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Activation of the Retinoid X Receptor Suppresses Appetite in the Rat

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The retinoid X receptor (RXR), a ubiquitously expressed intracellular receptor, regulates pathways controlling glucose, triglycerides, cholesterol, and bile acid metabolism. In addition to its role in those metabolic pathways, we reported that RXR activation with a pan agonist [*e.g.* LG100268 (LG268)] decreases both body weight gain (BWG) and food consumption (FC) in obese, insulin-resistant rodents. In parallel with those changes in energy balance, we show here that activation of RXR pathways results in adipose tissue remodeling, particularly within sc fat where the rate of apoptosis is increased 5-fold. This change may underlie the selective decrease in fat mass observed in Zucker fatty rats treated with LG268 for 6 wk. Because FC is strongly correlated with BWG in treated animals, we hypothesized that regulation of FC might be the primary mechanism underlying reduced BWG during RXR agonist administration. Importantly, decreased FC is due to decreased meal size, suggestive of induced satiety rather than malaise and/or aversion to food. Furthermore, administration of LG268 directly into the brain via intracerebroventricular injection also reduces FC, BWG, and insulin, whereas the elevation in triglycerides observed after oral administration is absent. The latter observation suggests that RXR actions on energy balance and lipid homeostasis are separable. Therefore, ligand-mediated activation of either an RXR homodimer or an unidentified heterodimeric complex regulates pathways controlling energy balance at least in part via a central nervous system-mediated mechanism. (*Endocrinology* 145: 565–573, 2004)

TOGETHER WITH ITS receptor partners, the retinoid X receptor (RXR) has been shown to control numerous key homeostatic pathways. A ubiquitously expressed intracellular receptor, RXR functions as a transcriptional regulator while heterodimerized with other members of the nuclear hormone receptor supergene family. Among the receptors that regulate transcription as obligate heterodimers with RXR are the peroxisome proliferator-activated receptors (PPARs), PPARα, PPARγ, and PPARδ; the farnesoid X receptor (FXR); and the liver X receptors (LXRs), LXRα and LXRβ. These receptors play major roles in glucose (PPARγ), triglycerides (PPARα), cholesterol (PPARδ, LXR), and bile acid (FXR) metabolism (1, 2). In addition to its role in those metabolic pathways, we have reported that RXR activation decreases both body weight gain (BWG) and food consumption (FC) in obese, insulin-resistant rodents (3, 4). Here we further describe the role that RXR plays in energy balance and investigate the mechanisms underlying these novel activities of RXR pathways. We have chosen an *in vivo* pharmacological approach, using LG100268 (LG268), a potent RXR agonist that activates all three receptor subtypes (α, β, and γ) in cotransfection experiments (5–7). Using Zucker fatty rats, our experiments confirm the insulin-sensitizing actions of LG268 as well as its antiobesity activity. Furthermore, we demonstrate that RXR activation both remodels adipose tissue and acts within the central nervous system (CNS) to suppress feeding.

Abbreviations: BWG, Body weight gain; CNS, central nervous system; FC, food consumption; FXR, farnesoid X receptor; icv, intracerebroventricular; LG268, LG100268; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TZD, thiazolidinedione.

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Materials and Methods

Animals and treatments

Ligand's Institutional Animal Care and Use Committee approved all *in vivo* procedures. Seven- to eight-week-old female Zucker fatty rats (*fa/fa*; Harlan, Indianapolis, IN) were housed individually on a 12-h light:12-h dark light cycle (lights on at 0600 h) with food (Purina 5008) and tap water continuously available. Blood samples were obtained from the tail vein 3 h after dosing on the indicated days. For chronic treatment, rats were dosed daily by oral gavage with vehicle, BRL 49653 (rosiglitazone, 3 mg/kg) or LG268 (3, 10, 30, or 100 mg/kg). We chose to use BRL 49653 as a reference; it is a thiazolidinedione (TZD) PPARγ agonist that is a potent insulin sensitizer in both rodents and type 2 diabetic patients (8–10). For clarity and brevity, data from fatty rats dosed with 30 mg/kg of LG268, the threshold dose for effects on BWG and FC in this experiment are shown in Fig. 1. Carcass composition was determined by gravimetric techniques after lipid extraction (11).

Compounds administered orally were suspended in an aqueous vehicle (1% carboxymethylcellulose/0.25% Tween 80 or 9.95% polyethylene glycol/1% carboxymethylcellulose/0.05% Tween 80) for daily dosing by gavage. No vehicle-dependent differences in the potency or efficacy of either LG268 or BRL 49653 were observed.

To test the effects of central administration of LG268, indwelling intracerebroventricular (icv) cannulae, aimed at the right lateral ventricle (12), were implanted under isoflurane anesthesia 7–10 d before experimentation. Animals were treated daily with 5 μl of vehicle (dimethylsulfoxide) ± LG268 (30 μg).

LG268 and BRL 49653 were synthesized at Ligand Pharmaceuticals, Inc.

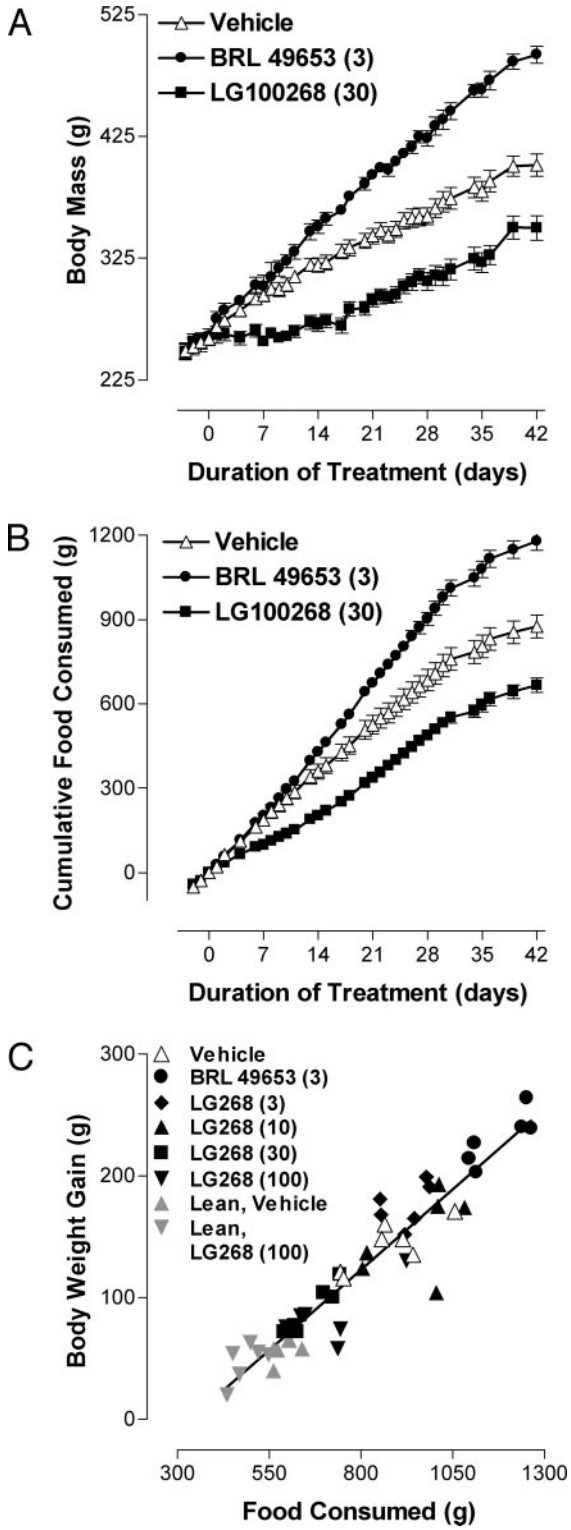

FIG. 1. Body weights and cumulative FC in Zucker fatty rats treated with the RXR agonist LG268 (30 mg/kg), the PPARγ agonist BRL 49653 (3 mg/kg), or vehicle beginning on d 1 (n = 6–7/group). A, Body weights were significantly decreased by LG268 from d 4 to 42, whereas body weights were increased by BRL 49653 from d 10 to 42 ($P < 0.05$, *t* test for both comparisons with vehicle-treated controls). B, Cumulative FC was decreased by LG268 (30 mg/kg) and increased by BRL 49653 (3 mg/kg) beginning with the first dose of compound. C, Individual BWGs are plotted against cumulative food consumed during 6 wk of treatment for all animals in the experiment. Dose levels of compound are indicated in *parentheses* within the legend (mg/kg). There was a significant correlation between food consumed and body weight gained ($P < 0.0001$; BWG = 0.26 × FC − 88.1 g; $r^2 = 0.90$).

In situ detection of adipogenesis and apoptosis

Female Zucker fatty rats were dosed for 4 d; killed; and inguinal, mesenteric, and ovarian fat samples frozen in liquid nitrogen. Tissues were embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin. Two-dimensional areas of adipocytes were measured using National Institutes of Health Image for PC. Apoptotic nuclei were identified by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling analysis (no. 1684809, Roche Molecular Biochemicals, Indianapolis, IN) and expressed as the percentage of all nuclei identified by propidium iodide staining.

Dynamic food intake behavior

Female Zucker fatty rats were caged individually in an automated food intake apparatus obtained from Research Diets, Inc. (New Brunswick, NJ). Briefly, hanging wire cages outfitted with spill-proof hoppers were connected to electronics that monitored movement of the food hopper (*i.e.* feeding activity) and measured its weight (FC). Time of day, duration, and size of each meal were recorded for individual animals. In our lab, we defined the initiation of a meal by the first movement of the hopper. A meal ended anytime the hopper did not move for at least 2 min after any feeding activity. Furthermore, the rat had to consume at least 0.25 g of food for the meal to be counted. Average meal size was defined as the total food consumed in 24 h divided by the number of meals recorded. Because we dosed each morning at approximately 0800 h, a day was defined as a 24-h period beginning at 0800 h. Soft-pelleted food (D12450B, Research Diets, Inc.) was used to further minimize wastage. Before using this apparatus to determine the effects of the RXR agonist on feeding behavior, we confirmed that Zucker fatty rats housed in the apparatus consumed a similar number of calories and gained weight at rates similar to animals housed in our standard cages and fed our standard food (data not shown). Because of the limited number of cages available to us (n = 4), we chose to collect data during a baseline period (animals dosed with vehicle for 4 d), during treatment with LG268 (100 mg/kg for 5 d), and during a 3-d recovery period (no dosing). To obtain a large sample size, four cohorts were used (final n = 15).

Biochemical and hormone measurements

Glucose and triglycerides were measured colorimetrically using commercially available kits (nos. 315–300 and 339–500, Sigma, St. Louis, MO) adopted for use in 96-well plates. Insulin was measured in diluted plasma samples (1:10) by double-antibody RIA using reagents for rat (Linco, St. Charles, MO). The sensitivity of this assay is 7.8 pg/tube, with an intraassay coefficient of variation of 7.5% that was determined using control plasmas provided by the manufacturer. Total T_4 was measured by RIA using commercially available kits (Diagnostic Products Corp., Los Angeles, CA). The assay range was 5–240 ng/ml, with a detection limit of about 2.5 ng/ml. Intra- and interassay variations ranged from 2.8 to 3.8% and 4.2 to 14.5%, respectively. To minimize the effects of interassay variation on the interpretation of results, all samples from each experiment were assayed at the same time.

Data analysis

All data were analyzed in Excel. Groups were compared using *t* test. $P < 0.05$ was accepted as significantly different.

Results

Oral administration of LG268 significantly reduced BWG, compared with control animals (Fig. 1A), an effect that was

evident within the first week of treatment. This reduction of body weight was maintained throughout the experiment and occurred in tandem with a decrease in FC (Fig. 1B). There was a tight correlation between BWG and FC, as confirmed by linear regression analysis of these two parameters at the end of the treatment period ($y = 0.26 \times FC - 88.1$ g; $r^2 = 0.90$) for animals treated with a broad dose range of LG268 (0–100 mg/kg; Fig. 1C). Not only did LG268 decrease FC in obese, hyperphagic animals, but it also reduced FC in lean animals (100 mg/kg; Fig. 1C). Although we reported previously that treatment with RXR agonists at one dose level (20 mg/kg) decreased BWG and FC in obese animals (3, 4), the data from this dose response strengthen the conclusion that pharmacological activation of RXR pathways regulates FC and BWG concurrently in both obese and lean animals.

To further examine this novel activity of LG268, we determined body composition of four animals from the six in each group. Animals that had body weights in the middle of the range for the group were chosen for the analysis. Because there were no significant differences in water or fat-free dry mass weights, we summed the two for each animal and labeled it lean mass. LG268 caused a selective decrease in fat mass in both obese (LG268, 136.7 ± 9.8 g *vs.* vehicle, 173.6 ± 7.5 g, a difference of 21%) and lean (LG268, 10.4 ± 0.6 g *vs.* vehicle, 18.7 ± 0.4 g, a difference of 44%) animals (Fig 2). In contrast, BRL 49653 caused a selective increase in fat mass in obese rats (249.0 ± 6.0 g, a difference of 43%).

BRL 49653 and LG268 lowered insulin levels by approximately 75% (Fig. 3), indicating insulin sensitization in these normoglycemic animals. Note that insulin lowering was observed at all doses of the rexinoid, whereas a significant effect on body weight was not (*i.e.* at 3 and 10 mg/kg). Chronic oral treatment with BRL 49653 reduced triglycerides in Zucker fatty rats (similar to previous reports of TZDs in various

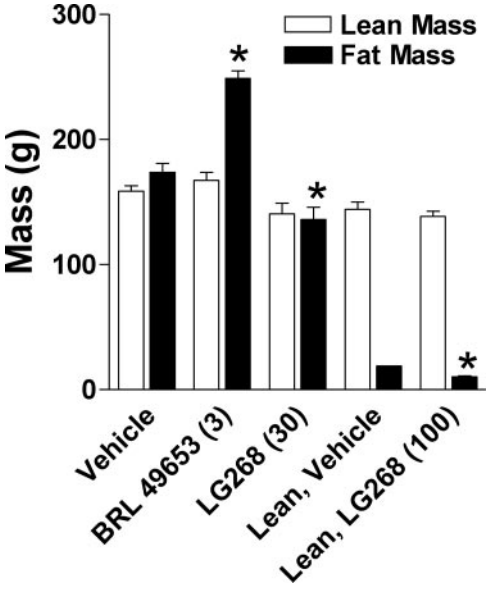

FIG. 2. Effects of the RXR agonist LG268 and the PPARγ agonist BRL 49653 on body composition in Zucker fatty and lean rats (n = 4/group). Dose levels are indicated in *parentheses* within the legend (mg/kg). BRL 49653 caused a significant increase (*, $P < 0.05$, *t* test) in fat mass, whereas LG268 decreased fat mass in both obese and lean Zucker rats. Compounds did not significantly affect lean mass.

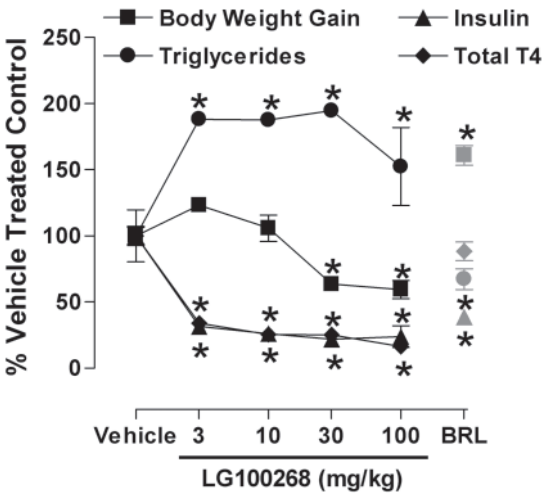

FIG. 3. Effects of ascending doses of the RXR agonist LG268 (*black symbols*, dose levels on x-axis) and the PPARγ agonist BRL 49653 (*gray symbols*, 3 mg/kg) on BWG (*squares*), insulin (*triangles*), triglycerides (*circles*), and total T_4 (*diamonds*) in Zucker fatty rats (n = 6–7/group). Data were obtained on d 40 and are represented as a percentage of vehicle-treated obese animals. LG268 decreased BWG, insulin, and total T_4 and increased triglycerides (*, $P < 0.05$, *t* test). BRL 49653 also reduced insulin but had opposite effects to LG268 on triglycerides and BWG. Total T_4 levels were not altered by BRL 49653.

animal models) (13–17) and had no significant effect on levels of T_4. Conversely, LG268 produced a sustained elevation in triglycerides at all doses tested and also profoundly suppressed T_4. These effects of RXR agonists have been reported to occur in lean animals (7, 18–20) and oncology patients treated with the selective RXR agonist LGD1069 (21–23).

Administration of TZDs increases the proportion of small adipocytes present in fat tissue, a finding that has been interpreted to indicate that these compounds are adipogenic *in vivo* (16, 24, 25). Based on those data and the effects of BRL 49653 and LG268 on carcass lipid content, we hypothesized that these compounds exerted different effects on fat mass by remodeling adipose tissue. Fat was sampled from sc and visceral (ovarian and mesenteric) depots to determine whether the effects on fat mass were exerted globally or regionally. No significant differences in either the percentage of small adipocytes or the rate of apoptosis were observed in mesenteric fat (data not shown). In ovarian fat, LG268 and BRL 49653 induced apoptosis and increased the prevalence of small adipocytes to a similar extent (Fig. 4). In sc fat, however, LG268 dramatically increased the rate of apoptosis (Fig. 4A) without altering the proportion of small adipoctyes (Fig. 4B), indicating that rexinoid-induced decreases in fat mass could be due to adipocyte deletion specifically within the sc compartment. BRL 49653, on the other hand, significantly increased the percentage of small adipocytes in sc fat (Fig. 4B), an effect that could eventually increase the size of that depot. Although BRL 49653 also increased apoptosis (Fig. 4A), the change was small and unlikely to overcome its adipogenic action.

Despite the healthy appearance of the animals and the preservation of lean mass in chronically treated animals, we wanted to determine empirically whether the activity of LG268 in rats treated with higher oral doses of the compound

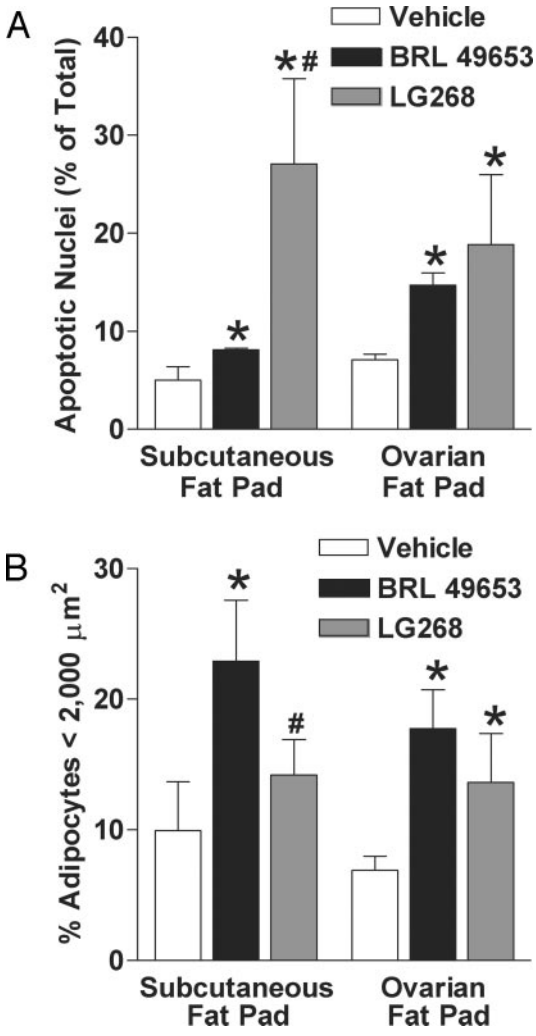

FIG. 4. BRL 49653 and LG268 alter the balance between adipogenesis and apoptosis within sc fat. A, Effects of vehicle, BRL 49653 (10 mg/kg), and LG268 (30 mg/kg) on apoptosis. B, Prevalence of small adipocytes (an index of *in vivo* adipogenesis) in sc and ovarian fat of female Zucker fatty rats dosed for 4 d (n = 3/group). Although both compounds increased the rate of apoptosis, LG268 had a greater effect in sc fat than did BRL 49653. In addition, BRL 49653 increased adipogenesis in sc fat, whereas LG268 did not. The compounds had similar effects in ovarian fat (see figure), whereas no significant differences in either the percentage of small adipocytes or the rate of apoptosis were observed in mesenteric fat (data not shown). Two-dimensional areas of adipocytes were measured in hematoxylin- and eosin-stained sections using NIH Image. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling analysis and expressed as the percentage of nuclei stained with propidium iodide. *, $P < 0.05$ *vs.* vehicle, #, $P < 0.05$ *vs.* BRL 49653 using *t* test.

were due to primary appetite suppression or might reflect an adverse response (*i.e.* toxicology and/or food aversion). We chose to observe dynamic feeding behavior before, during, and after oral treatment with LG268 (100 mg/kg). If the compound had adverse effects, we hypothesized that animals would forgo meals (*i.e.* decrease meal frequency). On the other hand, a decrease in meal size might indicate that the animals became satiated after consuming less food. Figure 5A shows a continuous record for an individual animal on 3 consecutive days. The pattern on d −1 (*i.e.* the day before the first dose of the rexinoid) is typical of these fatty rats. The majority of the meals takes place during the dark half of the light cycle, and this animal consumes an average of 2.7 g of food per meal. Immediately after the first dose of LG268, average meal size is decreased (to 1.1 g), whereas meal frequency is not reduced. Summary data from all of the treated animals are shown in Figs. 5, B–D. Again, LG268 decreased total food consumed during each 24-h period of treatment. The reduction in total food consumed per day was entirely due to a significant reduction in average meal size (Fig. 5C). Meal frequency was not significantly changed by the treatment, although there was a marked trend toward an increase in this end point largely driven by high meal frequency in approximately one third of the treated animals (Fig. 5D). These data suggest that the activation of RXR suppresses appetite in these Zucker fatty rats. Unexpectedly, we observed that 24-h FC and meal size remained partially suppressed for the 3 d during which we made measurements after the cessation of compound dosing.

The lack of a rapid recovery from the reduction in FC prompted us to ask how long this effect persisted after discontinuation of dosing with LG268. We treated Zucker fatty rats housed in our standard shoebox cages on aspen chip bedding with either vehicle or LG268 (100 mg/kg). Animals were treated for a period of 5 d, and both BWG and FC were followed for a total of 45 d after the initial dose. As shown in Fig. 6A, daily BWG reverted to control levels immediately after dosing with LG268 was discontinued. FC, on the other hand, returned to control levels more slowly, requiring 4 d for full recovery (Fig. 6B). Most interestingly, when FC was examined on a daily basis, we never observed significant compensatory hyperphagia in these animals. However, both cumulative food consumed and cumulative BWG in animals previously treated with LG268 reached control levels beginning 20 (data not shown) and 24 d (Fig. 6C), respectively, after the rexinoid was withdrawn.

Given the rapid effect of LG268 on FC and the presence of RXRs in hypothalamic satiety centers (26, 27), we thought that LG268 might act primarily in the brain. Therefore, we administered LG268 directly into the cerebral ventricles. Rats treated daily with icv injections of vehicle had rates of BWG and FC indistinguishable from those of rats treated orally with vehicle (Fig. 7). Intracerebroventricular treatment with a small amount of LG268 (30 μg/rat, which was the threshold dose in a pilot experiment) decreased both BWG and FC. The results are similar to those obtained after oral administration of LG268. Intracerebroventricular treatment with LG268 also decreased plasma insulin (LG268, 7.23 ± 1.59 ng/ml *vs.* vehicle, 12.63 ± 0.65 ng/ml). However, the decrease was not as large as that measured in orally treated animals (LG268, 3.06 ± 0.45 ng/ml *vs.* vehicle, 13.36 ± 0.88 ng/ml) and may be a consequence of decreased fat mass (especially visceral fat) (28) and/or FC (29). Importantly, icv injection of LG268 did not increase triglycerides (Fig. 8A) or decrease T_4 (Fig. 8B), suggesting that these actions of the rexinoid are mediated peripherally.

It is known that molecules introduced to the cerebral ventricles are subsequently cleared via the peripheral circulation (30, 31). To test whether the effects of icv treatment with

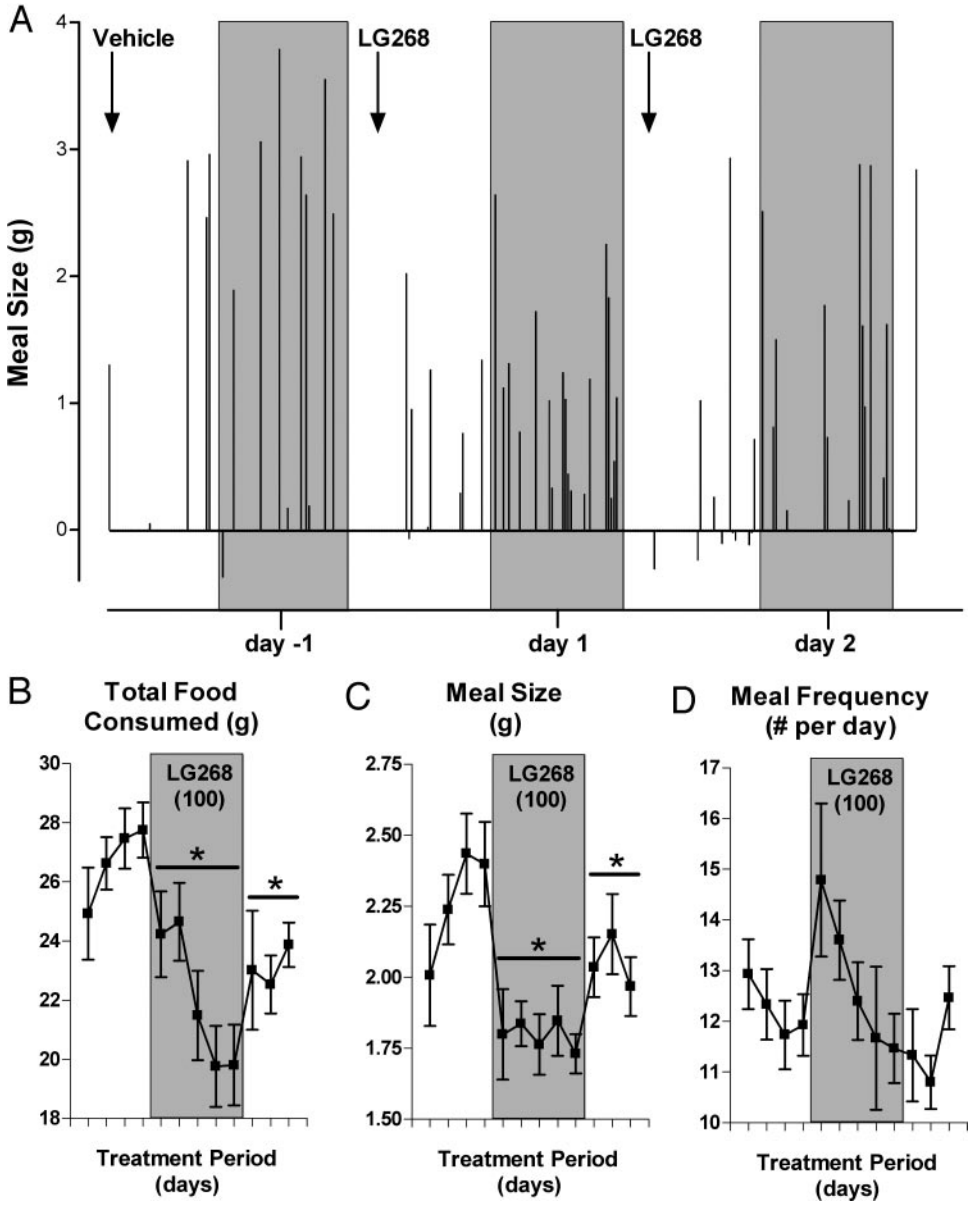

FIG. 5. LG268 reduces 24-h FC by decreasing meal size. A, Daily food intake pattern of one Zucker rat the day before (day −1) and during 2 d of treatment with LG268 at 100 mg/kg. The *shaded box* indicates the dark period and the *downward arrows* indicate the time of oral dosing. Note that meal size, indicated by the *height of the bars*, is decreased in this individual by treatment with LG268. B, Total food consumed (per day). C, Average meal size. D, Meal frequency of all animals before, during (*shaded boxes*), and after daily treatment with LG268 (n = 15). Total food consumed and meal size are significantly reduced during treatment with LG268 and do not recover to pretreatment levels within 3 d of compound withdrawal (*, $P < 0.05$ *vs.* pretreatment period).

LG268 were secondary to escape of the compound from the CNS, we treated animals iv with the same amount of compound that was efficacious when administered icv (30 μg). Zucker fatty rats injected iv with LG268 gained body weight and consumed food at rates indistinguishable from their vehicle-injected controls (14.2 ± 2.1, 14.3 ± 3.0 g BWG; vehicle and LG268, respectively; 21.2 ± 1.6, 21.5 ± 1.5 g daily FC; vehicle and LG268, respectively). These data confirm that LG268 administered icv must decrease FC and BWG by acting in the brain.

Discussion

Our data demonstrate that activation of RXR suppresses appetite in a rodent model of obesity, the Zucker fatty rat. Similar responses to oral administration have been obtained using other RXR ligands [*e.g.* LG100324 (Refs. 3 and 4; Ogilvie, K. M., M. S. Urcan, and M. D. Leibowitz, unpublished observations) and HX-531(32)] and/or alternate models of obesity [*e.g.* high fat-fed mice and rats; Ref. 32 and Ogilvie, K. M., M. S. Urcan, and M. D. Leibowitz, unpublished observations]. Furthermore, administration of an agonist directly into the cerebral ventricles reproduces the effects on both FC and BWG, suggesting a role for this nuclear hormone receptor in the regulation of feeding behavior and energy balance via CNS pathways. The presence of all three RXR subtypes in the brain (27, 33), including areas that are known to regulate feeding behavior (27), further supports the idea that RXRs may be an important endogenous regulator of satiety. Excitingly, RXR activation alters energy balance without provoking immediate compensatory hyperphagia, suggesting a long-lasting change in the set point for body mass in these rodents. In support of this concept, Ross *et al.* (34) reported that changed levels of RXR mRNA expression in the hypothalamus are associated with photoperiod-

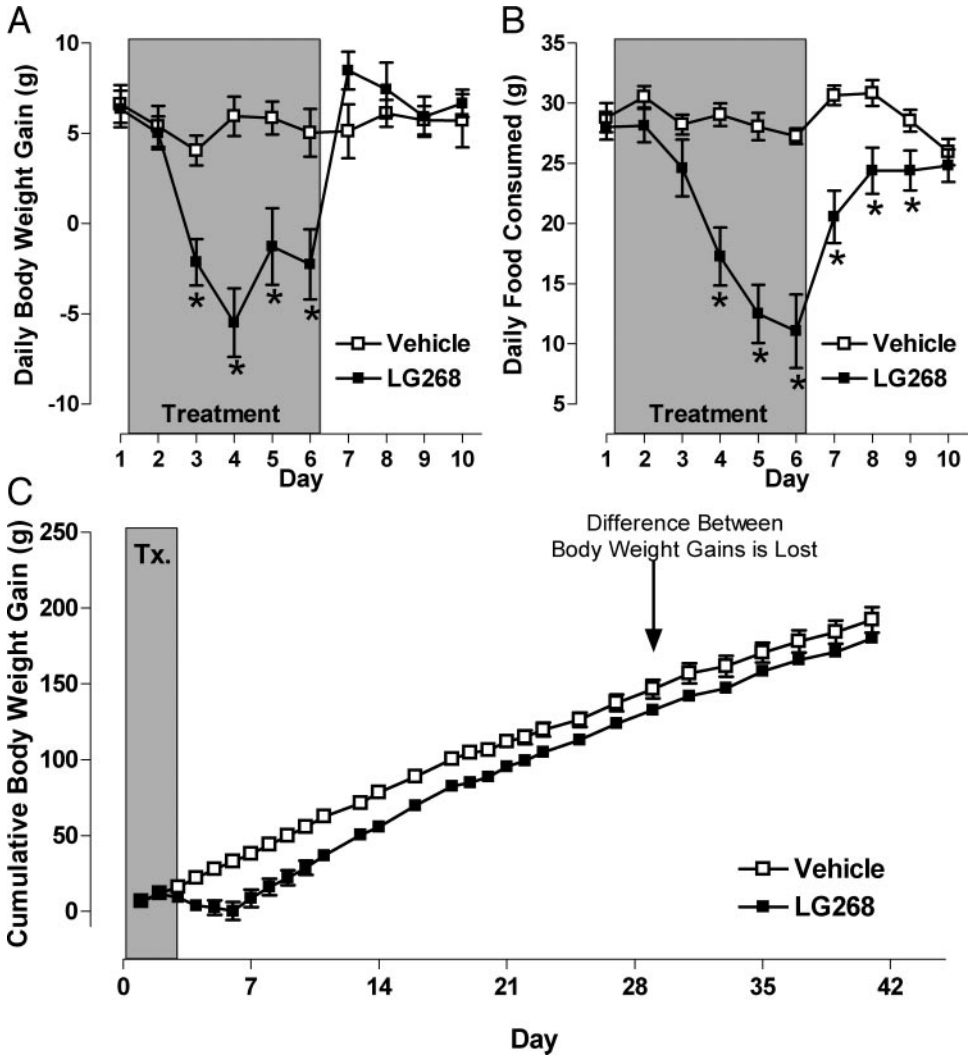

FIG. 6. Lack of immediate compensatory hyperphagia after withdrawal of LG268. Daily BWG (A) and food consumption (B) in rats (n = 9/group) treated on d 1–5 with LG268 (100 mg/kg). After withdrawal of the compound, BWG returns rapidly to control levels (P = 0.08 on d 7), whereas food consumption reverts more slowly (requires 4 d). We never observed a significant increase in daily food consumption (data not shown), but cumulative BWG reached control levels 24 d after compound withdrawal (C).

induced regulation of body weight set point in Siberian hamsters. The lack of immediate compensatory hyperphagia is similar to that observed after treatment with truncated ciliary neurotrophic factor (35), brain-derived neurotrophic factor (36), and a melanin-concentrating hormone-1 receptor antagonist (37); but contrasts with that measured after discontinuation of food restriction or administration of appetite suppressants [*e.g.* leptin (38) and sibutramine (39)]. Although we never measured a statistically significant increase in daily FC after discontinuation of LG268 treatment, we did find that cumulative FC returned to control levels shortly before cumulative BWG. Thus, a very modest increase in FC, occurring over a period of weeks, underlies the slow reversion of body weight to control levels. One might speculate that activation of pathways that directly alter transcription of genes (as is the case with nuclear hormone receptors) might result in long-lasting changes in physiological processes. Similarly prolonged after-effects have been described for the TZD troglitazone (40).

In concert with its effects on energy balance, RXR administration altered total body fat mass and adipose tissue structure in these rodents. We detected early changes in cell size and the prevalence of apoptosis in adipocytes, suggesting that LG268 remodels fat. The adipogenic activity of PPARγ ligands *in vivo* has been reported by a number of laboratories and is presumably via direct action on preadipocytes because PPARγ plays a central role in adipocyte differentiation (41). We observed that LG268 induced adipogenesis in ovarian fat in a manner similar to that observed after administration of BRL 49653, a finding that is not surprising, as RXR activation is also known to drive adipocyte differentiation *in vitro* (42) and *ex vivo* (43). However, the activity of LG268 was markedly distinct from that of BRL 49653 in sc fat. In this depot, LG268 treatment dramatically increased the rate of apoptosis without increasing adipogenesis, whereas BRL 49653 drove adipogenesis more than apoptosis. Because Zucker fatty rats store more than 50% of their fat sc (44), the changes we observed fit well with the opposite effects of BRL 49653 and LG268 on body fat content. Notably, patients and rats treated with TZDs also accumulate fat preferentially in sc regions (45–47). Although the role of adipocytes in the antidiabetic effects of PPARγ ligands remains a subject of debate, one might have predicted that RXRs, which in cooperation with PPARγ are involved in glucose homeostasis *in vivo* and adipogenesis *in vitro*, would have effects similar to PPARγ with regard to energy homeostasis. Although that is not the case,

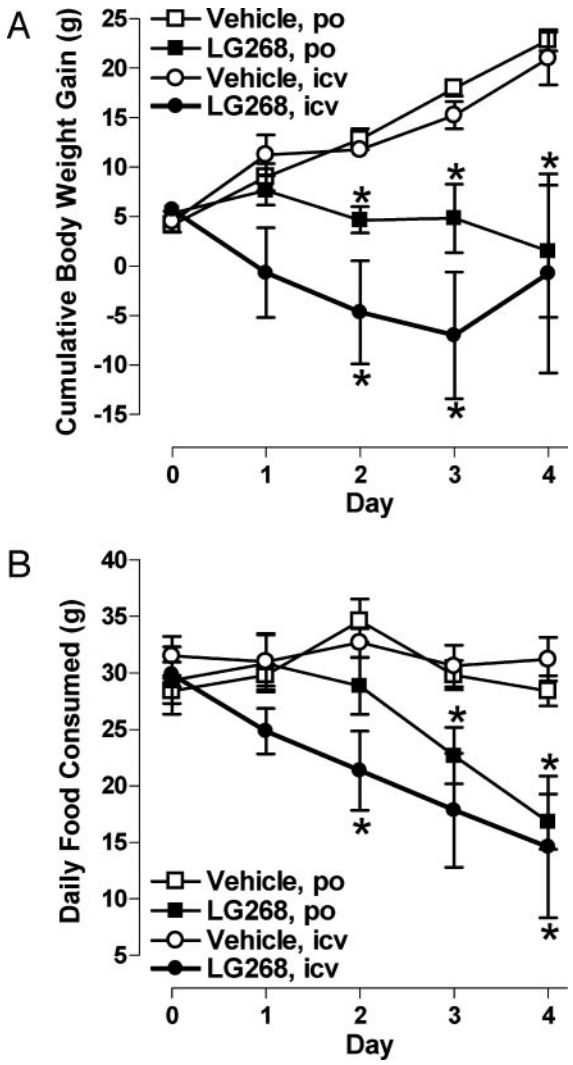

FIG. 7. Intracerebroventricular administration of LG268 decreases BWG and FC. BWG (A) and FC (B) in female Zucker fatty rats (n = 4–6/group) treated with vehicle (*open symbols*) or LG268 (*closed symbols*) administered orally (*squares*) or icv (*circles*). LG268 significantly reduced BWG and FC whether given orally (30 mg/kg) or icv (30 μg/animal; *, $P < 0.05$ *vs.* vehicle administered via same route, *t* test).

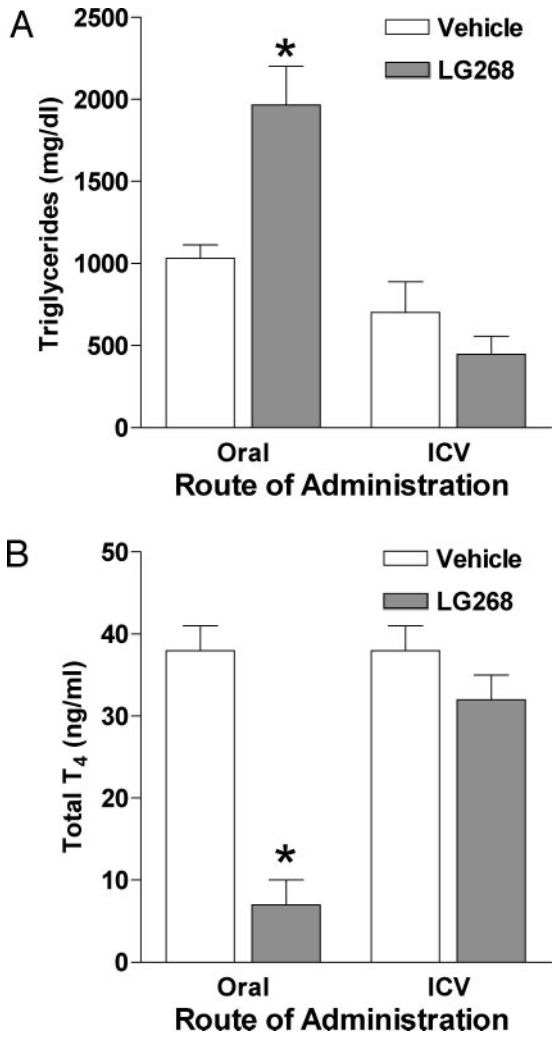

FIG. 8. LG268 administered icv does not alter triglycerides or T_4. Plasma triglycerides (A) and total T_4 (B) were measured in the animals represented in Fig. 7. Blood samples were obtained 3 h after treatment on d 4. *Open bars* represent vehicle-treated animals and *gray shaded bars* represent those treated with LG268 (100 mg/kg orally or 30 μg/rat, icv). *, $P < 0.05$ *vs.* vehicle administered via the same route. Statistical comparisons were made with *t* test.

the extent to which the effects of RXR activation on adipocytes *in vivo* are mediated directly or may arise secondary to alterations in FC has yet to be determined.

Although our data demonstrate a clear role of RXR pathways in the regulation of energy balance via modulation of food intake and fat mass, they do not exclude regulation of other pathways that contribute to energy balance (*e.g.* thermogenesis, physical activity). Indeed, the increased meal frequency that we observed in some animals when treated with LG268 might reflect more spontaneous activity. Further work is required to determine whether these other pathways are also altered by RXR ligands.

Activation of the PPARγ/RXR heterodimer with members of the TZD class of insulin sensitizers increases BWG and/or adipose tissue mass in both type 2 diabetic patients and rodents. Because increased BWG and adiposity also occur after treatment with insulin and sulfonylureas (48), part of this activity might be due to amelioration of the diabetic state (*i.e.* spared glucose is stored as fat). Our data suggest that increased fat mass is not a necessary attendant to improved insulin sensitivity. Nor is a decrease in FC and/or BWG necessary for the insulin-sensitizing effects of RXR pathways because insulin levels are lowered at doses of LG268 that do not alter BWG or FC (*e.g.* 3 mg/kg). Indeed, doses of LG268 and BRL 49653 that do not alter BWG and FC enhance insulin sensitization as demonstrated with an euglycemic hyperinsulinemic clamp (49). The greater potency of LG268 on insulin sensitization *vs.* appetite suppression is of interest, and we speculate that it might be due to inefficient penetration of the compound to the brain or to a difference in the ability of LG268 to activate PPARγ/RXR *vs.* an unknown heterodimeric partner that mediates appetite suppression.

RXR agonists have been reported to increase triglycerides and suppress thyroid hormone levels in a variety of animal models. The data we present here suggest that the elevation

in triglycerides is not a consequence of reduced adiposity because it occurs after oral doses of compound that do not decrease BWG and does not occur after icv treatments, which do decrease BWG. Interestingly, the effects of LG268 on circulating T_4 are also absent after central administration, which further supports our hypothesis that RXR pathways in the pituitary are responsible for suppression of the thyroid hormone axis during peripheral administration of RXR agonists (see Ref. 7).

We have demonstrated that the RXR agonist LG268 decreases FC, BWG, and adiposity while preserving lean mass. These biological effects of LG268 differ from those of TZDs, which mediate their activity via PPARγ, thus implying that these activities are distinct from PPARγ/RXR. Suppression of BWG and FC (or simply a lack of hyperphagia) are desirable attributes of an insulin sensitizer intended for an obese type 2 diabetic population. It is noteworthy in this regard that our data demonstrate that the positive characteristics of LG268 can be separated from its undesirable effects by administering the compound selectively to the CNS. Importantly, our data also suggest that ligand-dependent transcriptional regulation may play a role in the control of FC and BWG. Although the exact molecular or neuroanatomical sites of action are unknown, one can speculate that identification of a brain-specific rexinoid would provide a novel therapy for diabetes and/or obesity.

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