Sex-related difference 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

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Inductions by perfluoro-octanoic acid (PFOA) of hepatomegaly, peroxisomal β -oxidation, microsomal 1-acylglycerophosphocholine acyltransferase and cytosolic long-chain acyl-CoA hydrolase were compared in liver between male and female rats. Marked inductions of these four parameters were seen concurrently in liver of male rats, whereas the inductions in liver of female rats were far less pronounced. The sex-related difference in the response of rat liver to PFOA was much more marked than that seen with *p*-chlorophenoxyisobutyric acid (clofibric acid) or 2,2'-(decamethylenedithio)diethanol (tiadenol). Hormonal manipulations revealed that this sex-related difference in the inductions is strongly dependent on sex hormones, namely that testosterone is necessary for the inductions, whereas oestradiol prevented the inductions by PFOA.

INTRODUCTION

A number of hypolipidaemic agents which produce hepatomegaly and peroxisome proliferation have been reported [1,2]. These peroxisome proliferators are classified into three groups: (i) clofibrate [ethyl ester of clofibric acid (*p*-chlorophenoxyisobutyric acid)] and structurally related analogues, (ii) compounds structurally unrelated to clofibrate or plasticizer, and (iii) di(2-ethylhexyl) phthalate and related compounds. Regardless of the dissimilarities of their structures, these compounds have in common that they induce peroxisome proliferation and peroxisomal enzymes [1,2]. However, inductions of peroxisome proliferation and peroxisomal enzymes are merely one of a number of hepatic effects common to peroxisome proliferators. A cytochrome *P*-450 having high specificity for ω -hydroxylation of fatty acids was induced concomitantly with peroxisomal β -oxidation by treatment of rats with various peroxisome proliferators [3,4]. Peroxisome proliferators with widely varying structures also induced microsomal 1-acyl-GPC acyltransferase [5] and cytosolic long-chain acyl-CoA hydrolase [6]. Although the physiological significance of the inductions of these enzymes has not yet been fully understood, the inductions of these enzymes are due to peroxisome proliferators. These findings suggest that microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase are useful parameters by which to detect the effects of peroxisome proliferators.

With regard to the inductions of peroxisome proliferation and peroxisomal enzymes, sex-related differences have been reported in the response of rats to several peroxisome proliferators [7–11]. No information, however, is available about sex-related differences in the response of microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase to peroxisome

proliferators, except for our previous findings of the marked sex-related difference in the response of cytosolic long-chain acyl-CoA hydrolase to clofibric acid [12]. Many peroxisome proliferators have been shown to cause hepatocellular carcinoma in rodents [1,13,14]. Therefore information on differences in the response of these enzymes to peroxisome proliferators between male and female animals may be important.

Recently perfluorinated carboxylic acids, such as PFOA and perfluorodecanoic acid, were found to induce peroxisome proliferation [15,16]. The perfluorinated compounds were developed as industrial materials, and their structures are dissimilar from those of any known peroxisome proliferators. Owing to their metabolically inert properties [17], these compounds may be proximate inducers themselves. In this context, these compounds belong to a new class of peroxisome proliferators. In biochemical and toxicological senses, therefore, it is interesting to investigate the response of several biochemical parameters to PFOA in livers of male and female rats. In the present work, we studied the sex-related difference in effects of PFOA on the induction of relative liver weight, peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase in rat liver, and we found that the inductions of these parameters by PFOA are strongly dependent on sex hormones.

EXPERIMENTAL

Materials

Palmitoyl-CoA, myristoyl-CoA, oleoyl-CoA, clofibric acid and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); PFOA and tiadenol [2,2'-(decamethylenedithio)diethanol] were

Abbreviations used: 1-acyl-GPC, 1-acylglycerophosphocholine; PFOA, perfluoro-octanoic acid.

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from Aldrich (Milwaukee, WI, U.S.A.); 1-acyl-GPC was from Avanti Polar Lipid (Pelham, AL, U.S.A.); CoA and NAD⁺ were from Oriental Yeast Co. (Tokyo, Japan); testosterone propionate and oestradiol were from Wako Pure Chemical Co. (Osaka, Japan). All other chemicals were of analytical grade.

Animals and treatments

Male and female rats of Wistar strain, weighing 120–150 g and 100–130 g respectively, were used. Rats were fed *ad libitum* on a commercial diet (Sankyo Labo Service Co., Tokyo, Japan) or on that diet containing 0.5% (w/w) clofibrilic acid, 0.5% (w/w) tiadenol or 0.02% (w/w) PFOA for 1 week.

For the dose-response experiments, male rats were fed on a diet containing PFOA at doses of 0, 0.00125, 0.0025, 0.005, 0.01 and 0.02% (w/w) for 2 weeks. Female rats were fed on a diet containing PFOA at doses of 0, 0.005, 0.01, 0.02, 0.04 and 0.08% (w/w) for 2 weeks.

Some male rats, weighing 70–80 g, were castrated 28 days before being killed. These rats were subcutaneously administered with testosterone propionate (10 mg/kg) or oestradiol (10 mg/kg) once every 2 days for 3 weeks before being killed. These hormones were dissolved in corn oil. Non-treated controls were given subcutaneously corn oil (0.1 ml/100 g). These rats were fed on a commercial diet or on a diet containing PFOA (0.01%, w/w) for 2 weeks before being killed.

In some experiments, non-operated male rats, weighing 85–90 g, were treated with oestradiol (10 mg/kg) once every 2 days for 3 weeks before being killed. Intact female rats, weighing 80–90 g, were treated with testosterone propionate (10 mg/kg) once every 2 days for 3 weeks before being killed. Non-operated controls received corn oil (0.1 ml/100 g). These rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA for 2 weeks before being killed.

Subcellular fractionation

At the end of the treatments, rats were killed by decapitation. The livers were isolated, perfused with ice-cold 0.9% NaCl and chilled in 0.25 M-sucrose on ice. Part of the liver was frozen at -30°C until assayed for peroxisomal β -oxidation. The other part of the liver was homogenized in 3 vol. of 0.25 M-sucrose. The

homogenates were centrifuged at 18000 g (r_{av} , 7.0 cm) for 15 min. The supernatant obtained was re-centrifuged under the same conditions. The resulting supernatant was centrifuged at 105000 g (r_{av} , 5.9 cm) for 60 min. The supernatant was used as cytosol. The pellet was suspended in 0.25 M-sucrose, and the suspension was re-centrifuged at 105000 g for 60 min. The pellet obtained was resuspended in 0.25 M-sucrose and used as microsomal fraction. The frozen liver for the assay of peroxisomal β -oxidation was thawed and homogenized in 9 vol. of 0.25 M-sucrose. The resulting homogenates were centrifuged at 600 g (r_{av} , 10.5 cm) for 10 min to remove the nuclear fraction. The supernatant obtained was frozen at -30°C until use.

Enzyme assays

Cyanide-insensitive palmitoyl-CoA oxidation was assayed by the method of Lazarow & de Duve [18] with some minor modifications [19]. Post-nuclear supernatant was used as an enzyme source for the assay. Microsomal 1-acyl-GPC acyltransferase was assayed with oleoyl-CoA as substrate by the method of Lands & Hart [20] as previously described [5]. Cytosolic long-chain acyl-CoA hydrolase was assayed with myristoyl-CoA as substrate as described previously [21].

Other procedures

The protein concentrations in enzyme preparations were determined by the method of Lowry *et al.* [22], with bovine serum albumin as standard. Statistical analyses were performed by Student's *t* test for two means.

RESULTS

Comparison of peroxisome proliferators

Table 1 compares the inducing effects of peroxisome proliferators on hepatomegaly, peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase in livers of male and female rats. Treatment of male rats with clofibrilic acid, tiadenol or PFOA caused increases in relative liver weight of 1.74-, 1.86- and 1.65-fold (all $P < 0.001$) respectively over control. Administration of clofibrilic acid or tiadenol to female rats produced a significant hepatomegaly, although the effects were less marked than those observed

Table 1. Effects of peroxisome proliferators on relative liver weight, peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase in livers of male and female rats

Rats were fed on a diet containing 0.5% clofibrilic acid, 0.5% tiadenol or 0.02% PFOA for 1 week. Enzyme activities are expressed in nmol/min per mg of protein. Relative liver weight represents % of body weight. Results are means $\pm$ S.D. for three to six rats. Statistically significant effects are indicated by * $P < 0.01$ and ** $P < 0.001$.

Rats	Treatment	Relative liver weight	Peroxisomal β -oxidation	1-Acyl-GPC acyltransferase	Long-chain acyl-CoA hydrolase
Male	Control	5.3 $\pm$ 0.5	5.5 $\pm$ 0.3	63.4 $\pm$ 9.3	7.4 $\pm$ 1.5
	Clofibrilic acid	9.2 $\pm$ 0.2**	50.0 $\pm$ 10.2*	319.3 $\pm$ 0.5**	272.6 $\pm$ 7.0**
	Tiadenol	9.9 $\pm$ 0.5**	49.7 $\pm$ 3.5**	324.4 $\pm$ 17.7**	306.4 $\pm$ 29.6**
	PFOA	8.7 $\pm$ 0.6**	30.6 $\pm$ 7.4**	198.8 $\pm$ 22.4**	110.8 $\pm$ 15.2**
Female	Control	4.7 $\pm$ 0.2	5.3 $\pm$ 0.6	56.8 $\pm$ 12.9	13.7 $\pm$ 5.2
	Clofibrilic acid	6.8 $\pm$ 0.4**	28.2 $\pm$ 7.7*	166.4 $\pm$ 22.4*	92.4 $\pm$ 16.2*
	Tiadenol	7.4 $\pm$ 0.8**	31.9 $\pm$ 6.0**	203.9 $\pm$ 29.1**	117.0 $\pm$ 17.5**
	PFOA	4.8 $\pm$ 0.2	5.1 $\pm$ 0.7	68.8 $\pm$ 2.1	13.2 $\pm$ 3.2

Fig. 1. Effects of the dose of PFOA on relative liver weight and activities of peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase

Rats were fed a diet containing PFOA for 2 weeks. Results are means $\pm$ s.d. for three to six rats. Statistically significant effects of PFOA treatment are indicated by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (a) Relative liver weight, male rats; (b) relative liver weight, female rats; (c) peroxisomal β -oxidation, male rats; (d) peroxisomal β -oxidation, female rats; (e) 1-acyl-GPC acyltransferase, male rats; (f) 1-acyl-GPC acyltransferase, female rats; (g) long-chain acyl-CoA hydrolase, male rats; (h) long-chain acyl-CoA hydrolase, female rats.

in male rats. PFOA produced no hepatomegaly in female rats. Marked increases in the activities of peroxisomal β -oxidation and microsomal 1-acyl-GPC acyltransferase were observed in male rats treated with clofibrac acid or tiadenol, with a somewhat less marked increase with PFOA. Although clofibrac acid and tiadenol caused significant inductions of peroxisomal β -oxidation and 1-acyl-GPC acyltransferase in livers of female rats, the extent of the induction of these enzyme activities was far less pronounced in female rats than in male rats. PFOA did not change the activities of the enzymes. In good agreement with our previous findings [12], a marked sex-related difference was seen in the induction of cytosolic long-chain acyl-CoA hydrolase with clofibrac acid. Similarly, tiadenol showed a marked sex-related difference in the induction of long-chain acyl-CoA hydrolase. PFOA induced cytosolic long-chain acyl-CoA hydrolase greatly, but to a lesser extent compared with clofibrac acid or tiadenol in male rats. PFOA did not induce long-chain acyl-CoA hydrolase in livers of female rats.

Dose-response effects of PFOA

Fig. 1 shows the hepatic dose-response characteristics of the four parameters in male and female rats to PFOA. The administration of PFOA to male rats caused a great hepatomegaly and marked inductions of peroxisomal β -oxidation, 1-acyl-GPC acyltransferase and long-chain acyl-CoA hydrolase in a dose-dependent fashion (Figs. 1a, 1c, 1e and 1g). PFOA at 0.00125% significantly increased all four parameters. Treatment of female rats with PFOA at 0.08% significantly increased the relative liver weight and the activities of peroxisomal β -oxidation, 1-acyl-GPC acyltransferase and long-chain acyl-CoA hydrolase (Figs. 1b, 1d, 1f and 1h). Statistically significant inductions of hepatomegaly and 1-acyl-GPC acyltransferase were observed at a dose of 0.04%, but not in the activities of peroxisomal β -oxidation and long-chain acyl-CoA hydrolase. The extent of induction of hepatomegaly and the enzyme activities in female-rat liver with PFOA at a dose of 0.08% was comparable with that in male rats

at 0.00125 or 0.0025%. These results suggest that male rats are 32 or 64 times more sensitive to PFOA than are female rats.

Effects of sex hormones

The inductions of hepatomegaly and the enzyme activities with PFOA were prevented by castration of male rats (Table 2). A slight induction of 1-acyl-GPC acyltransferase in livers by dosing castrated rats with PFOA was abolished by pretreatment of castrated rats with oestradiol. Administration of PFOA to castrated rats which had been treated with testosterone produced a marked hepatomegaly, as was seen in sham-operated rats, and caused marked inductions of peroxisomal β -oxidation, 1-acyl-GPC acyltransferase and long-chain acyl-CoA hydrolase. The administration of PFOA to male rats that had been treated with oestradiol did not change any parameters tested. PFOA induced markedly hepatomegaly, peroxisomal β -oxidation, 1-acyl-GPC acyltransferase and long-chain acyl-CoA hydrolase in livers of female rats pretreated with testosterone. These inductions were comparable with those in sham-operated rats treated with PFOA.

DISCUSSION

There are several reports of sex-related differences in the response of rats to peroxisome proliferators. Treatment of female rats with di(2-ethylhexyl) phthalate, a typical peroxisome proliferator, caused a marked induction of peroxisomal β -oxidation in liver, but the induction was less pronounced than that in male rats [9,10]. Similarly, an induction of peroxisome proliferation was observed in livers of both sexes after treatment of rats with gemfibrozil [5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid], and the induction in male rats was 2-fold greater than that in female rats [11]. Administration of clofibrate to female rats at a dietary dose of 0.25% did not induce peroxisome proliferation and catalase in liver [7], and the inductions were shown

Table 2. Effects of sex hormones on the inductions by PFOA of hepatomegaly, peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase in rat liver

For experimental details see the text. Results are means $\pm$ S.D. for three to six rats. Enzyme activities are expressed by nmol/min per mg of protein. Relative liver weight represents % of body weight.

Rats	Treatment	PFOA treatment	Relative liver weight	Peroxisomal β -oxidation	1-Acyl-GPC acyltransferase	Long-chain acyl-CoA hydrolase
Male	Sham-operated	—	5.4 $\pm$ 0.3	5.5 $\pm$ 0.2	72.2 $\pm$ 12.9	9.3 $\pm$ 0.4
		+	10.0 $\pm$ 0.2	36.1 $\pm$ 1.8	232.0 $\pm$ 33.1	91.4 $\pm$ 16.4
	Castrated	—	5.2 $\pm$ 0.3	5.0 $\pm$ 0.6	64.7 $\pm$ 7.1	10.8 $\pm$ 0.8
		+	5.5 $\pm$ 0.2	5.9 $\pm$ 0.5	100.5 $\pm$ 11.0	18.0 $\pm$ 1.3
	Castrated plus testosterone	—	5.2 $\pm$ 0.4	4.0 $\pm$ 0.7	73.8 $\pm$ 8.5	8.1 $\pm$ 2.2
		+	9.8 $\pm$ 1.2	42.1 $\pm$ 5.2	417.1 $\pm$ 32.8	239.6 $\pm$ 24.6
	Castrated plus oestradiol	—	7.1 $\pm$ 0.2	5.0 $\pm$ 1.1	71.0 $\pm$ 13.5	12.5 $\pm$ 1.3
		+	8.1 $\pm$ 0.4	5.8 $\pm$ 0.6	76.8 $\pm$ 7.8	19.9 $\pm$ 0.7
Female	Non-operated plus oestradiol	—	7.3 $\pm$ 0.3	4.9 $\pm$ 1.0	67.0 $\pm$ 7.1	14.2 $\pm$ 0.9
		+	7.9 $\pm$ 0.5	5.0 $\pm$ 1.1	76.8 $\pm$ 11.5	13.5 $\pm$ 5.3
	Non-operated	—	4.9 $\pm$ 0.5	4.7 $\pm$ 0.6	62.1 $\pm$ 5.9	10.1 $\pm$ 1.1
		+	4.7 $\pm$ 0.2	5.5 $\pm$ 0.4	71.3 $\pm$ 3.6	9.8 $\pm$ 1.7
	Non-operated plus testosterone	—	4.9 $\pm$ 0.2	5.1 $\pm$ 0.3	67.0 $\pm$ 13.4	6.9 $\pm$ 0.9
		+	9.2 $\pm$ 1.0	28.4 $\pm$ 7.8	205.5 $\pm$ 47.2	113.7 $\pm$ 61.3

to be dependent on the state of sex hormones [8]. However, subsequent studies showed increases in relative liver weight and the activities of catalase and carnitine acetyltransferase in liver of female rats fed on a diet containing clofibrate at a dose higher than 0.5% [23]. No sex-related differences in induction of peroxisome proliferation and catalase were seen in rats treated with nafenopin {2-methyl-2-[*p*-(1,2,3,4-tetrahydroxy-1-naphthyl)phenoxy]propionic acid} [24]. However, most of these studies have been performed by using peroxisome proliferation and peroxisomal enzymes as the parameters.

The present study demonstrated a marked sex-related difference in the effects of PFOA on rat liver, by using four parameters: relative liver weight, peroxisomal β -oxidation, microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase. The concurrent inductions of these parameters by PFOA were clearly dependent on sex hormones. Testosterone stimulated the inductions of these parameters by PFOA, whereas oestradiol abolished the inductions. Compared with the sex-related difference seen with clofibric acid and tiadenol, that with PFOA was much more marked.

Because of a lack of understanding of the mechanism leading to peroxisome proliferation and enzyme inductions, it is difficult to know why male rats are more sensitive to peroxisome proliferators than are female rats. Conversely, however, a detailed study of sex-related differences in responses of rats to peroxisome proliferators may help to approach the mechanism by which peroxisome proliferators induce peroxisome proliferation and enzymes. To consider this problem, it is important to know whether the sex-related difference is responsible merely for differences in rates of absorption, metabolism and/or excretion, or for qualitative difference in intrinsic sensitivity of liver. For di(2-ethylhexyl) phthalate [25,26] and tiadenol [27] there is evidence that particular metabolites of these compounds act as the actual inducers. Hence, it is possible that the sex-related differences in the response of rats to these agents is due to differences in the rates at which the active metabolites are formed in male and female rats. However, this remains to be proven. In contrast with di(2-ethylhexyl) phthalate and tiadenol, clofibric acid seems to be a proximate inducer itself, because no metabolite of clofibric acid was found in rats, except for its glucuronide [28–30]. Similarly, it is likely that PFOA is a proximate inducer itself, owing to its metabolically inert properties, suggesting that, if there is a sex difference in metabolism of PFOA, it may be at the step of absorption and/or excretion. Recently, the renal elimination rate of PFOA in female rats was found to be roughly 10 times faster than in male rats, and correspondingly the plasma concentration of PFOA was higher in male rats than in female rats [31]. These facts might partially explain our present results. However, our rough calculation suggests that male rats are 32 or 64 times more sensitive to PFOA than are female rats. It is difficult to speculate from the presently available kinetic data to what extent intrahepatic PFOA contributes to the induction of the enzymes. Little is known at present about the difference in the rate of absorption or conjugation of PFOA between male and female rats, if any. No comparative information is available on intrinsic liver sensitivity to peroxisome proliferators between male and female rats. Recently, two different mechanisms by which peroxisome proliferators induce peroxisome proliferation have been

proposed. One is a receptor-mediated induction [32,33]; the other is an induction through lipid accumulation or perturbation of lipid metabolism in liver [26,34]. Further comparative studies on the receptor level or the effects on lipid metabolism in male and female rats may be needed to explain the sex-related difference in PFOA-mediated enzyme inductions.

Finally, it is noteworthy that the concurrent inductions of microsomal 1-acyl-GPC acyltransferase and cytosolic long-chain acyl-CoA hydrolase with peroxisomal β -oxidation showed clearly that the first two enzymes are good parameters by which to detect the effects of peroxisome proliferators as well as peroxisomal β -oxidation. Most peroxisome proliferators are known to cause hepatocellular carcinoma in rodents [1,13,14], and PFOA is used widely for industrial purposes. Therefore, the sex-related difference in the effects of PFOA on animals should be taken into consideration for human hazard prediction.

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REFERENCES

1. Reddy, J. K. & Lalwani, N. D. (1983) *CRC Crit. Rev. Toxicol.* **12**, 1–58
2. Hawkins, J. M., Jones, W. E., Bonner, F. W. & Gibson, G. G. (1987) *Drug Metab. Rev.* **18**, 441–515
3. Sharma, R., Lake, B. G., Foster, J. & Gibson, G. G. (1988) *Biochem. Pharmacol.* **37**, 1193–1201
4. Sharma, R., Lake, B. G. & Gibson, G. G. (1988) *Biochem. Pharmacol.* **37**, 1203–1206
5. Kawashima, Y., Hirose, A. & Kozuka, H. (1984) *Biochim. Biophys. Acta* **793**, 232–237
6. Katoh, H., Nakajima, S., Kawashima, Y., Kozuka, H. & Uchiyama, M. (1984) *Biochem. Pharmacol.* **33**, 1081–1085
7. Svoboda, D., Grady, H. & Azarnoff, D. (1967) *J. Cell Biol.* **35**, 127–152
8. Svoboda, D., Azarnoff, D. & Reddy, J. (1969) *J. Cell Biol.* **40**, 734–746
9. Osumi, T. & Hashimoto, T. (1978) *J. Biochem. (Tokyo)* **83**, 1361–1365
10. Mitchell, F. E., Price, S. C., Hinton, R. H., Grasso, P. & Bridges, J. W. (1985) *Toxicol. Appl. Pharmacol.* **81**, 371–392
11. Gray, R. H. & de la Iglesia, F. A. (1984) *Hepatology* **4**, 520–530
12. Kawashima, Y., Katoh, H. & Kozuka, H. (1982) *Biochim. Biophys. Acta* **712**, 48–56
13. Reddy, J. K., Reddy, M. K., Usman, M. I., Lalwani, N. D. & Rao, M. S. (1986) *Environ. Health Perspect.* **65**, 317–327
14. Rao, M. S. & Reddy, J. K. (1987) *Carcinogenesis* **8**, 631–636
15. Ikeda, T., Aiba, K., Fukuda, K. & Tanaka, M. (1985) *J. Biochem. (Tokyo)* **98**, 475–482
16. Pastoor, T. P., Lee, K. P., Perri, M. A. & Gillies, P. J. (1987) *Exp. Mol. Pathol.* **47**, 98–109
17. Clark, L. C., Jr., Becattini, F., Kaplan, S., Obrock, V., Cohen, D. & Becker, C. (1973) *Science* **181**, 680–682
18. Lazarow, P. B. & de Duve, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2043–2046
19. Kawashima, Y., Katoh, H. & Kozuka, H. (1983) *Biochim. Biophys. Acta* **750**, 365–372
20. Lands, W. E. M. & Hart, P. (1965) *J. Biol. Chem.* **240**, 1905–1911

21. Katoh, H., Kawashima, Y., Watanuki, H., Kozuka, H. & Isono, H. (1987) *Biochim. Biophys. Acta* **920**, 171–179
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
23. Reddy, J. K. & Kumar, N. S. (1979) *J. Biochem. (Tokyo)* **85**, 847–856
24. Reddy, J. K., Azarnoff, D. L., Svoboda, D. J. & Prasad, J. D. (1974) *J. Cell Biol.* **61**, 344–358
25. Mitchell, A. M., Lhuguenot, J.-C., Bridges, J. W. & Elcombe, C. R. (1985) *Toxicol. Appl. Pharmacol.* **80**, 23–32
26. Elcombe, C. R. & Mitchell, A. M. (1986) *Environ. Health Perspect.* **70**, 211–219
27. Facino, R. M., Carini, M. & Tofanetti, O. (1988) *Pharmacol. Res. Commun.* **20**, 265–276
28. Baldwin, J. R., Witiak, D. T. & Feller, D. R. (1980) *Biochem. Pharmacol.* **29**, 3143–3154
29. Cayen, M. N., Ferdinandi, E. S., Greselin, E., Robinson, W. T. & Dvornik, D. (1977) *J. Pharmacol. Exp. Ther.* **200**, 33–43
30. Emudianughe, T. S., Caldwell, J., Sinclair, K. A. & Smith, R. L. (1983) *Drug Metab. Dispos.* **11**, 97–102
31. Hanhijarvi, H., Ylinen, M., Kojo, A. & Kosma, V.-M. (1987) *Pharmacol. Toxicol.* **61**, 66–68
32. Lalwani, N. D., Fahl, W. E. & Reddy, J. K. (1983) *Biochem. Biophys. Res. Commun.* **116**, 388–393
33. Lalwani, N. D., Alvares, K., Reddy, M. K., Reddy, M. N., Parikh, I. & Reddy, J. K. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5242–5246
34. Milton, M. N., Elcombe, C. R., Kass, G. E. N. & Gibson, G. G. (1988) *Biochem. Pharmacol.* **37**, 793–798

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