Biochemical and Biophysical Research Communications **290,** 131–139 (2002)
doi:10.1006/bbrc.2001.6141, available online at http://www.idealibrary.com on IDEAL®

Fibrate and Statin Synergistically Increase the Transcriptional Activities of PPARα/RXRα and Decrease the Transactivation of NFκB

Ikuo Inoue,*[,1] Fumiaki Itoh,† Shigemi Aoyagi,† Shigeki Tazawa,† Hiroshi Kusama,† Masuo Akahane,† Toshiyuki Mastunaga,‡ Kenji Hayashi,* Takuya Awata,* Tugikazu Komoda,‡ and Sigehiro Katayama*

**Fourth Department of Internal Medicine and †First Department of Biochemistry, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan; and ‡Central Research Laboratory, Kissei Pharmaceutical, Nagano, Japan*

Received November 23, 2001

In this study, we used a coactivator-dependent receptor–ligand interaction assay (CARLA), which is a semifunctional *in vitro* assay, to determine whether hypolipidemic drugs are ligands for the three peroxisome proliferator-activated receptor isotypes (PPARα, δ, and γ). We also evaluated the transcriptional activities of the three PPAR isotypes by transient transfection assays. We found that bezafibrate was a ligand for PPARα, δ, and γ in the CARLA and that bezafibrate induced transcriptional activation of PPARα/RXRα, PPARδ/RXRα, and PPARγ/RXRα. Although the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors cerivastatin, fluvastatin, and pitavastatin were not ligands for these three nuclear receptors in the CARLA, they induced transcriptional activation of PPARα/RXRα, PPARδ/RXRα, and PPARγ2/RXRα. Moreover, cerivastatin, fluvastatin, and pitavastatin synergistically and dose-dependently increased the transcriptional activation of PPARα/RXRα induced by bezafibrate. In addition, the cerivastatin-induced transcriptional activation of PPARα/RXRα was decreased by addition of mevalonate, farnesol, geranylgeraniol, or cholesterol and by co-transfection with sterol regulatory element-binding protein-1 (SREBP-1). Moreover, concomitant administration of statins and fibrates also decreased the transactivation of nuclear factor κB (NFκB) and the activation of NFκB by mitogen-activated protein kinase kinase kinase (MEKK) also decreased the transactivation of PPARα/RXRα.

Key Words: **3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin); fibrate; coactivator-dependent receptor–ligand interaction assay (CARLA); cAMP-response element-binding protein (CREB)-binding protein (CBP/p300); bezafibrate; peroxisome proliferator-activated receptor α (PPARα); sterol regulatory element-binding protein-1 (SREBP-1); nuclear factor κB (NFκB).**

The peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor superfamily (1). Three types of PPARs have been described in rodents, humans, and amphibians: PPARα, PPARδ (also called FAAR, and PPARβ or Nuc1), and PPARγ. PPARα has been reported to be expressed most abundantly in the liver (2) and is associated with β-oxidation in the mitochondria and peroxisomes. The fibrates (bezafibrate, fenofibrate/fenofibric acid, clofibrate/clofibric acid, clinofibrate) are well-known synthetic ligand/activators of PPARα. Although PPARβ/δ/Nuc1 is expressed ubiquitously (3), its functional role remains unknown. Recently, the discovery of an agonist for PPARδ, compound L-165041, has been reported (4). PPARγ is a transcription factor expressed selectively in adipose tissue (5) and seems to be associated with differentiation of adipocytes. The thiazolidines (troglitazone, pioglitazone, rosiglitazone) are well-known synthetic ligand/activators of PPARγ.

The functional complex of these receptors is a heterodimer of PPAR and the retinoid X receptor (RXR) that binds to a consensus sequence in the promoter of target genes and can up-regulate transcription in the presence of PPAR ligand/activators. Steroid receptor coactivator-1 (SRC-1) and cAMP-response element-binding protein (CREB)-biding protein (CBP/p300), which are contained in the functional complex, play roles as coactivators for the signaling of PPARs and RXR.

[1] To whom correspondence should be addressed. Fax: 81-492-94-9752.

0006-291X/02 $35.00

We have previously reported that the protein and mRNA expression of PPARα in endothelial cells and hepatocytes was increased by hypolipidemic drugs such as fibrates (6) and statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) (7).

The statins inhibit the synthesis of cholesterol from mevalonic acid by suppressing the conversion of HMG-CoA and up-regulate the low density lipoprotein (LDL) receptor gene, which has been reported to be associated with a transcription factor, sterol regulatory element-binding protein (SREBP). There are two types of SREBP: SREBP-1 controls the transcription of genes involved in fatty acid synthesis (8, 9), whereas SREBP-2 is involved in the transcription of cholesterogenic enzymes (10). The SREBPs are thought to liberate their active nuclear form into the nucleus through cleavage of the membrane-bound precursor protein, and the activation domain of SREBP binds to the amino-terminal domains of CBP/p300 in the nucleus (11).

There have been no data indicating that the transcriptional activation of PPARα/RXRα could be regulated by statins or SREBP. By the use of transient transfection experiments or the coactivator-dependent receptor–ligand interaction assay (CARLA) (12), we determined whether the transcriptional activities of PPARs/RXRα could be induced by hypolipidemic drugs such as statins and fibrates, as well as by thiazolidines, known ligands for PPARs. Moreover, we determined whether the transcriptional activation of PPARα/RXRα could be synergistically induced by fibrates and statins.

MATERIALS AND METHODS

Reagents. Troglitazone, pioglitazone, rosiglitazone, bezafibrate, fenofibric acid, clinofibrate, and L-165041 were obtained from Kissei Pharmaceutical Co. (Matsumoto, Japan). Clofibric acid was purchased from Tocris (Bristol, UK). Fenofibrate and clofibrate were purchased from Sigma (Kirkland, WA). Pitavastatin, which was previously named itavastatin or nisvastatin, was generously provided by Kowa Co. Ltd. (Tokyo, Japan). Cerivastatin was generously provided by Bayer Yakuhin Ltd. (Osaka, Japan), and fluvastatin was generously provided by Novartis Co. (Tokyo, Japan).

In an additional experiment, 100 μM mevalonate, 10 μM farnesol, 10 μM geranylgeraniol, and 10 μM cholesterol, which were dissolved in ethanol, were added to medium containing statin dissolved in dimethylsulfoxide to observe the effects of statins on the mevalonate pathway. The cytotoxic effects of the statins were determined by trypan blue dye exclusion.

DNA sequencing. Direct sequencing of the PCR products was performed with an automated sequencer (ABI PRISM 310 Genetic Analyzer; Perkin Elmer, Foster City, CA). All DNA sequences were confirmed by reading both DNA strands.

Western blotting. Western blotting was performed and specific immunoreactivity detected with an Amersham ECL kit. Briefly, processed samples were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore) by semi-dry blotting. Antibodies against human PPARs and SREBP-1, 2 were obtained from Santa Cruz. We used two classes of SREBP-1: one reacts with the carboxy terminus of SREBP-1; the other reacts with an internal epitope of SREBP-1. Moreover, we also used two classes of SREBP-2: one reacts with the amino terminus of SREBP-2; the other reacts with the carboxy terminus of SREBP-2.

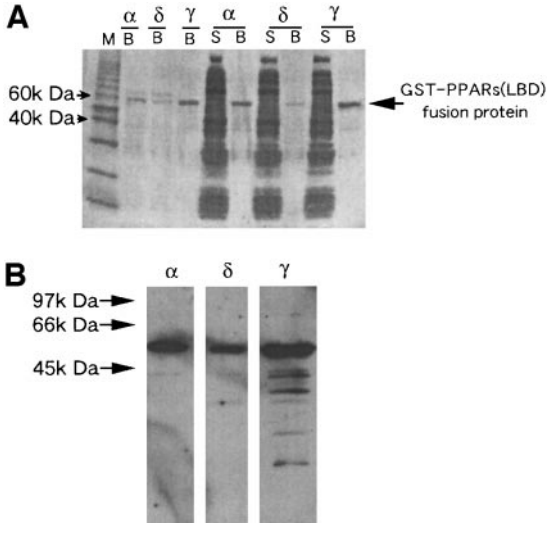


FIG. 1. (A) GST-hPPARα LBD, GST-hPPARδ LBD, and GST-hPPARγ LBD fusion proteins (arrow). Lane M: marker, S: Supernatant, B: Bound fraction for Glutathione Sepharose. (B) Western blotting analysis of GST-hPPARα LBD, GST-hPPARδ LBD, and GST-hPPARγ LBD fusion proteins.

Membranes were treated overnight with TBS-Tween/5% dry milk and incubated with goat anti-human PPARs and SREBP-2 antibodies and rabbit anti-human SREBP-1 antibodies for 1 h. After washing, membranes were incubated with horseradish peroxidase-conjugated rabbit anti-goat monoclonal antibodies. Antigen detection was performed with a chemiluminescence detection system.

Subcloning of full-length human PPARα, PPARδ, PPARγ2, and RXRα DNA fragments. Specific DNA fragments of full-length human PPARα, PPARδ, PPARγ2, and RXRα cDNAs were subcloned from human skeletal muscle (CLONTECH) or liver (CLONTECH) cDNA libraries and into the pCI-neo mammalian expression vector (Promega, WI). Direct sequencing of the PCR products was performed with an automated sequencer. For PPARα, the 1407 bp *Sal*I/*Not*I fragment was subcloned into the *Sal*I/*Not*I site of pCI-neo and the vector was named pCI-PPARα. PPARδ, PPARγ2, or RXRα was also subcloned into the *Sal*I/*Not*I site of the vector and the vector was named pCI-PPARδ, pCI-PPARγ2, or pCI-RXRα, respectively. The primers used for the N-terminal side of SREBP-1 were 5′-gctgaccgacatcgaagacatgct-3′ for the forward primer and 5′-gcttcaagagaggagctcaatgtg-3′ for the reverse primer. The PCR product was subcloned into the *Sal*I/*Not*I site of pCI-neo and the vector was named pCI-SREBP-1-N.

Transient transfection/co-transfection. Transient transfection of pCI-PPARα (3 μg), pCI-PPARδ (3 μg), pCI-PPARγ2 (3 μg), pCI-RXRα (3 μg), or pCI-SREBP-1-N (3 μg) into 1×10^6 cells was performed in 100-mm dishes using Tfx-50 reagent (Promega, Madison, WI) according to the instruction manual, with an internal control reporter (pRL-TK vector) (2 μg) (Promega) to correct for transfection efficiency. After 24 h, cells were stimulated with test drugs for a further 24 h, lysed, and centrifuged to prepare the sample for Western blotting and for reporter transient assay.

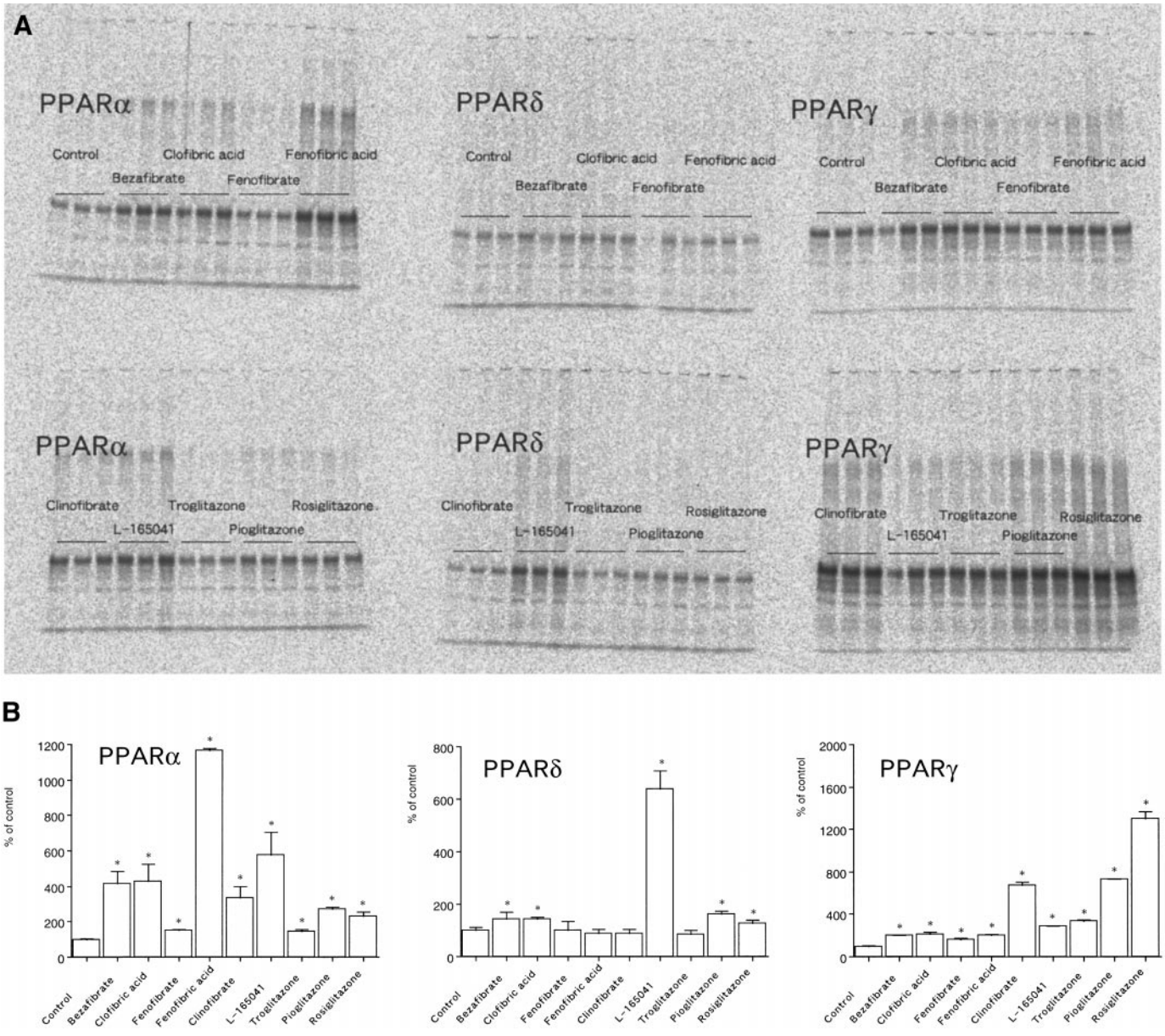


FIG. 2. (A) Semifunctional *in vitro* assay by coactivator-dependent receptor ligand interaction assay (CARLA). Experiments were performed in triplicate, and four independent experiments were performed. All data are means ± SD. $*P < 0.05$ vs non-treated cells. (B) The signals in A were quantified by scanning densitometry. All drugs were used at 100 μM.

Reporter assay for PPAR activation and nuclear factor κB (NFκB)-driven transactivation. Transient transfections for the reporter assay were performed with Tfx-50 reagent (Promega) according to the manufacturer's recommended protocol. Human kidney 293T cells were used because of their high transfection efficiency. To measure activation of PPARs, we used the PPAR-responsive promoter region for cellular retinol-binding protein type II (CRBPII) (−670 to +60) (13) cloned into the *Kpn*I/*Nco*I sites of pGL3-Basic upstream of the luciferase gene (pGL3-Basic) (Promega), termed pCRBPII-Luc. The PPAR-responsive promoter region for CRBPII is similar to the well-known PPAR-responsive promoter region for acyl-coenzyme A oxidase. This promoter contains both CAAT and TATA sequence motifs. The bases between −639 and −605 represent the PPAR-response element. pNFκB-Luc was purchased as NFκB *Cis*-Reporting System from Stratagene Cloning Systems. The system concludes pNFκB-Luc and pFC-mitogen-activated protein kinase kinase kinase (pFC-MEKK). pCRBPII-Luc and pRL-TK, in the presence or absence of pCI-PPARα, pCI-PPARδ, or pCI-PPARγ2, were co-transfected with pCI-RXRα into human kidney 293T cells grown in 24-well plates. The total amount of DNA transfected (0.5 μg) was normalized with a carrier DNA (pCI-neo, Promega). After 24 h, cells were stimulated with test drugs for another 24 h. Finally, luciferase activity for pCRBPII-Luc or pNF-κB-Luc was normalized to Renilla luciferase activity. Both activities were measured according to the manufacturer's instructions (Promega). With regard to the reporter assay for PPARα activation by cerivastatin, pCI-PPARα and pCI-RXRα were co-transfected into human kidney 293T cells with cerivastatin in the presence or absence of pCI-SREBP-1-N.

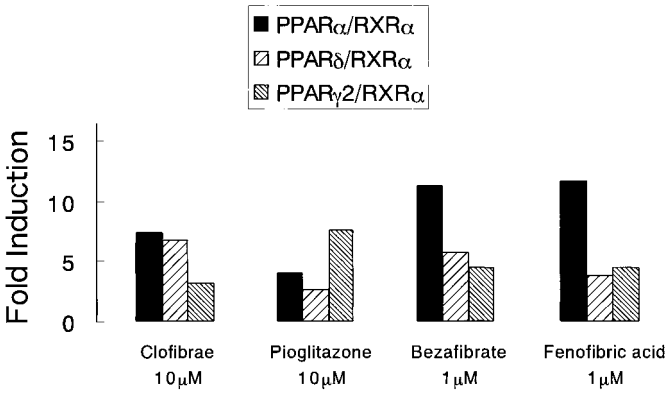


FIG. 3. Reporter gene assay for the effect of fibrates and thiazolidines on PPARα/RXRα-mediated, PPARδ/RXRα-mediated, and PPARγ2/RXRα-mediated transcription in human kidney 293T cells. Values shown are the fold-activation versus cells co-transfected with pGL3-Basic (empty vector), pRL-TK, and pCI-RXRα in the presence or absence of pCI-PPARα, pCI-PPARδ, or pCI-PPARγ2. Experiments were performed in triplicate, and four independent experiments were performed. All data are means.

Fusion protein constructs and protein expression. The ligand binding domain (LBD) fragments (D–E) for human PPARα (hPPARα), hPPARδ, and hPPARγ were amplified by PCR from clones containing the full-length sequences from a human liver cDNA library. The fragments were cloned into the *Bam*HI site of pGEX-4T for hPPARα, hPPARδ, and hPPARγ. The 1359-bp CBP/p300 (CBP/p300-partial) 5′-flanking sequence (199–1558) was amplified by PCR. The primer for CBP/p300-partial was 5′-agggatccatggctgagaacttgctggacggaccgc-3′ for the forward primer and 5′-ggcattctgttgccctgtgccaac-3′ for the reverse primer. The fragments were cloned into the *Bam*HI/*Eco*RI site of pcDNA3.1 (Invitrogen) for CBP/p300-partial. The GST-PPARs (LBD) fusion proteins were expressed in *Escherichia coli.* Briefly, 2 ml of overnight cultures of in *E. coli,* transformed with the GST-PPAR fusion plasmid, was used to inoculate 500 ml of LB medium. The bacteria were incubated at 37°C until the optical density of the culture medium reached A_{600} = 0.6 and were induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside at 35°C for 2 h for hPPARα, hPPARδ, and hPPARγ. After centrifugation, the bacterial pellets were equilibrated in NETN buffer (20 mM Tris–HCl, pH 8.0, 100 mmol/L NaCl, 1 mmol/L EDTA, 0.5% Nonidet P-40) supplemented with 0.5% skim milk and the bacterial cells were then lysed by mild sonication (output 2, 4–5 min) prior to use in CARLA. After centrifugation to remove the cell debris, fusion proteins were purified on glutathione–Sepharose beads (Amersham Pharmacia Biotech) at 4°C for 1 h, washed two or three times, and equilibrated in NETN buffer. The amount of protein was measured by BCA Protein Assay Kits (Pierce).

CARLA. Before CARLA was performed, protein was subjected to Western blotting to identify the fusion proteins. The CARLA was performed as described by Krey *et al.* (12), with the following modifications. Reactions were performed in 1000 μl of NETN buffer containing 3 mg of hPPAR fusion protein, 2 μl of L-[^{35}S]methionine-labeled *in vitro* translated (TNT T7 Coupled Reticulocyte Lysate System; Promega) CBP/p300-partial and 10 μl of a 100 mmol/L solution of the compound tested at 4°C overnight. After incubation, centrifugation was performed at 400–1000*g* for 3 min to remove the supernatant, and the immobilized fusion protein was washed three times in NETN buffer without milk. After washing, the glutathione–Sepharose-bound proteins were dried under nitrogen gas before being resuspended in SDS sample buffer and subjected to SDS–PAGE.

Statistical analysis. Parametric data are expressed as means ± SD. Differences between groups were evaluated by Scheffé's *F* test.

RESULTS AND DISCUSSION

We determined the expression proteins of the three PPAR subtypes purified as GST-hPPARα LBD, GST-hPPARδ LBD, and GST-hPPARγ LBD fusion proteins (Fig. 1A). As shown in Fig. 1B, we could identify hPPARα, hPPARδ, and hPPARγ by Western blotting methods.

Then, we tested the three PPAR subtypes with known PPAR ligand/activators to establish whether the CARLA performed appropriately. As shown in Figs. 2A and 2B, fibrates, thiazolidines, and L-165041 induced binding of *in vitro* translated and [^{35}S]-methionine-labeled CBP/p300 to purified GST-hPPARα and GST-hPPARγ LBD fusion protein immobilized on glutathione–Sepharose beads. Only L-165041 induced CBP/p300 and hPPARδ LBD interactions (Figs. 2A and 2B). This result is consistent with a previous report (14). All statins did not induce binding of CBP/p300 and GST-hPPARs LBD fusion protein immobilized on glutathione–Sepharose beads (data not shown).

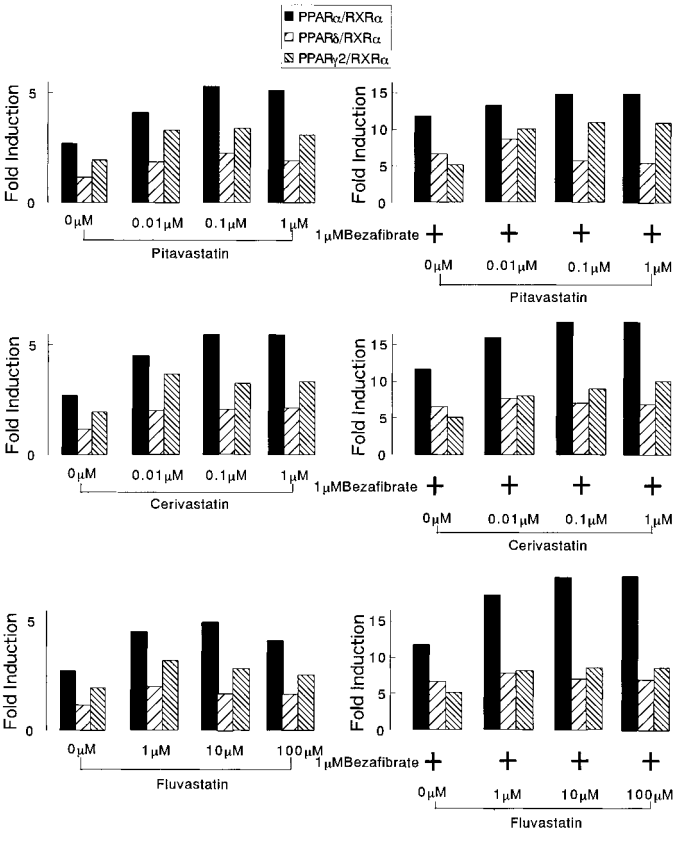


FIG. 4. Transcriptional activation of PPARα/RXRα, PPARδ/RXRα, and PPARγ2/RXRα by pitavastatin, cerivastatin, and fluvastatin. The activation of PPARα by bezafibrate was synergistically, dose-dependently, increased by statin. Values shown are the fold-activation versus cells co-transfected with pGL3-Basic (empty vector), pRL-TK, and pCI-RXRα in the presence or absence of pCI-PPARα, pCI-PPARδ, or pCI-PPARγ2. Experiments were performed in triplicate, and four independent experiments were performed. All data are means.

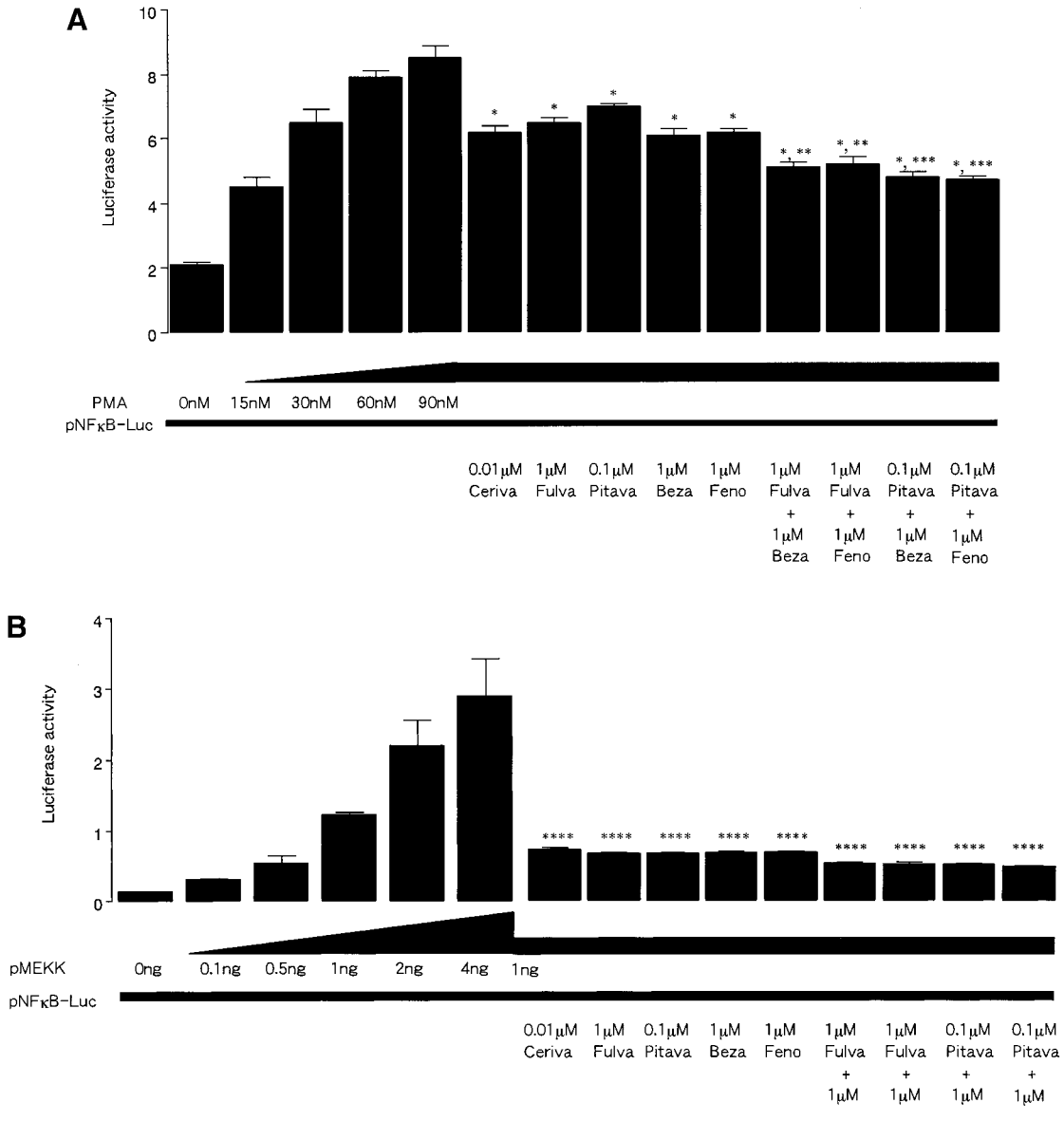


FIG. 5. Inhibition of transcriptional activation of NFκB by statin and/or fibrate. The activation of NFκB by phorbol myristate acetate (PMA) (A) and plasmid mitogen-activated protein kinase kinase kinase (pMEKK) (B) was decreased by statin (Ceriva: cerivastatin, Fluva: fluvastatin, Pitava: pitavastatin) and/or fibrate (Beza: bezafibrate, Feno: fenofibrate). Firefly luciferase activities were normalized to *Renilla* control activities and luciferase activities. Values show the ration of firefly luciferase activities/*Renilla* control activities in the human kidney 293T cells co-transfected with pRL-TK, pNFκB-Luc in the presence or absence of PMA and/or statin and/or fibrate (A). Values show the ration of firefly luciferase activities/*Renilla* control activities in the human kidney 293T cells co-transfected with pRL-TK, pNFκB-Luc, pMEKK in the presence or absence of statin and/or fibrate (B). Experiments were performed in triplicate, and four independent experiments were performed. All data are means ± SD. $*P < 0.05$ vs cells treated by 90 nM PMA, $**P < 0.05$ vs cells treated by 90 nM PMA plus 1 μM fluvastatin, $***P < 0.05$ vs cells treated by 90 nM PMA plus 1 μM pitavastatin, and $****P < 0.05$ vs cells transfected by 1 ng pMEKK.

The effects of fibrates and thiazolidines on PPARs were determined by a reporter gene assay in human kidney 293T cells (Fig. 3). In cells treated with fibrates and thiazolidines, PPARα/RXRα-mediated, PPARδ/RXRα-mediated, and PPARγ2/RXRα-mediated transcription of the reporter gene were enhanced although the grades were not same. PPARγ2/RXRα-mediated transcription of the reporter gene was strongly enhanced in cells treated with 10 μM pioglitazone. Furthermore, PPARα/RXRα-mediated transcription of the reporter gene was strongly enhanced in cells treated with 1 μM bezafibrate or 1 μM fenofibric acid. These data indicate that bezafibrate and fenofibric acid are used as the activators of PPARα, and pioglitazone is used as the activator of PPARγ2.

In addition, our results suggest that statin are activators of PPARα/RXRα-mediated, PPARδ/RXRα-mediated, and PPARγ2/RXRα-mediated transcription.

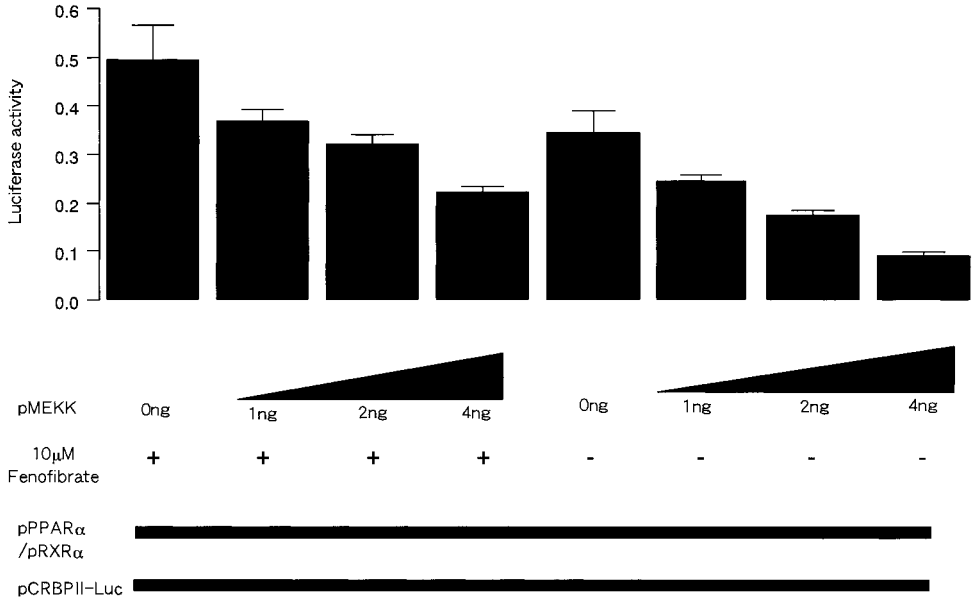


FIG. 6. Inhibition of transcriptional activation of PPARα/RXRα by co-transfection of plasmid mitogen-activated protein kinase kinase kinase (pMEKK). Firefly luciferase activities were normalized to *Renilla* control activities. Values show the ration of firefly luciferase activities/*Renilla* control activities in the human kidney 293T cells co-transfected with pRL-TK, pCI-RXRα, pCI-PPARα, pCRBPII-Luc in the presence or absence of pMEKK. Experiments were performed in triplicate, and four independent experiments were performed. All data are means ± SD.

Furthermore, the activation of PPARα by bezafibrate was synergistically and dose-dependently increased by 0.01, 0.1, and 1 μM pitavastatin and cerivastatin (Fig. 4). Fluvastatin also has the synergistical ability to increase the transcriptional activity of PPARα by bezafibrate. The levels of transactivation of NFκB, induced by phorbol myristate acetate (PMA) (Fig. 5A) or MEKK (Fig. 5B), were decreased by statin and/or fibrate. And the stimulation of transactivation of NFκB, induced by MEKK, was decreased by the transactivation of

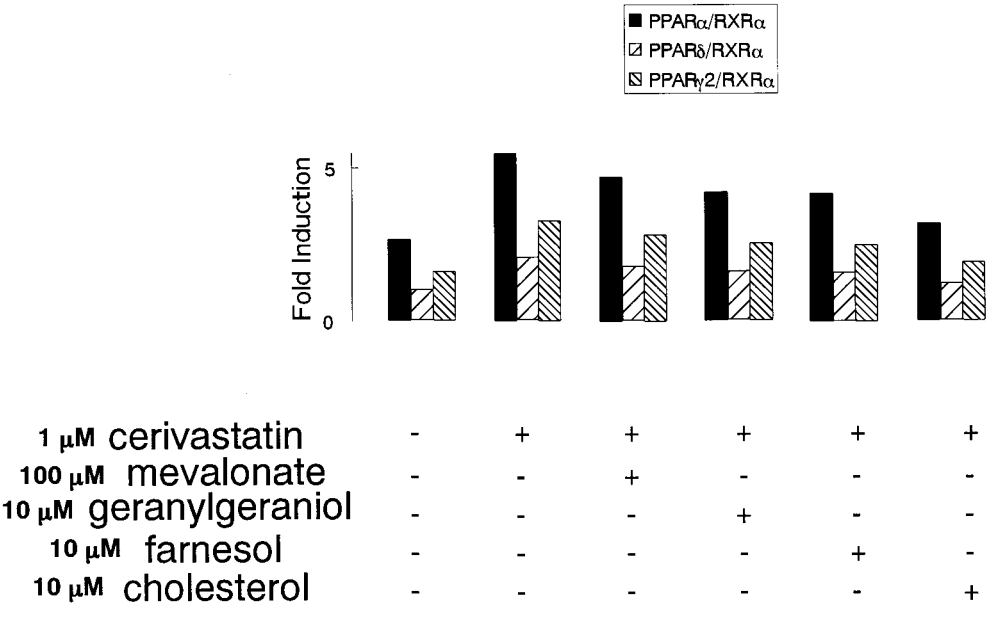
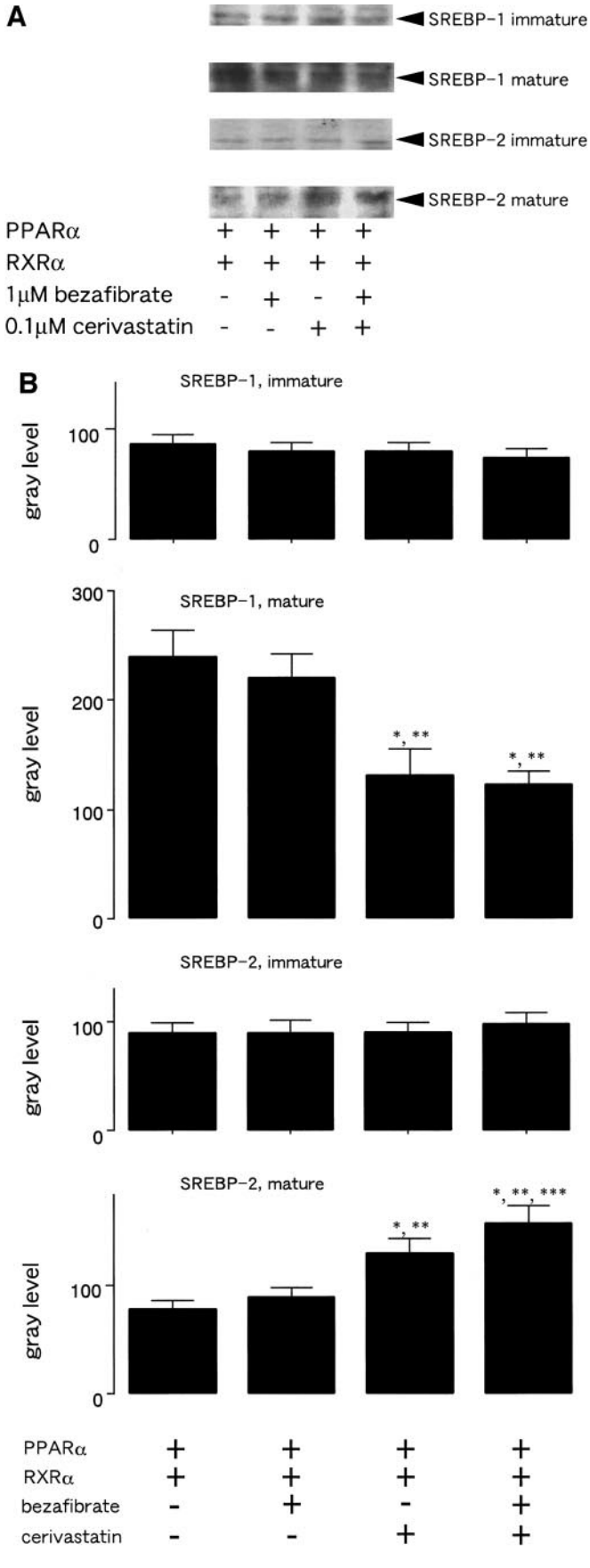


FIG. 7. Suppression by mevalonate, farnesol, geranylgeraniol, and cholesterol of induction by cerivastatin of PPARα/RXRα-mediated reporter gene transcription. Values shown are the fold-activation versus cells co-transfected with pGL3-Basic (empty vector), pRL-TK, and pCI-RXRα in the presence or absence of pCI-PPARα, pCI-PPARδ, or pCI-PPARγ2. Experiments were performed in triplicate, and four independent experiments were performed. All data are means.



PPARα/RXRα with/without ligands (Fig. 6). It is well known that statin and fibrate have the potency of anti-inflammatory effect. Our results suggest that statin induces inactivation of NFκB, resulting in an increase in the transactivation of PPARα/RXRα. Moreover, mevalonate, farnesol, geranylgeraniol, and cholesterol suppressed the induction by cerivastatin (Fig. 7), pitavastatin, and fluvastatin (data not shown) of PPARα/RXRα-mediated transcription of the reporter gene. Recently, it has been reported that these inhibitory effects were proved by their reversion by geranylgeranyl pyrophosphate (GGPP) but not by farnesyl pyrophosphate (FPP) (15). These data were not consistent with our results. Farnesol is a 15-carbon isoprenoid derived from farnesyl pyrophosphate, a key intermediate in cholesterol biosynthesis. Farnesol is the ligand for farnesoid X-activated receptor (FXR). FXR is a member of the nuclear hormone receptor superfamily and functions as a heterodimer with RXR in the presence of ligands such as PPARs. The heterodimer binds to specific DNA sequences in the promoters of target genes to regulate gene transcription. A potential FXR response element is an inverted repeat in which consensus receptor-binding hexamers are separated by one nucleotide (inverted repeat-1). In contrast, a potential PPAR response element is a direct repeat in which consensus receptor-binding hexamers are separated by one nucleotide (direct repeat-1). Recently, it has been reported that the effect of farnesol on the PPRE-luciferase reporter requires PPAR. Thus, there may be interaction/cross-talk between FXR and PPAR (16), although the detailed mechanism remains to be clarified. Such clarification might reveal other mechanisms for the induction of PPARs-mediated reporter gene transcription by statins. Thus, we investigated whether FPP or farnesol inhibits the PPRE binding activity. PPRE–protein complex by gel shift assay was markedly decreased by FPP or farnesol (data not shown). Our data demonstrated that FPP or farnesol had an effect on the transactivation of PPARα/RXRα.

However, the effect of mevalonate-derived isoprenoids on induction of PPARα by statin was weak (Fig. 7), suggesting that other specific effects of statin are involved in its induction of PPARα/RXRα-mediated transcription of the reporter gene.

FIG. 8. (A) Reduction of SREBP-1 protein expression level by bezafibrate and/or cerivastatin in human kidney 293T cells overexpressing PPARα/RXRα. Experiments were performed in triplicate, and four independent experiments were performed. All data are means ± SD. $*P < 0.05$ vs cells overexpressed by PPARα/RXRα, $**P < 0.05$ vs cells overexpressed by PPARα/RXRα and treated with bezafibrate, $***P < 0.05$ vs cells overexpressed by PPARα/RXRα and treated with cerivastatin. (B) The signals in A were quantified by scanning densitometry and showed as gray level analyzed by NIH Image.

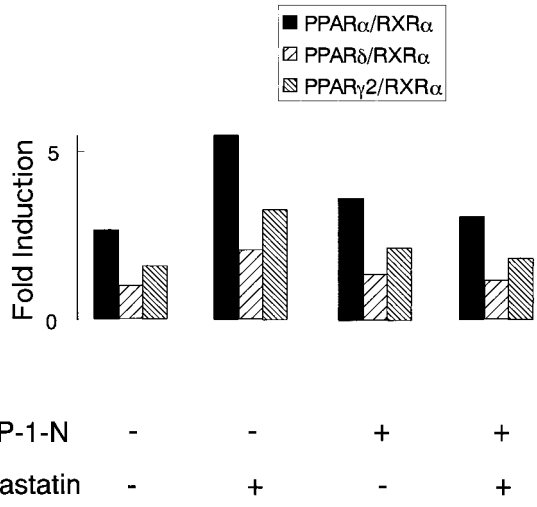


FIG. 9. Suppression of PPARα/RXRα-mediated transcription by pCI-SREBP-1-N. pCI-SREBP-1-N contain the N-terminal side of SREBP-1 as mentioned under Materials and Methods. Values shown are the fold-activation versus cells co-transfected with pGL3-Basic (empty vector), pRL-TK, and pCI-RXRα in the presence or absence of pCI-PPARα, pCI-PPARδ, or pCI-PPARγ2. Experiments were performed in triplicate, and four independent experiments were performed. All data are means.

Statins inhibit the synthesis of cholesterol from mevalonic acid by suppressing the conversion of HMG-CoA. We thought that the SREBP pathway might be involved in the action of PPARs of statins. SREBP-1 is associated with induction of fatty acid synthesis, and it has been reported recently that statins are able to reduce SREBP-1 transcript levels (17). Thus, we investigated whether SREBP-1 protein expression levels were altered in human kidney 293T cells overexpressing PPARα/RXRα. As shown in Figs. 8A and 8B, the SREBP-1 (mature type) protein expression level was decreased by cerivastatin in human kidney 293T cells overexpressing PPARα/RXRα. The SREBP-2 (mature type) protein expression level was increased by cerivastatin in human kidney 293T cells overexpressing PPARα/RXRα. The effect was greater with cerivastatin and bezafibrate than with cerivastatin alone. The induction by cerivastatin of PPARα/RXRα-mediated reporter gene transcription was markedly decreased by co-transfection with SREBP-1 (Fig. 9). However, the effect of co-transfection with SREBP-1 was not complete. Thus, we need to consider other mechanisms for the induction by cerivastatin of PPARα-mediated reporter gene transcription.

We speculated the cross-talk between statin (SREBP) and fibrate/thiazolidine (PPAR) (Fig. 10) although the detailed mechanisms were not unknown.

In conclusion, our results demonstrate that statins increase the transcriptional activity of PPARα/RXRα, PPARδ/RXRα, PPARγ/RXRα, that this effect is stimulated synergistically by addition of fibrates, and that the grade of transcriptional activity of PPARα/RXRα is strong. Moreover, concomitant administration of statins and fibrates decreases the transactivation of NFκB. These observations may at least in part explain the synergistic clinical benefits of combination therapy with statins and fibrates (18–20).

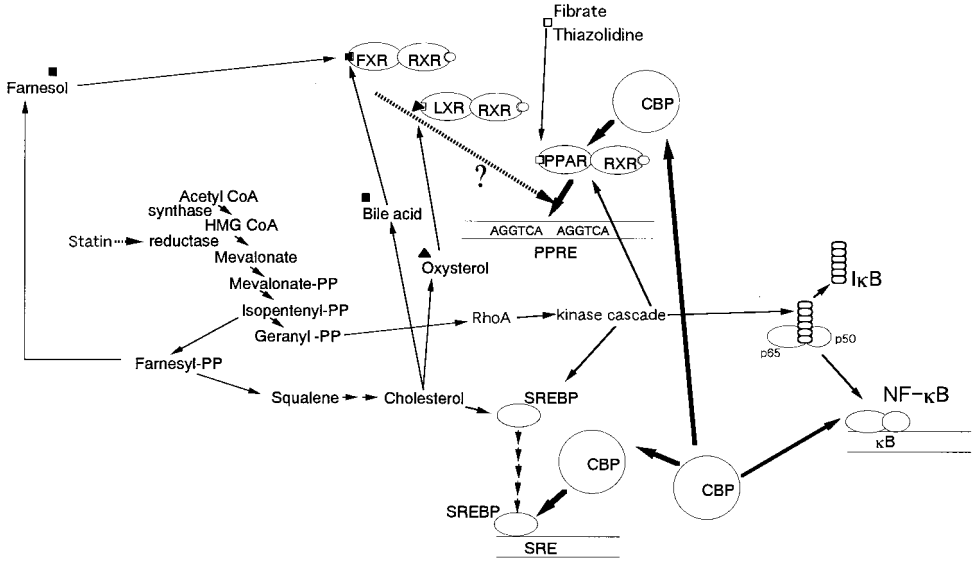


FIG. 10. Cross-talk between the SREBP (sterol regulatory element-binding protein) and PPAR (peroxisome proliferator-activated receptor) pathways. LXR, liver X receptor; FXR, farnesoid X-activated receptor; RXR, retinoid X receptor; CBP, CREB binding protein; NFκB, nuclear factor κB, IκB, inhibitor of NFκB; κB, response element for NFκB; PPRE, response element for PPAR; SRE, response element for SREBP; pp, pyrophosphate. (○) Ligand for RXR; (□) ligand for PPAR; (■) ligand for FXR; (▲) ligand for LXR.

ACKNOWLEDGMENTS

We are grateful to Ms. Sawako Satoh, Ms. Kozue Watanabe, and Ms. Ayako Goh (Saitama Medical School) for their technical assistance. This work was supported by a Grant-in-Aid for Encouragement of Young Scientists, Ministry of Education, Science and Culture of Japan, and by Japan Health Sciences Foundation.

REFERENCES

1. Issemann, I., and Green, S. (1990) *Nature* **347,** 645–650.
2. Lemberger, T., Braissant, O., Juge-Aubry, C., Keller, H., Saladin, R., Staels, B., Auwerx, J., Burger, A. G., Meier, C. A., and Wahli, W. (1996) *Ann. N.Y. Acad. Sci.* **486,** 231–251.
3. Schoonjans, K., Staels, B., and Auwerx, J. (1996) *Biochim. Biophys. Acta* **1302,** 93–109.
4. Leibowitz, M. D., Fievet, C., Hennuyer, N., Peinado-Onsurbe, J., Duez, H., Bergera, J., Cullinan, C. A., Sparrow, C. P., Baffic, J., Berger, G. D., Santini, C., Marquis, R. W., Tolman, R. L., Smith, R. G., Moller, D. E., and Auwerx, J. (2000) *FEBS Lett.* **473,** 333–336.
5. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234.
6. Inoue, I., Noji, S., Awata, T., Takahashi, K., Nakajima, T., Sonoda, M., Komoda, T., and Katayama, S. (1998) *Life Sci.* **63,** 135–144.
7. Inoue, I., Goto, S., Mizotani, K., Awata, T., Mastunaga, T., Kawai, S., Nakajima, T., Hokari, S., Komoda, T., and Katayama, S. (2000) *Life Sci.* **67,** 863–876.
8. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997) *J. Clin. Invest.* **99,** 846–854.
9. Shimano, H., Horton, J. D., Hammer, R. E., Shimomura, I., Brown, M. S., and Goldstein, J. L. (1996) *J. Clin. Invest.* **98,** 1575–1584.
10. Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., and Shimano, H. (1998) *J. Clin. Invest.* **101,** 2331–2339.
11. Oliner, J. D., Andresen, J. M., Hansen, S. K., Zhou, S., and Tjian, R. (1996) *Genes Dev.* **10,** 2903–2911.
12. Desvergne, B., Ijpenberg, A., Devchand, P. R., and Wahli, W. (1998) *J. Steroid Biochem. Mol. Biol.* **65,** 65–74.
13. Mangelsdorf, D. J., Umesono, K., Kliewer, S. A., Borgmeyer, U., Ong, E. S., and Evans, R. M. (1991) *Cell* **66,** 555–561.
14. Leibowitz, M. D., Fievet, C., Hennuyer, N., Peinado-Onsurbe, J., Duez, H., Bergera, J., Cullinan, C. A., Sparrow, C. P., and Baffic, J. (2000) *FEBS Lett.* **473,** 333–336.
15. Martin, G., Duez, H., Blanquart, C., Berezowski, V., Poulain, P., Fruchart, J. C., Najib-Fruchart, J., Glineur, C., and Staels, B. (2001) *J. Clin. Invest.* **107,** 1423–1432.
16. Hanley, K., Komuves, L. G., Ng, D. C., Schoonjans, K., He, S. S., Lau, P., Bikle, D. D., Williams, M. L., Elias, P. M., Auwerx, J., and Feingold, K. R. (2000) *J. Biol. Chem.* **275,** 11484–11491.
17. Sheng, Z., Otani, H., Brown, M. S., and Goldstein, J. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 935–938.
18. Gavish, D., Leibovitz, E., Shapira, I., and Rubinstein, A. (2000) *J. Intern. Med.* **247,** 563–569.
19. Papadakis, J. A., Ganotakis, E. S., Jagroop, I. A., Winder, A. F., and Mikhailidis, D. P. (1999) *Int. J. Cardiol.* **69,** 237–244.
20. Athyros, V. G., Papageorgiou, A. A., Hatzikonstandinou, H. A., Didangelos, T. P., Carina, M. V., Kranitsas, D. F., and Kontopoulos, A. G. (1997) *Am. J. Cardiol.* **80,** 608–613.