Pergamon

Biochemical Pharmacology, Vol. 47, No. 5, pp. 918–921, 1994.
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0006-2952/94 $6.00 + 0.00

0006-2952(93)E0069-J

Effects of testosterone, hypophysectomy and growth hormone treatment on clofibrate induction of peroxisomal β-oxidation in female rat liver

(*Received* 24 *July* 1993; *accepted* 12 *October* 1993)

Abstract—Induction of peroxisomal β-oxidation by clofibrate under altered hormonal states was investigated in female rat liver. Treatment of rats with clofibric acid (CPIB) caused a significant increase in hepatic peroxisomal β-oxidation, with female rats being less responsive than males (4.2- vs 12.2-fold increase). However, testosterone treatment following ovariectomy of female rats resulted in an enhanced response to CPIB, giving an induction (11.7-fold) comparable to that seen in male rats. Hypophysectomy of female rats also enhanced the induction (8.2-fold compared with 5.1-fold), suggesting a suppressive effect of a pituitary-dependent factor on CPIB induction of peroxisomal β-oxidation. Continuous infusion of growth hormone to the hypophysectomized female rats suppressed the enhanced induction nearly to the initial level (6.1-fold). The stimulatory effects of testosterone and hypophysectomy on the enzyme induction were additive. These findings suggest the involvement of growth hormone, as well as male sex hormone, in regulating the responsiveness to CPIB induction of peroxisomal β-oxidation in rat liver.

Key words: testosterone; growth hormone; peroxisome; clofibrate; enzyme induction; rat liver

Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) is a hypolipidemic peroxisome proliferating drug that induces, in rodents, a pleiotropic response characterized by hepatomegaly, hepatic peroxisome proliferation, and peroxisomal enzyme induction [1, 2]. A sex-related difference has been observed in the responsiveness of rat liver to clofibrate and several other peroxisome proliferators [1–5]. The hepatic effect of clofibrate is produced in both sexes, but it is less pronounced in female rats. In this context, early studies [3] indicated that the sex difference in catalase inducibility and peroxisome proliferative response depends on the male sex hormone. Kawashima *et al.* [4] also reported androgen-dependent induction of peroxisomal β-oxidation with respect to perfluorooctanoic acid, another peroxisome proliferator. However, investigation of possible pituitary control of the responsiveness to clofibrate has been limited. Therefore, we examined such a possibility, using the hypothalamo-pituitary-liver axis model [6] which has been proposed for a hormonal system to control certain sex-related events in the liver, such as sex-specific expression of some cytochrome P450s in rat liver microsomes [7, 8].

This paper reports the alteration of response to clofibrate induction of peroxisomal β-oxidation in female rat liver by hormonal manipulation, such as hypophysectomy, and treatments with testosterone and growth hormone.

Materials and Methods

Materials. CPIB and testosterone propionate were purchased from Sigma (St. Louis, MO, U.S.A.). Recombinant human growth hormone (Norditropin, 3 IU/mg) was a gift from Yamanouchi (Tokyo, Japan). Other chemicals were of the highest grade commercially available.

Animals and treatment. Wistar-Imamichi rats (intact and hypophysectomized at 7 weeks of age) were obtained from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan). CPIB, the free acid form of clofibrate, was used. In the experiment shown in Fig. 1, rats (8 weeks old) were treated with CPIB for 5 days. Ovariectomy was performed at 6 weeks of age. In Fig. 2, CPIB was given to rats (10 weeks old) for 3 days, and ovariectomy was performed at 9 weeks of age. Testosterone and growth hormone were given every day from 1 day before CPIB treatment was started.

CPIB was dissolved in 0.5% carboxymethylcellulose as a sodium salt and was administered to rats by gastric intubation once a day, at a dose of 300 mg/kg. Control animals were similarly given just the vehicle (5 mL/kg). Testosterone propionate was dissolved in corn oil and subcutaneously injected once a day (5 mg/kg). Human growth hormone was given by subcutaneous injection (2 IU/kg) twice a day, or by osmotic infusion (0.01 IU/hr) using an osmotic minipump implanted on the back of rats, as described in Ref. 8. Liver homogenates were prepared 24 hr after the last administration of CPIB, as previously described [5].

Enzyme assay. Peroxisomal β-oxidation was assayed in liver homogenates, by following lauroyl-CoA-dependent NAD-reduction in the presence of cyanide [5, 9]. Units of enzyme activity (U) are expressed as micromoles of NAD reduced per minute. Protein was determined by the method of Lowry *et al.* [10]. Statistical analysis was performed using Student's *t*-test.

Immunoblotting. Liver homogenates (10 μg protein/lane) were subjected to SDS–PAGE (10% gel) [11], followed by immunoblotting [12] with antiserum against peroxisomal bifunctional enzyme [5]. Peroxidase-anti-peroxidase staining was performed using 3,3′-diaminobenzidine [13].

Results and Discussion

Treatment of rats with CPIB caused a significant increase in hepatic peroxisomal β-oxidation, with female rats being less responsive than males (4.2- vs 12.2-fold increase) (Fig. 1). However, testosterone treatment of female rats following ovariectomy resulted in an enhanced response to CPIB, giving an induction of peroxisomal β-oxidation (11.7-fold) comparable to that seen in male rats. This stimulatory effect of testosterone on CPIB induction was also demonstrated by immunoblot analysis of peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (Fig. 1, upper panel). These findings are compatible with previous studies on catalase induction [3] and those with perfluoro-octanoic acid [4].

* Abbreviation: CPIB, clofibric acid (*p*-chlorophenoxyisobutyric acid).

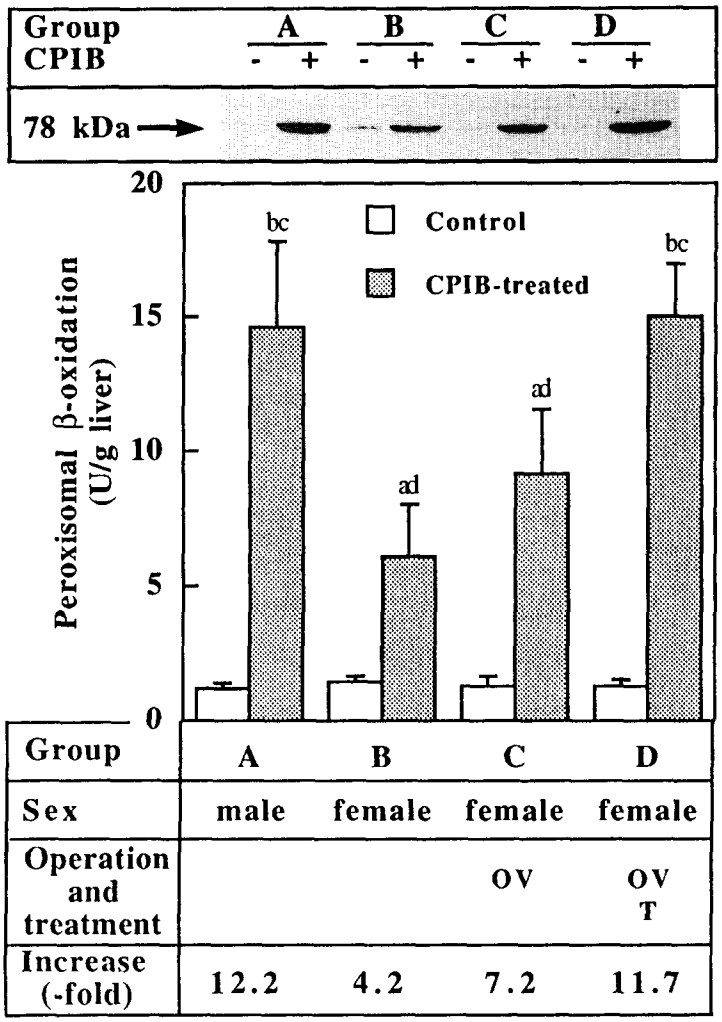

Fig. 1. Effect of testosterone treatment on CPIB induction of peroxisomal β-oxidation in female rat liver. Rats were divided into four experimental groups (A–D), with the treatment for each group indicated at the bottom of the figure (see "Animals and treatment"). CPIB was administered at 300 mg/kg for 5 days, and peroxisomal β-oxidation activity was measured in the liver. Abbreviations: OV, ovariectomy; and T, testosterone treatment. Values are means ± SD of four rats. Control activities in the four groups (1.2 to 1.5 U/g liver) did not differ significantly. Differences in the treated groups are indicated by lower case letters at the top of the columns. Like combinations of letters denote no statistical difference. Groups with different combinations of letters were significantly different from each other at $P < 0.05$. Fold-increases in enzyme activity (CPIB-treated/control) are also shown. Upper panel: immunoblots of liver homogenates, probed with antibody against peroxisomal bifunctional enzyme.

On the other hand, hypophysectomy of female rats also enhanced the CPIB induction of peroxisomal β-oxidation (8.2-fold in group B, compared with 5.1-fold in group A, Fig. 2). This suggests a suppressive effect of some pituitary-dependent factor on the induction. When the effect of growth hormone, as a candidate of such a pituitary factor, was examined in hypophysectomized female rats, growth hormone intermittently injected into rats had no effect, whereas that given by continuous infusion suppressed the induction to nearly the level in rats without hypophysectomy (8.1- and 6.1-fold in groups C and D, respectively, Fig. 2). The secretory pattern of growth hormone from the pituitary gland is sex-related in adult rats [14], and intermittent injection and continuous infusion of growth hormone to rats can mimic the secretory patterns characteristic of males and females, respectively. Thus, growth hormone given to rats by the female secretory pattern, but not by the male pattern, had a suppressive effect (Fig. 2), suggesting the involvement of growth hormone in regulating the responsiveness to CPIB induction of peroxisomal β-oxidation in the female rat liver. Testosterone may have exerted its stimulatory effect on CPIB induction (Fig. 1) by altering the secretory pattern of growth hormone in a sex-related manner. However, the effects of testosterone and hypophysectomy were additive (as indicated by the 10.3-fold increase in β-oxidation, group E in Fig. 2), indicating an independent effect of testosterone from the hypothalamo-pituitary-liver system.

These findings suggest the involvement of growth hormone, as well as male sex hormone, in regulating the

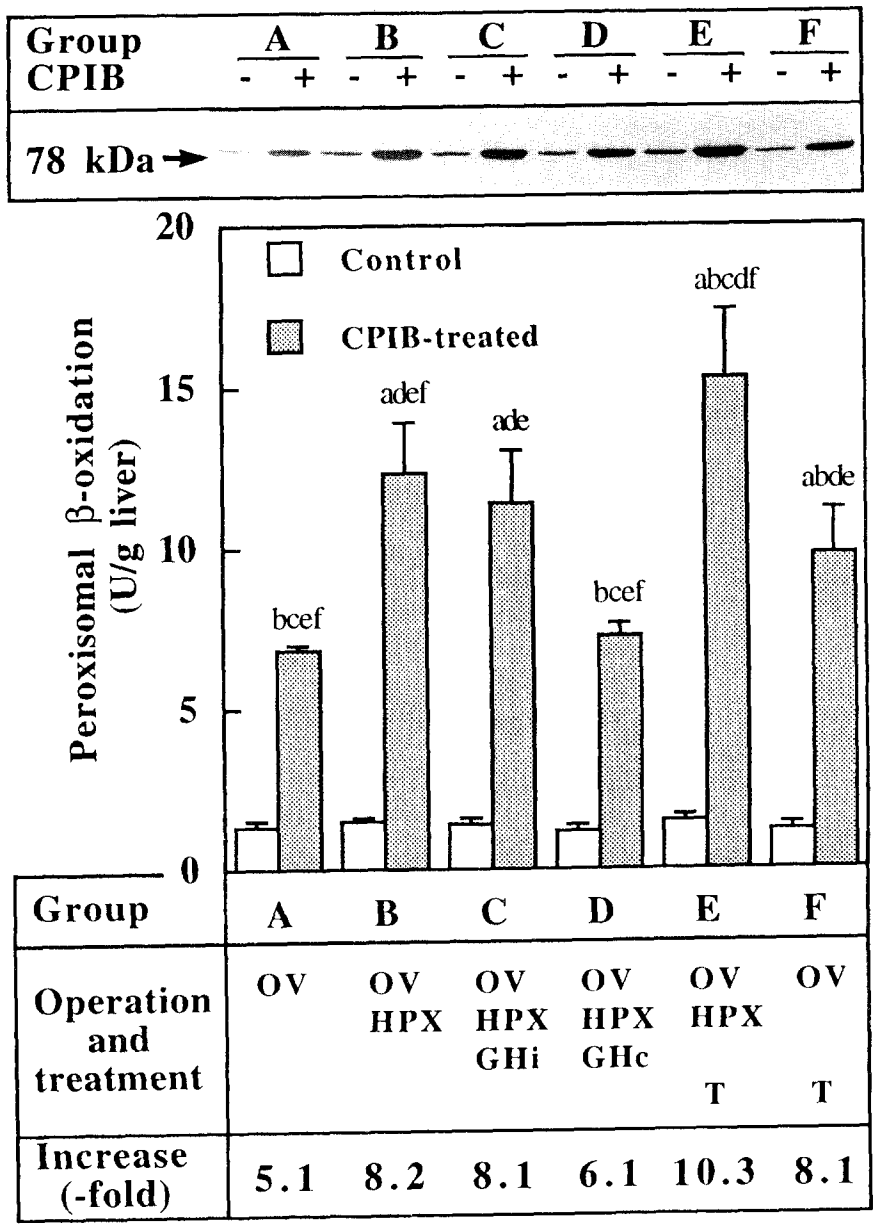

Fig. 2. Effects of hypophysectomy, growth hormone and testosterone treatment on CPIB induction of peroxisomal β-oxidation in female rat liver. Ovariectomized female rats that had received the treatment indicated were administered CPIB (300 mg/kg) for 3 days, and peroxisomal β-oxidation activity was measured in the liver. Abbreviations: OV, ovariectomy; HPX, hypophysectomy; GH, growth hormone treatment (GHi, intermittent injection; GHc, continuous infusion); and T, testosterone treatment. Values are means ± SD of five rats. Control activities in the six groups (1.2 to 1.5 U/g liver) did not differ significantly. Differences in the treated groups are indicated by lower case letters at the top of the columns. Like combinations of letters denote no statistical difference. Groups with different combinations of letters were significantly different from each other at $P < 0.05$. Fold-increases in enzyme activity (CPIB-treated/control) are also shown. Upper panel: immunoblots of liver homogenates, probed with antibody against peroxisomal bifunctional enzyme.

CPIB induction of peroxisomal β-oxidation in the rat liver. The lower inducibility of female rats appears to reflect the suppressive effect of growth hormone and the lack of a stimulatory testosterone effect. However, the mechanism by which these hormones exert their effects is unknown. The target of the hormone action may possibly be the peroxisome proliferator-activated receptor (PPAR)-mediated signal transduction pathway [15], or a certain CPIB-inducible enzyme system causally linked to peroxisome induction, such as cytochrome P4504A fatty acid ω-hydroxylases, whose induction has been proposed to mediate peroxisome induction via dicarboxylic acid

formation [16, 17]. The possibility that the alteration in pharmacokinetics of CPIB elimination, which could occur, may have been related to the altered inducibility observed cannot be excluded. Possible roles of other pituitary-dependent factors and systemic endocrine interactions should also be considered. Further studies are required.

Acknowledgements—This work was supported, in part, by a grant from the Research Foundation for Pharmaceutical Sciences.

Department of Clinical Biochemistry
Tokyo College of Pharmacy
Tokyo 192-03, Japan

HIROYUKI SUGIYAMA
JUNJI YAMADA*
TETSUYA SUGA

REFERENCES

1. Reddy JK and Lalwani ND, Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* **12**: 1–53, 1983.
2. Hawkins JM, Jones WE, Bonner FW and Gibson GG, The effect of peroxisome proliferators on microsomal, peroxisomal, and mitochondrial enzyme activities in the liver and kidney. *Drug Metab Rev* **18**: 441–515, 1987.
3. Svodoba D, Azarnoff D and Reddy J, Microbodies in experimentally altered cells. II. The relationship of microbody proliferation to endocrine glands. *J Cell Biol* **40**: 734–746, 1969.
4. Kawashima Y, Uy-Yu N and Kozuka H, Sex-related differences in the inductions by perfluoro-octanoic acid of peroxisomal β-oxidation, microsomal 1-acylglycerophosphocholine acyltransferase and cytosolic long-chain acyl-CoA hydrolase in rat liver. *Biochem J* **261**: 595–600, 1989.
5. Yamada J, Sakuma M, Ikeda T, Fukuda K and Suga T, Characteristics of dehydroepiandrosterone as a peroxisome proliferator. *Biochim Biophys Acta* **1092**: 233–243, 1991.
6. Gustafsson J-A, Mode A, Norstedt G, Hokfelt T, Sonnenschein C, Eneroth P and Skett P, The hypothalamo-pituitary-liver axis: A new hormonal system in control of hepatic steroid and drug metabolism. *Biochem Actions Horm* **7**: 47–89, 1980.
7. Kamataki T, Shimada M, Maeda K and Kato R, Pituitary regulation of sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem Biophys Res Commun* **130**: 1247–1253, 1985.
8. Yamazoe Y, Shimada M, Murayama N and Kato R, Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J Biol Chem* **262**: 7423–7428, 1987.
9. Lazarow PB and de Duve C, A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc Natl Acad Sci USA* **73**: 2043–2046, 1976.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
12. Towbin H, Staehelm T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
13. Guengerich FP, Wray P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Biochemistry* **21**: 1698–1701, 1982.
14. Edén S, Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105**: 555–560, 1979.
15. Issemann I and Green S, Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**: 645–650, 1990.
16. Sharma R, Lake BG, Foster J and Gibson GG, Microsomal cytochrome P-452 induction and peroxisome proliferation by hypolipidaemic agents in rat liver. A mechanistic inter-relationship. *Biochem Pharmacol* **37**: 1193–1201, 1988.
17. Sundseth SS and Waxman DJ, Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid ω-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. *J Biol Chem* **267**: 3915–3921, 1992.

* Corresponding author: Dr. Junji Yamada, Department of Clinical Biochemistry, Tokyo college of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan. Tel. (81) 426-76-5111; FAX (81) 426-75-2605.