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Retinoid X receptor agonist elevation of serum triglycerides in rats by potentiation of retinoic acid receptor agonist induction or by action as single agents

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Abstract

Hypertriglyceridemia is a major side-effect of retinoid therapy in humans. We previously reported that agonists for the retinoic acid receptors (RARs), but not the retinoid X receptors (RXRs), elevate serum triglycerides in male Fischer rats, and that, surprisingly, the RAR/RXR pan-agonists 9-*cis*-retinoic acid and AGN 191659 {(*E*)-5-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylic acid} induce 2- to 3-fold higher levels of serum triglycerides than the RAR-selective agonists alone. We have now demonstrated that hypertriglyceridemia induced by an RAR agonist, AGN 190121 [4-[4-(2′,6′,6′-trimethylcyclohex-1-enyl)-but-1-yne-3-enyl]benzoic acid], is substantially potentiated by the RXR-selective agonists AGN 191701 [(*E*) 2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophene-carboxylic acid] and AGN 192849 [(3,5,5,8,8,-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl) (5 carboxypyrid-2-yl)sulfide] in a dose-dependent manner. RXR-specific retinoids, as previously reported, had no independent effect on serum triglycerides when tested at 24 hr after final dosing, but did elicit a reversible hypertriglyceridemia at 2.5 and 5 hr. This induction of serum triglycerides could not be blocked by the potent RAR-specific antagonist AGN 193109 {4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)-ethynyl] benzoic acid}. The RXR ligand-induced hypertriglyceridemia was independent of the effect of feeding or fasting. The relative potencies of RXR-specific retinoids for acute triglyceride elevation (AGN 194204 {3,7-dimethyl-6*S*,7*S*-methano-7-[1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphth-7-yl] 2(*E*),4(*E*) heptadienoic acid} > AGN 192849 ~ AGN 191701) approximately correlated with potencies in the activation of the RXR receptors. The RAR/RXR pan-agonist effect included >50% inhibition of total heparin-releasable lipase activity in serum, consistent with inhibition of lipase-mediated triglyceride disposal. These data also indicate that RAR and RXR ligands can act synergistically to induce hypertriglyceridemia through distinct mechanisms of action.

Keywords: Triglycerides; Retinoic acid receptor; Retinoid X receptor; Fischer rats; Heparin-releasable lipase

1. Introduction

Retinoids have multiple biological activities and many proven clinical applications in dermatology and oncology [1]. Retinoids bind to two distinct groups of structurally homologous nuclear receptors, the RARs and the RXRs, thereby regulating gene transcription. Heterodimers of an RAR and an RXR, which can then bind DNA, appear to form the predominant, if not exclusive, targets for the actions of RAR-specific retinoids [2,3]. Several studies suggest that the RXR partner is silent based on the inability of RXR-specific ligands to bind RAR/RXR heterodimers *in vitro* or to elicit known retinoid effects *in vivo* [4,5], while other data demonstrate that RXR ligands can have synergistic effects with RAR ligands in several cell culture models [6–9]. The effects of ligands specific for the RXR receptor *in vivo* are, however, quite distinct from RAR ligands and include suppression of hyperglycemia in animal models of type 2 diabetes, liver hyperplasia and increased

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Abbreviations: RARs, retinoic acid receptors; and RXRs, retinoid X receptors.

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fatty acid oxidation, inhibition of intestinal cholesterol uptake, and central hypothyroidism, among others [10–15]. This RXR biology is probably mediated by heterodimerization of RXR with several other nuclear receptors, excluding RAR, in which RXR appears to be a pharmacologically active heterodimeric partner, or by the formation of RXR homodimers [2,13,15,16].

A major limitation on the wider clinical use of retinoids has been the associated side-effects, including mucocutaneous toxicity, bone toxicity, hypertriglyceridemia, and teratogenicity [17]. Hypertriglyceridemia occurs in at least 25% of patients treated orally with 13-*cis*-retinoic acid, etretinate, and acitretin [18–20] and has also been observed in initial clinical trials of *9-cis*-retinoic acid [21] and LGD1069 [14], which have selective but not specific interactions with the RXR as opposed to the RAR nuclear receptors. Hypertriglyceridemia was also the dose-limiting toxicity in a cancer chemotherapy trial of all-*trans*-retinoic acid [22]. Our previous data using receptor-selective agonists and antagonists demonstrated that RAR agonists induce hypertriglyceridemia in rats treated for 3 consecutive days and tested 24 hr after the final dose [23]. However, it was noteworthy that two RAR/RXR pan-agonists tested in this system, namely 9-*cis*-retinoic acid and AGN 191659, induced higher levels of hypertriglyceridemia than the RAR agonists alone [12,23]. In light of these findings, and the aforementioned *in vitro* data that RXR agonists can synergize the effects of RAR agonists [6–9], we tested the hypothesis that RXR agonists can potentiate hypertriglyceridemia induced by RAR agonists in rats. The results indicated that RXR-selective agonists can indeed potentiate the hypertriglyceridemic effect of RAR-selective agonists. Moreover, we observed that RXR agonists have an acute and transient hypertriglyceridemic effect that is clearly distinct from the RAR-mediated hypertriglyceridemic effect. These findings also suggest that hypertriglyceridemia may be more of a problem in the clinical use of RAR/RXR pan-agonists than in the use of RAR- or RXR-selective agonists.

2. Materials and methods

2.1. Chemicals

The retinoids used in this study were synthesized according to the corresponding published procedures: AGN 192849 [(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl) (5 carboxypyrid-2-yl)sulfide] [24]; AGN 191701, (*E*) 2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophene-carboxylic acid and AGN 191659 {(*E*)-5-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylic acid} [25]; AGN 190121 [4-[4-(2′,6′,6′-trimethylcyclohex-1-enyl)-but-1-yne-3-enyl]benzoic acid] [26]; and AGN 193109 {4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)-ethynyl] benzoic acid} [27]. AGN 194204 {3,7-dimethyl-6*S*,7*S*-methano-7-[1,1,4,4-tetramethyl1,2,3,4-tetrahydronaphth-7-yl] 2(*E*),4(*E*) heptadienoic acid} was synthesized as described [28]. Corn oil and heparin were obtained from the Sigma Chemical Co. [^{3}H]Triolein was obtained from NEN Dupont. Unless otherwise indicated, all other reagents were obtained from the Sigma Chemical Co.

2.2. Animals

Male Fischer rats [CDF (F-344)/CrlBR] were obtained from Charles River Laboratories and were acclimated for at least 6 days prior to experimentation. Rats were housed individually in stainless steel wire mesh cages. Food (Purina Rodent Chow 5001) and water purified by reverse osmosis were provided *ad lib*. Rats were exposed to a 12-hr light/dark cycle, with the light period extending from 6:00 a.m. to 6:00 p.m. Rats were 6- to 7-weeks-old at the initiation of treatments.

2.3. Animal treatments

Rats were randomized by weight into groups of 5–8 for each experiment. Unless otherwise indicated, test compounds were suspended in corn oil and administered by gavage (5 mL/kg) at the indicated doses once or daily for up to 3 days. Dosing suspensions were prepared fresh daily and were routinely administered between 10:00 a.m. and 11:00 a.m. In one experiment, AGN 193109 was suspended in corn oil and administered by gavage 2 hr prior to retinoid treatment. For studies of the effects of fasting, an RXR-specific retinoid was dissolved in DMSO and injected (5 mL/kg, i.p.) in the following vehicle: 3% DMSO, 2% Tween-80, 95% phosphate-buffered saline. In a study of plasma post-heparin lipolytic activity, rats were injected i.v. (2 mL/kg) in the tail vein with heparin (500 U/kg) 5 min prior to the collection of blood. Rats were euthanized by exsanguination under carbon dioxide anesthesia. Blood collected from the inferior vena cava was centrifuged at 8000 *g* for 15 min at 4° to collect serum, and serum was stored at 4° for up to 24 hr prior to the assay of triglycerides. Heparinized plasma was stored at −80° prior to the assay of lipolytic activity.

2.4. Biochemical assays

Serum or plasma samples were assayed for triglycerides using a colorimetric endpoint assay (Sigma No. 337) that was modified to a 96-well format. Spectrophotometric analysis was conducted with a Thermomax microtiter plate reader (Molecular Devices). Total triglyceride levels (triglyceride + glycerol) were determined by reference to glycerol standard curves run concurrently. Plasma lipolytic activity was assayed as described by Nilsson-Ehle and Schotz

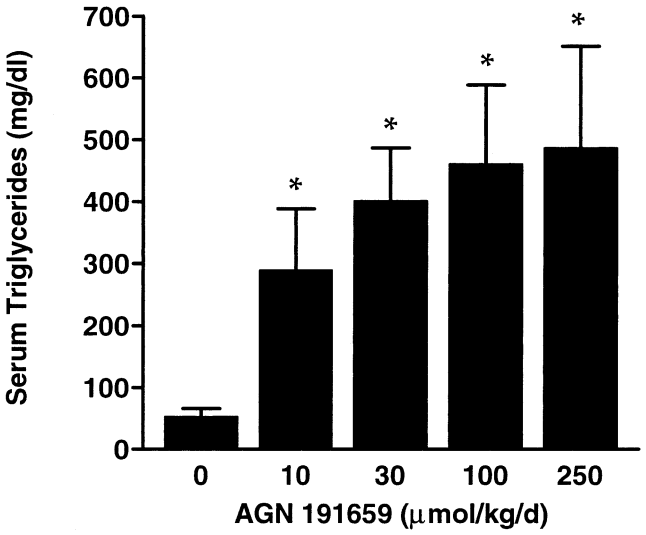

Fig. 1. Dose–response of AGN 191659-induced hypertriglyceridemia in rats. Male Fischer rats were treated orally by gavage for 3 consecutive days with corn oil or the indicated doses of AGN 191659 in corn oil. Serum was collected 24 hr after the last dose and assayed for serum triglycerides. Values are the means ± SD of 5 rats per group. Key: (*) significantly different ($P < 0.05$) from vehicle control.

Table 1
Effect of AGN 190121 alone or in combination with AGN 191701 or AGN 192849 on serum triglyceride levels in rats

Treatment[a]	Dose (μmol/kg)	Serum triglyercides (mg/dL)	% of Control
Experiment 1			
Corn oil		104 ± 16	100
AGN 190121	10	177 ± 35*	170
AGN 191701	60	73 ± 23	70
AGN 190121+	10	312 ± 100*,**	300
AGN 191701	60		
Experiment 2			
Corn oil		70 ± 11	100
AGN 190121	10	155 ± 27*	221
AGN 192849	25	73 ± 11	104
AGN 190121+	10	319 ± 76*,**	456
AGN 192849	25		

[a] Male Fischer rats were treated orally by gavage with corn oil or retinoid in corn oil for 3 consecutive days. Twenty-four hours after the last treatment, the rats were euthanized and blood was collected for the assay of serum triglerycides. Values are the means ± SD of 6 rats.

* Significant difference from control ($P < 0.05$).

** Significantly greater than AGN 190121 alone ($P < 0.05$).

[29] using [^{3}H]triolein as the substrate. Assay time was 10 min.

2.5. Statistics

Values are expressed as means ± SD. Pair-wise comparisons were made using an unpaired, two-tailed t-test. Multiple comparisons were made by one-way analysis of variance followed by Scheffe's test if significant differences were found. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Dose–response of AGN 191659

Our standard experimental protocol consisted of measuring serum triglycerides in rats 24 hr after three daily oral doses of retinoids. Using this protocol, maximal serum triglyceride elevations of 3- to 4-fold were observed for RAR agonists, while RXR agonists did not elevate triglyceride levels [23]. We had unexpectedly observed that AGN 191659, an RAR/RXR pan-agonist [25], induces a much higher level of triglyceridemia in this model than RAR agonists [12]. When evaluated in a dose–response study, AGN 191659 caused a 6-fold elevation of serum triglycerides at the lowest dose tested (10 μmol/kg), and ~9-fold increases in serum triglycerides at doses of 100 or 250 μmol/kg (Fig. 1). It should be noted that the large increase in serum triglycerides at the latter doses was observed in those two groups of animals, despite statistically significant weight losses of 13 and 23%, respectively.

3.2. Potentiation of AGN 190121-induced hypertriglyceridemia by AGN 192849

To determine if RXR agonists could modulate the hypertriglyceridemic activity of RAR agonists, rats were treated for 3 days with an RAR-selective agonist (AGN 190121) [26], an RXR-selective agonist (AGN 191701) [25], or the combination, and serum triglycerides were measured 24 hr after the last dose. As expected [23], AGN 190121 increased serum triglycerides, whereas AGN 191701 did not (Table 1). However, rats treated with both AGN 190121 and AGN 191701 had significantly higher serum triglyceride levels than rats treated with AGN 190121 alone (Table 1). AGN 192849, an RXR-selective agonist from a different structural class than AGN 191701 [24], also potentiated hypertriglyceridemia by AGN 190121 (Table 1). AGN 192849 had no effect on serum triglycerides by itself at a dose of 25 μmol/kg (Table 1). Indeed, in a dose–response study, AGN 192849 caused no elevation of serum triglycerides in the same 3-day dosing protocol at doses of 10, 30, or 100 μmol/kg (102, 94, and 84% of control, respectively).

To ascertain the dose-dependence of potentiation, rats were treated with AGN 190121 (10 μmol/kg) alone or in combination with increasing doses of AGN 192849. The increase in serum triglycerides induced by AGN 190121 alone did not reach statistical significance in this experiment, but was potentiated in a dose-dependent fashion by AGN 192849 co-treatment (Fig. 2). Serum triglycerides

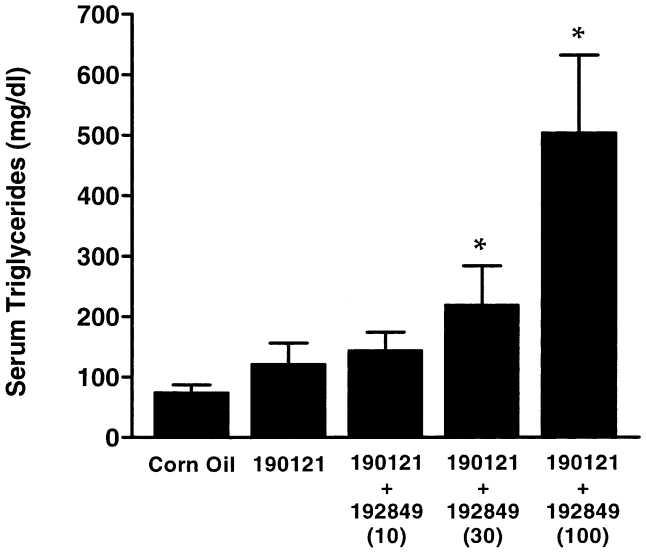

Fig. 2. Potentiation of AGN 190121-induced hypertriglyceridemia by AGN 192849 in rats. Male Fischer rats were treated orally by gavage once daily with corn oil, AGN 190121 (10 μmol/kg), or AGN 190121 (10 μmol/kg) in combination with increasing doses of AGN 192849 for 3 consecutive days. The dose of AGN 192849 in μmol/kg is indicated in parentheses. Serum was collected 24 hr after the last dose and assayed for serum triglycerides. Values are the means ± SD of 5 rats per group. Key: (*) significantly different ($P < 0.05$) from corn oil control.

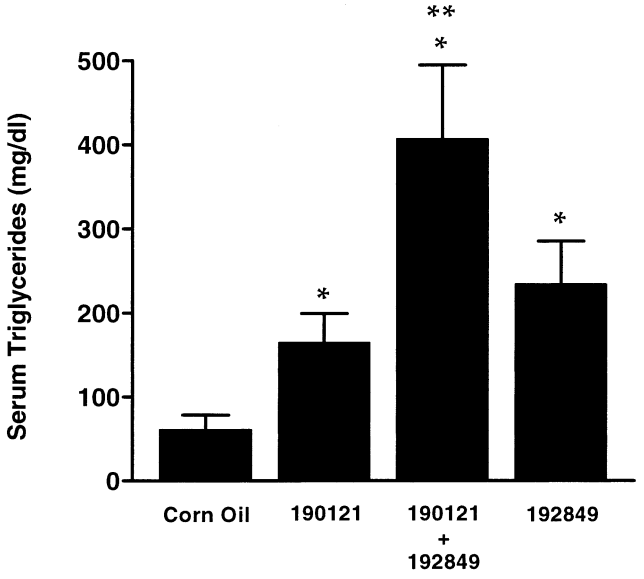

Fig. 3. Acute effect of AGN 190121 and 192849 on serum triglycerides in rats. Male Fischer rats were treated orally by gavage with corn oil, AGN 190121 (20 μmol/kg), AGN 192849 (60 μmol/kg), or AGN 190121 (20 μmol/kg) in combination with AGN 192849 (60 μmol/kg). Serum was collected 5 hr after treatment and assayed for serum triglycerides. Values are the means ± SD of 7 rats per group. Significant differences ($P < 0.05$) from corn oil control are indicated by a single asterisk (*) and from single treatment groups, AGN 190121 or AGN 192849 alone, by a double asterisk (**).

were significantly higher in rats treated with AGN 190121 in combination with either 30 or 100 μmol/kg of AGN 192849 (Fig. 2) than in control or rats treated with AGN 190121 alone.

3.3. Acute hypertriglyceridemic effect of the RXR agonist AGN 192849

The time course of RXR agonist-induced potentiation of hypertriglyceridemia was evaluated. Serum triglycerides were examined 5 hr after a single treatment with AGN 190121, AGN 192849, or the combination. AGN 190121 increased serum triglycerides, and this effect was significantly greater in combination with the RXR agonist AGN 192849 (Fig. 3). Unexpectedly, AGN 192849 by itself also significantly elevated serum triglycerides at this time point (Fig. 3).

Based on the unexpected finding of hypertriglyceridemia 5 hr after AGN 192849 treatment, we hypothesized that AGN 192849 might induce a transient hypertriglyceridemia. Accordingly, the time course of serum triglycerides after the last of three daily doses of AGN 192849 (60 μmol/kg) was examined. Indeed, serum triglycerides were dramatically elevated relative to vehicle controls at both 2.5 and 5 hr after the third treatment, but had returned to control levels at the 24-hr time point (Fig. 4a). This time course was markedly distinct from that of the RAR agonist AGN 190121, which produced prolonged hypertriglyceridemia after treatment with a single dose (Fig. 4b).

3.4. Dose responsiveness of multiple RXR ligands

To confirm and extend these findings, the dose responses of serum triglycerides 3 hr after a single treatment with AGN 192849, AGN 191701, or the highly potent RXR agonist AGN 194204 [28] were determined. AGN 192849 and AGN 191701 each caused dose-dependent elevations of serum triglycerides with comparable potency, while AGN 194204 was at least 10-fold more potent. Statistically significant elevations of the triglycerides were observed at doses as low as 10 μmol/kg of AGN 192849, 30 μmol/kg of AGN 191701, and 1 μmol/kg of AGN 194204 (Fig. 5). Additional data (not shown) demonstrate that the ED_{50} of AGN 194204 for triglyceride elevation was about 0.5 μmol/kg.[1]

3.5. Effect of the RAR antagonist AGN 193109

Since RXRs heterodimerize with RARs [2], the ability of the RAR antagonist AGN 193109 [23,27] to inhibit acute RXR agonist-induced hypertriglyceridemia was tested. Rats were pretreated for 2 hr with vehicle or AGN 193109 prior to challenge with AGN 192849. As a positive control, additional groups of rats were pretreated with vehicle or AGN 193109 prior to treatment with the RAR agonist AGN 190121. AGN 192849 treatment significantly increased se-

[1]Thacher SM and Escobar M, unpublished data.

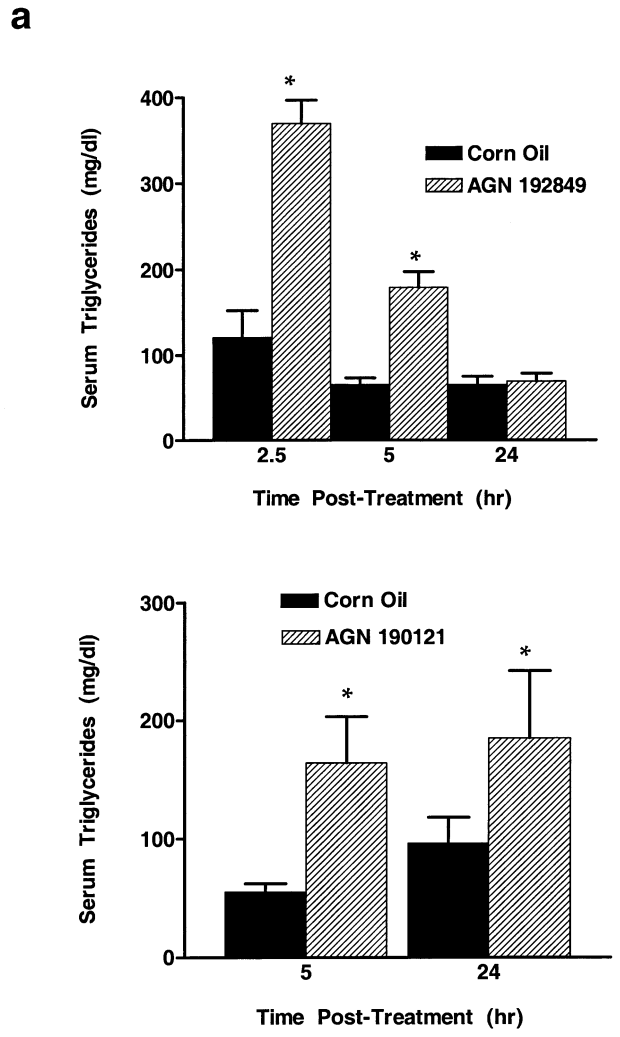

Fig. 4. (a) Time course of serum triglycerides after treatment of rats with AGN 192849. Male Fischer rats were treated orally by gavage with corn oil or AGN 192849 (60 μmol/kg) for 3 consecutive days. Groups of rats were euthanized 2.5, 5, or 24 hr after the third dose, and serum triglycerides were measured. (b) Time course of serum triglycerides after a single dose of AGN 190121 (20 μmol/kg) in corn oil or corn oil vehicle alone. Groups of rats were euthanized at 5 or 24 hr after dosing. Values are the means $\pm$ SD of 4–5 rats per group. Key: (*) significantly different ($P < 0.05$) from concurrent corn oil control.

rum triglycerides in the presence or absence of AGN 193109 pretreatment (Table 2). In contrast, a statistically significant acute increase in serum triglycerides induced by AGN 190121 was greatly attenuated in the presence of AGN 193109 (Table 2). Pretreatment with AGN 193109 alone had no effect on basal triglyceride levels.

3.6. Effect of fasting on action of AGN 194204

Triglycerides enter the bloodstream either as part of chylomicrons derived directly from dietary fat or as a major component of the very-low-density lipoprotein complex synthesized in liver. To exclude the possibility that the chylomicron fraction of serum lipid was primarily respon-

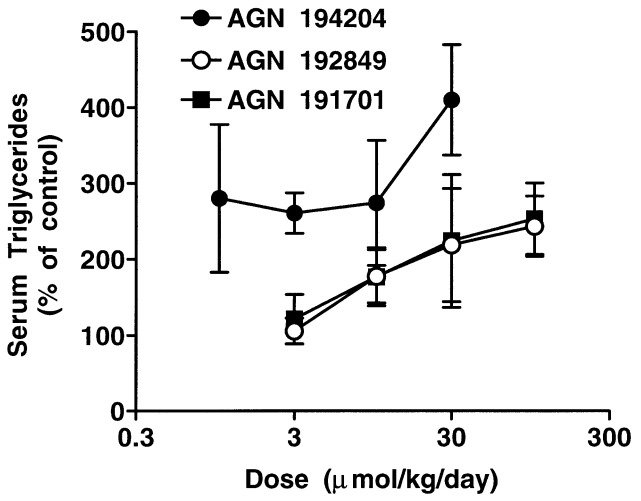

Fig. 5. Dose–response of acute hypertriglyceridemia induced by AGN 191701, AGN 192849, and AGN 194204 in rats. Male Fischer rats were treated orally by gavage with corn oil or 3, 10, 30, or 100 μmol/kg of AGN 191701 or AGN 192849 and corn oil or 1, 3, 10, and 30 μmol/kg of AGN 194204 in three separate studies. Serum was collected 3 hr after treatment and assayed for triglycerides. The percentage change was calculated based on serum triglyceride levels of the vehicle controls for each study, which were 89, 129, and 124 mg/dL for AGN 191701, AGN 192849, and AGN 194204, respectively. Values are the means $\pm$ SD of 5 rats per group.

sible for RXR-inducible triglyceride elevation, we tested the effect of overnight fasting on RXR-agonist activity. AGN 194204 was injected i.p. at a concentration of 2.84 μmol/kg, and serum triglycerides were measured 3 hr later. Animals fasted for both 4 and 16 hr had robust triglyceride induction (control: 51 $\pm$ 12 and treated: 173 $\pm$ 19 mg/dL with 4 hr of fasting versus 39 $\pm$ 9 and 115 $\pm$ 21 mg/dL after 16 hr of fasting, means $\pm$ SD). Although AGN 194204-induced triglycerides were higher in the minimally fasted rats in contrast to the 16-hr fasted animals, the ratio of serum triglycerides in treated and untreated animals was about 3-fold in each case.

3.7. RAR/RXR pan-agonist inhibition of plasma post-heparin lipolytic activity

To address the mechanism of the combined effects of RAR and RXR agonists on serum triglycerides, plasma post-heparin lipolytic activity was measured 27 hr after three daily treatments with AGN 191659 or the combination of AGN 190121 and AGN 192849. Both AGN 191659 and the AGN 190121/AGN 192849 combination dramatically reduced post-heparin lipolytic activity by 89 and 57%, respectively (Table 3). This effect correlated with the severe hypertriglyceridemia induced by the two treatments.

4. Discussion

The present data clearly demonstrate that RXR agonists can augment the hypertriglyceridemic response induced by

Table 2
Effect of RAR antagonist pretreatment on acute AGN 192849-induced hypertriglyceridemia in rats

Pretreatment[a]	Pretreatment dose (μmol/kg)	Treatment[a]	Treatment dose (μmol/kg)	Serum triglycerides (mg/dL)	% of control
Corn oil		Corn oil		108 ± 12	100
Corn oil		AGN 192849	20	318 ± 35*	294
Corn oil		AGN 190121	20	312 ± 116*	289
AGN 193109	20	Corn oil		123 ± 33	114
AGN 193109	20	AGN 192849	20	287 ± 106*	266
AGN 193109	20	AGN 190121	20	187 ± 46	173

[a] Male Fischer rats were pretreated orally by gavage with vehicle or AGN 193109 at the indicated doses for 2 hours. Rats were then treated orally by gavage with corn oil or AGN 191021 or AGN 192849 in corn oil. Three hours later, rats were euthanized and blood was collected for the assay of serum triglycerides. Values are the means ± SD of 5–6 rats.

* Significantly different from vehicle/vehicle treatment alone ($P < 0.05$).

co-administered RAR agonists. AGN 191701 and AGN 192849, RXR-selective agonists from distinct structural classes, each increased the hypertriglyceridemic effect of the RAR-selective agonist AGN 190121 in animals bled 24 hr after 3 consecutive days of dosing. At the same doses, or at doses up to 3- or 4-fold higher, these RXR agonists had no effect on serum triglycerides following the same dosing protocol [12,23]. The potentiation of RAR agonist-induced hypertriglyceridemia is dose-dependent, as increasing doses of AGN 192849 co-administered with a fixed dose of AGN 190121 for 3 days resulted in increasing serum triglyceride levels.

We had observed previously that the natural RAR/RXR pan-agonist 9-*cis*-retinoic acid and the synthetic RAR/RXR pan-agonist AGN 191659 induce higher levels of hypertriglyceridemia than RAR-selective agonists in rats [12,23]. AGN 191659, in particular, induces massive hypertriglyceridemia, with a 6-fold elevation at doses as low as 10 μmol/kg. The relatively large effect of RAR/RXR pan-agonists may be a consequence of the same potentiation observed with combinations of individual RAR and RXR agonists.

A novel and unexpected finding was that RXR agonists alone can cause hypertriglyceridemia acutely (within 2.5 hr or less). Previously, we observed that three RXR-selective agonists, AGN 191701, LGD1069, and SR11237, failed to cause hypertriglyceridemia 24 hr after two or three daily treatments [12,23]. This finding was confirmed for a fourth RXR selective agonist, AGN 192849, in this study. When earlier time points were examined, however, AGN 192849 was found to elevate triglycerides significantly at 2.5 and 5 hr after three daily treatments. In contrast, the effects of RAR-specific ligands such as AGN 190121 persist out to 24 hr or greater [23], in spite of the fact that AGN 190121 has a short half-life *in vivo* [30].

AGN 192849, AGN 191701, and a higher potency RXR-specific retinoid, AGN 194204, also caused dose-dependent increases in serum triglycerides 3 hr after treatment with a single dose. AGN 191701 and AGN 192849 have an approximately equal potency in receptor transactivation assays of the RXR receptors [24,25], while AGN 194204 is considerably more potent [28]. In addition, although triglyceride induction by the RAR agonist AGN 190121 was blocked by the RAR-specific antagonist AGN 193109, the elevation of triglycerides induced by the RXR-specific ligand AGN 192849 was not. These findings provide pharmacological evidence that ligand binding to the RXR receptor causes the acute hypertriglyceridemia. Thus, although RAR- and RXR-specific retinoids may both cause hypertriglyceridemia, their mechanisms of action appear to be very different based on their different time courses and sensitivity to an RAR receptor antagonist. The relatively rapid effects of RXR-specific ligands do not appear to be the result of increased intestinal uptake of lipids since AGN 194204 also induced serum triglycerides in animals fasted overnight.

These data suggested that triglyceride clearance might be affected by retinoid treatment. Lipoprotein lipase is bound to heparin sulfate chains on endothelial walls, particularly in adipose and muscle capillary beds, and cleaves triglycerides to monoacylglycerols and fatty acids [31]. Lipoprotein lipase plays a critical role in the removal of triglycerides from chylomicrons and very-low-density lipoproteins present in plasma. Hepatic lipase, which is found only in

Table 3
Plasma post-heparin lipolytic activity is inhibited by simultaneous activation of RAR and RXR receptors

Treatment[a]	Dose (μmol/kg)	Plasma lipolytic activity (nmol/min/mL)	Serum triglycerides (mg/dL)
Corn oil		43.5 ± 9.0	99 ± 10
AGN 191659	20	4.7 ± 5.0*	589 ± 244*
AGN 190121+	10	18.5 ± 4.7*	422 ± 72*
AGN 192849	30		

[a] Male Fischer rats were treated as described in the legend of Table 1. Serum triglycerides were measured 24 hours after the last treatment; at 27 hours, the animals were injected with heparin, and plasma was obtained 10 min later for the determination of lipolytic activity. Values are the means ± SD for 6–7 animals.

* Significantly different from control animals ($P < 0.001$).

liver, may play a similar but quantitatively less important role [31]. Both enzymes are displaced from the capillary wall by the infusion of heparin, and thus quantitation of heparin-releasable lipolytic activity has been used as a measure of triglyceride clearance capacity in the body [32,33]. Both the RAR/RXR pan-agonist AGN 191659 and the combination of the RAR agonist AGN 190121 and the RXR agonist AGN 192849 dramatically lowered plasma heparin-releasable lipolytic activity 27 hr after the third of three daily treatments. These data suggest that simultaneous activation of RAR and RXR receptors increases serum triglyceride levels by substantially impairing triglyceride clearance. 13-*cis*-Retinoic acid has been shown previously to decrease clearance of very-low-density lipoproteins in rats and to decrease lipoprotein lipase activity in humans, potentially accounting for its hypertriglyceridemic activity [32,33].

The biological activities of RAR ligands are thought to be mediated by binding of the RAR ligands to the RAR portion of RAR/RXR heterodimers. These heterodimers bind to retinoic acid response elements and activate transcription from retinoid-responsive genes [2,3]. Recent data suggest that in some cases RXR ligands can bind to the RXR portion of the RAR/RXR heterodimer and have additive, if not synergistic, effects with RAR agonists in a number of *in vitro* systems [6–9]. The potentiation of RAR agonist-induced hypertriglyceridemia by an RXR agonist may be an example of such an interaction. However, RXR ligands also appear to be silent under most circumstances in which the RAR partner is responsive [5] and have no significant effect on a sensitive RAR-responsive tissue such as skin. In contrast to the RARs, other nuclear receptors that function as RXR heterodimers do appear to be regulated by RXR ligands [2,15,34]. One of these RXR partners could, in principle, mediate the RXR ligand-induced hypertriglyceridemia, but the best-studied of these receptors do not appear to be involved. Ligands to vitamin D receptor (VDR) and thyroid receptor (TR) are not known to cause hypertriglyceridemia, and there is no evidence that PPARγ and PPARα ligands cause *in vivo* triglyceride elevation in rodents as reported here [35]. In fact, the fibrate ligands of PPARα reduce circulating triglycerides. A synthetic LXR ligand, which induces cholesterol conversion to bile acids, was shown recently to activate liver fatty acid synthesis and to elevate serum triglyceride levels [36], but it is unclear whether the mechanism of RXR ligands is similar.

At early times points (e.g. 5 hr after treatment), both RAR and RXR agonists caused hypertriglyceridemia, and it appears that RXR agonists have an additive rather than a synergistic effect with an RAR agonist. Potentiation was only apparent at longer time points (e.g. 24 hr after three treatments) where RXR agonists alone were without effect on serum triglycerides. These findings are consistent with our conclusion that the RXR ligand acts by a distinct mechanism of action, in this case possibly altering gene expression in such a way that exacerbates the RAR-mediated hypertriglyceridemia. Ongoing studies are directed at determining the mechanistic basis of this interaction.

An obvious clinical implication of the current findings is that hypertriglyceridemia may be a greater issue for RAR/RXR pan-agonists than for either RAR-selective or RXR-selective agonists. Initial studies in cancer patients of oral 9-*cis*-retinoic acid, which binds both the RXRs and the RARs, found that 4 of 29 patients developed severe (“grade 3”) hypertriglyceridemia [21]. Further study will be required to determine if the incidence or severity of RAR/RXR pan-agonist-induced hypertriglyceridemia is actually greater than that of RAR- or RXR-selective agonists in humans. Such comparisons are made difficult by the fact that some nominally RXR-selective agonists may have RAR activity at high dose levels, e.g. LGD1069 [37], and that the RAR-selective compound all-*trans*-retinoic acid can be converted spontaneously to 9-*cis*-retinoic acid [22,38].

The elevation of triglycerides reported here contrasts with three recent reports on the effect of RXR ligands on diabetic *db/db* mice. Both LGD1069 and LGD100268 lead to lowering of serum triglycerides when compared with untreated control mice in animals treated for more than 1 week [13,39,40]. This effect is in addition to a reduction in elevated blood glucose levels. We have also demonstrated a substantial, long-term reduction of serum triglycerides by AGN 194204 in *db/db* mice, but we found that, as in normal rats, serum triglycerides were elevated at shorter time periods (e.g. 2 days) [1]. We have reported previously that RXR ligands induce liver acyl-CoA oxidase [12]. In addition, the messenger RNA (mRNA) for acyl-CoA oxidase is elevated in rat liver along with the mRNA for bifunctional enzyme [39]. Fatty acid oxidation is highly elevated in liver lysates of *db/db* mice that are chronically treated with LGD100268 [40]. These data suggest that there are multiple steps at which RXR ligands can regulate lipid metabolism, and that their action may depend upon both the species tested and the metabolic state induced by a particular disease condition.

In conclusion, these data demonstrate that RXR-selective agonists can potentiate the hypertriglyceridemic activity of RAR agonists in normal rats. This effect may explain the high hypertriglyceridemic efficacy of RAR/RXR pan-agonists in rats, and suggests that hypertriglyceridemia may be a greater issue for RAR/RXR pan-agonists than for RAR- or RXR-selective agonists in the clinic. These data also show that RXR agonists alone can induce transient hypertriglyceridemia, but the potential clinical significance of this finding is unclear because of the rapid reversibility of the effect following a single dose.

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