Leptin Receptor–Deficient Obese Zucker Rats Reduce Their Food Intake in Response to a Systemic Supply of Calories From Glucose

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It has been established that leptin exerts a negative control on food intake, allowing one to maintain stable caloric intake over time. The aim of the present study was to investigate whether leptin regulates food intake when a supply of calories is provided by the systemic route. Experiments were carried out in leptin receptor–deficient obese *fa/fa* rats and lean *Fa/fa* controls. In both groups, 48 h of glucose infusion reduced food intake in proportion to caloric supply, resulting in virtually no change in total caloric intake as compared to before the infusion. This hypophagic response was reproduced without adding systemic calories, but by increasing glucose and insulin concentrations specifically in the brain through carotid artery infusion. Concomitant intracerebroventricular administration of 5-(tetradecyloxy)-2-furoic acid, an acetyl CoA carboxylase inhibitor that precludes malonyl-CoA synthesis, abolished the restriction of feeding in carotid-infused lean and obese rats. These data indicate that a supply of calories via glucose infusion induces a hypophagic response independent of leptin signaling in the rat, and support the hypothesis that a rise in central malonyl-CoA, triggered by increased glucose and insulin concentrations, participates in this adaptation. This process could contribute to the limiting of hyperphagia, primarily when leptin signaling is altered, as in the obese state. *Diabetes* 52:277–282, 2003

A stable body weight over time requires that caloric intake closely match energy expenditure. When excess calories are taken in, a state of positive energy balance occurs, resulting in weight gain. Tightly controlled food intake is a crucial component of energy homeostasis. This is demonstrated experimentally in rats submitted to overfeeding either by direct gastric loading (1,2) or by systemic infusion of glucose (3–5). In both situations, animals spontaneously reduce their food intake to avoid a drift in total caloric intake. A lack of adaptation to calorie overload might participate in the development of obesity. Indeed, when obesity-prone SD rats have free access to palatable food, they increase their daily food intake and fail to adapt to enhanced caloric intake (6). In this model, hyperphagia occurs despite increased circulating levels of leptin, an adipose-derived hormone that negatively controls food intake (rev. in 7). Thus, the mechanisms driving the regulatory reduction of food consumption appear to be blunted in these hyperphagic rats; part of this defect might rely on leptin resistance.

A lack of leptin response is well described in obese Zucker rats (8), which bear a mutation (*fa*) in the leptin receptor gene (9,10). We used this rat model to address the following issues: Does a systemic supply of calories from glucose result in a decrease in food intake in the absence of leptin signaling? If so, what mechanisms are involved? In the first series of studies, age-matched obese *fa/fa* and lean *Fa/fa* rats were submitted to systemic glucose infusion with variable caloric input. In a second series of studies, infusions were performed through the carotid artery to increase glucose concentration specifically within the brain, with no systemic supply of calories. In both protocols, daily food intake was monitored throughout the infusion period. Finally, in an attempt to investigate the molecular mechanisms involved in the feeding response to glucose infusion, we tested a possible role of central malonyl-CoA, based on the recent proposal that this intermediate of the fatty acid synthetic pathway exerts a leptin-independent anorexic effect in mice (11).

RESEARCH DESIGN AND METHODS

Animals. Animal studies were conducted according to the French Guidelines for the Care and Use of Experimental Animals. Male lean (*Fa/fa*) and obese (*fa/fa*) rats of the Zucker strain, ~2 months old, weighing 180–220 g and 250–330 g, respectively, and bred in our animal facility were used. They had free access to water and standard laboratory diet pellets (A04; UAR, Villemoisson-sur Orge, France) and were housed under conditions of controlled temperature (23°C) and light (light from 7:00 A.M. to 7:00 P.M.).

Systemic and central infusions. The long-term infusion technique under unrestrained conditions was used, as previously described (12,13). Briefly, 3 days before the experiments were begun, rats were anesthetized with ketamine (125 mg/100 g of body weight intraperitoneally) for the placement of a catheter in the jugular vein (systemic infusion) or in the carotid artery toward the brain (central infusion). In both cases, the catheters were exteriorized at the vertex of the head and attached to a swiveling infusion device, allowing the animal free access to water and diet.

Hyperglycemia, hyperinsulinemia protocol. Hyperglycemia (HG) and hyperinsulinemia (HI) were obtained by infusing glucose (30% wt/vol providing 1.2 cal/μl; Chaix et Du Marais, Paris, France) through the jugular vein. The infusion rate was set at 20 $\mu l \cdot min^{-1} \cdot 100\ g^{-1}$ of body weight in lean rats. Obese rats were administered glucose at half the infusion rate of lean rats (10 $\mu l \cdot min^{-1} \cdot 100\ g^{-1}$) to achieve a similar level of hyperglycemia in both groups.

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ACC, acetyl CoA carboxylase; EUG, euglycemia; FAS, fatty acid synthase; HG, hyperglycemia; HI, hyperinsulinemia; ICV, intracerebroventricular; TOFA, 5-(tetradecyloxy)-2-furoic acid.

TABLE 1
Systemic concentrations of glucose, insulin, and leptin in lean and obese Zucker rats submitted to glucose infusions

Infusion protocol	Glucose (mmol/l)		Insulin (pmol/l)		Leptin (ng/ml)	
	Lean	Obese	Lean	Obese	Lean	Obese
Systemic HG-HI (n = 9)						
0 h	4.83 ± 0.33	4.44 ± 0.39	570 ± 64.5	2,418 ± 342‡	2.19 ± 0.37	34.8 ± 1.6‡
24 h	21.5 ± 1.78*	18.3 ± 0.88*	3,307 ± 245*	9,990 ± 556*‡	4.14 ± 0.46*	32.9 ± 1.2‡
48 h	19.5 ± 0.83*	16.7 ± 1.17*	3,178 ± 238*	9,432 ± 834*‡	7.65 ± 0.94*	37.4 ± 1.8‡
Systemic EuG-HI (n = 6)						
0 h	4.72 ± 0.11	5.05 ± 0.22	563 ± 71.2	3,132 ± 429‡	1.79 ± 0.33	34.1 ± 1.5‡
24 h	5.83 ± 0.94	5.88 ± 0.50	7,446 ± 1,416*	16,920 ± 3068*§	4.44 ± 0.79†	37.5 ± 1.8‡
48 h	4.33 ± 0.50	5.11 ± 0.17	7,524 ± 1,338*	10,842 ± 2556*	8.26 ± 1.1*	38.1 ± 1.5‡
Central HG-HI (n = 6)						
0 h	5.10 ± 0.33	5.44 ± 0.78	630 ± 72.0	4,428 ± 408‡	2.93 ± 0.42	37.0 ± 3.7‡
24 h	4.66 ± 0.33	5.83 ± 0.67	762 ± 132	3,570 ± 498‡	3.20 ± 0.34	36.7 ± 3.0‡
48 h	5.44 ± 0.28	5.66 ± 0.72	690 ± 54.0	2,940 ± 522‡	3.47 ± 0.44	38.3 ± 2.7‡

Data are means ± SE for the indicated number of rats of each genotype (n). Glucose blood concentrations and plasma insulin and leptin concentrations were measured on blood samples obtained at tail tip before starting the infusions (0 h) and after 24 and 48 h of infusion. Systemic HG-HI, infusion of glucose alone through the jugular vein; systemic EuG-HI, infusion of insulin plus glucose through the jugular vein; central HG-HI, infusion of glucose plus insulin through the carotid artery towards the brain. *$P < 0.01$, †$P < 0.05$ vs. baseline (0 h) by paired t test; ‡$P < 0.01$, §$P < 0.05$ vs. lean rats by unpaired t test.

Euglycemia, hyperinsulinemia protocol. In a second series of experiments, HI and euglycemia (EuG) were obtained by the concomitant infusion of glucose and insulin (Novo Nordisk, Copenhagen, Denmark) through the jugular vein. The rate of insulin infusion was adjusted to induce hyperinsulinemia in the same range as in rats infused with glucose alone. Insulin infusion rates averaged 8.4 and 5.4 pmol · min^{-1} · 100 g^{-1} of body weight in lean and obese rats, respectively. Euglycemia was achieved by glucose infusion at 12 μl · min^{-1} · 100 g^{-1} of body weight in the lean rats, whereas a markedly lower rate of 2 μl · min^{-1} · 100 g^{-1} was sufficient to maintain normoglycemia in the obese rats.

Central infusion. Infusions were performed through a catheter inserted in the carotid artery, with the tip being directed toward the brain. Insulin and glucose were infused, either alone or combined, at a rate of 2.5 pmol/min and 0.50 mg/min, respectively, in both groups of rats. In preliminary experiments, it was determined that these were the highest rates of infusion possible that did not elevate the systemic concentrations of glucose or insulin. The flow rate for carotid infusion was set at 7 μl/min.

Intracerebroventricular administration of TOFA. Some rats submitted to central infusion were concomitantly administered 5-(tetradecyloxy)-2-furoic acid (TOFA; Merck Sharp & Dohme-Chibret, Rahway, NJ). Before insertion of the carotid catheter, the rats were stereotactically implanted with a chronic stainless steel cannula in the right lateral cerebral ventricle, using the following coordinates from Bregma: anterior-posterior, −0.8 mm; dorsal-ventral, −3.5 mm; and medial-lateral, −1.5 mm. The cannula was connected via a polyethylene catheter to a subcutaneous osmotic minipump (Alza Corporation, Palo Alto, CA) filled with either TOFA at a concentration of 20 mg/ml in DMSO (Sigma, St Louis; MO) or vehicle. Rats received 10 μg/h of the compound, starting at the time of insertion of the minipump (3 days before carotid infusion).

Food intake and blood sampling. Daily food intake was measured by weighing the pellets between 9:00 and 10:00 A.M. A basal rate was determined for each rat the day before starting the infusion. Food intake was then measured after 24 or 48 h or longer, as indicated. In some experiments, food intake was determined during the 3 days of recovery from surgery. Caloric intake from food was calculated on the basis of 3 kcal/g of pellet, according to the manufacturer's specifications. Arteriovenous blood samples were obtained from the tail vessels. Plasma insulin and leptin levels were measured by radioimmunoassay using commercial kits from CIS Bio International (Gif sur Yvette, France) and Linco Research (St Louis, MO), respectively. Blood glucose was determined by a glucose analyzer (Glucotrend; Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. Statistical analysis was performed using Student's t test for paired samples when the effect of infusion was tested in the same rat or for unpaired samples when comparing different groups, as indicated in the figure and table legends. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Blood parameters. Characteristics of the rats used in this study are given in Table 1. The obese *fa/fa* rats were hyperinsulinemic, but normoglycemic, thus displaying an insulin-resistant state without overt diabetes, as has been previously described in this model (14). They were also markedly hyperleptinemic. Glucose infusion via the jugular vein (HG-HI protocol) resulted in a rapid rise in the levels of glucose (approximately fourfold) and insulin (approximately four to fivefold). Both concentrations remained steady over the time interval 4–48 h in lean and obese rats. Concomitantly, there was a rise in plasma leptin in the lean rats, to levels two- to fourfold higher than baseline. By contrast, leptinemia was unchanged in glucose-infused obese rats. In the EuG-HI protocol, plasma insulin levels increased within 4 h and remained elevated in both groups, with euglycemia readily maintained throughout the infusion period. In lean rats, circulating leptin exhibited a pattern strikingly similar to that observed in rats infused with glucose alone. Again, plasma leptin concentrations remained unchanged in the obese rats. During saline infusion (NaCl 0.9%), glucose, insulin, and leptin systemic levels were stable in both groups of rats (data not shown).

Caloric intake during HG-HI and EuG-HI infusions. Hyperphagia is a prominent feature of obese Zucker rats, as was shown by the greater amounts of caloric intake from food as compared to lean rats (31.4 ± 0.77 vs. 25.4 ± 1.00 kcal · 100 g^{-1} · 24 h^{-1}; n = 9; $P < 0.001$). In the lean rats, 24 h of systemic glucose infusion induced a marked reduction of food intake that persisted throughout the infusion period (Fig. 1). Over the 48-h period, food intake averaged 37.5 ± 2.6 and 15.4 ± 1.6% of basal values in EuG-HI− and HG-HI−infused rats, respectively. In the obese group, food intake was also reduced, but at a less pronounced rate than in lean rats. Caloric intake from food was decreased to 50.4 ± 2.8% of baseline in response

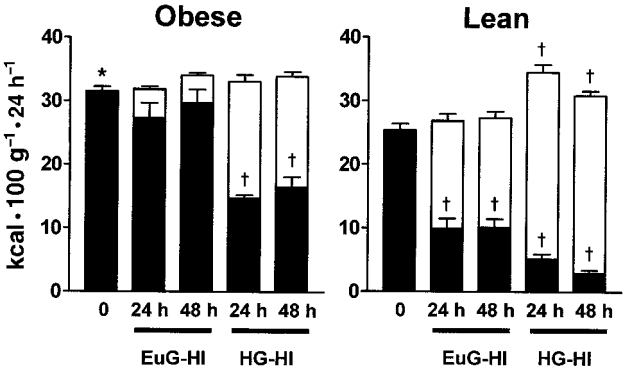

FIG. 1. Caloric intake in lean and obese Zucker rats in response to systemic infusion of glucose alone (HG-HI protocol) or glucose with insulin (EuG-HI protocol). Daily food intake was measured between 9:00 and 10:00 A.M. before starting the infusion to determine basal caloric intake for each rat (0) and again after 24 and 48 h of infusion, as indicated. Variable amounts of glucose were infused, depending on the infusion protocol and genotype. The caloric intake from food (■) was calculated on the basis of 3 kcal/g of pellet. The calories supplied by glucose (□) were calculated according to infusion rates, given that the 30% wt/vol solution of glucose infused provides 1.2 cal/μl. Both bars are added to display the total caloric intake during infusion. Data are means ± SE for nine (HG-HI) and six (EuG-HI) rats of each genotype. *P < 0.01 vs. lean by unpaired t test; †P < 0.01 vs. basal caloric intake by paired t test.

to HG-HI infusion, and by <10% in response to EuG-HI infusion in obese rats. Although variable amounts of calories were provided by the glucose infusion, the total caloric intake from glucose plus pellet was not significantly different from caloric intake before infusion, except in HG-HI−infused lean rats, where it was slightly increased (Fig. 1). These data indicate that both lean and obese rats were able to maintain stable caloric intake during glucose infusion by reducing their food intake in a dosage-dependent manner with the amount of calories supplied by glucose. When individual values of caloric intake from pellets were plotted as a function of caloric input from glucose, all data fit on an exponential curve (r^2 = 0.91), thereby demonstrating that the greater the supply of systemic calories, the lower the food intake (Fig. 2).

Food intake in response to central infusion. During HG-HI infusion, systemic insulin and glucose levels are increased. We sought to determine whether elevation of insulin and/or glucose specifically within the brain could mimic the anorectic effect of systemic glucose infusion.

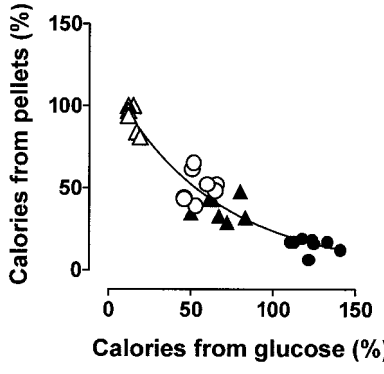

FIG. 2. Inverse relation between the caloric intake from food and glucose in lean (filled symbols) and obese (open symbols) Zucker rats submitted to HG-HI (circles) or EuG-HI (triangles) infusion for 48 h. Data are expressed as percent of basal caloric intake. Individual values best fitted an exponential curve (r^2 = 0.91).

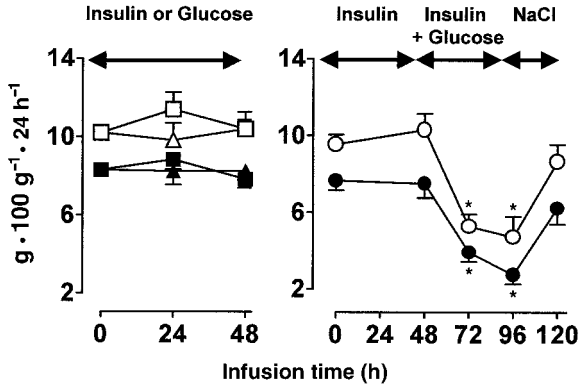

FIG. 3. Food intake in response to central infusion of glucose (squares) or insulin (triangles) and in response to successive central infusion of insulin, insulin plus glucose, and NaCl (circles) in lean (filled symbols) and obese (open symbols) Zucker rats. Data are means ± SE for six rats of each genotype. *P < 0.01 vs. basal food intake by paired t test. .

Infusions were performed via the carotid artery. Systemic concentrations of glucose, insulin, and leptin were unchanged (Table 1). Neither glucose nor insulin, when infused alone, significantly affected the rate of food intake (Fig. 3). By contrast, when glucose and insulin were combined, food intake dropped by ~50% in both lean and obese rats. This response was fully reversible; when the infusion was switched to saline, food intake returned to baseline within 24 h (Fig. 3).

Effect of intracerebroventricular administration of TOFA. It has been recently proposed that increasing the intracellular pool of malonyl-CoA in the brain generates a satiety signal (11). To test whether this mechanism could account for the reduction of food intake elicited by carotid infusion of glucose plus insulin, we sought to block the activity of acetyl CoA carboxylase (ACC) using TOFA, an allosteric inhibitor of this enzyme (15,16). Intracerebroventricular (ICV) administration of TOFA started at the time of surgery (3 days before carotid infusion). During this period, TOFA did not significantly affect daily food intake in rats of either genotype (Fig. 4). However, when carotid infusion of glucose and insulin was started to induce hypophagia, rats receiving TOFA maintained a food intake similar to basal, irrespective of genotype (Fig. 4). As shown in lean rats, vehicle (DMSO) infusion did not preclude the drop in food intake, demonstrating that the reversal of repressed feeding behavior relied specifically on an effect of TOFA.

DISCUSSION

Overconsumption of food is thought to contribute to the current epidemic of obesity. This tenet implies that the physiological mechanisms that should prevent an excess of energy intake over time are defective in obese individuals. The bulk of available evidence indicates that the satiating effect of leptin is altered in the obese state (7), and identifies leptin as a prime participant in the hypophagic response to overfeeding that normally occurs in lean individuals. The present study was designed to test whether food intake can be reduced in the absence of leptin signaling when calories are supplied through systemic glucose infusion. Of note, this experimental approach differs from cafeteria or high-fat feeding, as it

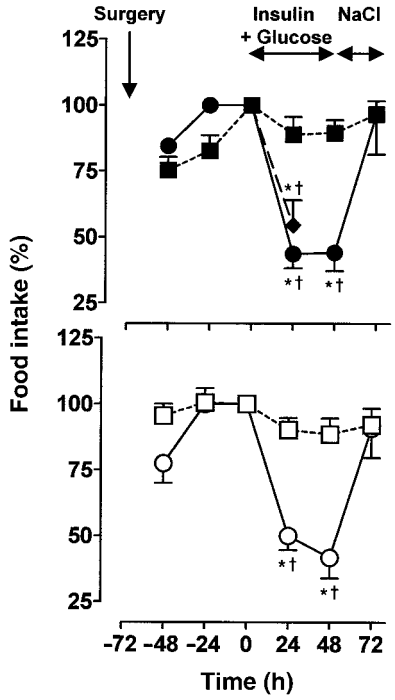

FIG. 4. Food intake in response to successive central infusion of insulin plus glucose and NaCl, without (circles, solid line) or with concomitant ICV administration of TOFA (squares, short dash line) or vehicle DMSO (♦, long dash line), in lean (filled symbols) and obese (open symbols) Zucker rats. TOFA was administered in the right lateral ventricle through an osmotic minipump delivering 10 μg/h, starting 3 days before carotid infusion. Data are expressed as percent of food intake on day 0 of infusion and represent the means ± SE for seven obese and eight lean rats. The effect of DMSO was assessed in three lean rats. Food intake before carotid infusion was measured in three rats of each genotype. The rate of food intake on day 0 in lean and obese rats was 7.40 ± 0.40 and 8.97 ± 0.32 g · 100 g^{-1} · 24 h^{-1} without TOFA, 7.40 ± 0.53 and 8.81 ± 0.62 g · 100 g^{-1} · 24 h^{-1} with TOFA, respectively, and 7.76 ± 1.27 · 100 g^{-1} · 24 h^{-1} in lean rats infused with DMSO. *P < 0.01 vs. basal food intake by paired t test; †P < 0.01 vs. TOFA by unpaired t test.

bypasses the digestive tract and the hedonic control of food intake. Rats received a supply of calories in variable amounts depending on the infusion protocol. More calories were provided during HG-HI infusion than during EuG-HI infusion because in the EuG-HI protocol, the rate of glucose infusion compensates for glucose utilization, whereas in the HG-HI protocol, higher rates of infusion are required to induce hyperglycemia.

A salient finding of the present study was that obese *fa/fa* rats decreased their food consumption while receiving calories from systemic glucose infusion, despite the absence of leptin signaling. At first glance, their reduction in food intake appeared to be less pronounced than that of lean rats. However, this difference was directly related to the amount of calories provided, given that the rates of glucose infusion required to achieve the same level of glycemia as in lean rats were systematically lower in insulin-resistant obese rats. Irrespective of genotype, total caloric intake (i.e., calories from food plus glucose) remained within the same range as that measured in the basal state (Fig. 1). Thus, when a supply of calories was experimentally imposed through the systemic route, lean and obese rats were capable of adjusting their food intake to meet their basal caloric requirements. This was further illustrated by the inverse relation between caloric intake from food and calories supplied by glucose (Fig. 2). In a situation where ~50% of the basal caloric intake was provided by glucose (i.e., HG-HI–infused obese rats and EuG-HI–infused lean rats values), rats reduced their food intake by half. These data clearly demonstrated that the calories supplied by systemic infusion elicited a signal independent of leptin, which participates in the regulation of energy intake. This finding was in keeping with the results of our previous study showing that long-term systemic lipid infusion reduces food intake by ~35% in normal rats (17). The similar response in the current study suggests that the signal(s) that inhibits feeding arises from the amount of calories rather than from the nutrient itself (glucose or free fatty acids). Alternatively, glucose and lipids may decrease food intake by distinct mechanisms. The question is whether leptin-resistant obese rats are able to adapt their food intake to a systemic supply of calories from lipids, as they do in response to calories from glucose.

By inducing a marked reduction in food intake of up to 85%, systemic glucose infusion in the lean rats mimicked a fasting state in some aspects. However, in contrast to fasted rats (18), circulating leptin was not decreased, but, instead, was consistently increased in glucose-infused rats. Several systemic factors that are decreased in fasting but increased in glucose-infused rats could account for the enhanced adipose leptin production. These include hyperinsulinemia (19–21), enhanced energy flux and glucose metabolism within adipose cells (22,23), and/or the elevation of uridine diphosphate-*N*-acetylglucosamine, the end product of the hexosamine biosynthetic pathway, which has been recently proposed as a link between availability of nutrients and leptin expression (24). The physiological relevance of elevated leptin concentrations in glucose-infused lean rats is not clear at present. It cannot be excluded that enhanced leptin participates in the reduction of food intake, although the observation that leptin receptor–deficient rats are responsive to the feeding inhibitory effect of glucose argues against this possibility. Interestingly, upregulation of leptin levels does not occur in hyperleptinemic obese rats, indicating that the *fa* mutation alters the regulation of adipose leptin production. In keeping with this idea, dysregulation of the leptin system and increased concentrations early in life in *fa/fa* rats, favor a role for leptin receptors in controlling adipose leptin release (25–28).

In this study, the mechanism mediating the fall in food intake in response to a supply of calories from glucose was investigated. We tested the hypothesis that elevation of blood insulin and/or glucose concentrations within the brain could contribute to this effect. Indeed, it is well established that specific hypothalamic neurons respond to changes in glucose concentration. As in pancreatic β-cells, glucokinase activity, glucose-induced changes in the ATP-to-ADP ratio modulating the activity of ATP-sensitive K^+ channels, as well as the production of NADH through glycolysis have been proposed as components of brain glucose-sensing mechanisms (rev. in 29). There is also compelling evidence that insulin exerts an anorectic effect via its receptors localized in various regions of the central nervous system (30–32). A recent study using an antisense oligodeoxynucleotide directed against the insulin receptor

showed that a selective decrease of insulin receptor protein within cells located in the arcuate nucleus of the hypothalamus is sufficient to produce this anorectic effect (33). In the current study, in rats infused through the carotid artery, neither glucose nor insulin when infused alone was sufficient to restrain feeding. In contrast, glucose produced a potent anorectic effect when infused with insulin, both in leptin-sensitive and leptin-unresponsive rats (Fig. 3). Therefore, this approach reproduces the feeding inhibitory signal induced by systemic glucose in the absence of a caloric supply, and unmasks a mechanism of control of food intake that is responsive to increased blood glucose and insulin concentrations in the brain and does not require intact leptin signaling.

In a recent work, it was postulated that central malonyl-CoA might be linked to feeding control in mice (11). This proposal arose from the observation that ICV administration of inhibitors of fatty acid synthase (FAS), an enzyme that converts malonyl-CoA into fatty acids, markedly inhibited feeding. Moreover, ICV administration of TOFA, which inhibits the enzyme (ACC) responsible for synthesizing malonyl-CoA, attenuated the anorectic effect of FAS inhibitor. Thus, although malonyl-CoA was not directly measured, the opposite effects of these two types of inhibitors strongly support the hypothesis that elevation of malonyl-CoA in yet unidentified region(s) of the brain reduces food intake. These observations prompted us to assess whether this mechanism could operate in glucose-infused rats. In our experimental conditions, ICV administration of TOFA did not alter food intake, unless the rat was challenged by carotid infusion of glucose plus insulin (Fig. 4), when the fall in food intake was fully prevented in the rats receiving TOFA. Although not a direct proof that glucose fuels the malonyl-CoA pathway in the brain, it can be assumed that TOFA acted by precluding the rise in central malonyl-CoA that would result from enhanced intracellular flux of glucose through the lipogenic pathway. Because increasing insulin concentration is required for this effect, it is likely that this process occurred in a subset of neurons where glucose uptake was sensitive to insulin. In support of this possibility, selective neurons of the hypothalamus have been shown to express the insulin-sensitive glucose transporter GLUT4 (34–36). How a change in the central pool of malonyl-CoA influences food intake is yet to be determined. It has been recently proposed that accumulation of long-chain fatty acids might generate an anorectic signal (37). It is possible that this mechanism accounts for the fall in food intake in glucose-infused rats. Indeed, because TOFA blocks an early step in the biosynthetic pathway of fatty acids from glucose, the reversal of repressed food intake caused by this compound could be accounted for by inhibition of the production of either malonyl-CoA or downstream products.

In conclusion, this study provided experimental evidence that an increase in the supply of calories via glucose infusion induces a hypophagic response independent of leptin signaling in the rat. Moreover, our data favor the hypothesis that a rise in central malonyl-CoA triggered by elevation of glucose and insulin concentrations within the brain participates in this regulatory process. Increased production of malonyl-CoA and/or downstream metabolites of the lipogenic pathway might serve as signals triggering an adaptation in feeding behavior in response to energy supply. Because this process operates in the obese Zucker rat, it could limit hyperphagia primarily when leptin signaling is altered, as in the obese state.

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