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INDUCTION BY PERFLUOROOCTANOIC ACID OF MICROSOMAL 1-ACYLGLYCEROPHOSPHOCHOLINE ACYLTRANSFERASE IN RAT KIDNEY

SEX-RELATED DIFFERENCE

YOICHI KAWASHIMA,* TOMOMI MATSUNAGA, NARCISA UY-YU and HIROSHI KOZUKA
Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

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Abstract—Response of rat kidney to the challenges by perfluorooctanoic acid (PFOA) was studied using microsomal 1-acyglycerophosphocholine (1-acyl-GPC) acyltransferase as a parameter. Marked induction of the enzyme was brought about in kidney of male rats, whereas the induction in kidney of female rats was far less pronounced. The sex-related difference in the response of kidney to PFOA was much more marked than those seen with *p*-chlorophenoxyisobutyric acid (clofibric acid) or 2,2'-(decamethylenedithio)diethanol (tiadenol). Hormonal manipulations revealed that the sex-related difference in the response of kidney to PFOA was strongly dependent on the state of gonadal hormones of rats. Even after a prolonged administration of PFOA for up to 26 weeks, this sex-related difference was still evident. Induction of peroxisomal β-oxidation was brought about concurrently with microsomal 1-acyl-GPC acyltransferase and a high correlation was confirmed between the inductions of these two parameters.

Extensive attempts have been made to evaluate the response of liver to peroxisome proliferators. Besides liver, kidney and small intestine have been shown to respond to the challenges by peroxisome proliferators, so that microsomal 1-acylglycerophosphocholine (1-acyl-GPC†) acyltransferase and peroxisomal β-oxidation were induced in these organs [1–4]. Considering the metabolism of peroxisome proliferators, most parts of peroxisome proliferators and their metabolites are thought to be excreted through the kidney, leading to the exposure of this organ to high concentrations of peroxisome proliferators or their metabolites. Compared with the liver, however, fewer studies have been done on the response of kidney to peroxisome proliferators, and information is lacking on whether there is a sex-related difference in the response of kidney to peroxisome proliferators. This is probably due to the lower response of the renal peroxisomal β-oxidation system to peroxisome proliferators compared with that of the hepatic system [3, 4]. However, this does not necessarily indicate that kidney is less responsive to peroxisome proliferators, since our previous study [4] showed that specific activity of microsomal 1-acyl-GPC acyltransferase in kidney was comparable to that in liver and that the extent of the increase in its activity in kidney by peroxisome proliferators was rather greater than that in liver. In this context, microsomal 1-acyl-GPC acyltransferase seems to be a suitable parameter to estimate the response of kidney to peroxisome proliferators.

Due to the chemical and thermal stability and the surfactant properties, the industrial production and applications of PFOA have been increasing recently, so that it is possible for the public to be exposed to these compounds. This is not the case for other peroxisome proliferators such as hypolipidaemic drugs (e.g. clofibrate), plasticizers (e.g. di-(2-ethylhexyl)phthalate) and herbicides (e.g. 2,4,5-trichlorophenoxyacetic acid). Namely, hypolipidaemic drugs are potent peroxisome proliferators, whereas the usage of the drugs is very limited to patients. On the other hand, plasticizers and herbicides are relatively weak peroxisome proliferators [5, 6], regardless of their wide applications. In contrast to these peroxisome proliferators, PFOA is very potent as a peroxisome proliferator [5, 7–9] and is used widely. Although kidney is one of the organs which respond sensitively to peroxisome proliferators and industrial applications of PFOA as wetting agents, lubricants, corrosion inhibitors and foam fire extinguishers are increasing recently, little biochemical and toxicological information about the effects of PFOA on kidney is available. In the present work, the response of kidney to the challenges by PFOA was studied using microsomal 1-acyl-GPC acyltransferase as a parameter.

MATERIALS AND METHODS

Materials. Oleoly-CoA, palmitoyl-CoA, clofibric acid and bovine serum albumin were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.); PFOA and tiadenol were from Aldrich (Milwaukee, WI, U.S.A.); 1-acyl-GPC from Avanti Poplar Lipid

* To whom correspondence should be addressed.

† Abbreviations: PFOA, perfluorooctanoic acid; clofibric acid, *p*-chlorophenoxyisobutyric acid; tiadenol, 2,2'-(decamethylenedithio)diethanol; 1-acyl-GPC, 1-acylglycerophosphocholine.

Table 1. Effects of peroxisome proliferators on microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation in rat kidney

Conditions	Male rats		Female rats	
	1-Acyl-GPC acyltransferase	Peroxisomal β-oxidation	1-Acyl-GPC acyltransferase	Peroxisomal β-oxidation
Control	38.2 ± 2.8	1.55 ± 0.35	39.7 ± 3.6	1.56 ± 0.13
0.5% Clofibric acid	287.5 ± 30.1†	8.87 ± 1.51†	194.7 ± 28.1†	6.29 ± 1.28†
0.5% Tiadenol	259.3 ± 27.9†	10.15 ± 1.83†	153.4 ± 25.8†	6.74 ± 0.61†
0.02% PFOA	209.1 ± 11.0†	5.15 ± 0.73*	58.1 ± 8.9	1.75 ± 0.10

Rats were fed for 1 week on a diet containing one of clofibric acid, tiadenol or PFOA. Enzymes were assayed as described in Materials and Methods.

Enzyme activities were expressed in nmol/min/mg protein. Values are mean ± SD for four separate experiments.

* Significantly different from control at $P < 0.05$.

† Significantly different from control at $P < 0.01$.

(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 on a commercial diet or a diet containing 0.5% (w/w) clofibric 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, 0.01, 0.02 or 0.04% (w/w) and female rats were fed on a diet containing PFOA at the doses of 0, 0.01, 0.02, 0.04 or 0.08% (w/w) for 2 weeks.

Some male rats, weighting 70–80 g, were castrated 28 days before being killed. These rats were subcutaneously administered testosterone propionate (10 mg/kg) or oestradiol (10 mg/kg) once every 2 days for 3 weeks before being killed. Corn oil was used as a vehicle. In some experiments, non-operated male rats were treated with oestradiol (10 mg/kg) once every 2 days for 3 weeks before being killed. Non-operated female rats, weighing 85–90 g, were administered with testosterone propionate (10 mg/kg) once every 2 days for 3 weeks before being killed. Non-treated controls were administered corn oil subcutaneously (1 mL/kg). The rats were fed on a commercial diet or a diet containing 0.01% (w/w) PFOA for 2 weeks before being killed.

For long-term treatments with PFOA, male and female rats aged 5 weeks at the initiation of the treatment were used. Male rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA and were killed at 2, 22 and 26 weeks after the initiation of the treatment. Some rats were fed on the diet containing 0.01% (w/w) PFOA for 22 weeks and then were fed on the control diet for 4 weeks. Female rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA for 2 or 26 weeks. Male and female rats aged 24 weeks were fed on a diet containing 0.01% (w/w) PFOA for 2 weeks as age-matched control experiments.

Subcellular fractionation. At the end of the treatments, rats were killed by decapitation and kidneys were isolated. The kidneys were homogenized with 9 vol. of 0.25 M sucrose. One part of the homogenate was centrifuged at 600 *g* for 10 min to prepare the post-nuclear supernatant. The other part of the homogenate was centrifuged at 18,000 *g* for 15 min and the supernatant obtained was recentrifuged under the same conditions. The resulting supernatant was centrifuged at 105,000 *g* for 60 min. The pellet was suspended in 0.25 M sucrose and then recentrifuged at 105,000 *g* for 60 min. The resulting pellet was resuspended in a small volume of 0.25 M sucrose (25–30 mg of protein/mL). Protein concentrations were determined by the method of Lowry *et al.* [10] with bovine serum albumin as standard.

Enzyme assays. Microsomal 1-acyl-GPC acyltransferase was assayed spectrophotometrically with oleoyl-CoA as substrate, as described previously [11]. Cyanide-insensitive palmitoyl-CoA oxidation was assayed according to Lazarow and de Duve [12] with some modifications as described previously [13]; post-nuclear supernatant was used for the assay as the enzyme source.

Statistical analysis. Statistical significance of mean differences between control and PFOA-treated groups was investigated by analysis of variance and by Scheffé multiple comparison method at $P < 0.05$ or $P < 0.01$. Statistical analyses were performed by Student's *t*-test or Welch's test for two means.

RESULTS

Comparison of peroxisome proliferators

Effects of three peroxisome proliferators on activity of microsomal 1-acyl-GPC acyltransferase in rat kidney were compared (Table 1). The administration of clofibric acid or tiadenol to male rats caused a marked increase in the activity of microsomal 1-acyl-GPC acyltransferase. Similarly, these two drugs increased considerably the activity of 1-acyl-GPC acyltransferase in kidney of female rats, with the extent of the increase being less

Table 2. Effects of gonadal hormones on PFOA-caused inductions of microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation in rat kidney

Conditions	PFOA treatment	1-Acyl-GPC acyltransferase	Peroxisomal β-oxidation
Male rats			
Sham-operated	−	42.2 ± 6.0	1.71 ± 0.33
	+	306.5 ± 68.2†	6.64 ± 0.13†
Castrated	−	44.4 ± 4.4	1.35 ± 0.09
	+	90.2 ± 14.9†	2.46 ± 0.19†
Castrated	−	35.6 ± 1.5	1.57 ± 0.33
plus testosterone	+	269.0 ± 7.4†	6.22 ± 0.58†
Castrated	−	39.5 ± 4.6	1.31 ± 0.13
plust oestradiol	+	64.7 ± 6.4†	1.82 ± 0.08†
Non-operated	−	47.4 ± 4.6	1.25 ± 0.34
plus oestradiol	+	70.9 ± 13.7*	1.63 ± 0.31
Female rats			
Non-operated	−	39.2 ± 4.3	1.56 ± 0.16
	+	58.8 ± 2.6†	1.75 ± 0.08
Non-operated	−	44.1 ± 0.9	1.67 ± 0.20
plus testosterone	+	192.2 ± 30.7†	4.09 ± 0.90*

The hormonal state of rats were altered as described in Materials and Methods. Rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA for 2 weeks.

Enzyme activities were expressed in nmol/min/mg protein. Values are mean ± SD for three separate experiments.

* Significantly different from control diet-fed groups at $P < 0.05$.

‡ Significantly different from control diet-fed groups at $P < 0.01$.

pronounced than in that of male rats. Treatment of male rats with PFOA resulted in a great increase in the activity of microsomal 1-acyl-GPC acyltransferase in kidney, whereas only a slight increase was observed in female rats. Likewise, the activity of peroxisomal β-oxidation increased in kidney of male and female rats following the treatment of rats with clofibric acid or tiadenol, and a moderate sex-related difference was seen in the response of peroxisomal β-oxidation to these drugs. PFOA showed a marked sex-related difference in the induction of peroxisomal β-oxidation in kidney, as was observed for microsomal 1-acyl-GPC acyltransferase.

Sex hormone dependent effect of PFOA

Dose–response characteristics of microsomal 1-acyl-GPC acyltransferase to PFOA were compared in kidneys of male and female rats. The administration of PFOA to male rats increased markedly the activity of microsomal 1-acyl-GPC acyltransferase in kidney (Fig. 1A). Although the administration of PFOA to female rats increased significantly the activity of the enzymes in kidney, the extent of the increases was far less pronounced than those of male rats. Changes corresponding to microsomal 1-acyl-GPC acyltransferase were seen in the activity of peroxisomal β-oxidation (Fig. 1B).

To study further the differential effects of PFOA on male and female rats, the states of gonadal hormones were altered in rats and the effects of PFOA on the induction of microsomal 1-acyl-GPC acyltransferase was studied (Table 2). Upon the castration of male rats, extent of the induction of microsomal 1-acyl-GPC acyltransferase was reduced extremely, compared to that of sham-operated male rats. The administration of PFOA to castrated male rats which were pretreated with testosterone resulted in a marked induction of the enzyme in kidney. Pretreatment of castrated male rats with oestradiol prevented kidney inducing the enzyme by PFOA. 1-Acyl-GPC acyltransferase was induced little by PFOA in kidney of non-operated male rats which had been treated with oestradiol. Considerable induction of microsomal 1-acyl-GPC acyltransferase was observed in kidney of female rats which were pretreated with testosterone. Upon alteration in the state of gonadal hormones, the activity of peroxisomal β-oxidation changed in a similar way to that of microsomal 1-acyl-GPC acyltransferase.

Effects of prolonged administration of PFOA

Effects of long-term administration of PFOA for 26 weeks to male and female rats are shown in Table 3. Male rats were fed on a diet containing 0.01% (w/w) PFOA for up to 26 weeks and renal activity of microsomal 1-acyl-GPC acyltransferase was measured. The increased activities in kidney of young male rats treated with PFOA for 2 weeks lasted until the 26th week of treatment. Upon withdrawal of PFOA from the diet at the 22nd week of treatment, the activity returned to control levels after feeding with control diet for 4 weeks. No considerable increase in the activity of the enzyme was observed after the treatment of female rats with PFOA for 26 weeks. Peroxisomal β-oxidation responded to the prolonged administration of PFOA in concert with microsomal 1-acyl-GPC acyltransferase.

Relationship between microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation

As presented in this study, the activity of

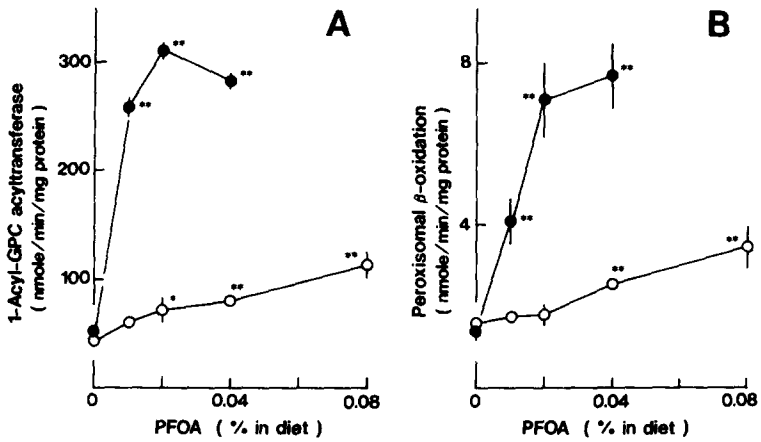

Fig. 1. Dose–response effects of PFOA on microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation in kidneys of male and female rats. Rats were fed on a diet containing PFOA for 2 weeks. Values are mean ± SD for three rats. (A) Microsomal 1-acyl-GPC acyltransferase; (B) peroxisomal β-oxidation. (●) Male rats; (○) female rats. *Significantly different from control at $P < 0.05$; **significantly different from control at $P < 0.01$.

Table 3. Effects of prolonged administration of PFOA on microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation

Conditions	1-Acyl-GPC acyltransferase	Peroxisomal β-oxidation
Young male rats		
Control	38.2 ± 3.6	1.61 ± 0.35
PFOA 2 weeks	274.5 ± 17.2*	4.39 ± 0.30*
PFOA 22 weeks	217.7 ± 35.5*§	4.18 ± 0.37*
PFOA 26 weeks	215.0 ± 9.7*‡‖	4.75 ± 0.58*‡**
PFOA/withdrawal‡‡	40.0 ± 4.0††	1.95 ± 0.20††
Aged male rats		
Control	32.9 ± 2.3	1.81 ± 0.26
PFOA 2 weeks	192.6 ± 19.5‡	3.82 ± 0.33‡
Young female rats		
Control	38.5 ± 3.5	1.47 ± 0.11
PFOA 2 weeks	70.5 ± 12.4*	2.29 ± 0.22*
PFOA 26 weeks	51.0 ± 2.8‡§¶	2.23 ± 0.17*
Aged female rats		
Control	30.7 ± 5.2	1.51 ± 0.31
PFOA 2 weeks	62.5 ± 7.2‡	2.39 ± 0.46†

Rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA during the indicated periods. The detailed conditions for the treatments of animals were described in Materials and Methods.

Enzyme activities were expressed in nmol/min/mg protein. Values are mean ± SD for four separate experiments.

* Significantly different from young control at $P < 0.01$.

† Significantly different from aged control at $P < 0.05$.

‡ Significantly different from aged control at $P < 0.01$.

§ Significantly different from young rats treated with PFOA for 2 weeks at $P < 0.05$.

‖ Significantly different from young rats treated with PFOA for 2 weeks at $P < 0.01$.

¶ Significantly different from aged rats treated with PFOA for 2 weeks at $P < 0.05$.

** Significantly different from aged rats treated with PFOA for 2 weeks at $P < 0.01$.

†† Significantly different from rats treated with PFOA for 26 weeks at $P < 0.01$.

‡‡ Rats were fed on a diet containing 0.01% (w/w) PFOA for 22 weeks and then fed on a control diet for 4 weeks.

peroxisomal β-oxidation changed correspondingly with that of microsomal 1-acyl-GPC acyltransferase, subsequent to the challenges by peroxisome proliferators. Regression analysis was performed on 43 sets of data from Fig. 1 and Tables 1, 2 and 3, and a high correlation with $r = 0.8935$ ($Y = -2.430 + 37.026X$, $P < 0.001$, $N = 43$) was revealed between peroxisomal β-oxidation and microsomal 1-acyl-GPC acyltransferase.

DISCUSSION

Although many investigations have been made into sex-related differences in the response of liver to peroxisome proliferators [14–18], little information is available about sex-related difference in the response taking place in kidney to peroxisome proliferators. Three chemicals having diverse structures are employed as peroxisome proliferators in this study, and the present results showed a significant sex-related difference in the response of kidney to these peroxisome proliferators. On the basis of the results in Fig. 1A and B, microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation in male rat kidney responded to PFOA approximately 25 times as sensitively as those in female rat kidney. Tiadenol is shown to be metabolized and its metabolite(s) is thought to be a proximate inducer in liver [19]. However, there is no evidence whether the proximate form of tiadenol for the kidney is formed in renal cells or derived from liver via blood. On the other hand, clofibric acid is thought to be an active principle in its pharmacological action [20]. PFOA is also believed to be the proximate form itself due to its metabolically inert properties [21]. Since these two chemicals seem to be excreted through the kidney [22, 23], this organ is conceivably exposed to high concentrations of these compounds. Therefore, the present results with regard to sex-related differences in enzyme induction suggest that there is a considerable difference in the response of kidney to peroxisome proliferators between PFOA and the other two drugs.

The present study showed that the induction by PFOA of microsomal 1-acyl-GPC acyltransferase in the kidney was dependent on testosterone. These results agree with toxico-kinetic findings of Ylinen and co-workers [23, 24] who showed that the rate of excretion of PFOA into urine was dependent on gonadal hormone and was much faster in female than in male rats. These findings led us to ask whether chronic treatment of female rats with PFOA results in a cumulative effect. Contrary to our expectations, however, the activities of the two biochemical parameters after the 26-week treatment with PFOA were almost the same as those after the 2-week treatment in male and female rats. These results suggest that the kidneys of female rats still excrete PFOA much faster than male rats even after chronic treatment with PFOA.

A high degree of correlation between microsomal 1-acyl-GPC acyltransferase and peroxisomal β-oxidation in kidney was confirmed in the present study. This finding may support our previous speculation [4, 5, 25] of co-induction of these two enzymes by peroxisome proliferators and the relevance of microsomal 1-acyl-GPC acyltransferase as a parameter to detect the response of kidney to peroxisome proliferators. In kidney, specific activity of peroxisomal β-oxidation and long-chain acyl-CoA hydrolase, which are typical parameters to detect the response of liver to peroxisome proliferators, are low [26]. Moreover, as far as we know, no chemicals other than peroxisome proliferators induce microsomal 1-acyl-GPC acyltransferase, and changes in hormonal and nutritional states did not affect the activity of the enzyme [4]. These facts strongly suggest that microsomal 1-acyl-GPC acyltransferase is a suitable parameter to measure renal response to PFOA.

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