytokines (reviewed in Ref. 1), which have potent effects on insulin action (44, 67). To this end, the effect of ICV OA on liver glucose-6-phosphatase expression and on hepatic glucose fluxes is reminiscent of those induced by infusion of recombinant adiponectin in mice (44). However, we did not detect changes in plasma leptin and plasma adiponectin (Table I) levels during the ICV injections in any of the experimental groups.

In conclusion, we have shown that the central effects of long-chain fatty acids in diet-induced obesity are nutritionally regulated. Under normal circumstances biological responses to long-chain fatty acids on food intake and GP are nutritionally regulated. Under normal circumstances biological responses to long-chain fatty acids on food intake and GP are nutritionally regulated. Under normal circumstances biological responses to long-chain fatty acids on food intake and GP are nutritionally regulated.
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