Expression of Putative Fatty Acid Transporter Genes Are Regulated by Peroxisome Proliferator-activated Receptor α and γ Activators in a Tissue- and Inducer-specific Manner*

Kiyoto Motojima‡§, Patricia Passilly¶, Jeffrey M. Petersa, Frank J. Gonzalezii, and Norbert Latruffee

From the ‡Department of Biochemistry, School of Pharmaceutical Sciences, Teho University, Funabashi, Chiba 274, Japan, the ¶Laboratoire de Biologie Moléculaire et Cellulaire, Université de Bourgogne, BP138, 21004 Dijon, France, and the iLaboratory of Metabolism, National Institutes of Health, Bethesda, Maryland 20892

Regulation of gene expression of three putative long-chain fatty acid transport proteins, fatty acid translocase (FAT), mitochondrial aspartate aminotransferase (mAspAT), and fatty acid transport protein (FATP), by drugs that activate peroxisome proliferator-activated receptor (PPAR) α and γ were studied using normal and obese mice and rat hepatoma cells. FAT mRNA was induced in liver and intestine of normal mice and in hepatoma cells to various extents only by PPARα-activating drugs. FATP mRNA was similarly induced in liver, but to a lesser extent in intestine. The induction time course in the liver was slower for FAT and FATP mRNA than that of an mRNA encoding a peroxisomal enzyme. An obligatory role of PPARs in hepatic FAT and FATP induction was demonstrated, since an increase in these mRNAs was not observed in PPARα-null mice. Levels of mAspAT mRNA were higher in liver and intestine of mice treated with peroxisome proliferators, while levels in hepatoma cells were similar regardless of treatment. In white adipose tissue of KKα obese mice, thiazolidinedione PPARγ activators (pioglitazone and troglitazone) induced FAT and FATP more efficiently than the PPARα activator, clofibrate. This effect was absent in brown adipose tissue. Under the same conditions, levels of mAspAT mRNA did not change significantly in these tissues. In conclusion, tissue-specific expression of FAT and FATP genes involves both PPARα and -γ. Our data suggest that among the three putative long-chain fatty acid transporters, FAT and FATP appear to have physiological roles. Thus, peroxisome proliferators not only influence the metabolism of intracellular fatty acids but also cellular uptake, which is likely to be an important regulatory step in lipid homeostasis.

Long-chain fatty acids (LCFAs) are an efficient energy source for many cells. Cells metabolize and/or store intracellular LCFAs, depending on cell type and energy requirement. In addition to their ability to biosynthesize LCFAs, cells can utilize plasma LCFAs released by lipoprotein lipase-catalyzed hydrolysis of triglycerides from circulating chyomicrons and very low density lipoproteins. Therefore, uptake of LCFAs into cells can be an important step in energy metabolism. However, the mechanism and regulation of uptake of extracellular LCFAs into mammalian cells is not well understood. Because of their hydrophobic properties, it has been suggested that LCFAs are transported across the plasma membrane by simple diffusion. However, other studies have suggested that this process is mediated by proteins. Three independent transport proteins have been identified that may contribute to this process, including fatty acid translocase (FAT)(4), mitochondrial aspartate aminotransferase (mAspAT)(5), and fatty acid transport protein (FATP)(6). The precise role of these proteins in mediating LCFA uptake is not known and the mechanisms of LCFA uptake into various mammalian cells may not be the same. Nevertheless, there is evidence that all three may, at least in part, contribute to LCFA cellular uptake.

Peroxisome proliferator-activated receptors (PPARs) have unique roles in lipid homeostasis. PPARs are part of the nuclear hormone receptor superfamily, and there are three subtypes that have been described, α, β(δ), and γ. Each is encoded by a separate gene and have unique tissue distribution patterns. Furthermore, their roles in mediating changes in gene expression appear to be cell- and tissue-specific. For example, PPARα mediates fibrate and dietary polysaturated fatty acid induction of hepatic peroxisomal lipid-metabolizing enzymes, including acyl-CoA oxidase a key enzyme in regulating peroxisomal lipid catabolism (7, 8). Furthermore, PPARα mediates hepatic gene expression of apolipoprotein A-I and C-III in response to the peroxisome proliferator Wy 14,643 (9). It is of interest to note that recently, polysaturated fatty acids Wy 14,643 and leukotriene B4 have been shown to bind to PPARγ (10–12). Upon binding, PPARα-dependent gene transcription is activated, and these effects are most pronounced in the liver, a tissue with a high capacity for β-oxidation of fatty acids. Compounds that bind to another subtype, PPARγ, have also been identified. The antidiabetic drugs, thiazolidinediones and prostaglandin J2 derivatives, have been shown to preferentially bind to and activate PPARγ, and these effects occur predominantly in adipose cells (11–15). In summary, roles for PPARα include those involved in fatty acid catabolism, while those for PPARγ include those involved in adipogenesis.

Since PPARα are known to be key transcription factors of different genes participating in lipid homeostasis (16), we examined the effects of specific activators of PPARα on mRNA levels of the three putative fatty acid transporters.
Three Fatty Acid Transporters and PPARs

EXPERIMENTAL PROCEDURES

Materials—2-p-Chlorophenoxyisobutyric acid ethyl ester (clofibrate), 4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thioacetic acid (Wy 14,643), di(2-ethylhexyl) adipate (DEHA), and di(2-ethylhexyl) phthalate (DEHP) were purchased from Tokyo-Kasei (Tokyo, Japan). Troglitazone was a generous gift from Sankyo Co. Ltd. (Tokyo, Japan).

Animals and Treatment—Normal male NZB mice (5–6 weeks of age) were kept on a 12-h light-dark cycle and provided with food and water ad libitum. Mice were fed either a control diet (Clea Japan) or one containing 0.05% Wy 14,643, 0.5% clofibrate, 2% DEHA or 2% DEHP for 1–5 days. Animals were euthanized at 1330 h to minimize the effect of diurnal rhythms. Male PPARα wild-type (+/+) or homozygote-null (−/−) mice (F3 generation, 10–12 weeks of age) (7) were fed either a control diet (Bisserv, France, New Jersey, NJ) or one containing 0.1% Wy 14,643 for 14 days as described previously (17). Male KK-Ay obese mice (11 weeks of age) were obtained from Clea Japan (18) and fed either a control diet (MF, Oriental Kobe, Japan) or one containing 0.5% clofibrate, 0.05% pioglitazone, or 0.1% or 0.3% troglitazone for 5 days.

Cell Culture—The Fao cell line, a subclone of rat hepatoma HII4E, were cultured under conditions as reported previously (19). When a PPAR activator was added, a concentrated solution in Me2SO was added to medium before adding serum and sonicated to dissolve completely prior to adding to the cells. Treatment was initiated by changing the medium to a prewarmed drug-containing medium.

RNA Preparation and Northern Blot Analysis—Total RNA was prepared from the liver, intestine, adipose tissue, or the cultured hepatoma cells by the acid guanidium thiocyanate-phenol-chloroform extraction method (20). Northern blot analysis was carried out essentially as described previously (17). Among the cDNAs used for probes included peroxisomal HD, α2u-globulin, apolipoprotein E (apoE), and acyl-CoA oxidase, which have been described previously (17). The remaining cDNAs used for probes were obtained by cloning of PCR products of cDNA synthesized from poly(A) RNA isolated from the liver of Wy 14,643-fed mice. Their identities were confirmed by sequencing from both ends to 300–400 bases inside after cloning into the Smal site of pUC18. The synthetic oligonucleotides used to amplify respective cDNA sequences were 5'-TCTGACATTTGCGGTTCACTTATCTG and 5'-ATCTCAACCGGCAGGAGCACAATT for FAT (corresponding to nucleotide number from 873 to 1410 of the published rat sequence (4) (GeneBank™ accession number L19658); 5'-CTACTGCTCTCCAGAGTGTCGAGGAG and 5'-GGAGGAGGACACTGCTCTCTGGGATT for FATP (corresponding to nucleotide number from 134 to 538 of the published mouse sequence (5) (GeneBank™ accession number U82470); 5'-GGATTTGGTGGGTACACCACTTA and 5'-TCTGGTCCCTAATGCAGTCA for mAspAT (corresponding to nucleotide number from 775 to 1285 of the published mouse sequence (6) (U15976)); 5'-GGAGGTTTGCCTGGCAATTGACC and 5'-GGAAACTTTGTTGAGGATCTGAGGAC for lipoprotein lipase (corresponding to nucleotide number from 8 to 1000 of the published mouse sequence (21) (GeneBank™ accession numbers J03302 and J02740). SmaI-FAB

RESULTS AND DISCUSSION

All Three Fatty Acid Transporters, FAT, FATP, and mAspAT mRNA induction by Wy 14,643 in the liver and intestine. NZB mice were fed either a control diet or one containing 0.05% Wy 14,643 for 1–5 days as indicated. Total RNA (5 μg) isolated from individual livers (Liver) and proximal (Intest.1) and distal (Intest. 2) intestine was subjected to Northern blot analysis using the cDNAs for FAT, FATP, mAspAT, peroxisomal HD, intestinal fatty acid binding protein (I-FAB), and apoA1.

Fig. 1. Time course of FAT, FATP, and mAspAT mRNA induction by Wy 14,643 in the liver and intestine. NZB mice were fed either a control diet or one containing 0.05% Wy 14,643 for 1–5 days as indicated. Total RNA (5 μg) isolated from individual livers (Liver) and proximal (Intest.1) and distal (Intest. 2) intestine was subjected to Northern blot analysis using the cDNAs for FAT, FATP, mAspAT, peroxisomal HD, intestinal fatty acid binding protein (I-FAB), and apoA1.

Three Fatty Acid Transporters and PPARs

16711

LIVER

| Wy Treatment | FAT | FATP | mAspAT | HD | I-FAB | apoA1 |
|--------------|-----|------|--------|----|-------|-------|
| 0 1 2 3 5 | 0 1 2 3 5 | 0 1 2 3 5 | 0 1 2 3 5 | 0 1 2 3 5 | 0 1 2 3 5 | days |
| Liver |  |  |  |  |  |  |
| Intest.1 |  |  |  |  |  |  |
| Intest.2 |  |  |  |  |  |  |
FAT and FATP mRNAs Are Induced in Mouse Liver by Various PPARα Activators—To examine whether PPARα activators other than Wy 14,643 induce FAT and FATP in the liver, the effects of three other peroxisome proliferators were compared. The mRNA levels in liver from mice treated with these compounds for 5 days were determined by Northern blot analysis as shown in Fig. 2. All four PPARα activators induced liver fatty acid binding protein compared with controls. In addition, peroxisomal HD mRNA was markedly increased in response to all 4 PPARα activators consistent with previous work (not shown; see Ref. 17). Similarly, FAT mRNA was markedly induced by all four PPARα activators compared with controls. Interestingly, mRNA of FATP and lipoprotein lipase were higher, and α2u-globulin was lower, as a result of exposure to Wy 14,643, clofibrate, or DEHA, while these effects were less pronounced or absent in mice treated with DEHP. This could be due to the fact that DEHP is less effective at activating PPAR-dependent processes, including increasing reporter gene activity in transient co-transfections (27), peroxisome proliferation (28), increasing replicative DNA synthesis (29), and formation of hepatic tumors (29). In contrast, mAspAT mRNA levels were not significantly different in response to any of the PPARα activators, although Wy 14,643 did cause a small increase in this mRNA compared with controls. These results suggest that expression of FAT and FATP, but not mAspAT, mRNA in the liver is under the control of PPARα.

FAT and FATP mRNAs Are Induced by Various PPARα Activators but Not by a PPARγ Activator in Rat Hepatoma Cells—To further examine the effects of a wide variety of PPAR activators on the induction of FAT and FATP mRNAs, we used the rat hepatoma Fao cells line that is responsive to peroxisome proliferators (19). Fao cells were treated with various PPAR activators, including Wy 14,643, ciprofibrate, troglitazone, carbamylc acid, indomethacin, ibuprofen, and perfluorooctoleate for 6 or 24 h, and the levels of FAT, FATP, and mAspAT mRNAs, together with control mRNAs, were measured by Northern blots as shown in Fig. 3. Fao cells responded to most PPARα activators examined (Wy 14,643, ciprofibrate, indomethacin, ibuprofen, and perfluorooctoleate) as assessed by the increased levels of peroxisomal acyl-CoA oxidase and HD mRNAs. Time courses of induction were different among the activators, and indomethacin was the least effective. FAT and FATP mRNAs were increased by PPARα activators similarly as the peroxisomal mRNAs were while the effect of indomethacin on these mRNAs was negligible. Thus the relative abilities of various PPARα activators to induce FAT and FATP mRNAs were of similar magnitude to those observed with peroxisomal enzyme mRNAs. Levels of mAspAT and apoE mRNA were not affected by any treatment. The results obtained using cultured hepatoma cells are consistent with those observed in the liver of mice treated with PPARα activators. In contrast to the PPARα activators, a typical PPARγ activator Troglitazone did not cause any change in the levels of all the mRNAs examined. Combined, these results suggest that both the FAT and FATP genes are PPARα target genes.

Induction of FAT and FATP in the Liver Is Mediated by PPARα—To directly examine the role of PPARα in the induction of FAT and FATP mRNAs, we utilized the PPARα-null mouse (7). Northern blot analyses of liver RNA from (+/+) and (−/−) mice fed either a control diet or one containing 0.1% Wy 14,643 (+). Total RNA was isolated from individual livers of mice treated with various PPAR activators. Confluent Fao cells were cultured in a normal medium (−) or that containing Wy 14,643 (50 μM), ciprofibrate (Cip, 300 μM), troglitazone (Trg, 10 μM), carbamylc acid (PG, 25 μM), indomethacin (Ind, 300 μM), ibuprofen (Ibu, 300 μM), or perfluorooctoleate (PFO, 100 μM) for 6 or 24 h. At the time indicated, total RNA was prepared from the cells and analyzed by Northern blot using the cDNAs for FAT, FATP, mAspAT, peroxisomal acyl-CoA oxidase (AOx), peroxisomal HD, and apoE.

| Genotype | (+/+) | (−/−) |
|----------|-------|-------|
| Wy14,643 | −     | +     |
HD mRNA were higher in the wild-type mice (+/+) fed Wy 14,643, and this effect was not observed in the (−/−) mice fed Wy 14,643. In addition, α2u-globulin mRNA was significantly lower in (+/+)-mice fed Wy 14,643 but unaffected by Wy 14,643 feeding in (−/−) mice as reported previously (17). These results indicate that PPARα has an obligatory role in Wy 14,643 induction of FAT and FATP mRNAs in the mouse liver. This could be due to direct interaction of PPARα with peroxisome proliferator responsive elements, although peroxisome proliferator responsive elements have not been indentified in these genes to date. Alternatively, the increase in gene transcription of FATP as a result of PPARα activators (30) could be the result of other PPARα-dependent events that indirectly result in altering gene expression of fatty acid transport proteins to compensate for the increase in fatty acid catabolism.

**FAT and FATP Are Induced by PPARγ Activators in White Adipose Tissue**—Although FAT and FATP mRNAs were induced by PPARγ activators in hepatoma cells and in a PPARα-dependent manner in the liver, the possible involvement of PPARγ in other tissues where the γ-type receptor is predominant over the α-type cannot be excluded (31). Therefore, we examined the effect of PPARγ activators in adipose tissue of mice. We used KKα obese mice (18) for this purpose to facilitate adipose tissue preparation. We utilized two white adipose tissue stores, interintestinal and subcutaneous, and brown adipose tissue. Northern blots from the three adipose tissues are shown in Fig. 5. Changes in the levels of several mRNAs were different among the three adipose tissues. Brown adipose did not respond to the PPARα or PPARγ activators compared with controls. Basal levels of FAT, FATP, A-FAB (adipose-type fatty acid-binding protein), lipoprotein lipase, and leptin mRNAs were lower in interintestinal white adipose than those in subcutaneous white adipose tissue. The greatest effect induced by PPAR activators was observed in interintestinal adipose.

In both white adipose tissues, FAT and FATP were induced most efficiently by pioglitazone followed by troglitazone and then clofibrate, just as other characterized PPARγ target genes such as A-FAB, lipoprotein lipase, and leptin. Thus, both FAT and FATP were induced by PPARγ activators in white adipose tissue.

**Comparison of FAT and FATP**—The responses of both putative LCFA transporter mRNAs to PPAR activators were similar, but not exactly coordinated. The differences were: 1) FATP was not induced as much in the intestine as FAT (see Fig. 1); 2) most peroxisome proliferators induced both mRNAs in the liver, but DEHP only induced FAT (see Fig. 2); and 3) FAT responded by rapid or transient induction, but FAT did not (see the lanes of RNA samples from the cells treated for 6 h with Wy 14,643, carbacyclin, and perfluoroooleate in Fig. 3). These results suggest a complexity in the PPAR-mediated transcriptional activation (32) and/or differences in their physiological roles.

During the completion of this manuscript, another paper concerning FATP and PPAR activators was published (30). Their conclusion on regulation of the expression of FATP is essentially the same as ours. We extend these observations by suggesting that FAT, in addition to FATP, may have an important role in fatty acid uptake and lipid homeostasis. Furthermore, we demonstrated that in liver, PPARα is required for the induction of FAT and FATP, since these effects were absent in PPARα-null mice fed Wy 14,643. Functional analysis of both proteins will be necessary to better understand their roles in lipid homeostasis.

**REFERENCES**

1. Goldberg, I. J. (1996) J. Lipid Res. 37, 693–707
2. Deevich, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Auwerx, J. (1997) J. Biol. Chem. 272, 27307–27312
3. Tigratti, B. L., and Gerber, G. E. (1996) Biochem. J. 313, 487–494
4. Abumrad, N. A., el-Maghrabi, M. R., Amri, E. Z., Lopes, E., and Grimaldi, P. A. (1993) J. Biol. Chem. 268, 17665–17668
5. Isola, L. M., Zhou, S.-L., Kiang, C.-L., Stume, D. D., Bradbury, M. W., and Berk, P. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9866–9870
6. Schaffer, J. E., and Lodish, H. F. (1994) Cell 78, 457–466
7. Lee, S. S. T., Pineasu, T., Drago, J., Lee, E. J., Owens, J. W., Krozetz, D. I., Fernandez-Salgueiro, P. M., Westphal, H., and Gonzalez, F. J. (1995) Mol. Cell. Biol. 15, 3012–3022
8. Ren, B., Thelen, A. P., Peters, J. M., Gonzalez, F. J., and Jump, D. B. (1997) J. Biol. Chem. 272, 26827–26832
9. Peters, J. M., Hennuyer, N., Stauds, B., Fruchart, J.-C., Fiovet, C., Gonzalez, F. J., and Auwerx, J. (1997) J. Biol. Chem. 272, 27307–27312
10. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
11. Lehmann, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3296–3297
12. Lehmann, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2670–2674
13. Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahl, W. (1996) Nature 384, 34–43
14. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3212–3217
15. Forman, B. M., Chen, J., and Evans, R. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4318–4323
16. Lemberger, T., Desbergne, B., and Wahli, W. (1996) Mol. Cell. Biol. 16, 3356–3363
17. Kliever, S. A., Sutdend, S. S., Jones, S. A., Brwon, P. J., Wisely, G. B., Cobb, C. S., Devchand, P. R., Wahli, W., Wilson, T. M., Lenhard, J. M., and Lehmann, J. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 813–819
18. Shi, T. M., and Kliewer, S. A. (1995) Cell 83, 803–812
19. Kliewer, S. A., Lenhard, J. M., Patel, I., Morris, D. C., and Lehmann, J. M. (1995) Cell 83, 813–819
20. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkinson, W. O., Willson, T. M., and Kliewer, S. A. (1995) J. Biol. Chem. 270, 12953–12956
21. Lemberger, T., Desbergne, B., and Wahli, W. (1996) Annu. Rev. Cell Dev. Biol. 12, 335–363
22. Motogiwa, K., Peters, J. M., and Gonzalez, F. J. (1997) Biochem. Biophys. Res. Commun. 235, 155–158
23. Saha, A. K., Kurowski, T. G., and Ruderman, N. B. (1994) Am. J. Physiol. 267, E95–E101
Three Fatty Acid Transporters and PPARs

25. Phillips, M., Djian, P., and Green, H. (1986) *J. Biol. Chem.* **261**, 10821–10827.
26. Motojima, K., Goto, S., and Imanaka, T. (1992) *Biochem. Biophys. Res. Commun.* **188**, 799–806.
27. Issemann, I., Prince, R. A., Tugwood, J. D., and Green, S. (1993) *J. Mol. Endocrinol.* **11**, 37–47.
28. Reddy, J. K., Reddy, M. K., Usman, M. I., Lalwani, N. D., and Rao, M. S. (1986) *Environ. Health Perspect.* **85**, 317–327.
29. Marsam, D. S., Cattley, R. C., Conway, J. G., and Popp, J. A. (1988) *Cancer Res.* **48**, 6739–6744.
30. Martin, G., Schoonjans, K., Lefebvre, A.-M., Staels, B., and Auwerx, J. (1997) *J. Biol. Chem.* **272**, 28210–28217.
31. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.-M., Heyman, R. A., Briggs, M., Deeb, S., Staels, B., and Auwerx, J. (1996) *EMBO J.* **15**, 5336–5348.
32. Motojima, K. (1997) *Biochimie (Paris)* **78**, 101–106.
Expression of Putative Fatty Acid Transporter Genes Are Regulated by Peroxisome Proliferator-activated Receptor α and γ Activators in a Tissue- and Inducer-specific Manner

Kiyoto Motojima, Patricia Passilly, Jeffrey M. Peters, Frank J. Gonzalez and Norbert Latruffe

J. Biol. Chem. 1998, 273:16710-16714.
doi: 10.1074/jbc.273.27.16710

Access the most updated version of this article at http://www.jbc.org/content/273/27/16710

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 32 references, 16 of which can be accessed free at http://www.jbc.org/content/273/27/16710.full.html#ref-list-1