Induction of the Fatty Acid Transport Protein 1 and Acyl-CoA Synthase Genes by Dimer-selective Rexinoids Suggests That the Peroxisome Proliferator-activated Receptor-Retinoid X Receptor Heterodimer Is Their Molecular Target*

(Received for publication, January 21, 2000)

Geneviève Martin‡‡, Hélène Poirier‡, Nathalie Hennuyer, Diane Crombie, Jean Charles Fruchart‡, Richard A. Heyman**, Philippe Besnard§, and Johan Auwerx‡‡ § §

From the ‡Département d’Athérosclérose, INSERM U325, Institut Pasteur de Lille, 1 rue du Prof. Calmette, 59019 Lille, France, ‡Laboratoire de physiologie de la nutrition, EP1777/CNRS/CESEG, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l’Alimentation, Université de Bourgogne, Campus Universitaire, 1 Espanade Erasme, 21000 Dijon, France, §Ligand Pharmaceuticals, 10255 Science Center Drive, San Diego, California 92121, and §§Institut de Génétique et Biologie Moléculaire et Cellulaire, CNRS, INSERM, Université Louis Pasteur 1 rue Laurent Fries, 67400 Illkirch, France

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

The intracellular fatty acid content of insulin-sensitive target tissues determines in part their insulin sensitivity. Uptake of fatty acids into cells is a controlled process determined in part by a regulated import/export system that is controlled at least by two key groups of proteins, i.e. the fatty acid transport protein (FATP) and acyl-CoA synthetase (ACS), which facilitate, respectively, the transport of fatty acids across the cell membrane and catalyze their esterification to prevent their efflux. Previously it was shown that the expression of the FATP-1 and ACS genes was controlled by insulin and by peroxisome proliferator-activated receptor (PPAR) agonists in liver or in adipose tissue. The aim of this investigation was to determine the effects of retinoic acid derivatives on the expression of FATP-1 and ACS. In several cultured cell lines, it was shown that the expression of both the FATP-1 and ACS mRNAs was specifically induced at the transcriptional level by selective retinoid X receptor (RXR) but not by retinoic acid receptor (RAR) ligands. This effect was most pronounced in hepatoma cell lines. A similar induction of FATP-1 and ACS mRNA levels was also observed in vivo in Zucker diabetic fatty rats treated with the RXR agonist, LGD1069 (4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoyl acid). Through the use of heterodimer-selective compounds, it was demonstrated that the modulatory effect of these rexinoids on FATP-1 and ACS gene expression was mediated through activation of RXR in the context of the PPAR-RXR heterodimer. The observation that both RXR and PPAR agonists can stimulate the transcription of genes implicated in lipid metabolism, suggest that rexinoids may also act as lipid-modifying agents and support a role of the permissive PPAR-RXR heterodimer in the control of insulin sensitivity.

Free fatty acids can be released from adipocytes by hormone-sensitive lipase or they can be generated from triglycerides-rich lipoproteins by the action of lipoprotein lipase. Circulating fatty acids can then cross the plasma membrane either by virtue of their lipid solubility (1) or by being actively taken up by cells in a process mediated by proteins of the fatty acid transport protein (FATP)1 family. The best characterized of these FATPs is FATP-1, a plasma membrane protein of 63 kDa, which has been shown to actively transport long chain fatty acids (2). Another protein found to be indirectly involved in the process of long chain fatty acid uptake is the acyl-CoA synthetase (ACS). Whereas FATP acts as a transporter of fatty acids, the role of ACS is confined to prevent the efflux of fatty acids through an esterification process. Two other specialized membrane transport system for fatty acids have been described. The first one is the fatty acid translocase (FAT) or glycoprotein CD36 (3, 4), which acts in a coordinate manner with low molecular weight intracellular fatty acid-binding protein (FABPs) (5, 6). The second is a membrane bound fatty acid-binding protein, FABP1P4 closely related to the mitochondrial aspartate aminotransferase (7–10).

Nutritionally derived vitamin A derivatives are affecting metabolism by changing gene expression secondary to activation of receptors of the nuclear receptor superfamily (11, 12). Three retinoic acid receptors (RARs), termed RARα, -β, and -γ, and three retinoid X receptor (RXR), designated RIXα, -β, and -γ, are classically thought to transduce most of the effects of retinoic acid (RA) on gene expression. Both 9-cis RA (9c-RA) and all-trans-RA (at-RA) can directly bind and activate RARs, whereas RXR only binds 9c-RA. Recently, new specific ligands

---

1 The abbreviations used are: FATP, fatty acid transport protein; RA, retinoic acid; at-RA, all-trans-RA; 9c-RA, 9-cis RA; RAR, retinoic acid receptor; RXR, retinoid X receptor; TTNFβ, ethyl-β-[E]-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl]-1-propanoyl]benzoyl acid; LGD1069, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoyl acid; LGI00268, 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydrooctaphenanthre-2-yl)cyclopentyl][nicotinyl]acid; LG100754, 2E,4E,6Z,7-3-n-propoxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl-3 methylcoumarin-2-yl-4-trienoic acid; PPAR, peroxisome proliferator-activated receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ACS, acyl-CoA synthase; FABP, fatty acid-binding protein; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; ZDF rats, Zucker diabetic fatty rats.

---

* This work was supported by grants from CNRS INSERM CHUR, Association de Recherche contre le Cancer (Grant 6403), Janssen Research Foundation, Ligand Pharmaceuticals, and Fondation pour la Recherche Médicale (to J.A.) and Conseil regional de Bourgogne (to P.B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** Current address: Xceptor Pharmaceuticals, 4757 Nexus Centre Drive, Suite 200, San Diego, CA 92121.

*** Supported by the Janssen Research Foundation.

† † A research director with CNRS. To whom correspondence should be addressed: Fax: 33-388-65-32-01; E-mail: auwerx@igbmc.u-strasbg.fr.
for RXR and/or a particular dimers have been developed (13). These selective RXR agonists, called retinoids, function as insulin sensitizers and have beneficial effects on the hyperglycemia and hyperinsulinemia in mouse models of noninsulin-dependent diabetes mellitus and obesity (14). Some of these compounds, such as LGD1069 and LG100268 (15), selectively induce the activity of the RXR homodimer and the RXR-LXR and RXR-peroxisome proliferator-activated receptor (PPAR) heterodimers, but not of RXR-RAR or RXR-TR heterodimers. By contrast, LG100754 has been identified as an antagonist of the RXR homodimer but it activates RXR when it is paired as an heterodimer with PPAR or RAR (13). This ligand, hence, is different from the other activators of RAR-RXR heterodimers, because it was previously thought that retinoids can only activate RAR in the RAR-RXR heterodimer, whereas RXR was believed to be a silent partner in this setting. Because retinoid effects on gene expression can be determined by multiple dimer combinations, including but not limited to RXX-RAR, RXRXR, and RXRX-PPAR, these retinoids make the determination possible of which heterodimer is specifically involved in gene regulation and, hence, they provide tools for a better understanding of RXR action and the development of new drugs.

We suggested previously that thiazolidinedione PPARα activators might improve glucose secondary to tissue-specific co-regulation of the several proteins involved in fatty acid uptake such as lipoprotein lipase (16), FATP-1, and ACS target genes (17), which ultimately alter fatty acid partitioning. RXX-RXR heterodimers, however, have been shown to respond to both RXR and PPAR ligands (18). Therefore, we speculated that RXR ligands could have similar effects and be potential modulators of the expression of the FATP-1 and ACS genes through activation of RXR in the RXR-PPAR heterodimer. In order to confirm the implication of the PPAR-RXR heterodimer in the improvement of glucose homeostasis, we studied the regulation of the FATP-1 and ACS genes by several synthetic retinoid acid analogues. This allowed us to determine the composition of the dimer that was involved in this process and could provide us with a better understanding of the regulation of lipid uptake by nuclear receptors. Our results implicate the PPAR-RXR heterodimer as the molecular target mediating the effects of rexinoids on fatty acid partitioning leading to an improvement of insulin sensitivity.

**EXPERIMENTAL PROCEDURES**

**Materials—**at-RA was purchased from Sigma, 9c-RA, TTNPB (ethyl-p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-1-pro- nyl]benzoic acid) (19), LGD1069 [4-(1-[3,5,5,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl]ethenyl)benzoic acid], LG100754 [(2E,4E,5Z)-7-(-3-n-propoxy-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-3-methyl-octa-2,4,6-trienoic acid], and LG100268 [6-(1-[3,5,5,8-,pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-cylopropyl]nicotinic acid] (20, 15) were kind gifts from Dr. M. Boehm at Ligand Pharmaceuticals.

**Cell Culture and Treatments—**FAO cells are a well-differentiated subclone, derived from the rat hepatoma H4 IIEC3 cell line. They were maintained at 37 °C in a humidified atmosphere of 5% CO2, 95% air in Ham’s F-12 medium (Life Technologies, Inc.) containing 10% fetal calf serum, penicillin (200 IU/mL), and streptomycin (50 mg/mL) according to previously published procedures (21). Culture medium was changed every 48 h. The human hepatoma cell line HepG2 was obtained from ECACC (Porton Down, Salisbury, United Kingdom), and the mouse preadipocyte cell line 3T3-L1 was from ATCC (Manassas, VA). These cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% lipoprotein-depleted and dextran charcoal-treated fetal calf serum, penicillin, and antibiotics, unless stated otherwise. 3T3-L1 cells were differentiated initially by a 2-day treatment with dexamethasone (0.1 μM), isobutyl methyl xanthine (0.25 mM), and insulin (0.4 μM). Subsequently, the cells were maintained for an additional 8 days with insulin until complete differentiation. Caco2 cells were maintained in DMEM supplemented with 10% fetal calf serum and 1% of essential amino acids. Differentiation occurred spontaneously, and cells were treated after 7 days of differentiation process. Experiments were performed on subconfluent hepatoma cells maintained in culture under serum-free conditions. at-RA and 9c-RA were dissolved in dimethyl sulfoxide, whereas oleic acid was complexed with bovine serum albumin (BSA) before addition to the cells. Actinomycin D treatment was performed 45 min before the treatment of the cells with retinoid acid treatment.

**In Vivo Study—**Male Zucker diabetic fatty (ZDF) rats were purchased from Harlan at 7 weeks of age. Animals were housed four per cage and were maintained in a 12-h light/12-h dark cycle (lights were turned on at 7 a.m.). All animals had free access to laboratory chow and tap water. 9c-RA, LG1069, 0.3, 1, 3, 10, and 30 mg/kg/die was added to the diet of ZDF rats during 50 days. There were four animals per treatment group. Lean rats fed with either normal chow or chow supplemented with 30 mg/kg/day of LGD1069 served as nondiabetic controls. Animals were fasted overnight before sacrifice. Animals were anesthetized with ether and sacrificed by cervical dislocation. Tissues (liver, epidydimal fat pad, skeletal muscle, and heart) were collected and immediately frozen in liquid nitrogen until analysis.

**RNA Analysis—**RNA preparation, Northern blot hybridizations, and quantification of total cellular RNA were performed as described previously (22). A mouse FATP-1 cDNA probe was obtained after cloning an RT-PCR fragment from mouse adipose tissue RNA (primers, 5'-ATG CGG GCT CCT GGA GCA GAG CC-3' and 5'-CTG GTC AGG CAG CAC CCA-3') into pBlueScript was sequenced and found to be identical to the reported mouse FATP-1 sequence (2). The rat ACS probe corresponds to the EcoRV restriction fragment of the rat ACS cDNA (23), and β-actin was used as a control probe (24).

**Isolation of Nuclei and Transcription Rate Assay—**Nuclei were prepared from FAO and differentiated 3T3-L1 cells that were treated either with 9c-RA or vehicle. Transcription run-on assays were performed as described by Nevins (25). Equivalent counts of nuclear RNA labeled with [α-32P]UTP (3000 Ci/mmol) were hybridized for 36 h at 65 °C to 5 μg of FATP-1, ACS, glyceroldehyde-3-phosphate dehydrogenase (GAPDH), and vector DNA (pBlueScript) immobilized on Hybrid-C Extra filters (Amersham Life Science, Buckinghamshire, U.K.). After washing filters were washed once at 21 °C, twice at 37 °C, and 0.1% SDS and twice at 65 °C for 30 min and subsequently exposed to x-ray film (BIOMAX-M, Eastman Kodak company, Rochester, NY). Quantitative analysis was performed using scanning densitometry (Bio-Rad GS670 densitometer, Hercules, CA).

**Preparation of Albumin-bound Fatty Acids and Fatty Acid Uptake Assay—**Radiolabeled [14C]oleate was added to water at 40 °C. Albumin was added, and the mixture was then heated at 50 °C, fatty acid-free Sigma, fatty acids-free Sigma was then added by gentle mixing from a concentrated stock (20 g/100 mL) to give a final molar ratio of 1/1, thereafter 2× Hanks’ solution was added to obtain a 1× final solution. The radioactive fatty acid was allowed to equilibrate with the albumin for 45 min at 37 °C. The measurement of uptake of [14C]oleate (about 50 mCi/mmol, NEN Life Science Products) was carried out in 24- or 6-well plates with 106 cells/mL of medium. Before treatment, the cells were washed with 1× Hanks’ solution. Retinoids (doses ranging from 10-9 to 10-6 M) were added in fresh DMEM medium containing 10% fetal calf serum. After 48 h of treatment, cells were washed with 1× Hanks’ solution and incubated for one additional hour in serum-free, glucose-free medium. Cells were then washed once at 37 °C and twice at 37 °C with 1× Hanks’ solution containing BSA. Hanks’ solution without BSA was then added before the assay. A volume corresponding to 1 μL of [14C]oleate albumin-bound solution was added in each well, and cells were incubated for 1 min at 21 °C. Incubation was stopped after 1 min with three washes of ice-cold 1× Hanks’ solution without BSA. A complementary experiment has been performed to verify whether a specific cell surface binding of [14C]oleate could interfere with the assay. For this second assay, the cells were washed under more stringent conditions in 1× Hanks’ solution containing 0.5% BSA. Cells were then lysed in 400 μL of 0.1% SDS solution. The lysate was counted for 5 min with 4 mL of scintillation solution. All assays were performed on triplicate points.

**RESULTS**

9c-RA Induces FATP-1 and ACS mRNA Levels in Vitro—We examined the regulation of FATP-1 and ACS gene expression upon retinoidic acid treatment over a dose range from 10-8 to 10-6 M in FAO cells cultured during 6 h. 9c-RA produced a dose-dependent increase of both FATP-1 and ACS mRNA levels in this hepatic cell line (Fig. 1A). A maximal increase for FATP-1 (>9-fold) and ACS (14-fold) was observed with a dose
of $10^{-6}$ M 9c-RA. FATP-1 and ACS mRNA were also up-regulated by at-RA but to a much lesser extent than with 9c-RA (data not shown). We next determined whether FATP-1 levels were modified by retinoids in the human hepatoma cell line HepG2. 9c-RA also induced FATP-1 and ACS (data not shown) mRNA after 24 h in HepG2 cells. This effect was already evident at a dose of $10^{-3}$ M of 9c-RA (Fig. 1B). However, no effect of at-RA on the expression of FATP-1 and ACS mRNA could be detected in HepG2 cells (data not shown).

The strong induction of FATP-1 in hepatoma cells led us to investigate the regulation in other rat and human cell lines representative of tissues with a high fatty acid metabolism (i.e. adipose tissue and small intestine). 3T3-L1 preadipocyte cells were therefore induced to differentiate, with a mixture of dexamethasone, isobutyl methyl xanthine, and insulin, and then treated with 9c-RA ($10^{-9}$ to $10^{-3}$ M during 24 h). 9c-RA induced both FATP-1 and ACS (data not shown) mRNA expression in a dose-dependent manner in these differentiated 3T3-L1 cells. A maximal increase was observed at $10^{-6}$ M 9c-RA (Fig. 1C).

The expression of FATP-1 and ACS mRNA was evaluated after retinoid treatment in the human colon adenocarcinoma cells Caco2. Both FATP-1 and ACS gene expression increased after 6 h of treatment with 9c-RA at $10^{-6}$ M in this cell line. Under similar conditions, however, higher levels of induction of both mRNA species are nevertheless observed in FAO cells (Fig. 1A) relative to Caco2 cells (Fig. 1D).

The Induction of FATP-1 and ACS mRNA Levels by 9c-RA Is At the Transcriptional Level—We next determined the relative contribution of transcriptional and post-transcriptional processes to the increase in mRNA levels of FATP-1, by using the transcriptional inhibitor actinomycin D. Prior addition of actinomycin D completely abolished the increase of FATP-1 (Fig. 2A) and ACS (data not shown) mRNA in 3T3-L1 and FAO cells.

To assess the role of mRNA stability in the observed changes of the FATP-1 mRNAs, RNA synthesis was blocked with actinomycin D 6 h after 9c-RA induction in FAO cells, and the relative rates of disappearance of FATP-1 mRNA were determined (Fig. 2, B and C). Disappearance rates of FATP mRNA were very similar before and after 9c-RA treatment, suggesting that 9c-RA did not change the stability of FATP-1 mRNA.

Nuclear run-on analysis was then carried out to determine whether the induction of FATP-1 and ACS expression by 9c-RA was a direct consequence of enhanced gene transcription. FAO cells were treated for 4 h with 9c-RA ($10^{-6}$ M) (Fig. 3), whereas differentiated 3T3-L1 adipocytes were treated for 12 h with 9c-RA ($10^{-5}$ M) (Fig. 3). Control cells received vehicle only. Transcription rates for the FATP-1 and ACS genes were induced 2-fold and 2.8-fold, respectively, in FAO cells and 4- and 3-fold, respectively, in differentiated 3T3-L1 cells. In the retinoic acid-treated cells, transcription of the GAPDH gene was not affected in neither of the two cell lines. Because the stability of FATP mRNA is not altered by 9c-RA treatment, the induction of FATP-1 mRNA is primarily the result of an increase in the transcription rate rather than being caused by alterations in mRNA stability.

Retinoids, but Not RAR Agonists, Induce FATP-1 and ACS Gene Expression in Differentiated 3T3-L1 Cells—To determine whether the effect of retinoids on the regulation of FATP-1 and ACS gene expression was mediated by RAR or RXR, both 3T3-L1 preadipocytes and differentiated 3T3-L1 adipocytes were treated either with at-RA or TTNPB (specific RAR agonists) or 9c-RA (an RAR/RXR panagonist). Interestingly, no effect of these retinoids on FATP-1 or ACS mRNA levels was observed in undifferentiated 3T3-L1 preadipocyte cells (Figs. 4 and 5). In differentiated 3T3-L1, no activation of FATP-1 and ACS gene expression was detectable after 24 h of treatment.
with at-RA or TTNPB. In contrast, a dose-dependent induction of both FATP-1 (Fig. 4) and ACS (Fig. 5) mRNA expression was observed 24 h after treatment with 9c-RA in the differentiated 3T3-L1 cells. Maximum induction of mRNA expression occurred at 10^{-6} M 9c-RA (3.5- and 3-fold for FATP-1 and ACS, respectively). Furthermore, the fold induction of steady-state mRNA levels (Fig. 5) corresponds to the increase in transcription rates (Fig. 5) observed in 3T3-L1 cells.

To specify which RXR heterodimer combination induced FATP-1 and ACS gene expression, a number of specific RXR ligands were used: 1) LGD1069 and LG100268, specific activators of RXR homodimers and PPAR-RXR or LXR-RXR, but without activity on RAR-RXR heterodimers; and 2) LG100754, an activator of PPAR-RXR and RAR-RXR heterodimers, which is unable to activate RXR homodimers. All these compounds enhance FATP-1 in a significant and dose-dependent manner in differentiated 3T3-L1 adipocytes expression (Fig. 4; 4-fold activation at 10^{-6} M with LGD1069, 3.6-fold induction at 10^{-6} M with LG100268, and a 4-fold induction at 10^{-5} M with LG100754). In contrast, ACS expression was not affected by LG100268 and LGD1069, whereas LG100754 induced ACS mRNA levels significantly (Fig. 5). Once again, none of these specific rexinoids have an effect on undifferentiated 3T3-L1 preadipocytes (Figs. 5 and 6).

**RXR Agonists, but Not RAR Agonists, Induce Oleate Uptake in Differentiated 3T3-L1 Cells**—To establish whether the induction of the FATP-1 and ACS was accompanied by a concomitant increase in fatty acid uptake into the cells, we measured [^{14}C]oleate uptake in differentiated 3T3-L1 cells exposed to increasing doses of two prototypic retinoids, i.e. LG100754, an RXR agonist, and TTNPB, an RAR agonist (dose range 10^{-9} to 10^{-4} M). LG100754 induces [^{14}C]oleate uptake dose-dependently. A maximal induction of fatty acid uptake by 2-fold was observed at a concentration of 10^{-7} M. No effect on [^{14}C]oleate uptake...
uptake was observed after TTNPB, confirming that this effect was specific for the rexinoids (Fig. 6).

LGD1069 Induces FATP-1 mRNA Levels in the Liver and Adipose Tissue of Zucker Diabetic Fatty Rats—We wanted to know whether these strong effects on FATP-1 and ACS gene expression in vitro could be translated into changes in mRNA expression in vivo. ZDF rats were therefore treated during 50 days with either no or increasing amounts of LGD1069 (0.3, 1.0, 3.0, 10 to 30 mg/kg/day; Fig. 7). In the liver, FATP-1 mRNA levels were significantly induced in a dose-dependent fashion by LGD1069 with a maximal effect at 30 mg/kg/day (Fig. 7). Interestingly, in adipose tissue, FATP-1 was only induced at the dose of 1 mg/kg/day (an effect lost at higher doses of LGD1069) in the ZDF rats. In the heart or skeletal muscle the expression of the FATP-1 gene was not affected by LGD1069 therapy. Similarly to FATP-1 mRNA, ACS mRNA levels were dose-dependently induced in the liver. No effect of LGD1069 was detected in adipose tissue, muscle, and heart. Also in lean animals, FATP and ACS mRNA levels were only induced in the liver, whereas the changes observed in the other tissues, such as adipose tissue, did not reach significance.

DISCUSSION

The results of this study demonstrate that RXR ligands control both FATP-1 and ACS gene expression in several cell types. In liver cell lines, FATP-1 and ACS mRNA levels have been shown previously to be strongly induced by PPARα activators, such as the fibrates (25–27). The transcriptional induction of the ACS gene is mediated by the interaction of the activated PPARα-RXR heterodimer with a cognate response element in the promoter region of the ACS (25). Hence, it was not too surprising to observe a strong induction of FATP-1 and ACS expression in hepatoma cell lines by specific RXR activators, which activate the other partner of the PPARα-RXR heterodimer. Long chain fatty acid transport in hepatoma cells seems to be tightly controlled by retinoic acid, because L-FABP, a cytoplasmic protein implicated in long chain fatty acid uptake, is also regulated by 9c-RA (28). The co-induction of FATP-1 and ACS expression by rexinoids, which is similar to the co-induction of both these genes by fibrates furthermore indicates that rexinoids and fibrates may have similar metabolic effects on rat and human liver. This has already been suggested by the observation that both retinoids and peroxisome proliferators stimulate transcription of genes encoding the peroxisomal β-oxidation enzymes (29, 30), most likely through activation of the PPARα-RXR heterodimer (18). In adipose cell lines, which are highly insulin-sensitive, FATP-1 and ACS expression are also co-regulated by rexinoids in a dose-dependent manner, although the maximum level of induction appears lower. In contrast to the liver cells, which mainly express PPARα, PPARγ is the predominant PPAR isotype expressed in adipocytes and PPARγ activators have been shown to induce both FATP-1 and ACS expression (27). Hence, the
induction of FATP-1 (and to a lesser extent ACS) expression in adipose cell lines by RXR agonists, suggest that RXR is also an active partner in the PPARg-RXR heterodimer. In Caco2, FATP-1 and ACS genes are also co-regulated by retinoids. This is the first time that FATP-1 is shown to be expressed in a human intestinal cell line. In view of the co-regulation by retinoic acids of several fatty acid transporters, such as L-FABP (28), ACS and FATP-1, in this tissue, a potential important role for retinoic acid in absorptive processes in the gut is suggested. It furthermore underscores the potential implication of FATP-1, in addition to FATP-4 in fatty acid uptake in the gut (31).

Interestingly, the strong regulatory effects of retinoids on FATP-1 and ACS expression in the liver-derived cell lines (FAO and HepG2; Fig. 1) seems to be translated in vivo (Fig. 7), underscoring the physiological relevance of these findings. In contrast, the apparent difference between the regulation of FATP-1 and ACS gene expression between our in vitro studies in adipocyte cell lines and our in vivo studies in adipose tissue seems puzzling. Whereas we observe a ±4-fold induction of the expression of both genes in adipocyte cell lines, a much smaller effect was observed in vivo in ZDF adipose tissue, suggesting that the in vivo response in ZDF adipose tissue is somehow impeded. This could be linked to differences either in the expression or activity of one of the heterodimer components (RXR/PPAR) or of one of the cofactors required for transcriptional activity. Further studies in our laboratory are addressing this issue at present.

Our studies with more selective ligands confirm the importance of the RXR-PPAR heterodimer in mediating the effects of PPAR and/or RXR agonists on gene expression. Because LG100754 induces only the activity of the RXR-RAR and RXR-PPAR heterodimers, the up-regulation of FATP-1 and ACS by LG100754 implicates one or the other heterodimer in mediating the effects. LG100268, which is specific for the RXR homodimer or RXR-LXR and RXR-PPAR heterodimers, but not RAR-RXR heterodimers, activates FATP-1 expression. Finally, TTNPB, which is an activator of RAR in the context of RXR-RAR heterodimer, has no effect on FATP-1 and ACS. The combined results of studies using these synthetic retinoids
implicates the PPAR-RXR heterodimer as the molecular target of the regulation of FATP-1 and ACS by retinoic acids. Because PPAR and RXR are both active components of this heterodimer and because we have previously demonstrated that the FATP-1 and ACS genes are both regulated by PPAR activators (27), it appears that retinoids cooperate synergistically with PPAR ligands to control the expression of these two genes involved in fatty acid metabolism. Furthermore, the fact that the co-regulation of FATP-1 and ACS genes by retinoids depends on the differentiated state of 3T3-L1 cells points to the importance of PPARγ in this process. In preadipocyte cells, PPARγ is expressed at low levels and its expression increases upon adipocyte differentiation. The absence of a retinoid response in undifferentiated 3T3-L1 cells, expressing almost no PPARγ, hence also suggests that PPARγ is an obligatory partner of RXR in the retinoid-dependent regulation of FATP-1 and ACS genes in adipose tissue.

It is interesting to note that RXR agonists such as LG100268 and LGD1069 function as insulin sensitizers in a similar way as PPARγ agonists (14). Glucose homeostasis is improved when fatty acid concentration decreases in the circulation and more particularly in muscle tissue. The beneficial effect of PPARγ activators on glucose homeostasis has been previously shown to be part due to a redistribution of fatty acids toward adipose tissue with a relative depletion of fatty acids in the muscle (reviewed in Ref. 17). As known since Randle’s work (32–34), depletion of muscle fatty acid content will result in an improvement of glucose homeostasis. Hence, it is tempting to speculate that the improvements of glucose homeostasis observed with retinoids are, similar to the effects of PPARγ agonists, linked to an altered partitioning of fatty acids, with a relative depletion of muscle fatty acid uptake and metabolism. As observed in vivo in this study, the attenuated regulation of FATP-1 and ACS expression in both heart and skeletal muscle is in fact suggestive of a role for these two genes in reducing fatty acid uptake in muscle tissue, which could contribute to the insulin-sensitizing effects of these retinoids. In view of the distinct tissue distribution of PPARγ and the various RXRα, RXR ligands might have a different tissue-specific activity, affecting mainly the liver, whereas PPARγ activators will affect mainly adipose tissue. RXR agonists could, hence, provide an alternative or complimentary way to regulate the expression of genes implicated in fatty acid distribution and that ultimately affect glucose homeostasis.

This study further underscores distinct metabolic effects of retinoids versus retinoids and demonstrates that RXR is an important signaling molecule in the regulation of two genes implicated in fatty acid metabolism. Combined with our previous studies on the effects of PPAR agonists on FATP-1 and ACS expression, the data obtained with dimer-specific retinoids in this manuscript suggest that the PPAR-RXR complex is the molecular target by which retinoids regulate FATP-1 and ACS gene expression. The observation, that both retinoids and PPAR agonists control the transcription of the same genes implicated in lipid metabolism, suggests that retinoids also act as hypolipidemic and hypoglycemic agents through activation of PPAR-RXR complex and are consistent with the hypothesis that the PPAR-RXR heterodimer is a molecular target for insulin sensitivity.

REFERENCES

1. Higgins, C. F. (1994) Cell 79, 393–395
2. Schaffer, J. E., and Lodish, H. F. (1994) Cell 79, 427–436
3. Abumrad, N. A., Forest, C. C., Regen, D. M., and Sanders, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6008–6012
4. Abumrad, N. A., el-Maghrabi, M. R., Amri, E.-Z., Lopez, E., and Grimaldi, P. A. (1993) J. Biol. Chem. 268, 17655–17668
5. Spitsberg, V. L., Mattiashvili, E., and Goreswit, R. C. (1995) Eur. J. Biochem. 230, 872–878
6. Van Nieuwenhoven, F. A., Verstijnen, C. P., Abumrad, N. A., Willemsen, P. H., Van Eys, G. J., Van Der Vusse, G. J., and Glaz, J. F. (1995) Biochem. Biophys. Res. Commun. 207, 747–752
7. Stremmel, W., Strohmeyer, G., Border, S., Kochwa, S., and Berk, P. D. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4–8
8. Schwieterman, W. D., Sorrentino, D., Potter, B. J., Rand, J., Kiang, C. L., Stump, D., and Berk, P. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 359–363
9. Berk, P. D., Wada, H., Horio, Y., Potter, B. J., Sorrentino, D., Zhou, S. L., Isola, L. M., Stump, D., and Kiang, C. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3484–3488
10. Zhou, S. L., Stump, D., Kiang, C. L., Isola, L. M., and Berk, P. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 263–270
11. Mangelsdorf, D. J., and Evans, R. M. (1995) Cell 83, 841–850
12. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Rastner, P., Mark, E., Chambon, P., and Evans, R. M. (1995) Cell 83, 839–859
13. Lala, D. S., Mukherjee, R., Schulman, I. G., Canan Koch, S. S., Dardashili, L. J., Nautanen, A. M., Croston, G. E., Evans, R. M., and Heyman, R. A. (1996) Nature 383, 450–453
14. Mukherjee, R., Davies, P. A. J., Crambie, D. L., Bischoff, E. D., Cesario, R. M., Lew, L., Hamann, L. G., Boehm, M. F., Monden, C. E., Nadzan, A. M., Patteniti, J. R., and Heyman, R. A. (1997) Nature 386, 407–410
15. Boehm, M. F., Zhang, L., McCurg, M. R., Berger, E., Wagoner, M., Mais, D. E., Suto, C. M., Davies, P. A. J., Heyman, R. A., and Nadzan, A. M. (1995) J. Med. Chem. 38, 3146–3145
16. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A. M., Heyman, R., Briggs, M., Cayet, D., Deeb, S., Staels, B., and Auwerx, J. (1996) EMBO J. 15, 5336–5348
17. Martin, G., Schoonjans, K., Staels, B., and Auwerx, J. (1998) Atherosclerosis 11, 35–47
18. Kriever, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) Nature 358, 771–774
19. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., and Evans, R. M. (1990) Nature 345, 224–229
20. Boehm, M. F., Zhang, L., Badea, B. A., White, S. K., Mais, D. E., Berger, E., Suto, C. M., Goldman, M. E., and Heyman, R. A. (1994) J. Med. Chem. 37, 2930–2941
21. Meunier-Dumort, C., Poirier, H., Forest, C., and Besnard, P. (1996) Biochem. J. 319, 483–487
22. Auwerx, J., Deeb, S., Brunazzi, J. D., Peng, R., and Chait, A. C. (1988) Biochemistry 27, 2651–2655
23. Schoonjans, K., Staels, B., Grimaldi, P., and Auwerx, J. (1993) Eur. J. Biochem. 216, 615–622
24. Cleveland, D. W., Iopista, M. A., McDonald, R. J., Cowan, M. J., Rutter, W. J., and Kirschner, M. W. (1980) Cell 20, 95–105
25. Nevin, J. R. (1987) Methods Enzymol. 152, 234–241
26. Schoonjans, K., Watanabe, M., Suzuki, H., Mahfoudi, A., Krey, C., Wahli, W., Grimaldi, P., Staels, B., Yamamoto, T., and Auwerx, J. (1995) J. Biol. Chem. 270, 19269–19276
27. Martin, G., Schoonjans, K., Lefebvre, A., Staels, B., and Auwerx, J. (1997) J. Biol. Chem. 272, 29210–29217
28. Poirier, H., Brassant, O. N., I., Wahli, W., and Besnard, P. (1997) FEMS Lett. 412, 480–484
29. Reedy, J. K., and LaVal, N. D. (1983) CRC Crit. Rev. Toxicol. 12, 1–58
30. Hertz, R., and Bar-Tana, J. (1992) Biochem. J. 281, 41–43
31. Stahl, A., Hirsch, D. J., Gimeno, R. E., Frayn, S., Ger, P., Watson, N., Patel, S., Koller, M., Raimondi, A., Tartaglia, L. A., and Lodish, H. F. (1999) Mol. Cell. 4, 299–309
32. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963) Lancet I, 785–789
33. Randle, P. J., Newsholme, E. A., and Garland, P. B. (1964) Biochem. J. 93, 652–663
34. Boden, G., Chen, X., Ruiz, J., White, J. V., and Rossetti, L. (1994) J. Clin. Invest. 93, 2438–2446
Induction of the Fatty Acid Transport Protein 1 and Acyl-CoA Synthase Genes by Dimer-selective Rexinoids Suggests That the Peroxisome Proliferator-activated Receptor-Retinoid X Receptor Heterodimer Is Their Molecular Target

Geneviève Martin, Hélène Poirier, Nathalie Hennuyer, Diane Crombie, Jean Charles Fruchart, Richard A. Heyman, Philippe Besnard and Johan Auwerx

J. Biol. Chem. 2000, 275:12612-12618.
doi: 10.1074/jbc.275.17.12612

Access the most updated version of this article at http://www.jbc.org/content/275/17/12612

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 10 of which can be accessed free at http://www.jbc.org/content/275/17/12612.full.html#ref-list-1