

Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor

(arachidonic acid/peroxisomal proliferation/nuclear receptor superfamily/transcription factors/alkaline phosphatase)

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ABSTRACT Peroxisome proliferators such as clofibric acid, nafenopin, and WY-14,643 have been shown to activate PPAR (peroxisome proliferator-activated receptor), a member of the steroid nuclear receptor superfamily. We have cloned the cDNA from the rat that is homologous to that from the mouse [Issemann, I. & Green, S. (1990) *Nature (London)* 347, 645–650], which encodes a 97% similar protein with a particularly well-conserved putative ligand-binding domain. To search for physiologically occurring activators, we established a transcriptional transactivation assay by stably expressing in CHO cells a chimera of rat PPAR and the human glucocorticoid receptor that activates expression of the placental alkaline phosphatase reporter gene under the control of the mouse mammary tumor virus promoter. Testing of compounds related to lipid metabolism or peroxisomal proliferation revealed that 150 μ M concentrations of arachidonic or linoleic acid but not of dehydroepiandrosterone, cholesterol, or 25-hydroxycholesterol, activate the receptor chimera. In addition, saturated fatty acids induce the reporter gene. Shortening the chain length to $n = 6$ or introduction of an ω -terminal carboxylic group abolished the activation potential of the fatty acid. In conclusion, the present results indicate that fatty acids can regulate gene expression mediated by a member of the steroid nuclear receptor superfamily.

Unsaturated fatty acids induce peroxisomal proliferation and lower blood triglyceride levels (1–3). Similar effects are evoked by a number of man-made compounds which are either considered for therapy of hyperlipidemia—e.g., clofibric acid, nafenopin, WY-14,643, or sulfur-substituted fatty acids—or are in use as industrial plasticizers (4–6). The steroid dehydroepiandrosterone (DHEA) also induces peroxisomal proliferation but increases blood triglyceride and cholesterol levels (7, 8). Two main hypotheses have been developed to explain the complex response of peroxisomal proliferation to this wide variety of inducers. According to one theory the intracellular accumulation of fatty acids is the key stimulus for triggering peroxisomal proliferation (4, 9). The other theory postulates the involvement of a receptor protein (4, 10) and an as-yet-unknown intracellular messenger—e.g., the ligand for this receptor.

The latter idea gained substantial support from the discovery of mouse peroxisome proliferator-activated receptor (mPPAR), a member of the steroid nuclear receptor superfamily (11, 12). The gene encoding PPAR belongs to a number of genes cloned in the last few years by means of their homology with steroid receptors. In general, ligands or physiologically occurring activators have been identified for only a few of these so-called orphan receptors (13–15). In the case of mPPAR, transactivation studies using chimeric proteins composed of the putative ligand-binding domain of the

novel receptor and DNA-binding domains of known steroid receptors showed that mPPAR could be activated by peroxisome proliferators (11). However, the identity of the ultimate ligand of the receptor protein, the nature of physiological activators, and how the receptor might relate to the concept of fatty acids as inducers of peroxisomal proliferation remain unclear.

We now describe the cloning from rat liver of a gene homologous to that encoding mPPAR. To search for physiological activators of the encoded receptor protein, we developed a transactivation assay that is suitable for testing large numbers of potential ligands. A chimeric construction consisting of cDNA encoding the putative ligand-binding domain of rat PPAR (rPPAR)[‡] and the amino-terminal and DNA-binding domains of the human glucocorticoid receptor (hGR) was stably expressed in CHO cells together with the gene for placental alkaline phosphatase (PAP) under the control of the mouse mammary tumor virus (MMTV) promoter as a reporter for receptor activation. Screening compounds related to lipid metabolism revealed that fatty acids like linoleic (C18:2), arachidonic (C20:4), or lauric acid, but not DHEA, DHEA sulfate, cholesterol, or 25-hydroxycholesterol, activate the expressed chimeric receptor.

MATERIALS AND METHODS

Cloning of rPPAR cDNA and Construction of Mammalian Expression Vectors. Eight oligonucleotides, each 69 base pairs (bp) in length, were synthesized (Applied Biosystems 380B DNA synthesizer), matching homologous parts of the cDNAs from the receptors for glucocorticoids, mineralocorticoids, estrogen, progesterone, androgens, thyroid hormones, vitamin A, and 1,25-dihydroxycholecalciferol/vitamin D₃. The chosen fragments code for a stretch of 23 amino acids starting with the amino acid preceding the second conserved cysteine residue of the ligand-binding domain (12). The oligonucleotides were pooled, labeled with ³²P, and used for low-stringency screening (16) of 500,000 clones in a commercially available 20% oligo(dT)/80% random-primed cDNA library from Sprague–Dawley rat liver in the λ ZAP vector (Stratagene). Clones corresponding to known steroid receptors were eliminated by high-stringency hybridization, and the remaining three independent clones were converted into plasmids by the use of R408 helper phage (Stratagene). The isolation of overlapping clones and sequence analysis (Sequenase, United States Biochemical) generated a clone encompassing a full open reading frame homologous to mPPAR cDNA (11) which we termed rPPAR cDNA.

Abbreviations: hGR, human glucocorticoid receptor; PPAR, peroxisome proliferator-activated receptor; mPPAR and rPPAR, mouse and rat PPAR; PAP, placental alkaline phosphatase; DHEA, dehydroepiandrosterone; MMTV, mouse mammary tumor virus.

[‡]The sequence of the rPPAR cDNA reported in this paper has been deposited in the GenBank data base (accession no. M88592).

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The cDNA encoding the putative ligand-binding domain of rPPAR was isolated as a 942-bp fragment spanning codons 168–468 by 20 cycles of PCR (Perkin-Elmer/Cetus). *Xho* I restriction sites were introduced by using the mutated oligonucleotide primers CCCATTCCTCGAGCACACAATGC AATCCGTTTTGGAAGA (5') and AAAGCCACTC-GAGTGGCTACGGCCTACCATCTCAGGAAA (3'). A *Bam*HI-*Eco*RI fragment of pMT-hGR (17) coding for amino acids 1–500 of hGR was subcloned into pGEM-7Zf(+) (Promega), and the *Xho* I-digested PCR product was cloned into the adjacent *Xho* I site in the pGEM-7Zf(+) multiple cloning site by standard techniques (18). The sequence of the PCR-amplified DNA and the ligation of hGR DNA with rPPAR-derived DNA was confirmed by dideoxy sequencing (19) with Sequenase. A 2.6-kilobase (kb) *Bam*HI-*Xba* I fragment containing the hGR-and rPPAR-derived elements was reisolated and inserted into pMT-hGR in place of the sequence coding for hGR (17). The resulting plasmid termed pMT-GR/PPAR contains the open reading frame for the chimeric receptor under the control of the human metallothionein (MT) AII promoter and the human growth hormone polyadenylation signal (20). The reporter gene construct pMMTV-AF (ref. 17; provided by Stefan Nilsson, Karo Bio, Stockholm) contains a *Sph* I-*Xho* I fragment of the cDNA for a secreted form of human PAP (21) under the control of the MMTV promoter (pMSG, Pharmacia).

Cell Culture and Introduction of Foreign DNA. CHO cells were kept in Ham's F-12 medium supplemented with 10% fetal bovine serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO/BRL). The reporter construct pMMTV-AF was integrated by electroporating 3 × 10⁶ cells suspended in 0.5 ml of serum-free Ham's F-12 medium containing 1 μg of pSV2neo (22) and 10 μg of pMMTV-AF at 1500 V/cm and τ = 9 ms. Stably transfected cells were

selected in the presence of 0.5 mg of G418 per ml. Ten colonies of 120 were expanded and tested for maximum induction of the reporter gene by dexamethasone after transient transfection with pMT-hGR. The clone yielding the highest response was cotransfected with 1 μg of pKSV-Hyg (S. Nilsson), 10 μg of either pMT-hGR or pMT-GR/PPAR, and 1 μg of pSV2-cat (23) as an indicator of transfection efficiency, by using lipofectin (GIBCO/BRL) according to the manufacturer's instructions. Stably transfected cells were selected in medium containing 0.5 mM hygromycin and used for further experiments as a pool.

Assay for PAP Induction. Stably transfected cells were seeded at a density of 20,000–40,000 per 2-cm² culture on 24-well multidishes (Nunc) in 0.5 ml of medium and grown for 24 hr prior to adding 0.5 ml of medium containing the inducing compound. Lauric, stearic, petroselinic, elaidic, and 1,12-dodecanedioic acid, dexamethasone, and WY-14,643 (Chem-syn Science Laboratories, Lenexa, KS) were dissolved as 1000-fold concentrated stock solutions in dimethyl sulfoxide. Cholesterol and 25-hydroxycholesterol were dissolved in ethanol. Other fatty acids were added to the cell culture medium at a concentration of 0.4–2 mM, suspended by sonication, and further diluted in cell culture medium to the desired concentration. After 48 hr of treatment, cell culture supernatants were heated to 65°C for 30 min. PAP activity was determined as the increase of A₄₀₅ at 30°C in a 1-ml reaction mixture containing 0.75 ml of supernatant, 200 mM Tris (pH 8.8), 275 mM NaCl, 0.5 mM MgCl₂, and 5 mM *p*-nitrophenyl phosphate.

RESULTS

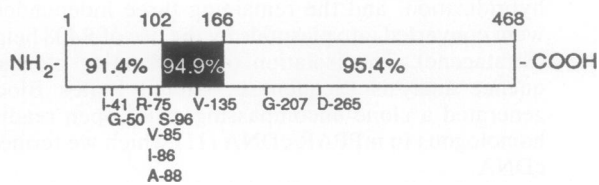
Low-stringency screening of a rat liver cDNA library with a mixture of synthetic oligonucleotide probes derived from

A

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1 CGAACTGTCGGCTACTTCGAGTCCCCTTGAGCGCCGTGTGCCGCTCTGAACATTGGCGTTCGCAGCTGTTTTGTGGCTGGAGGTTCTGTGGAGTCTCGAACTGAAGCGACCTGGGT
121 CCTCTGGTGTCCCTTGAAGGGGGCACACAGCGGGGGACATCGGGCGCTCCCTTCCCACAGCGTGGTGCATTTGGGCGTAACACACCGGGAGGCGTTTCTTGAGACCTCGGGGATC
241 TTAGAGCGGAGCCCAAGACTGAAGTTCAAGGCCCTGCCCTTCCCTGTGAACACTGACATTTGTACTGGTCAAGCTCAGGACACAGACCTGTGCATCACAGATGGTCTGTGGCCCGCCT
361 GGGCACACCACTTCAACATGGTGACACAGAGAGCCCACTGTCTCTCTCCCACTTGAAGCAGATGACCTGGAAAGTCTCCCTTATCTGAAGAATCTTACAAGAGATGGGAAACATTC
1 M V D T E S P I C P L S P L E A D D L E S P L S E E F L Q E M G N I Q
481 AAGAGATTCTCAGTCCCTCGGAGAGGAGTTCGGAAAGCTTAAAGTTTTCGGGACTACAGTACTTAGGGAGCTGTCCAGGCTCGGAGGCGCTCTCATCACAGACCTCTCTCCAG
36 E I S O S G S L G E E S S G S F A D Y Q Y L G S C P G S E G S V I T D T L S P A
601 CTTCAGCCCTCTCAGTCACTGCGCTGTCCCAACAGTACAGATGAGTCCCTGGCAATGCCTGAACATCGAGTGTGAAATATGTGGGCAAGGCTCAGGATACCACACTATG
76 S S P S V S C P A V P T S T D E S P G N A L N I E C R I C G D K A S G G Y H Y G
721 GAGTCCACGCAATGTGAAGGCTGCAAGGCTTCTTTCCGGCAACTATTCCGGCTAAAGCTGGCGTACGCAAGTGTGATCGAAGCTGCAAGATTGAGAAAAGAACCGAAACAAATGCCAGT
116 V H A C E G C K G F F R R T I R L K L A Y D K C D R S C K I Q K K M R N K C O Y
841 ACTGCCCTTCCACAAGTCCGTGCCGTCCGGATGTCACACAATGCAATCCGTTTGGAAAGATGCCAAGATCTGAGAAAAGCAAAATGAAGGCGAGAAATCCCTTACCTGTGAACAGATC
156 C R F H K C L S V G M S H N A I R F G R M P R S E K A K L K A E I L T C E H D L
961 TGAAGATTTCGAAACTGCAGACCTCAAATCTCTGGCCAAAGAGAATCCAGGAAGCTTCAACATGAACAAAGTCAAGGCCCGGTCATCCGAGGAAAGATAGCA
196 K D S E T A D L K S L A K R I H E A Y L K N F N M N K V K A R V I L A G K T S N
1081 ACAATCCGCTTTTGCATACATGACATGGAGACCTTGTGCATGGCTGAGAAGACGCTTGTGGCCAAAGATGGTAGCCAAAGGCTTGAAGGCAAGGAGGAGGAGGTCGATCTTCCACT
236 N P P F V I H D M E T L C M A E K T L V A K M V A N G V E N K G E A E V R F F H C
1201 GCTGCAAGTGCAGTCCGTGGAGACCTCAGGAAATTTGCCAAGGATATCCAGGCTTGAACAACTGGACTTGAATGACAGGTTACCTTGTGAGTACGGTGTGTATG
276 C Q C M S V E T V T E L T E F A K A I P G F A N L D L N D Q V T L L K Y G V Y E
1321 AAGCCATCTCAGTACGCTGCTGATGAACAAAGAGGATGCTGATCGCGTACGGGCTTCAACCCGAGAGTTCATCAAGAACCTGAGGAAAGGCAATTCGCGACATCA
316 A I F T M L S S L M K D G M L I A Y G N G F I T R E F L K N L R K P F C D I M
1441 TGAACCCAAAGTTTGAATGAAGTTCAATCCCTGCAACTGATGACATGACATTTCCCTTTTGTGGCTGCTATAATTTGCTGTGGAGATCGGCTCGGCTTCAAAATATG
356 E P K F D F A M K F N A L E L D D S D I S L F V A A I I C C G D R P G L L N I G
1561 GATACATTGAAAGTTGCAAGGAGGAGTGTGCACGTGCTCAAGCTCCAGCTGCAGAGAACCATCCGGATGATACCTTTCTTCCCAAAACTCTTCAAAAATGTTGGACCTCCGG
396 Y I E K L Q E G I V H V L K L H L Q S N H P D D T F L F P K L L Q K M V D L R Q
1681 AGCTGGTACGGAGCATCGCCAGCTCGTGCAGGTCATCAAGAAGACCGAGTACAGCGGGGCTGACCCACTGTTGCAAGAGATCAGAGAGACTGACTGATTTTCTGAGATGGT
436 L V T E H A Q L V Q V I K K T E S D A A L H P L L Q E I Y R D M Y *
1801 AGCCGTTGCCACTGTTCAGGACCTCTGAGGCTCGCGCCCAATACAGGAGAGCAGGGATTGCACAGATGGCCCTCCCTTACCTTGGAGATGAAGAGGCGTAGCCCTAGGCAATG
1921 CAGGCTCTCCACATCTTACTTTCTGAATGAGCACTTCAAGACTCTCTGACTAGTAATGGTGGTATCAGAGGCTAGTAGGATTGAGCAATTACAGG
    
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B



C

GMNLEARKTKKKIKGIprAHNAI
 485 500 168

FIG. 1. Primary structure of rPPAR and its chimera with hGR. (A) Nucleotide sequence of the cDNA and predicted amino acid sequence in single-letter code of the open reading frame. (B) The putative domain structure of rPPAR is indicated by boxes. Cys-102 and Met-166 indicate the boundaries between the putative N-terminal, DNA-binding, and ligand-binding domains. Nucleotide identity to the gene from the mouse is given inside the boxes, and unmatched amino acids are indicated below the diagram. (C) Amino acid sequence of the chimeric receptor at the fusion region. Capital letters indicate hGR-derived sequences; underlined letters, rPPAR-derived sequences; and lowercase letters, linker-encoded amino acids.

known steroid receptors revealed a cDNA species homologous to mPPAR cDNA (11) (Fig. 1A). The cloned fragment spans 2020 bp and contains a predicted open reading frame encoding 468 amino acids. Although a poly(A) tail was not isolated, the clone appears to contain almost the full-length cDNA as judged from Northern blot analysis of rat liver RNA (data not shown). The cDNAs from rat and mouse are 91.4%, 94.9%, and 95.4% identical in the regions coding for the N-terminal, DNA-binding, and putative ligand-binding domains, respectively (Fig. 1B).

A chimeric receptor encompassing the N-terminal and DNA-binding domains of the hGR and the putative ligand-binding domain of rPPAR (Fig. 1C) was stably expressed in CHO cells that had the PAP gene under the control of the MMTV promoter integrated into their genome. The reporter activity in these cells is 50- and 20-fold inducible by the known activators of mPPAR, WY-14,643 and clofibrilic acid, respectively (Fig. 2B). In agreement with the presence of endogenous, albeit low, levels of hamster glucocorticoid receptor in CHO cells (17, 24), the reporter is slightly inducible (4- to 6-fold) by dexamethasone even in the absence of coexpressed hGR (Fig. 2A and B). Stable expression of hGR increases the response to dexamethasone to a >100-fold induction (Fig. 2C). Thus, induction by dexamethasone and WY-14,643 provided the control for the integrity of the reporter system in all subsequent experiments.

Screening through potential activators of rPPAR revealed that exposure to fatty acids like linoleic (C18:2) and arachidonic (C20:4) acid, but not DHEA, DHEA sulfate, cholesterol, or 25-hydroxycholesterol, activated the reporter gene in cells expressing the receptor chimera (Fig. 2B). Only dexamethasone induced the reporter gene activity in cells

expressing either no additional receptor (Fig. 2A), the full-length hGR (Fig. 2C), or a truncated hGR lacking the ligand-binding domain (data not shown). Fatty acids at 300 μ M, 25-OH-cholesterol at 25 μ M, and DHEA at 100 μ M were toxic to the cells as indicated by cell detachment or, in the case of DHEA, decreased cell number.

Fig. 3 shows the dose-response relationship for the induction of the reporter gene. The highest tested concentration of fatty acids (300 μ M) caused complete detachment of cells. The maximum induction level by linoleic (C18:2) or arachidonic (C20:4) acid was comparable to that of WY-14,643 and was achieved at a concentration of 150 μ M. Though maximum induction occurred at fatty acid concentrations just below toxic levels, 40 μ M of arachidonic acid and 20 μ M of linoleic were sufficient to induce the reporter gene significantly.

To probe the requirement for specific structural properties of fatty acids, we tested saturated caproic acid (C6:0), lauric acid (C12:0), 1,12-dodecanedioic acid, and stearic acid (C18:0) and unsaturated petroselinic acid (*cis*- Δ 6-C18:1), oleic acid (*cis*- Δ 9-C18:1), elaidic acid (*trans*- Δ 9-C18:1), and linolenic acid (all-*cis*- Δ 9,12,15-C18:3) (Fig. 4) in addition to linoleic acid (all-*cis*- Δ 9,12-C18:2) and arachidonic acid (all-*cis*- Δ 5,8,11,14-C20:4). The unsaturated fatty acids activated the hGR/PPAR receptor chimera to an extent similar to that of WY-14,643, and the activation by unsaturated fatty acids was independent of specific structural requirements with regard to degree of unsaturation, position of the double bonds, or steric conformation. Among the saturated fatty acids, C12:0 was the best inducer of the reporter gene activity, whereas the solubility of C18:0 might have limited its effect. C6:0 and 1,12-dodecanedioic acid did not substantially

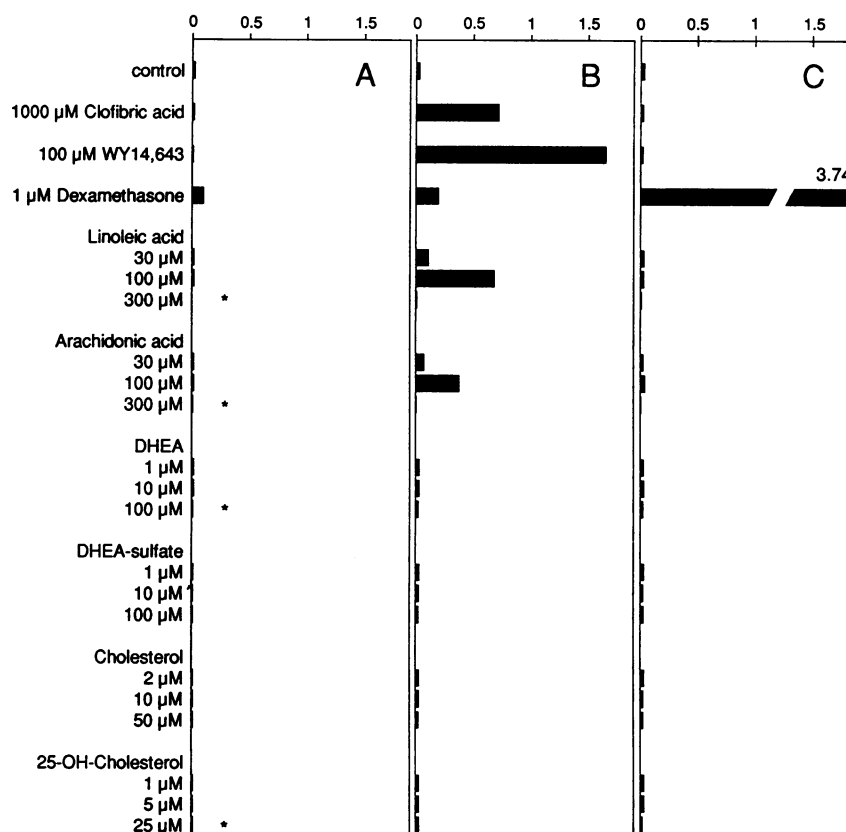


FIG. 2. Activation of the hGR/PPAR chimera by unsaturated fatty acids. Relative PAP activity in culture supernatants was measured by the increase in A_{405} per hr per culture, as indicated by the numbers at the top. CHO cells containing only the MMTV-AF reporter gene (A) or possessing the reporter gene together with the stably expressed hGR/PPAR chimera (B) or full-length hGR (C) were seeded at a density of 40,000 per 2-cm² culture well and grown for 24 hr. Cells were grown for an additional 48 hr in the presence of test compounds prior to assaying the culture supernatants for PAP activity. Asterisks in A indicate treatments that led to decreased cell numbers. Values represent means from double determinations that did not differ by more than 15%. Similar results were obtained in two further independent experiments.

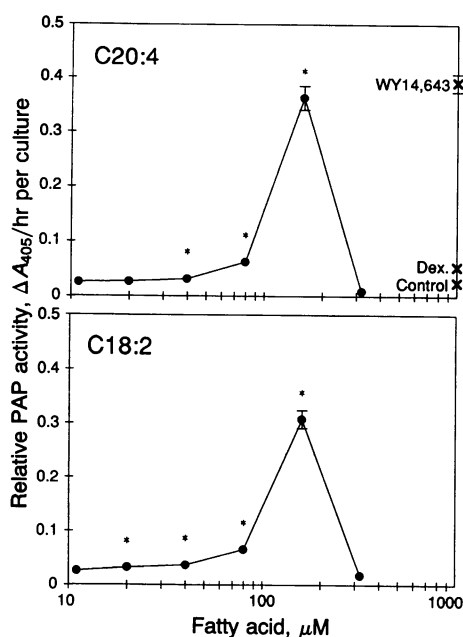


FIG. 3. Dose dependency of hGR/PPAR chimera activation by arachidonic acid (Upper) and linoleic acid (Lower). CHO cells harboring the MMTV-AF reporter gene and pMT-hGR/PPAR were seeded at a density of 20,000 per 2-cm² culture well and grown for 24 hr prior to addition of arachidonic acid or linoleic acid. PAP activity in the culture supernatant was determined after an additional 48 hr of culture. Marks on the right ordinate indicate PAP activity from control cultures and cultures exposed to 1 μM dexamethasone (Dex.) or 100 μM WY-14,643, respectively. Values represent means ± SD from triplicate determinations, with asterisks indicating a significant difference ($P < 0.005$; Student's *t*-test) from untreated cultures. Similar results were obtained in a second independent experiment.

activate the receptor chimera even at 1 mM concentration. None of the fatty acids tested in this experiment activated the reporter gene when the cells expressed the full-length hGR instead of the hGR/PPAR chimera (data not shown).

DISCUSSION

The present study describes cloning and functional analysis of a rat gene homologous to the mouse gene encoding mPPAR. The putative ligand-binding domain of this nuclear receptor-type protein is surprisingly well conserved between mouse and rat, with only two amino acid substitutions, whereas the much smaller N-terminal and DNA-binding domains carry seven and one substitutions, respectively.

To identify physiologically occurring activators of PPAR, we chose an approach that utilizes a chimeric receptor (11, 25) that activates the expression of an easily detectable reporter gene. Known peroxisomal proliferators such as WY-14,643, nafenopin, and clofibric acid significantly induced the reporter system at concentrations of 1, 10, and 100 μM, respectively (data not shown). Thus, the results with our stably transfected cells faithfully reproduce the specificity of mPPAR (11) and, furthermore, suggest that stably transfected cells and an easily detectable reporter gene activity might be generally useful in attempts to identify activators for orphan receptors.

The activation of PPAR by peroxisomal proliferation-inducing drugs prompted us to investigate intermediates of lipid metabolism as potential activators of PPAR. In fact, the unsaturated fatty acids arachidonic acid (C20:4) and linoleic acid (C18:2) were found to induce the reporter gene. The induction clearly depended on the presence of the putative ligand-binding domain of rPPAR in the chimeric receptor because fatty acids did not activate the reporter gene in cells

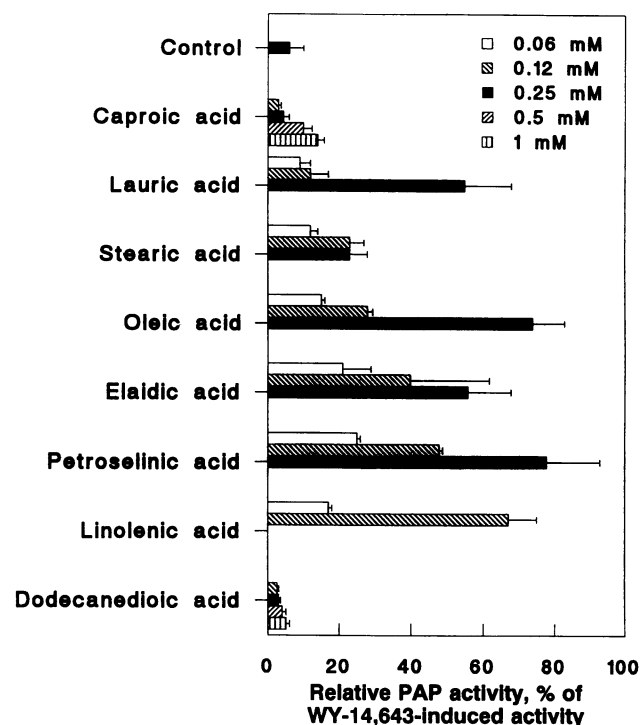


FIG. 4. Activation of the hGR-PPAR chimera by saturated and cis or trans unsaturated fatty acids. CHO cells containing the MMTV-AF reporter and pMT-hGR/PPAR were seeded at a density of 20,000 per 2-cm² culture well and treated with the indicated fatty acids. Relative PAP activity was determined as described in the legend to Fig. 2. PAP activities at fatty acid concentrations that were toxic, exceeded solubility, or were well below biologically active levels are not indicated. Values are presented relative to the PAP activity induced by 100 μM WY-14,643 in each particular experiment (means ± SD from 4–12 determinations in two to four independent experiments).

either expressing only the endogenous hamster glucocorticoid receptor or overexpressing the full-length hGR. Stable expression of a truncated hGR lacking the ligand-binding domain increased basal activity of the reporter gene. However, neither WY-14,643 nor fatty acids induced the reporter gene in these cells (data not shown), arguing against an unspecific effect of fatty acids on the MMTV promoter or other transcription factors controlling the activity of this promoter.

The structural requirements for fatty acids to activate the hGR/PPAR chimera are not very strict because only the reduction of the chain length to $n = 6$, the introduction of a second carboxylic group at the ω -position, or, in a trivial case, the limited solubility of saturated fatty acids with $n > 18$ abolished the inducing activity. However, some specificity among the tested compounds is discernible in that DHEA does not activate the receptor chimera, although it has been reported to induce peroxisomal proliferation *in vivo* (7). An unspecific effect of fatty acids on the reporter gene activity related to cytotoxicity seems unlikely because 25-hydroxycholesterol and DHEA are toxic but do not induce the reporter gene. Furthermore, fatty acid concentrations clearly below toxic levels activate the receptor chimera significantly. However, the lack of pronounced structural specificity is in close agreement with previous comparisons of peroxisomal proliferators, which have revealed the presence or easy formation of a poorly metabolized acidic group as the predominant common structural property of these compounds (1, 4, 5, 26).

If fatty acids themselves are considered ligands to PPAR, the ligand specificity appears surprisingly low as compared

with that of other members of the nuclear receptor superfamily. Thus, other or additional pathways of receptor activation should be considered. Metabolism of fatty acids could give rise to common intermediates that may activate the receptor. In the case of arachidonic acid, for example, the formation of biologically active compounds involves a complex cascade of events in the cyclooxygenase pathway (27), the lipoxygenase system (28), and the cytochrome P450-dependent metabolism. Alternatively, the ultimate activation of PPAR after formation of secondary effectors, which are structurally unrelated to the fatty acids, is also possible. The second-messenger cAMP, for example, has been suggested to play a role in the activation of COUP-TF, another member of the nuclear receptor superfamily (14).

So far, physiologically occurring activators have been identified for only a few of the recently discovered members of the nuclear receptor superfamily, which have been called orphan receptors because their activators have not been identified (11, 14, 15). A potential physiological role of fatty acids as activators of PPAR gains support from the finding that, at least in the case of linoleic acid, effective concentrations are within the range of serum levels of the nonesterified acid (29). Phenomenologically, the activation of PPAR by fatty acids is in close agreement with the findings that fatty acids (1) and poorly metabolized derivatives thereof (5, 6) evoke similar physiological responses as peroxisomal proliferation-inducing drugs. Our present results show that fatty acids, in principle, can regulate gene expression by activating a member of the nuclear receptor superfamily. It is tempting to speculate that this receptor, the peroxisome proliferator-inducible and fatty acid-metabolizing cytochrome P450IV family (30, 31), and the peroxisomal β -oxidation pathway might constitute an autoregulatory loop in lipid or fatty acid homeostasis (9, 32–34). However, a more complete understanding of the physiological role of this receptor awaits isolation of its ultimate ligand, biochemical characterization of its mechanism of activation, determination of its DNA-recognition motif, and identification of its target genes.

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