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Metabolic Effects of Rexinoids: Tissue-Specific Regulation of Lipoprotein Lipase Activity

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ABSTRACT

Hypertriglyceridemia is a frequent complication accompanying the treatment of patients with either retinoids or rexinoids, [retinoid X receptor (RXR)-selective retinoids]. To investigate the cellular and molecular basis for this observation, we have studied the effects of rexinoids on triglyceride metabolism in both normal and diabetic rodents. Administration of a rexinoid such as LG100268 (LG268) to normal or diabetic rats results in a rapid increase in serum triglyceride levels. LG268 has no effect on hepatic triglyceride production but suppresses post-heparin plasma lipoprotein lipase (LPL) activity suggesting that the hypertriglyceridemia results from diminished peripheral processing of plasma very low density lipoproteins particles. Treatment of diabetic rats with rexinoids suppresses skeletal and cardiac muscle but not adipose tissue LPL activity. This effect is independent of changes in LPL mRNA. In C2C12 myocytes, LG268 suppresses the level of cell surface (i.e., heparin-releasable) LPL activity without altering LPL mRNA. This effect is very rapid ($t_{1/2}$ = 2 h) and is blocked by the transcriptional inhibitor actinomycin D. These studies demonstrate that RXR ligands can have dramatic effects on the post-translational processing of LPL and suggest that skeletal muscle may be an important target of rexinoid action. In addition, these data underscore that the metabolic consequences of RXR activation are distinct from either retinoic acid receptor or peroxisome proliferator-activated receptor activation.

Retinoids, naturally occurring and synthetic derivatives of vitamin A, are nuclear receptor ligands that have therapeutic applications in a variety of dermatologic and oncologic diseases. Retinoids are morphogens that regulate the growth and differentiation of embryonic cells. In addition to these effects on cell differentiation, retinoids also can have dramatic effects on serum lipid metabolism. Administration of retinoids to both experimental animals (Gerber and Erdman, 1981b; McMaster et al., 1989; Oliver and Rogers, 1993; Standeven et al., 1996) and humans (Lyons et al., 1982; Bershad et al., 1985) often results in increases in serum triglyceride levels. Although the modest hypertriglyceridemia most frequently induced by retinoid therapy may be innocuous, the extreme elevations occasionally encountered in retinoid-treated patients can precipitate pancreatitis (McCarter and Chen, 1992). For this reason understanding of the cellular and molecular basis of retinoid-induced hypertriglyceridemia will aid in efforts to develop retinoids suitable for prolonged therapy.

The hypertriglyceridemia induced by retinoic acid isomers (either all-*trans*-RA or 13-*cis*-RA) is due to alterations in several aspects of serum triglyceride metabolism. In fasted animals, plasma triglyceride levels represent an equilibrium between the rate of hepatic secretion of very-low-density lipoproteins (VLDL), and the rate of their clearance. VLDL particles are cleared by lipoprotein lipase (LPL) activity present in the tissue vascular beds and hepatic lipase present in the liver. In rats, retinoic acid-induced hypertriglyceridemia is due to both increased hepatic production of VLDL (Gerber and Erdman, 1981a) and a suppression of LPL activity in peripheral tissues (Gerber and Erdman, 1981a; Ol-

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ABBREVIATIONS: RA, retinoic acid; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; LPL, lipoprotein lipase; RAR, retinoic acid receptor; RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptor; ZDF, Zucker diabetic fatty; DMEM, Dulbecco's modified Eagle's medium; HDL, high-density lipoprotein; TTNPB, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid; Act D, actinomycin D.

iver and Rogers, 1993). In humans there are no data on the effects of retinoids on hepatic VLDL production, however, 13-*cis*-RA-treated patients have a reduced capacity to clear infused triglycerides (Vahlquist et al., 1987), suggesting reduced tissue lipolytic activity. Furthermore, in humans, 13-*cis*-RA increases the levels of apolipoprotein (apo)-CIII a hepatic protein that binds to VLDL and interferes with their lipolytic processing (Vu-Dac et al., 1998).

The multiple effects of retinoids on serum triglyceride metabolism may be due to the distinct activities of the different retinoid receptors, all members of the nuclear receptor superfamily (Mangelsdorf et al., 1993). All-*trans*-RA is the physiological ligand for the retinoic acid receptors (RARα, β, and γ), which mediate many of the effects of retinoids on morphogenesis and connective tissue metabolism. In addition, all-*trans*-RA readily isomerizes to 9-*cis*-RA, a ligand for not only the RARs but also for the retinoid X receptors (RXRs) (Heyman et al., 1992; Levin et al., 1992). 13-*cis*-RA binds to neither the RARs nor the RXRs, but in vivo, it spontaneously isomerizes to both all-*trans*-RA and 9-*cis*-RA and thereby indirectly activates both RARs and RXRs (Chen and Juchau, 1998). Ligand-dependent activation of the RXRs is particularly interesting because RXRs form both homodimers and serve as obligate heterodimeric partners for a number of nuclear receptors, such as RARs, peroxisome proliferator-activated receptors (PPARs), thyroid hormone receptors, the vitamin D receptor, and several other orphan nuclear receptors (Mangelsdorf and Evans, 1995). Thus, the pharmacologic effects of all-*trans*-RA, 9-*cis*-RA, or 13-*cis*-RA can be due to activation of both RAR- and RXR-dependent signaling pathways.

Retinoid-induced hypertriglyceridemia was initially encountered in patients receiving retinoic acid isomers that either directly or indirectly activate both RARs and RXRs. RAR-specific retinoids induce hypertriglyceridemia in both experimental animals (Standeven et al., 1996) and humans (Takeuchi et al., 1998). The situation with RXR-selective retinoids (rexinoids) is less clear-cut. In humans, rexinoids cause hypertriglyceridemia (Miller et al., 1997; Rizvi et al., 1999), but in experimental animals, they have been reported either to have no effect (Standeven et al., 1996) or to lower serum triglyceride levels (Mukherjee et al., 1997a, 1998; Lenhard et al., 1999). In the studies reported here on the acute effects of rexinoids in diabetic rats, we found that rexinoids markedly increased serum triglyceride levels by suppressing LPL activity in both skeletal and cardiac muscle. We have further demonstrated that rexinoids suppress the expression of LPL activity on the surface of differentiated myocytes. Taken together these findings suggest that RXR-dependent signaling pathways are intimately involved in the regulation of triglyceride metabolism via their specific effects on the LPL activity of cardiac and skeletal muscle.

Materials and Methods

Animals and Treatments. Harlan Sprague-Dawley rats (8-weeks old, approximately 220 g) were obtained from Harlan Laboratories, Inc. (Indianapolis, IN) and Zucker diabetic fatty (ZDF) rats (8-weeks old, approximately 350 g) were obtained from Genetic Models Inc. (Indianapolis, IN). Rats were housed individually on a 12:12 light/dark cycle (lights on at 6:00 AM) with food (Teklad 7012a or Purina 5008, respectively) and tap water continuously available. Blood samples were obtained via the tail vein, 3 h after dosing on the indicated day. For chronic treatment, rats were gavaged with vehicle, rosiglitazone (3 mg/kg), LGD1069, or LG268 (3, 10, 30, or 100 mg/kg). At the end of the experiments, animals were weighed and anesthetized. Blood was collected by cardiac puncture before euthanization with CO_2. Serum was separated and used within 1 week for analysis of lipids, lipoproteins, and apolipoproteins. The liver, muscle, and adipose tissue were removed immediately, weighed, frozen in liquid nitrogen, and stored at −80°C until further analysis.

Cultured Cells. Control C2C12 cells, stably transfected with a β-galactosidase reporter construct, (C2-β-gal) and C2C12 cells transfected with pMCKhLPL (Poirier et al., 2000) were maintained in 100-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum, penicillin, streptomycin, and G-418 (200 μg/ml). For individual experiments, cells were plated in 12-well plates at 10^5 cells per well in DMEM + 20% fetal calf serum. After 3 days, when the cell achieved confluency, the medium was changed to DMEM + 2% horse serum + cytosine arabinoside (10 μM), and thereafter, the medium was changed every 3 days until >95% myotubes formed (6 days).

Lipid, Lipoprotein, and Lipase Measurements. Serum cholesterol and triglycerides were determined by enzymatic assay adapted to microtiter plates using commercially available reagents (Roche Molecular Biochemicals, Mannheim, Germany). Lipoprotein triglyceride and cholesterol profiles, separating the three major lipoprotein classes (VLDL, LDL, and HDL) were obtained by fast protein liquid chromatography and analyzed by the Millennium 20/0 program (Waters, Milford, MA) (Duverger et al., 1993). LPL and hepatic lipase activity was measured in tissue extracts and serum according to the procedure of Ramirez and coworkers (Grinberg et al., 1995). The heparin-releasable LPL activity of differentiated C2C12 cells was measured by washing the cells once with 0.4 ml of DMEM and then incubating them at room temperature for 10 min with 0.4 ml of DMEM + heparin (100 μg/ml). An aliquot (100 μl) of the cell extract was assayed for LPL activity (Nilsson-Ehle and Schotz, 1976). One unit of enzyme activity is the amount of enzyme that releases 1 μmol of oleate/min at 25°C.

RNA Analysis. RNA was isolated from tissues by the acid guanidinium thiocyanate/phenol/chloroform method and Northern and Dot blot analysis of total cellular RNA with LPL, and apoCIII cDNA probes were performed as described previously (Auwerx et al., 1989).

Results

Effect of Rexinoids on Serum Triglyceride Levels. Rexinoids have been reported to lower both the hyperglycemia and hypertriglyceridemia of genetically obese and insulin-resistant (*db*/*db*) mice (Mukherjee et al., 1997a, 1998; Lenhard et al., 1999). We were therefore surprised to find that in ZDF rats, LG268, a prototypic rexinoid (Boehm et al., 1995), lowered serum glucose levels (Fig. 1A) but raised serum triglycerides from a baseline value of 4.08 ± 0.5 g/liter to a peak of 14.81 ± 2.0 g/liter (Fig. 1B). The rise is serum triglycerides is not directly linked to the fall in serum glucose levels since rosiglitazone (BRL49653), a PPARγ agonist that lowers serum glucose levels in these animals, also lowered triglyceride levels (Fig. 1, A and B).

In earlier studies of the effects of rexinoids on serum lipid levels, triglycerides were measured 24 h following administration of the last dose of drug (Standeven et al., 1996; Mukherjee et al., 1997a, 1998). To determine the kinetics of rexinoid-induced hypertriglyceridemia, a single dose of either vehicle or LG268 was administered (by gavage) to non-obese, non-diabetic rats. At specific times following drug or vehicle administration, animals were sacrificed, and blood was collected (terminal bleeds) for serum triglyceride determinations. Administration of LG268 (but not the vehicle) to

these normal rats resulted in a rapid but transient increase in serum triglyceride levels from a baseline value of 1.54 ± 0.3 g/liter to a peak of 3.03 ± 0.5 g/liter (Fig. 1C). After a lag of 60 min, serum triglyceride levels rose rapidly and remained elevated for at least 6 h. Serum triglyceride levels returned to normal 24 h following administration of the rexinoid. These results demonstrated that normal rats were as sensitive as diabetic rats to rexinoid-induced hypertriglyceridemia and that the hypertriglyceridemic effect of the rexinoid was as prominent in the naïve animals (Fig. 1C) as it was in those that had been subjected to chronic administration (Fig. 1B).

Rexinoids are presumed to produce their biological effects via RXR-dependent activation of gene expression. The remarkably rapid effects of rexinoids on triglyceride levels raised the question of whether this response was due to their transcriptional activity or whether it might be due to some previously unrecognized post-transcriptional effect. To address this issue, we administered LG268 to non-diabetic rats in which transcription had been blocked by prior administration of the RNA polymerase II inhibitor actinomycin D (Act D). Terminal blood samples were collected 3 h following administration of LG268 to either control or Act D-treated animals. Act D had no effect on basal triglyceride levels, but it completely blocked the acute hypertriglyceridemic effect of the rexinoid (Fig. 1D).

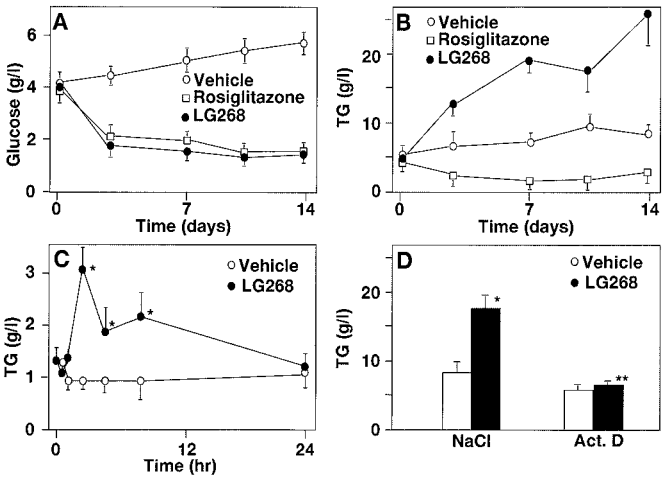

Fig. 1. Effect of LG268 and rosiglitazone on serum glucose and triglyceride levels in Zucker diabetic fatty (A and B) and in non-obese, non-diabetic (C) rats. A and B, LG268 (3 mg/kg/day), rosiglitazone (3 mg/kg/day), or vehicle (1% carboxymethyl cellulose/9.95% polyethylene glycol) was administered daily by gavage to groups ($n = 5$) of male ZDF rats. At intervals up to 14 days, serum was collected 3 h after drug administration, and glucose (A) and triglyceride (B) concentrations were determined. Values shown are the means ± S.E.M. C, time course of serum triglyceride levels following administration of a single dose of LG268 (3 mg/kg/day) or vehicle to groups ($n = 6$) of non-obese, non-diabetic rats (Harlan Sprague-Dawley). Drug or vehicle was administered by gavage at time 0, and at the indicated time, groups of rats were euthanized, and blood was collected for the determination of serum triglyceride levels. Single asterisk reflects significant difference ($p < 0.05$) versus vehicle control. D, groups ($n = 8–10$) of non-obese, non-diabetic rats (Harlan Sprague-Dawley) were either administered Act D (2 mg/kg) or normal saline solution (NaCl) via tail vein injection. Two hours later both control and Act D-treated rats received either LG268 (3 mg/kg/day) or vehicle by gavage. Three hours later serum was collected for triglyceride determinations. Values shown represent the mean ± S.E.M., single asterisk reflects significant difference ($p < 0.05$) versus vehicle control; double asterisk reflects significant difference ($p < 0.05$) Act D versus NaCl control.

To characterize the effects of rexinoids on serum lipid metabolism, we examined the dose-dependent effects of a second rexinoid, LGD1069 (Targretin) (Boehm et al., 1994), on triglyceride and cholesterol levels in diabetic rats. Like LG268, LGD1069 caused marked dose-dependent hypertriglyceridemia. Over the same dose range, total serum cholesterol levels remained virtually unchanged (Fig. 2A). The dose-response range is very broad, doses as low as 0.3 mg/kg/day caused significant elevations in serum triglyceride levels, and the maximum effect occurred at doses equal to or higher than the highest dose tested (30 mg/kg/day). To determine which lipoproteins accounted for the increase in total triglycerides, we performed lipoprotein profile analysis on pooled serum from three untreated animals (total cholesterol level in the pool of 0.92 g/dl) and three animals that received LGD1069 (30 mg/kg/day; total cholesterol level of 1.4 g/dl). The increase in triglyceride levels in LGD1069-treated animals was entirely due to increased levels of VLDL as evidenced by size exclusion chromatography of lipoproteins and subsequent analysis of both triglyceride (Fig. 2B) or cholesterol distribution (Fig. 2C) in the different fractions. LGD1069 administration also resulted in a slight change in HDL particle characteristics. HDL particles became less heterogeneous and more bulky relative to control HDL (Fig. 2C). No significant changes in intermediate density lipoprotein and LDL fractions were observed upon fast protein liquid chromatography.

Effect of Rexinoids on VLDL Metabolism and LPL in Vivo. Increased steady-state plasma VLDL levels can arise from either increased hepatic production or decreased peripheral clearance or both. To determine the mechanism of rexinoid-induced hypertriglyceridemia in rats, we measured the effects of rexinoids on hepatic triglyceride production and heparin-releasable plasma LPL activity. Administration of Triton WR-1339 blocks the clearance of triglyceride-rich lipoproteins by inhibiting their degradation. The resulting increase in triglyceride levels provides an indirect measurement of hepatic triglyceride secretion (Gerber and Erdman, 1981a). The triglyceride secretion rate of the control and rexinoid-treated (LG268 at 3 mg/kg/day) rats were similar,

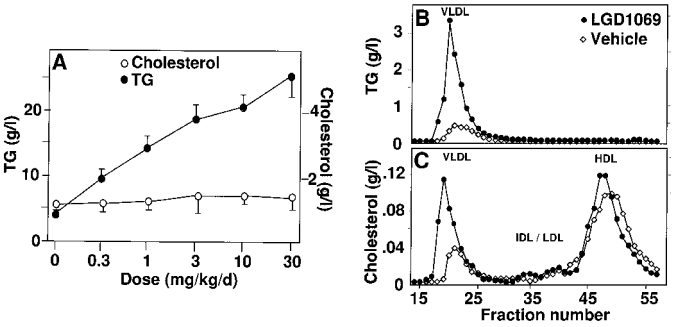

Fig. 2. Effect of LGD1069 on serum lipid (A) and lipoprotein (B) levels in ZDF rats. A, either vehicle or LGD1069 (0.3–30 mg/kg/day) were administered to groups ($n = 4–6$) of male ZDF rats by gavage for 14 days. Three hours after the last dose, serum was collected for triglyceride and cholesterol determinations. Values are mean ± S.E.M. B, serum from three animals treated either with vehicle or LGD1069 (30 mg/kg/day) was pooled, and lipoproteins were fractionated by size exclusion chromatography. Triglycerides and cholesterol were determined in the fractions. C, serum from three animals treated either with vehicle or LGD1069 (30 mg/kg/day) was pooled, and lipoproteins were fractionated by size exclusion chromatography. Cholesterol was determined in the fractions.

410.47 ± 14.9 and 407.29 ± 33.02 mg/dl/h, respectively (Fig. 3A).

The activity of LPL on the surface of endothelial cells regulates the rate of plasma VLDL clearance. Heparin-releasable LPL activity can be used to assess endothelial cell surface LPL activity. The post-heparin plasma LPL activity following the administration of LG268 to non-diabetic rats demonstrated that the increase in triglyceride levels 3 h following LG268 administration (Fig. 1) is matched by a reciprocal decline in post-heparin plasma LPL activity (Fig. 3B). By 24 h, LPL activity had returned to near normal levels (Fig. 3B).

The preceding studies suggested that rexinoid-induced hypertriglyceridemia could be linked to alterations in the LPL activity present on the surface of endothelial cells. Endothelial cells do not synthesize LPL; their LPL activity is derived from the underlying parenchymal cells, particularly muscle cells (both skeletal and cardiac muscle) and adipocytes (Goldberg, 1996). To determine whether rexinoids affected the level of expression or activity of LPL in tissues, RNA and heparin-treated tissue extracts were prepared from white adipose tissue, skeletal muscle (quadriceps), and cardiac muscle (ventricle) of ZDF rats that had been treated with LGD1069 for 14 days (Fig. 4). Rexinoid treatment resulted in a marked decline of LPL activity in both skeletal and cardiac muscle. Skeletal muscle was the most sensitive, doses of LGD1069 as low as 0.3 mg/kg/day produced a significant decrease in tissue LPL activity, and 3 mg/kg/day was a maximally effective dose. There was also a marked suppression of cardiac LPL activity with a minimally effective dose of 1 mg/kg/day and a maximal effect at 10 to 30 mg/kg/day. The decrease in LPL activity in both skeletal and cardiac muscle was not a reflection of decreased LPL expression since the level of LPL mRNA in cardiac muscle was unaffected, and the level of LPL mRNA in skeletal muscle was only slightly depressed by the highest dose of rexinoid. There was no significant decrease in the LPL activity of adipose tissues taken from animals treated with LGD1069 in doses ranging from 0.3 to 30 mg/kg/day. There does appear to be a small decrease in the LPL activity of the animals treated with the highest doses of LGD1069 (10–30 mg/kg/day), but this decrease did not achieve statistical significance. Rexinoids have no effect on the level of LPL mRNA in the adipose tissue.

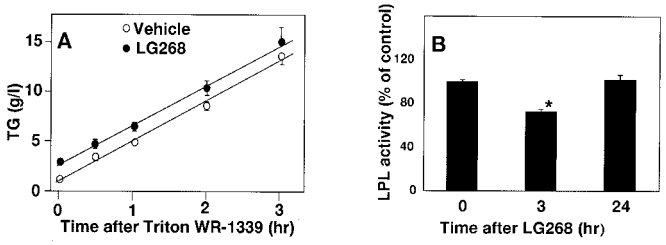

Fig. 3. Effect of LG268 on triglyceride production rates (A) and plasma LPL activity (B) in non-obese, non-diabetic rats. A, non-obese, non-diabetic rats (Harlan Sprague-Dawley) (n = 4 per group) received either vehicle or LG268 (3 mg/kg/day) for 3 days. Three hours after the last dose, Triton WR1339 solution (500 mg/kg, 20% solution) was administered to all the animals via tail vein injection. At the indicated times, animals were euthanized, and serum was collected for determination of triglyceride levels. Values shown are the mean ± S.E.M. B, groups of non-obese, non-diabetic rats (Harlan Sprague-Dawley) (n = 6) received a single dose of either vehicle or LG268 (3 mg/kg/day) by gavage. All animals received heparin (1000 U/kg) 3 and 24 h later by tail vein injection, and serum was collected 15 min later for the determination of post-heparin LPL activity. Values shown are mean ± S.E.M. Asterisk indicates a significant difference ($p < 0.01$) versus vehicle control.

Effect of Rexinoids on LPL Metabolism in Cultured Myocytes. The preceding studies demonstrated that rexinoids could have dramatic effects on muscle LPL metabolism in vivo. To determine whether these same compounds could have direct effects on the metabolism of LPL in vitro, we examined the effects of LG268 on the expression and activity of the enzyme in differentiated mouse C2C12 myocytes (Fig. 5). LG268 induced a dose-dependent suppression in the heparin-releasable LPL activity of the C2C12 cells with an EC_{50} of 1×10^{-9} M (Fig. 5A). To further characterize the effects of the rexinoid on the expression of LPL activity, we used C2C12 cells stably transfected with an LPL expression vector, pMCKhLPL (Duverger et al., 1993) that contains the human LPL cDNA under the control of a creatine kinase promoter. The basal LPL activity of these transfected cells is 10-fold higher than either non-transfected cells (data not shown) or cells transfected with an irrelevant β-galactosidase expression vector. LG268 (10^{-9}–10^{-6} M) produces an equivalent suppression in the LPL activity of both the mock-transfected (C2-β-gal) and LPL-transfected (C2-LPL) cells (Fig. 5A). The onset of the inhibitory effect of LG268 on LPL activity in the transfected cells is very rapid. Following a reproducible 1 to 2 h lag, there is a precipitous decline in heparin-releasable LPL activity such that within 4 to 6 h the activity had decreased to less than 10% of that present in control cells (Fig. 5B).

The effect of LG268 on myocyte LPL activity was also dependent on transcription since coaddition of Act D with LG268 completely blocked the decrease in heparin-releasable LPL activity (Fig. 5C). Even though transcription was required, the effect of LG268 on myocyte LPL activity was not due to an effect on LPL expression since the levels of LPL mRNA in LG268-treated cells were similar to those in controls (data not shown).

RXRs form homodimers and heterodimers with several

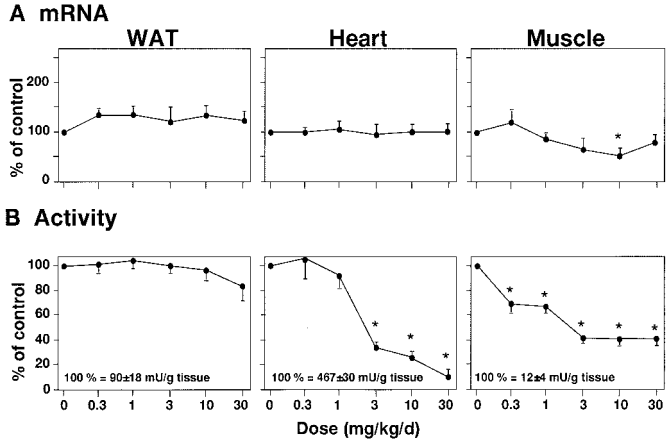

Fig. 4. Effect of LGD1069 on LPL mRNA (A) and activity (B) in skeletal muscle, cardiac muscle, and white adipose tissues (WAT) of ZDF rats. Vehicle or LGD1069 (0.3–30 mg/kg/day) was administered to ZDF rats (n = 4–6 per group) for 14 days. Animals were sacrificed 3 h after the last dose, and quadriceps muscle (muscle), ventricular myocardium (heart), and epididymal fat pads (WAT) were collected for preparation of RNA and heparinized tissue extracts. LPL mRNA and activity were determined as described under *Materials and Methods*. Values shown are the level of LPL activity and LPL mRNA as a percentage of the control (vehicle-treated) animal values. Values shown are mean ± S.E.M.; asterisks identify LPL activities significantly different ($p < 0.05$) from controls.

nuclear receptors. To determine whether the effect of the rexinoid on LPL activity in differentiated myocytes was attributable to activation of a specific RXR-containing heterodimer, we compared the effects of the rexinoids with a series of ligands specific for the various RXR "partner" receptors present in myocytes. Differentiated C2C12 cells were treated for 24 h with either LG268 or TTNPB (a pan-RAR ligand), rosiglitazone (a PPARγ ligand), WY14,643 (a PPARα ligand), 4β-hydroxycholesterol (a liver X receptor ligand), 1,25-dihydroxycholecalciferol (a vitamin D receptor ligand), or triiodothyronine (a thyroid hormone receptor ligand) (Fig. 5D). The effect of the rexinoid was very specific. LG268 induced a reproducible suppression in heparin-releasable LPL activity, whereas none of the other nuclear receptor ligands tested had any inhibitory effect on the level of enzyme activity. Thus, none of the ligands for receptors that can partner with RXR replicated the activity of the RXR ligand.

Discussion

Retinoid- and Rexinoid-Induced Hypertriglyceridemia. Activation of RXR is associated with insulin sensitization and has therefore been proposed as a novel strategy for the treatment of type II diabetes (Mukherjee et al., 1997a; Lenhard et al., 1999). In human subjects, however, rexinoid therapy is associated with hypertriglyceridemia (Miller et al., 1997; Rizvi et al., 1999), and the results presented here show that the same effects can be observed in both normal and diabetic rodents. Rexinoid-induced hypertriglyceridemia developed rapidly after administration of LG268 to normal rats; it reached peak levels in 2 to 3 h and was resolved in 24 h. This transient effect is compatible with the short half-life of both LG268 and LG1069 in rodents (E. Ulm, personal communication). To develop RXR agonists with a favorable therapeutic index for long-term treatment of impaired glucose homeostasis, it is important to understand the molecular basis of this unwanted side effect.

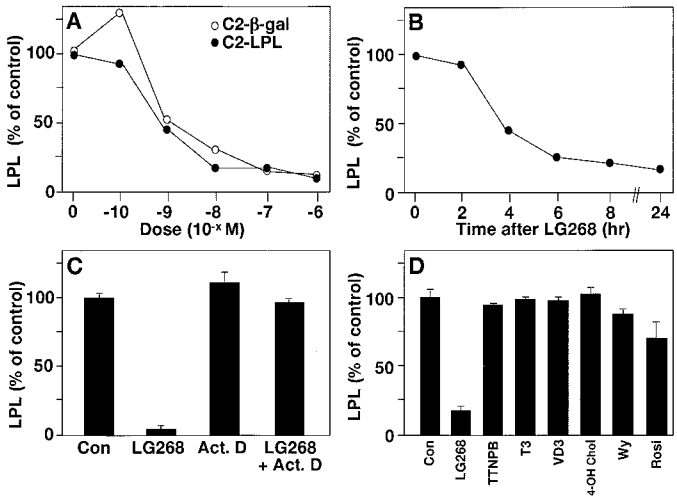

Fig. 5. Effect of LG268 on heparin-releasable LPL activity in differentiated C2C12 myocytes. A, differentiated C2C12 cells (C2-β-gal) and LPL-transfected C2C12 cells (pMCKhLPL; C2-LPL) were treated with either medium alone or medium containing LG268 (10^{-10}–10^{-6} M) for 16 h. Then heparin-releasable LPL activity was determined by enzymatic assay as described. Values shown (% of control) are the mean and range of LPL activity in duplicate wells. B, LPL-transfected differentiated C2C12 cells (C2-LPL) were treated with LG268 (1 μM) for 4 to 25 h, and heparin-releasable LPL activity was determined as described. Values shown (% of control) are the mean and range of LPL activity in duplicate wells. C, LPL-transfected C2C12 cells were treated with either solvent alone (Con; control), actinomycin D (Act D; 1 μM), LG268 (1 μM), or LG268 + actinomycin D for 3 h. Heparin-releasable LPL activity was then determined by enzymatic assay. Values shown are the mean ± S.D. of the LPL activity from triplicate wells. D, differentiated LPL-transfected C2C12 myocytes (C2-LPL) were treated with vehicle or LG268 (1 μM), TTNPB (0.1 μM), triiodothyronine (T3; 0.1 μM), 1,25$(OH)_2$ vitamin D_3 (VD3; 1 μM), 4β-hydroxycholesterol (4-OH Chol; 10 μM), WY14,643 (Wy; 10 μM), and rosiglitazone (rosi; 1 μM) for 16 h. Heparin-releasable LPL activity was determined by enzymatic assay and is expressed as a percentage of the vehicle control. Values shown represent the mean ± S.D. of three separate experiments in which LPL activity was assessed in duplicate wells.

Retinoid-Induced Hypertriglyceridemia. Previous studies in experimental animals have shown that *retinoid*-induced hypertriglyceridemia is due to both alterations in hepatic lipoprotein secretion (Gerber and Erdman, 1981a) and peripheral lipoprotein catabolism (Gerber and Erdman, 1981a; Oliver and Rogers, 1993). In contrast, the *rexinoid* effect on triglyceride metabolism is restricted to effects on clearance. The studies with Triton WR-1339 demonstrated that hypertriglyceridemia develops in the absence of alterations in the rate of hepatic triglyceride secretion. Although our studies did not measure triglyceride clearance rates directly, elevations in the steady-state triglyceride levels in the absence of alterations in production strongly suggest a primary defect in lipoprotein catabolism. Triglyceride-rich lipoproteins are catabolized by two lipases, LPL and hepatic lipase. Defects in hepatic lipase activity lead to the accumulation of intermediate density lipoprotein and HDL particles (Gehrisch et al., 1999), whereas defects in LPL leads to increased levels of VLDL (Merkel et al., 1998). The hypertriglyceridemia of the rexinoid-treated animals consisted entirely of elevated levels of VLDL suggesting a primary defect in LPL-dependent catabolism.

Alterations in LPL-dependent catabolism of VLDL can be due either to changes in the level of the enzyme in tissues or to changes in the level of regulatory apoproteins in the plasma. In experimental animals, all-*trans*-RA and 13-*cis*-RA lower LPL activity in both adipose tissue and skeletal muscle but have no effect on LPL activity in the heart (Gerber and Erdman, 1981a; Oliver and Rogers, 1993). Rexinoids also suppress LPL activity in skeletal muscle but they differ from retinoids in their effects on LPL activity in adipose tissue and cardiac muscle. Rexinoids have no effect on adipose tissue LPL activity whereas retinoids do, and reciprocally, while rexinoids suppress cardiac LPL activity, retinoids have no effect. The results we have obtained suggest skeletal muscle LPL activity can be affected by both RAR- and RXR-specific ligands, whereas RAR ligands also affect LPL metabolism in adipose tissue and RXR ligands affect it in the heart. It is possible that the lack of effect of rexinoids on LPL activity in adipose tissue reflects an unusual pattern of tissue distribution for the drug but we do not think this is the case. The synthetic rexinoids used in these studies, LG1069 and LG268, are very lipophilic drugs that have marked effects on gene expression in muscle, liver, and adipose tissue in both normal and diabetic rodents (H. S. Ahuja, S. Liu, D. L. Crombie, M. Boehm, M. D. Leibowitz, R. A. Heyman, C. Depre, L. Nagy, P. Tontonoz, and P. J. A. Davies, submitted for publication). It is more likely that the tissue-specific effects of retinoids and rexinoids on LPL metabolism reflect differences in the types and amounts of receptors and their various heterodimeric partners or in the coactivator and bridging factors present in the different tissues.

Alteration in the level of LPL activity is not the only factor controlling the rate of VLDL catabolism in vivo. ApoCII and apoCIII are serum proteins that bind to the VLDL and either enhance (apoCII) or depress (apoCIII) its susceptibility to hydrolysis by LPL (Jong et al., 1999). Retinoids have been reported to increase apoCIII expression and secretion by hepatocytes both in vitro and in vivo via an RXR-dependent pathway (Vu-Dac et al., 1998). In our studies, LGD1069 induced a minor increase in hepatic apoCIII mRNA; there was no increase in plasma apoCIII levels 3 h after LG268 administration at a time when there was both marked suppression of muscle LPL activity and marked hypertriglyceridemia (data not shown).

Mechanisms of Rexinoid-Induced Hypertriglyceridemia. It has been suggested that the metabolic effects of rexinoids are attributable to their ability to activate RXR/PPAR heterodimers (Mukherjee et al., 1997a, 1998; Lenhard et al., 1999). The RXR serves as an obligate partner for all three members of the PPAR family of receptors (Mukherjee et al., 1998) and in transfection studies, rexinoids transactivate PPAR heterodimers as effectively as PPAR ligands (Mukherjee et al., 1997a,b, 1998). In diabetic mice, rexinoids replicate the ability of PPARγ ligands such as thiazolidinediones to lower serum glucose levels (Mukherjee et al., 1997a; Lenhard et al., 1999). The overlapping pharmacological activities of rexinoids, fibrates, and thiazolidinediones has led to the suggestion that RXR/PPAR heterodimers are capable of being equivalently activated by ligands of either of the heterodimeric partners (Mukherjee et al., 1998). In spite of these overlapping activities in some metabolic responses, we do not believe that the hyperlipidemic effects of rexinoids reflect the activation of PPARs. In contrast to rexinoids, PPARα and PPARγ ligands lower triglyceride levels (Auwerx et al., 1996). Unlike PPARγ ligands, which increase LPL expression in adipose tissue (Schoonjans et al., 1996; Lefebvre et al., 1997), rexinoids have no effect on either the expression or activity of LPL in adipose tissue. PPARγ ligands can suppress heparin-releasable LPL activity in cultured 3T3L-1 adipocytes (Ranganathan and Kern, 1998) but they do not inhibit myocyte LPL activity whereas rexinoids do. Thus, as far as we can tell, the hypertriglyceridemic activity of rexinoids is independent of their ability to activate PPARs.

If the PPARs are not the partners for RXRs in their effects on triglyceride metabolism, then what receptors are? One set of candidate partners are the RARs, which form obligate heterodimers with RXRs. Both RAR and RXR ligands induce hypertriglyceridemia in vivo (Standeven et al., 1996). However, in cultured myocytes, rexinoids suppress LPL activity whereas a potent pan-RAR agonist such as TTNPB does not. Furthermore, as discussed above, the profile of tissues in which LPL activity is suppressed by the rexinoid is different from the tissues affected by RAR agonists. These results suggest that rexinoids and retinoids share the ability to induce hypertriglyceridemia in vivo but that they do so via the activation of distinct and different receptor complexes.

Having excluded PPARs and RARs as RXR partners responsible for the hyperlipidemic activity of rexinoids, the question of which partner receptors are responsible remains. Ligands for the thyroid hormone and vitamin D receptors neither produce acute hypertriglyceridemia in vivo nor do they suppress LPL activity in cultured myocytes. The hyperlipidemic activity of rexinoids could be due either to the activation of an unrecognized RXR-containing heterodimeric complex or to activation of RXR homodimers. RXR receptors form stable homodimers on DNA (Holmbeck et al., 1998) that are effective activators of transcription (Mangelsdorf et al., 1991; Vu-Dac et al., 1996). It is possible that the unique ability of rexinoids, among nuclear receptor ligands, to induce hypertriglyceridemia is due to their unique ability to serve as ligands for RXR homodimers.

Even though we are not certain of the receptor complexes that mediate the effects of rexinoids on triglyceride metabolism, these effects require the induction of new gene expression. Both the LPL-suppressive effects of the rexinoids in cultured cells and rexinoid-induced hypertriglyceridemia are blocked by actinomycin D. The time courses for the effects of the rexinoids in vitro and in vivo are also compatible with the induction of gene expression and the accumulation of a specific gene product (or products) that subsequently perturb LPL metabolism. Our data suggest that the effects of rexinoids on LPL metabolism occur primarily at a post-translational level. Treatment of either cells or animals with rexinoids has no effect on the levels of LPL mRNA even though there are large changes in LPL activity. Furthermore rexinoids are as effective in suppressing the LPL activity of cells transfected with an LPL expression construct as they are in suppressing the activity of endogenous LPL. Chronic administration of thyroid hormone suppresses LPL activity via regulatory elements encoded in the 3′-untranslated region of the LPL transcript (Kern et al., 1996). However, rexinoids are fully effective in suppressing LPL actvity in cells transfected with an LPL construct that contains a heterologous 3′-untranslated region so it is unlikely that this type of mechanism accounts for these inhibitory effects. The abolition of the hypertriglyceridemic effect of rexinoids by actinomycin D suggests that their effect on LPL metabolism depends on the induction of RXR-regulated genes, which affect the complex post-translational processing, dimerization, and export pathways that are necessary for the expression of cell surface LPL activity.

Physiological Implications. Rexinoid-induced suppression of the lipolytic activity of skeletal muscle and heart are likely to have physiological consequences beyond the induction of hypertriglyceridemia. Since LPL is a gatekeeper enzyme, controlling the delivery of fatty acids to tissues, decreased LPL activity will, in the long-run, result in a depletion of lipid stores in muscle and heart, which in turn will lead to improved muscle insulin sensitivity. PPARγ agonists induce a preferential delivery of fatty acids to adipose tissue via the induction of LPL and enzymes and transport proteins that facilitate fatty acid uptake into adipose tissue (Schoonjans et al., 1996). The resulting "fatty acid steal" by adipose tissue, diminishes the quantity of fatty acids taken up by the muscle and may contribute to the insulin-sensitizing effect of PPARγ agonists in muscle. Rexinoids may cause a depletion of muscle lipid stores not by increasing the delivery of fatty acids to adipose tissue but by directly reducing their delivery to muscle. It is possible that this modulation of fatty acid metabolism in skeletal muscle cells is an important contributor to the therapeutic effects of these compounds in animal models of type II diabetes.

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