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Mitogenic Effect of Retinoid X Receptor Agonists in Rat Liver

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ABSTRACT. (*E*)-2-[2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophenecarboxylic acid (AGN 191701) and other retinoid X receptor (RXR)-selective agonists were observed to cause hepatomegaly in rats. The purpose of the present study was to understand the biochemical basis of RXR agonist-induced hepatomegaly. Male Fischer rats were implanted s.c. with osmotic pumps containing 5-bromo-2′-deoxyuridine (BrdU) and treated by gavage with 0, 60, or 180 μmol/kg/day of AGN 191701 for 3 days. AGN 191701 caused dose-dependent hepatomegaly in the absence of hepatic necrosis and increased hepatocyte BrdU labeling index (LI). To determine if AGN 191701-induced hepatic hyperplasia was sustained, rats were treated by gavage with 60 μmol/kg of AGN 191701 for up to 7 days and exposed to BrdU via osmotic pump on days 1–3 or on days 6–8. Hepatocyte LI and mitotic index were increased only in rats exposed to BrdU on days 1–3, indicating that AGN 191701-induced hepatocyte proliferation was transient. The receptor specificity of this mitogenic effect was tested by co-treating rats for 2 days with various retinoids and BrdU. 2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2-(4-carboxylphenyl)-1,3-dioxolane (SR11237), an RXR-selective agonist, and (*E*)-5-[2-(5,6,7,8-tetrahydro-3,5,5,8-pentamethyl-2-naphthyl)propen-1-yl]-2-thiophenecarboxylic acid (AGN 191659), a retinoic acid receptor (RAR)/RXR pan-agonist, both increased hepatocyte LI. Two RAR-selective agonists, all-*trans*-retinoic acid and (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propen-1-yl] benzoic acid (TTNPB), did not affect hepatocyte LI. To determine if RXR agonists have biochemical effects in common with a peroxisome proliferator, various endpoints were measured 24 hr after two daily treatments with AGN 191701, SR11237, or clofibrate. While all three compounds induced hepatic acyl CoA oxidase activity, only clofibrate increased hepatic carnitine acyl transferase activity and lowered serum triglycerides. Taken together, these data show that RXR-selective agonists but not RAR-selective agonists cause hepatomegaly accompanied by hepatocyte mitogenesis in rats. The fact that RXR agonists have some biological effects distinct from RAR agonists and clofibrate suggests that RXR-selective agonists may have unique therapeutic applications. BIOCHEM PHARMACOL **54**;4:517–524, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. RAR; RXR; retinoid; liver; mitogenesis; hepatomegaly

RXRs[‖] are members of the superfamily of nuclear receptors and include three subtypes: RXR-α, -β, and -γ. RXRs show no more sequence homology to RARs than to a variety of other receptors with which RXRs are known to form heterodimers, including thyroid, vitamin D, and PPARs. These heterodimers act as ligand-dependent transcription factors when bound by ligands for the heterodimeric partner of RXR [1]. RXR ligands generally do not transactivate through RXR heterodimers [2, 3], although a PPAR–RXR heterodimer mediates transactivation in response to both PPAR activators and RXR ligands in co-transfection assays [4–7]. RXRs can also form homodimers and activate gene transcription in *in vitro* co-transfection assays, but since RXR homodimers bind to DNA with much lower affinity than RAR-RXR heterodimers, the physiological relevance of RXR homodimers is not clear [1, 8].

While RXRs clearly are important as heterodimeric partners for other receptors, the role of RXR ligands is less clear. 9-*cis*-Retinoic acid, an activator of both RARs and RXRs [9, 10], has a number of effects in cells and whole animals, including inhibition of T-cell driven apoptosis and inhibition of mammary carcinogenesis, respectively [11, 12]. However, the extent to which these activities are mediated by 9-*cis*-retinoic acid's RAR agonist, rather than RXR agonist, properties has not been determined. Recently, selective RXR agonists have been developed [13–16]. RXR selective agonists have been demonstrated to have biological effects in relatively few cellular systems, e.g. the induction of apoptosis in HL-60 cells [17]. RXR agonists also induce 25-hydroxyvitamin D_3-24-hydroxylase mRNA in mice and hepatic acyl CoA oxidase in rats, although these are the only effects reported for RXR agonists in whole animals [2, 18–20]. Thus, the biological

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‖ *Abbreviations:* BrdU, 5-bromo-2′-deoxuridine; H & E, hematoxylin and eosin; LI, labeling index; PPARs, peroxisome proliferator-activated receptors; RARs, retinoic acid receptors; and RXRs, retinoid X receptors.

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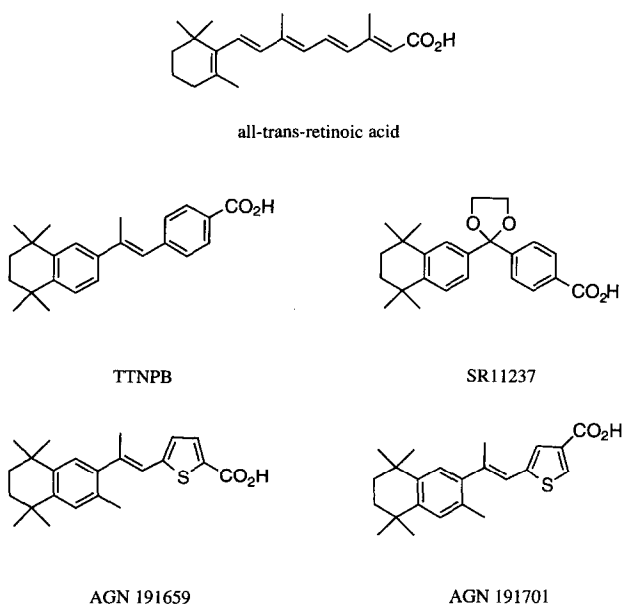

FIG. 1. Chemical structures of AGN 191701, AGN 191659, SR11237, TTNPB, and all-*trans*-retinoic acid.

activity of RXR-selective agonists *in vivo* remains to be defined.

The present studies were initiated by the observation that several RXR-selective agonists, including AGN 191701 [16], cause dose-dependent hepatomegaly in rats. Hepatomegaly can be a pathological or merely adaptive response of the liver to xenobiotics [21]. Since the RXR agonist-induced hepatomegaly was not accompanied by overt toxicity, we hypothesized that RXR agonists act as hepatic mitogens and tested this hypothesis in a rat model. The results indicate that RXR agonists, but not RAR agonists, induce hepatic mitogenesis and have additional biochemical effects in rat liver. These biological effects represent potential markers of RXR exposure *in vivo*.

MATERIALS AND METHODS

Chemicals

(*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl) propen-1-yl]benzoic acid (TTNPB) was synthesized as described by Locliger *et al.* [22]. SR11237, 2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2-(4-carboxylphenyl)-1, 3-dioxolane, was synthesized essentially as described by Gendimenico *et al.* [2]. AGN 191701, (*E*)-2-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophenecarboxylic 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, were synthesized as described by Beard *et al.* [16]. Structures of all retinoids used in the present experiments are given in Fig. 1. Phosphate-buffered saline was obtained from Gibco-BRL (Bethesda, MD). Isoflurane was obtained from Anaquest Inc. (Madison, WI). Sources of reagents for BrdU immunohistochemistry were as indicated previously [23]. All-*trans*-retinoic acid, clofibrate, BrdU, corn oil, and all other reagents were obtained from the Sigma Chemical Co. (St. Louis, MO).

Animals

Male Fischer rats [CDF (F-344)/CrlBR] were obtained from Charles River Laboratories (Hollister, CA) and were acclimated for at least 6 days prior to experimentation. Rats were housed singly 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 and were randomized according to weight for each experiment.

Osmotic Pump Implantation

Osmotic pumps (Alzet model 2ML1, Alza Corp., Palo Alto, CA) were loaded with an 8.0 mg/mL solution of BrdU in phosphate-buffered saline that was adjusted to pH 7.4 with sodium hydroxide. These pumps deliver fluid continuously at a rate of 10 μL/hr for up to 7 days. Prior to surgery, animals were anesthetized by isoflurane inhalation (3%, v/v). Dorsal hair was shaved, and the skin was rinsed sequentially with chlorhexidine (Novalsan, Fort Dodge Laboratories, Fort Dodge, IA), 0.9% sodium chloride solution (Lens Plus, Allergan, Inc.), and 70% isopropanol. A single incision was made in the dorsal skin, osmotic pumps were implanted s.c., and the incision was closed with surgical clips. Osmotic pumps were routinely implanted from ~9:00 to 11:00 a.m.

Animal Treatments

Test compounds were suspended in corn oil (vehicle) and sonicated on ice to decrease particle size. Suspensions were prepared fresh each day. Treatments were administered routinely once daily from 12:00 noon to 2:00 p.m. by gavage in a volume of 5 mL/kg. Specific experimental protocols are described below:

EXPERIMENT NO. 1. Groups of rats (N = 6) were treated with vehicle or AGN 191701 (60 or 180 μmol/kg/day) on days 1, 2, and 3. Rats were implanted with osmotic pumps on day 1 and euthanized on day 4.

EXPERIMENT NO. 2. Rats were treated with vehicle or AGN 191701 (60 μmol/kg/day) for up to 7 days. Groups of rats (N = 5) in each treatment group were euthanized on days 3 and 8. An additional group of rats (N = 5) was treated with AGN 191659 (60 μmol/kg) on days 1 and 2 and euthanized on day 3. Rats euthanized on days 3 and 8 had osmotic pumps implanted on days 1 and 6, respectively.

EXPERIMENT NO. 3. Groups of rats (N = 4–5) were treated with corn oil, SR11237 (60 μmol/kg/day), TTNPB

(0.6 μmol/kg/day), or all-*trans*-retinoic acid (60 μmol/kg/day) on days 1 and 2. Rats were implanted with osmotic pumps on day 1 and euthanized on day 3.

EXPERIMENT NO. 4. Groups of rats (N = 5) were treated with corn oil, AGN 191701 (60 μmol/kg/day), SR 11237 (60 μmol/kg/day), or clofibrate (200 mg/kg/day) on days 1 and 2. Rats were euthanized on day 3.

Necropsy

Necropsy was performed routinely from ~1:00 to 3:00 p.m. on the indicated days. Rats were weighed, anesthetized with CO_2, bled from the inferior vena cava under CO_2 narcosis, and euthanized by exsanguination. Livers were rinsed with saline, blotted dry, and weighed. Sections of the left, right median, and right anterior lobes of the liver as well as a section of the duodenum were taken and fixed in 10% buffered formalin. In experiment No. 4, sections of liver were frozen on dry ice for subsequent cytosol preparation as described below. Blood was centrifuged for 10 min at 4° to prepare serum. Liver and duodenum specimens were embedded in paraffin and sectioned at 5 μm for H & E staining and/or immunohistochemistry.

Preparation of Hepatic Cytosols

Liver stored at −80° was thawed and combined with 4 vol. of ice-cold 0.154 M KCl/0.05 M Tris–HCl (pH 7.4). Samples were homogenized with a Polytron homogenizer for 5 sec on ice. Homogenates were centrifuged for 20 min at 4° to pellet nuclei. The post-nuclear supernatant (cytosol) was used for the carnitine acyltransferase and acyl CoA oxidase assays.

Biochemical Assays

Serum alanine aminotransferase activity was determined at ambient temperature using a kinetic spectrophotometric assay (Sigma, Kit No. 259). Serum triglycerides were assayed as total triglycerides (triglycerides plus glycerol) using a spectrophotometric endpoint assay (Sigma, Kit No. 337-B). Carnitine acyltransferase activity was assayed using a spectrophotometric method that measured the rate of appearance of free CoA [24]. Acyl CoA oxidase activity was measured by the fluorometric method of Poosch and Yamazaki [25] using lauroyl CoA as a substrate.

Determination of Hepatocyte BrdU LI

Liver and duodenum sections were stained immunohistochemically for BrdU as described previously [23]. Duodenum served as a positive control for BrdU delivery to the animal [26]. BrdU-stained liver sections were examined by light microscopy with the experimenter blind to the treatment group. Random fields were identified to score nuclei for BrdU incorporation. At least 1500 hepatocellular nuclei in the right anterior lobe were scored. BrdU labeling did not appear to differ among the hepatic lobes sampled. Hepatocyte LI was calculated by dividing the number of labeled nuclei by the total number of nuclei scored, and multiplying by 100.

Determination of Mitotic Index

H & E-stained liver sections were examined by light microscopy for the presence of mitotic figures with the experimenter blind to the treatment group. Random fields were identified to score nuclei, and at least 1500 hepatocellular nuclei in the right anterior lobe were scored. Mitotic index was calculated by dividing the number of mitotic figures by the total number of nuclei scored, and multiplying by 100.

Statistics

Values are expressed as means ± SD. Labeling indices and mitotic indices were log-transformed prior to statistical analysis to fulfill the requirement of homogeneity of variance. Pair-wise comparisons were made using an unpaired, two-tailed *t*-test. Multiple comparisons were made by one-way analysis of variance followed by Dunnett's test if significant differences were found. Differences were considered significant at $P < 0.05$.

RESULTS

Dose–Response of AGN 191701-Induced Hepatomegaly and Hyperplasia

In preliminary studies, it was observed that several RXR-selective agonists, including AGN 191701, caused hepatomegaly in rats in the absence of weight loss when administered by gavage for 3 days (data not shown). To determine if RXR agonists induce hepatic hyperplasia, hepatocyte BrdU LI was studied in rats in Experiment 1. As shown in Table 1, AGN 191701 caused a dose-dependent increase in liver weight and an approximately 3-fold increase in hepatocyte LI at either dose tested. While BrdU-labeled nuclei were pan-lobular in distribution in control rats (Fig. 2, top), BrdU-labeled nuclei were predominantly periportal and midzonal in distribution in AGN 191701-treated rats (Fig. 2, bottom). It is noteworthy that control hepatocyte LI was high relative to literature values for male rats [27–29] and relative to later experiments, although the occasional high background hepatocyte LI has been reported previously [27]. The reason for the high background in this particular experiment was not clear. AGN 191701 did not affect weight gain (Table 1) or show any histological evidence of hepatic necrosis.

Time–Course of AGN 191701-Induced Hyperplasia

To determine if AGN 191701-induced hepatocyte proliferation was sustained, rats were treated by gavage with 60

TABLE 1. Effect of AGN 191701 on body weight, liver weight, and hepatocyte BrdU LI in rats

Dose* (μmol/kg)	Final body weight (g)	Absolute liver weight (g)	Relative liver weight† (%)	Hepatocyte BrdU LI (%)
0	136 ± 4	5.51 ± 0.43	4.03 ± 0.19	13.5 ± 9.1
60	135 ± 5	6.20 ± 0.36‡	4.56 ± 0.23‡	36.7 ± 9.2‡
180	136 ± 4	6.91 ± 0.35‡	5.08 ± 0.14‡	43.3 ± 5.9‡

* Male Fischer rats were treated i.g. with corn oil or AGN 191701 in corn oil for 3 consecutive days and administered BrdU continuously via osmotic pump. Twenty-four hours after the last treatment, rats were euthanized and livers were removed for analysis. Values are the means ± SD of 6 rats.

† Liver weight as a percent of body weight.

‡ Significantly different from control ($P < 0.05$).

μmol/kg/day of AGN 191701 for 2 or 7 days and exposed to BrdU via osmotic pump either on days 1–3 or days 6–8, respectively. Additional rats were treated for 2 days with AGN 191659, an RAR/RXR pan-agonist, and euthanized on day 3 with the other treatment groups. AGN 191701 and AGN 191659 both caused a ~7-fold increase in hepatocyte LI after only 2 days (Fig. 3). However, rats exposed to BrdU beginning on day 6 of AGN 191701 treatment showed no increase in hepatocyte LI relative to vehicle controls (Fig. 3). To confirm that the increased hepatocyte LI at day 3 reflected an increase in cell proliferation and not merely an increase in DNA synthesis, mitotic figures were quantitated on H & E-stained sections. The mitotic index of vehicle controls was quite low (0.10 ± 0.05%), but was increased significantly by both AGN 191701 (0.57 ± 0.39%) and AGN 191659 treatment (0.58 ± 0.40%). Thus, both AGN 191701 and AGN 191659 caused hepatocyte proliferation.

While neither AGN 191701 nor AGN 191659 caused histological evidence of hepatic necrosis, AGN 191659 induced mild periportal vacuolization on day 3. The latter finding probably represented fat deposition, since AGN 191659 caused hypertriglyceridemia, a typical effect of RAR but not RXR agonists in this dosing regimen [20]. Serum triglycerides on day 3 were 51 ± 15 mg/dL in control rats, 64 ± 10 mg/dL in AGN 191701-treated rats, and 620 ± 169 mg/dL in AGN 191659-treated rats.

Receptor Selectivity of Retinoid-Induced Hepatocyte Proliferation

To further define the retinoid receptor selectivity of the hepatocyte proliferative effect, groups of rats were treated for 2 days with another RXR-selective agonist, SR11237, or one of two RAR-selective agents, all-*trans*-retinoic acid or TTNPB. Retinoids were tested at the same dose level as used above (60 μmol/kg/day) except for TTNPB, which was tested at 0.6 μmol/kg/day. TTNPB is known to be a potent retinoid [18], and a preliminary dose-ranging study had indicated that as little as 0.6 μmol/kg/day of TTNPB caused modest weight suppression after three daily treatments (data not shown). Rats were co-administered BrdU via osmotic pump and were euthanized on day 3. As shown in Table 2, none of the retinoids caused a significant increase in liver weight. The apparent increase in relative liver weight in SR11237-treated rats was not statistically significant ($P = 0.07$, analysis of variance) in this experiment. However, SR11237 did induce a greater than 4-fold increase in hepatocyte LI (Table 2) that was predominantly periportal/midzonal in distribution, as observed for AGN 191701. SR11237 did not induce any significant liver histopathology except for an increase in mitotic figures. Neither all-*trans*-retinoic acid nor TTNPB affected hepatocyte LI (Table 2) or caused liver histopathology.

Additional Biochemical Effects of RXR Agonists

Since several RXR agonists induced hepatic mitogenesis in our animal model, it was of interest to determine whether or not these agents belonged to known classes of hepatic mitogens. The fact that RXRs form heterodimers with PPARs [4–7] made a comparison to a known peroxisome proliferator of particular interest. Thus, various serum and hepatic biochemical parameters were measured 24 hr after two daily treatments with AGN 191701, SR11237, or clofibrate, a classical peroxisome proliferator agent [30]. Both AGN 191701 and SR11237 increased hepatic fatty acyl CoA oxidase activity but did not affect significantly hepatic carnitine acyl transferase activity or serum triglyceride levels (Table 3). In contrast, clofibrate induced both hepatic acyl CoA oxidase and carnitine acyl transferase activity and significantly decreased serum triglyceride levels (Table 3). None of the compounds elevated serum alanine aminotransferase activity.

DISCUSSION

This investigation was initiated by the observation that several RXR agonists cause hepatomegaly in rats when administered orally. In the present study, the RXR selective agonist AGN 191701 [16] was used to illustrate hepatomegaly by this class of compounds and to study the mechanism of this effect. Hepatomegaly is a common response of liver to xenobiotics and can reflect hyperplasia and/or hypertrophy [21]. Hyperplasia of even a transient nature can be detected sensitively by continuous delivery of BrdU via osmotic pump [26, 27, 31], and this method was used to determine if AGN 191701 causes hepatic hyperplasia in rats. Treatment of rats for as little as 2 days with AGN 191701 dramatically increased hepatocyte BrdU LI, reflect-

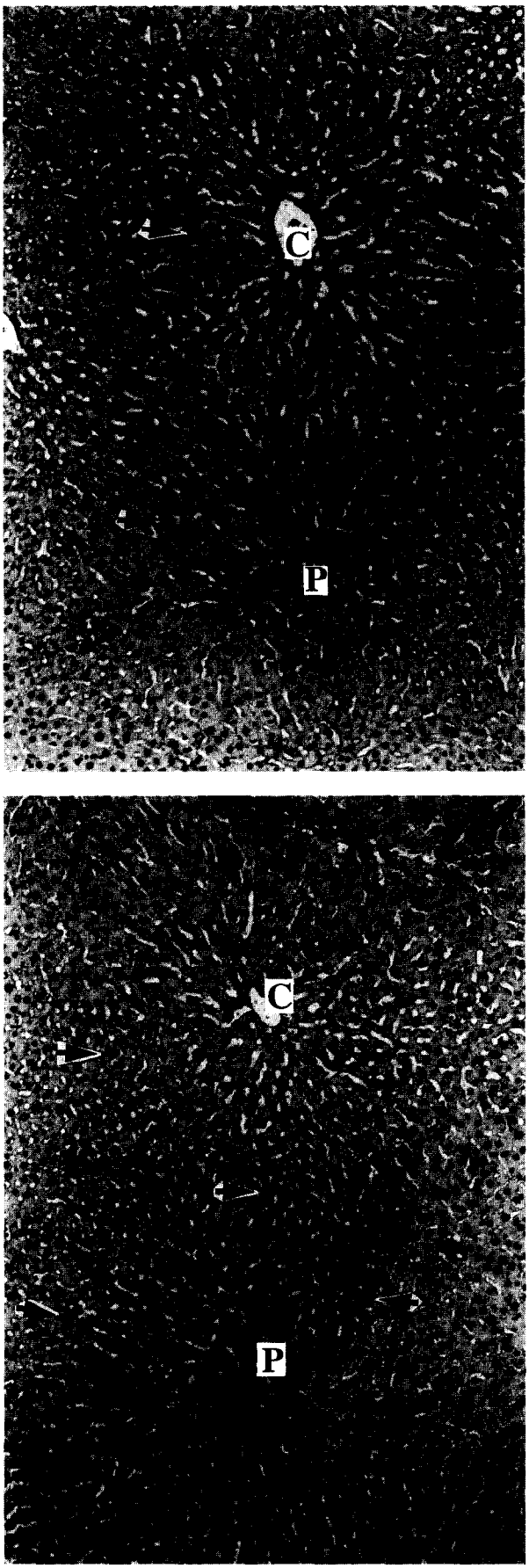

FIG. 2. Photomicrograph of a BrdU-stained liver section from vehicle (top panel) and AGN 191701 (bottom panel)-treated rats. Male Fischer rats were treated i.g. for 3 consecutive days with corn oil (vehicle) or AGN 191701 (180 μmol/kg/day). Rats were euthanized 24 hr after the last treatment, and liver sections were stained for BrdU incorporation. (Top) Relatively few BrdU-labeled, dark-staining nuclei (arrows) scattered in the parenchyma. (Bottom) Numerous BrdU-labeled, dark-staining nuclei in the parenchyma except in the area adjacent to the central vein. Key: (C) central vein; and (P) portal area.

ing an increase in DNA synthesis. The fact that this increased DNA synthesis was accompanied by an increase in mitotic figures on H & E sections of liver clearly demonstrates that AGN 191701 induces hepatocyte proliferation in rats. Thus, hepatocyte proliferation accounts, at least in part, for AGN 191701-induced hepatomegaly. SR11237, another RXR agonist [13], did not induce significant hepatomegaly in one of two experiments (Table 2), but it should be noted that treatment was for 2 days (not 3 days as in the AGN 191701 experiments) and that there was a trend toward increased liver weight in both experiments. Hepatocyte mitogenesis would not be expected to result in hepatomegaly until the daughter cells grow in size. Thus, 2 days may not be long enough to consistently observe hepatomegaly at low doses of RXR agonists.

Hepatocyte proliferation is probably a general effect of RXR agonists, since a structurally unrelated RXR-selective agonist, SR11237 [13], and an RAR/RXR pan-agonist, AGN 191659 [18], also induced hepatocyte proliferation in the rat. Significantly, two prototypical RAR-selective agonists, all-*trans*-retinoic acid and TTNPB [18], did not

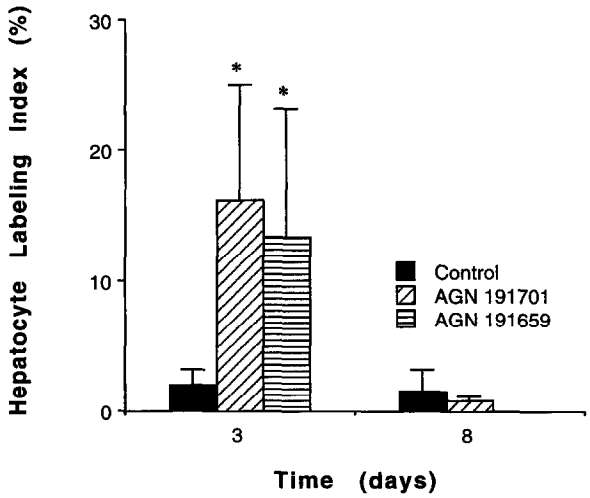

FIG. 3. Hepatocyte BrdU LI in AGN 191701- and AGN 191659-treated rats. Male Fischer rats were treated i.g. with corn oil, AGN 191701 (60 μmol/kg), or AGN 191659 (60 μmol/kg) for 2 or 7 consecutive days and euthanized on day 3 or 8, respectively. Rats euthanized on day 3 were exposed to BrdU via osmotic pump on days 1–3, and rats euthanized on day 8 were exposed to BrdU via osmotic pump on days 6–8. Values are the means ± SD of 5 rats. Significant ($P < 0.05$) differences from vehicle control are indicated by an asterisk (*).

TABLE 2. Effect of SR11237, TTNPB, and all-*trans*-retinoic acid (RA) on body weight, liver weight, and hepatocyte BrdU LI in rats

Treatment* (dose)	Final body weight (g)	Absolute liver weight (g)	Relative liver weight† (%)	Hepatocyte BrdU LI (%)
Corn oil (5 mL/kg)	157 ± 6	6.26 ± 0.54	3.98 ± 0.22	2.7 ± 1.5
SR11237 (60 μmol/kg)	158 ± 8	6.82 ± 0.51	4.32 ± 0.25	12.4 ± 5.6‡
TTNPB (0.6 μmol/kg)	151 ± 7	6.05 ± 0.54	4.00 ± 0.22	1.1 ± 0.4
All-*trans*-RA (60 μmol/kg)	152 ± 6	6.08 ± 0.41	3.99 ± 0.16	1.5 ± 0.8

* Male Fischer rats were treated i.g. once daily with corn oil or the indicated doses of various retinoids for 2 days. Twenty-four hours after the last treatment, rats were euthanized and livers were removed for analysis. Values are the means ± SD of 4–5 rats.

† Liver weight as a percent of body weight.

‡ Significantly different from corn oil control ($P < 0.05$).

induce hepatocyte proliferation in the same model. These findings clearly suggest that it is RXR agonist and not RAR agonist activity that is responsible for retinoid-induced hepatocyte proliferation. Recently, Ohmura *et al.* [32] reported that 9-*cis*-retinoic acid, an RAR/RXR pan-agonist, stimulated hepatocyte DNA synthesis in rats within 24 hr of a single dose. Based on the fact that 200 mg/kg of all-*trans*-retinoic acid was required to induce a DNA synthetic response comparable to that of 10 mg/kg of 9-*cis*-retinoic acid, the authors suggested that the all-*trans*-retinoic acid effect might be due to metabolic conversion to 9-*cis*-retinoic acid [32]. This interpretation is consistent with the present findings using RAR- and RXR-selective agonists, which show that the induction of hepatocyte proliferation is an RXR agonist effect.

Cell proliferation has been classified according to mode of action as either regenerative or mitogenic [33, 34]. Regenerative cell proliferation functions to replace lost cells, as occurs in liver following chloroform-induced hepatotoxicity or partial hepatectomy. Mitogenic cell proliferation refers to direct stimulation of hyperplasia in the absence of necrosis, as occurs after phenobarbital treatment. Mitogenic cell proliferation typically ceases after a few days despite continued exposure to the mitogen, presumably due to homeostatic growth control mechanisms [31, 33]. While both cytotoxicants and mitogens cause hepatic neoplasia in rodents, a causal relationship between hepatocyte proliferation and cancer is more clearly implicated for agents that cause cell proliferation by a regenerative mode of action [33, 34]. Thus, the mode of RXR agonist-induced hepatocyte proliferation was of interest. AGN 191701 treatment did not result in inhibition of weight gain, histological evidence of hepatic necrosis, or elevation of serum alanine aminotransferase activity, a marker of hepatotoxicity [21]. SR11237 also did not cause weight loss or evidence of hepatotoxicity under conditions in which it induced hepatic hyperplasia. Moreover, AGN 191701-induced hepatocyte proliferation was apparently transient in nature, since the compound did not increase BrdU LI during the last 2 days of seven daily treatments. These data suggest that RXR agonists induce hepatocyte proliferation by a mitogenic mode of action.

Acyl CoA oxidase is the rate-limiting step in fatty acid oxidation and is invariably induced in liver by known peroxisome proliferators [30, 35]. 9-*cis*-Retinoic acid, an RAR/RXR pan-agonist [9, 10], has been reported to transactivate the acyl CoA oxidase gene *in vitro* in the presence of co-transfected PPARs and RXRs [4, 5, 7]. Thus, it

TABLE 3. Effect of gavage treatment with RXR agonists or clofibrate on relative liver weight, serum alanine aminotransferase activity, serum triglycerides, hepatic acyl CoA oxidase activity, and hepatic carnitine acyl transferase activity

Treatment*	Relative liver weight (% of body weight)	Serum alanine aminotransferase activity (U/L)	Serum triglycerides (mg/dL)	Hepatic acyl CoA oxidase activity (nmol/min/mg)	Carnitine acyl transferase activity (nmol/min/mg)
Corn oil (5 mL/kg)	3.99 ± 0.20	8.9 ± 3.2	81 ± 42	4.9 ± 1.3	1.0 ± 0.4
AGN 191701 (60 μmol/kg)	4.79 ± 0.16†	11.3 ± 2.7	99 ± 23	31.3 ± 4.4†	2.2 ± 0.2
SR 11237 (60 μmol/kg)	4.53 ± 0.39†	11.7 ± 1.5	104 ± 3	13.8 ± 4.2†	1.6 ± 0.2
Clofibrate (200 mg/kg)	4.76 ± 0.31†	11.1 ± 1.7	26 ± 5†	36.3 ± 2.9†	9.7 ± 2.1†

* Male Fischer rats were treated i.g. once daily for 2 days with corn oil or the indicated retinoids in corn oil (5 mL/kg). Twenty-four hours after the last treatment, blood was collected, rats were euthanized, and livers were removed and weighed. Livers were used to prepare hepatic cytosols. Values are the means ± SD of 4–5 rats.

† Significantly different from corn oil control ($P < 0.05$).

seemed plausible that RXR agonists might belong to the peroxisome proliferator class of hepatic mitogens that are thought to transactivate through a PPAR-RXR heterodimer [4–7]. This possibility was supported by the fact that both AGN 191701 and SR11237 induced hepatic fatty acyl CoA oxidase activity in rats. Moreover, RXR agonist-induced increases in DNA synthesis were distinctly periportal and midzonal in distribution, which is similar to the zonation of DNA synthesis in rats treated with Wy-14,643, a peroxisome proliferator [27].

Carnitine acyl transferase is another hepatic enzyme induced by known peroxisome proliferators and, frequently, to the greatest extent of any biochemical marker [30, 35]. Although carnitine acyl transferase was induced ~10-fold by clofibrate in the present study, neither AGN 191701 nor SR11237 significantly increased this enzyme activity at the dose level tested. The induction of hypotriglyceridemia is another common feature of known peroxisome proliferators, including industrial chemicals such as di(2-ethylhexyl) pthalate that are not used clinically for this purpose [30]. However, neither AGN 191701 nor SR11237 caused hypotriglyceridemia in rats in the same experiment in which clofibrate did lower serum triglycerides. The failure of RXR agonists to induce hepatic carnitine acyltransferase or cause hypotriglyceridemia suggests that the observed effects of RXR agonists may not be mediated through the PPARα-RXR heterodimer that is thought to mediate the pleiotropic actions of peroxisome proliferators in liver [36]. However, these findings might be explained if the PPARα-RXR heterodimer has different functional properties depending on whether a PPAR or an RXR ligand binds. Another possibility is that the RXR agonist activity observed in the present study is mediated by an RAR-RXR heterodimer. RAR and RXR agonists are reported to have synergistic effects in some cellular systems [37], and thus one could speculate that exogenous RXR agonists synergize with endogenous RAR agonists to produce the observed effects. Clearly, more studies will be needed to define the receptor pathway(s) by which RXR agonists act *in vivo*.

In conclusion, these data show that RXR-selective agonists cause hepatomegaly in rats that is secondary, at least in part, to hepatic mitogenesis. RAR-selective agonists do not cause hepatomegaly or hepatocyte proliferation under similar conditions. RXR agonists also induce acyl CoA oxidase *in vivo*, as observed previously *in vitro* and *in vivo* [4, 20]. Although these hepatic effects are of unknown therapeutic or toxicological significance, they provide evidence that RAR and RXR ligands, presently lumped together as "retinoids," have distinct activities *in vivo*. RAR agonists have already found numerous therapeutic applications in dermatology and oncology [38]. RXR agonists, which appear to lack some classical retinoid toxicities [18, 20], may have unique clinical applications.

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