フッ素化脂肪酸化合物を認識する新規細胞膜輸送体の検索とそのクローニング

（研究課題番号　09772029）

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工藤なをみ

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はしがき

現在、我々は膨大な数の化学物質を作り出して利用している。これを利用することによって我々は大変便利で豊かな生活を享受しているが、その反面、一部の化学物質による生態系への広範な影響が少しずつ明らかになりつつある。特に、微量の化学物質が内分泌などの生体機能調節に影響を与えるなど、従来の毒性検索では検討されてこなかった点が新たな問題として浮かび上がってきている。しかしながら、化学物質を完全に排除することは全く非現実的なことである。このような問題の対処が現在、様々に議論されているのは周知のことである。このような現状をふまえると、化学物質の将来にわたる利用あるいは規制を念頭におき、化学物質の生体に対する影響について多様な情報を蓄積していくことは非常に重要であろう。

フッ素化合物は他のハロゲン化合物とは大きく異なる性質を持つことから、化成品、医薬品を始めとして様々な分野で幅広く利用されている。本研究で取り上げたペルフルオロ脂肪酸は水素をすべてフッ素で置換した化合物で撥水、撥油などの特殊な性質を有することから幅広く利用されている化合物である。急性毒性は低く、催奇形性、発ガン性も認められないことから、安全な化合物として認識されてきた。しかしながら、齧歯類において種々の生体作用を持つことが明らかになるとともに、一口にペルフルオロ脂肪酸といっても生体影響は化合物ごとに異なっていることもわかってきた。本研究ではこのような現象を統一的に理解するために必要な基礎的な知見を得るとともに、生体が本来備えている物質認識の機構のなかでどのようにして化学物質が認識されるのかという点について明らかにしていきたいと考えた。本研究において当初計画した事の一部については未完成の部分もあるが、これまでに得られたいくつかの新しい知見を報告させて頂く。

本研究に対する文部省からの科学研究補助金の交付に対して深く感謝する次第である。

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Induction by perfluorinated fatty acids with different carbon chain length of peroxisomal β-oxidation in the liver of rats

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Abbreviations

BrAMC: 3-bromoacetyl-7-methoxycoumarin

clofibric acid: 2-(p-chlorophonoxy)-2-methylpropionic acid

PFCAs: perfluorinated fatty acids

PFDA: perfluorodecanoic acid

PFHA: perfluoroheptanoic acid

PFNA: perfluorononanoic acid

PFOA: perfluorooctanoic acid

ABSTRACT

The potency of the induction of peroxisomal β-oxidation was compared between perfluorinated fatty acids (PFCAs) with different carbon chain lengths in the liver of male and female rats. In male rats, perfluoroheptanoic acid (PFHA) has little effect, although perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanopic acid (PFDA) potentially induced the activity. By contrast, PFHA and PFOA did not induce the activity of peroxisomal β-oxidation in the liver of female rats while PFNA and PFDA effectively induced the activity. The induction of the activity by these PFCAs was in a dose-dependent manner, and there is a highly significant correlation between the induction and hepatic concentrations of PFCAs in the liver regardless of their carbon chain lengths. These results strongly suggest that the difference in their chemical structure is not the cause of the difference in the potency of the induction. Hepatic concentrations of PFOA and PFNA was markedly higher in male compared to female rats. Castration of male rats reduced the concentration of PFNA in the liver and treatment with testosterone entirely restored the reduction. In contrast to the results obtained from the *in vivo* experiments, the activity of peroxisomal β-oxidation was induced by PFDA and PFOA to the same extent in cultured hepatocytes prepared from both male and female rats. These results, taken together, indicate that difference in accumulation between PFCAs in the liver was responsible for the different potency of the induction of peroxisomal β-oxidation between PFCAs with different carbon chain lengths and between sexes.

INTRODUCTION

Perfluorinated fatty acids (PFCAs), straight chain fatty acid analogues whose aliphatic hydrogens are all replaced by fluorine, are commercially used as lubricants, anti-wetting agents, plasticizers and corrosion inhibitors in relation to their surfactant properties and their chemical and thermal stability (1,2). The effects of PFCAs on biological systems have been studied using perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) (3-24). The common effects of these chemicals on rodents were characterized by peroxisome proliferation (3-6), induction of peroxisomal enzymes (6-9), microsomal enzymes involved in lipid metabolism (6,7,10-12) and drug metabolism (13-15), and cytosolic proteins such as fatty acid binding protein (16,17), acyl-CoA hydrolase (18) and acyl-CoA binding protein (16,17). By contrast, PFDA has been shown to reduce the binding of norepinephrine to β-adrenoreceptor in rat heart (19,20), to lower the level of thyroid hormone in rat serum (21,22), to causes uncoupling of electron transport in isolated rat mitochondria (23) and to inactivate a channel for 2-aminopurine in L5178Y cells (24), but these biological effects have not been found for PFOA. These facts suggests that PFCAs with different carbon numbers have biologically diverse effects or greatly different potency of the effects on animals. To date, however, a little information is available about PFCAs except for PFOA and PFDA, therefore, the relationship between chain lengths of PFCAs and biological effects is unclear. In addition to the difference in biological properties of PFOA and PFDA which were found in male rats, a marked sex-related difference has been reported in the induction of peroxisomal β-oxidation by PFOA (25,26), no

difference being observed with PFDA between both sexes. It is of interest, therefore, to clarify the mechanism responsible for the difference in biological effects of PFCAs with different carbon chain lengths in female rats as well as male rats.

These strange properties of PFCAs stimulated our interest in studying how PFCAs having diverse carbon chain lengths cause biological changes with different potency in livers of male and female rats. To address this question, we studied the relationship between the induction of peroxisomal β-oxidation and hepatic accumulation of PFCA having carbon chain lengths of C7, C8, C9 and C10. The results obtained suggest that the accumulated amounts of PFCAs in the liver of rats is responsible for the induction of peroxisomal β-oxidation and that, contrary to expectations, this induction does not depend on their different carbon chain lengths. We report the results herein.

MATERIALS AND METHODS

Materials

Perfluoroheptanoic acid (PFHA), PFOA and PFDA were purchased from Aldrich Japan (Tokyo, Japan); Perfluorononanoic acid (PFNA) was from Lancaster Synthesis (Lancashire, UK). BSA, insulin (porcine), dexamethasone, 2-(*p*-chlorophenoxy)-2-methylpropionic acid (clofibric acid) and palmitoyl-CoA was purchased from Sigma (St. Louis, MO, U.S.A.). Wy 14,643 was purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA, U.S.A.). NAD and CoA were from Oriental Yeast Co. (Tokyo, Japan). Fetal calf serum and Dulbecco's modified Eagle's medium were purchased from Gibco Oriental (Tokyo, Japan). All reagents were of analytical grade.

Animals

Male and female Wistar rats of 5 week old were purchased from SLC (Hamamatsu, Japan). After 1 week acclimatization, rats were intraperitoneally administered with PFHA, PFOA, PFNA and PFDA at doses ranging from 2.5 to 20 mg/ kg body weight once a day for 5 days. PFCAs were dissolved into propyleneglycol:water (1:1, v/v) after neutralization with 1 M NaOH.

Some of male rats (24-26 day-old) were castrated 27 days before being killed. Half of them were subcutaneously administered with testosterone propionate (10 mg/ kg body weight) with corn oil as a vehicle once every 2 days for 3 weeks before being killed. These rats were administered with PFNA or PFDA at a dose of 20 mg/ kg body weight once a day for 5 days before being killed.

Rats were killed by decapitation under light ether anesthesia and blood samples were collected 24 h after the final administration with PFCAs. Livers were quickly isolated, perfused with ice-cold 0.9% NaCl, frozen in liquid nitrogen and stored at -80°C until use. The frozen liver was thawed on ice and homogenized with 9 volumes of 0.25M sucrose/ 1 mM EDTA/ 10 mM Tris Hcl, pH 7.4. Protein concentrations in the homogenates were determined by the method of Lowry *et al.* (27) with BSA as a standard.

Enzyme assays

Peroxisomal β-oxidation was assayed as the activity of cyanide-insensitive palmitoyl-CoA oxidation employing homogenates as an enzyme source (28). Acyl-CoA oxidase was assayed by measuring palmitoyl-CoA-dependent H_2O_2 production spectrophotometrically at 502 nm, as described by Small, *et al.* (29).

Determination of PFCAs

PFCAs were extracted from liver homogenates as an ion pair with tetrabutylammonium, derivertized with 3-bromoacetyl-7-methoxycoumarin (BrAMC) and quantified by HPLC with a fluorescence detection according to the method reported previously (30) with some modifications as follows. For the determination of PFNA and PFDA, 5-20 nmol of PFHA were added as an internal standard, and perfluorohexanoic acid and PFDA were used as internal standards for the measurement of PFOA and PFHA, respectively. After mixing 0.5 ml of liver homogenates with an internal standard and then with 0.5 M

tetrabutylammonium solution, PFCAs were extracted with ethylacetate:hexane (1:1, v/v). The extracts were dried by flushing nitrogen, and to the resulting residue was added 2 ml of 0.2% BrAMC acetone solution (w/v). The mixture was incubated at 70°C for 25 min, then cooled on ice and kept at -30°C for 2 h. The solution was filtered through a glass-wool filter and applied to HPLC with a reverse phase column (Wakosil-II 3C18, 4.6 mm ID x 50 mm, Wako Pure Chemicals, Osaka, Japan). Acetonitrile:water (3:1) was used for a mobile phase at a flow rate of 0.5 ml/min. The peaks of PFCAs were detected by fluorescent detector at 316 nm of excitation and 419 nm of emission.

Culture of hepatocytes

Hepatocytes were isolated from male or female Wistar rats (180 g) by collagenase perfusion (31). Hepatocytes were separated from other cells by centrifugation at 50 x g for 1 min. This procedure was repeated 4 times. Cell viability was in the range of 80-95%, determined by Trypan blue dye exclusion. Cells were cultured on collagen-coated plastic dish in Dulbecco's modified Eagles medium containing 10% fetal calf serum, 10^{-6} M insulin, , 10^{-6} M dexamethasone and 4 mg/l kanamycin in the presence or the absence of PFCA. PFCAs were dissolved in DMSO and added into the culture medium. The final concentration of DMSO in the medium was always 1% (v/v). At the end of the culture, the medium was discarded, and cells were washed with ice-cold PBS, scraped off from the dish and homogenized by sonication for 20 s (Astrason sonifier with ultramicrotip, level 4) in 0.25 M sucrose/ 1 mM EDTA/ 10 mM Tris-HCl, pH 7.4.

Statistical analysis

Analysis of variance was used to test the significance of differences between different doses of PFCAs, between different PFCAs at a same dose and between male, female, castrated male and castrated male treated with testosterone. Where differences were significant, the statistical significance between any two means was determined using Sheffe's multiple range test. Statistical significance between male and female rats was analyzed by Student's *t*-test or Welch's test after *F*-test for two means.

RESULTS

Induction of peroxisomal β-oxidation by PFCAs in the liver of male and female rats

Maximum induction of peroxisomal β-oxidation in livers was compared between PFCAs with 7-10 carbon chain lengths and clofibric acid (Figure 1). PFCAs having longer carbon chain showed more potent induction of the activity. PFCAs that contain carbon atoms more than 8 significantly induced peroxisomal β-oxidation in the livers of male rats. On the other hand, PFCAs with more than 9 carbons had a significant effect on female rats, whereas PFOA did not induce peroxisomal β-oxidation in female rats. The maximum induced level by PFDA was as high as that by clofibric acid, a typical peroxisome proliferator. The induction was in a dose-dependent manner by PFOA, PFNA and PFDA in male rats, and by PFNA and PFDA in female rats (Figure 2). A high dose (160 mg/ kg body weight) of PFHA slightly, but significantly, induced the activity in male rats, but not in female rats. The induction of peroxisomal β-oxidation by PFNA and PFDA was saturable in both male and female rats, and the maximum activities were calculated to be approximately 50 and 35 nmol/min/mg protein, respectively. The maximum induction by PFOA in male rats was calculated to be approximately 54 nmol/ min/ mg protein at the dose of 30 mg/kg body weight. When the activities were compared on the basis of % of the maximum induction, the effect of PFOA was stronger in male rats than female, whereas little difference was observed in the induction by PFDA between male and female. A

small, but significant, sex-related difference was observed with PFNA, although the extent was not large as that seen with PFOA.

Accumulation of PFCAs in the liver

Hepatic concentrations of PFCAs with different carbon chain lengths were determined in male and female rats (Figure 3). The concentrations of PFHA in the liver were less than the detection limit (<3 μg/g liver) in both male and female rats at any doses examined (unpublished data). In female rats, PFOA was not detected at any doses, while the accumulation of PFOA in male rat liver was significant and saturable (Figure 3A). Dose-dependent accumulation was observed with PFNA and PFDA in the liver of male rats (Figure 3B and C). Hepatic concentrations of PFOA and PFNA were calculated to be 15% and 80% of that of PFDA, respectively, when administered at a dose of 20 mg/kg body weight. The longer becomes the carbon chain length of PFCA, the more accumulated PFCA in the liver of male rats. Hepatic accumulation of PFDA was higher than that of PFNA. Hepatic concentrations of PFOA and PFNA in male rats were significantly higher than those in female rats, whereas no significant difference was observed in the case of PFDA.

Relationship between enzyme induction and PFCA concentration in the liver

The relationship between the induction of peroxisomal β-oxidation and the concentrations of PFCA on the basis of nmol/g liver was shown in Figure 4. The

activity of peroxisomal β-oxidation increased with increase in hepatic concentrations of PFCAs regardless of their carbon chain lengths. The induced activity reached to maximum level at the concentration of approximately 500 nmol/g liver in both sexes, although the maximum activity in male rats (Figure 4A) was higher than female (Figure 4B). To examine the relationship between the activity of peroxisomal β-oxidation and hepatic concentration of PFCAs, a linear regression analysis was carried out for the data obtained from PFCA concentration of less than 500 nmol/ g liver. To compensate the difference of the maximum activity of peroxisomal β-oxidation between male and female rats, the activity was expressed as a percentage of the maximum activity in male and female rats, respectively. This revealed that the correlation between the two parameters was significant, with $r=0.850$ ($P<0.001$, solid line in Figure 4A). When the data of PROA-treated male rats and PFNA-treated female rats were excluded from the analysis, the correlation becomes more highly significant, with $r=0.984$ ($P<0.001$) (Figure 4, dotted line). In male rats, PFOA induced more efficiently than did PFNA and PFDA at the concentration of 100 nmol/g liver; the activity of peroxisomal β-oxidation was about 30 nmol/min/mg protein, which was two times higher than those by PFNA and PFDA. Similarly, in female rats, PFNA induced more efficiently that did PFDA at the concentration of 200 nmol/g liver.

Induction of acyl-CoA oxidase by PFCAs in cultured hepatocytes

The potency of induction of peroxisomal β-oxidation was compared between PFHA, PFOA, PFNA and PFDA in cultured hepatocytes. When hepatocytes prepared from male rats were cultured in the presence of PFOA for 72 h, the activity of acyl-CoA oxidase, a rate limiting enzyme of peroxisomal β-oxidation, increased in a concentration-dependent manner (Figure 5A). The induction was maximum at 100 μM PFOA, and PFOA significantly reduced cell viability at higher concentrations (unpublished data). The induction of acyl-CoA oxidase in hepatocytes was compared between PFCAs with different carbon chain lengths in male and female rats (Figure 5B). Acyl-CoA oxidase was induced by Wy 14,643, a potent peroxisome proliferator, in both sexes to the same extent. In male hepatocytes, not only PFOA, PFNA and PFDA, but also PFHA induced acyl-CoA oxidase activity, whereas the induction by PFHA in vivo was very limited. PFOA induced acyl-CoA oxidase in female hepatocytes as was observed in male hepatocytes, despite that PFOA never induced peroxisomal β-oxidation in the liver of female rats *in vivo*. PFDA also induced acyl-CoA oxidase in hepatocytes prepared form male and female rats to the same extent, and there was no difference in the potency to induce acyl-CoA oxidase between PFDA and PFOA in cultured hepatocytes from both sexes.

Role of testosterone in the accumulation of PFCA

To elucidate the role of sex hormone in regulating hepatic accumulation of PFCAs, the effects of castration and testosterone treatment were tested (Table 1). Castration significantly reduced the level of PFNA in the liver of male rats, but

hepatic level of PFNA in castrated rat was still higher than that in female rats. Treatment of castrated rats with testosterone entirely restored the level of PFNA. Hepatic concentration of PFNA that was seen in the castrated rats was 200.9 ± 19.7 μg (433 nmol) /g liver, which is high enough for the maximum induction of peroxisomal β-oxidation (Figure 2). Therefore, the activity of peroxisomal β-oxidation was not changed by either castration or testosterone treatment despite the significant changes in the levels of PFNA in male liver. The activity of peroxisomal β-oxidation in livers of female rats was significantly lower than those of male rats, when treated with PFNA and PFDA (Table 1 and Figure 1). The difference seems to be due to the sex-related difference in the maximum response, but not to difference in the accumulation of PFCAs. In contrast to the case of PFNA, hepatic concentration of PFDA in male rats was not diverse from that in female rats and was affected by neither castration nor testosterone treatment. The activities of peroxisomal β-oxidation were the maximum in all experimental conditions as observed with PFNA.

DISCUSSION

Induction of peroxisomal β-oxidation by PFCAs correlates their hepatic accumulations.

PFOA and PFDA have been demonstrated to induce peroxisomal β-oxidation (6-9). The present study showed that PFNA was a strong inducer of peroxisomal β-oxidation, while PFHA had very week effect on peroxisomal β-oxidation in the liver of male rats. Compared the potency to induce peroxisomal β-oxidation between PFHA, PFOA, PFNA and PFDA, the longer was the carbon chain lengths of PFCA, the more potent was PFCA as an inducer of peroxisomal β-oxidation. These facts raised a question of what is responsible for the different potency between these PFCAs. Determination of hepatic concentrations of PFCAs showed that the activities of peroxisomal β-oxidation was increased with increase in hepatic concentrations of PFCA and reached maximum at approximately 500 nmol PFCA/ g liver (Figure 4). Regression analyses revealed that there was a highly significant correlation between the induction of peroxisomal β-oxidation and hepatic concentrations of PFCAs, regardless of their carbon chain length and sex. Consequently, we concluded that the difference in the potency of the induction of peroxisomal β-oxidation between PFCAs is essentially due to the different accumulation of PFCAs in the liver. This is strongly supported by the results that the potency to induce peroxisomal β-oxidation in cultured hepatocytes was not different between PFCAs and between sex (Figure 5, 32). The values of peroxisomal β-oxidation activity corresponding to the accumulation of more than 100 nmol PFOA /g liver in male rats and those of more than 200 nmol PFNA/g

liver were off the correlation curve (Figure 4). This is considered to be due to rapid clearance of PFOA in male rats and PFNA in female rats compared to PFCAs with longer carbon chain lengths. The half-life of PFOA in livers of male rats was less than one third of PFDA (33,34), leading to that the accumulation of PFOA was not proportional to the dose but saturable (Figure 3A). Since hepatic concentrations of PFOA was measured 24 h after the final injection, there is a possibility to have underestimated the concentration of PFOA which actually played a role in induction of peroxisomal β-oxidation.

Sex-related difference in the accumulation of PFCAs in the liver

Previous reports have demonstrated that hepatic concentrations of PFOA in male rats is significantly lower than that of female rats (33,35). The present study demonstrated that sex-related difference of hepatic concentration exists in not only PFOA but also PFNA. The induction of peroxisomal β-oxidation by PFOA showed significant difference between both sexes, which is thought to be due to the different accumulation of PFOA in the liver (Figures 2B and 3A). By contrast, there was no difference in the hepatic accumulation of PFHA and PFDA between both sexes. PFHA was not detected in the livers of male and female rats whereas PFDA highly accumulated in the livers of both sexes (Figure 3). There was a tendency that sex-related difference of the accumulation of PFCAs in the liver was gradually reduced with increase in carbon numbers of PFCA.

Several studies have demonstrated that urinary excretion of PFOA in female rats is faster than male rats (33,35,37). By contrast, PFDA is hardly excreted in

urine in both sexes and the elimination rate of PFDA in urine was not different between both sexes (34,38). These facts suggest that sex-related difference in hepatic concentrations of PFNA is due to different rate in urinary excretion of PFNA, which causes the sex-related difference in the accumulation of PFNA. Our preliminary study showed that PFNA was efficiently excreted in urine in female rats whereas male rats little excreted PFNA in urine (unpublished data). Biliary excretion rate can be responsible for the sex-related difference, but the rate of biliary excretion of PFOA and PFDA in male rat was not different from female rats (33,34). In addition, contribution of fecal excretion to the elimination was relatively small in the case of PFOA (33) and probably PFNA. Taken together, the difference in the rate of urinary excretion seems to be a principle determinant of sex-related difference in PFCA accumulation in the liver.

Hormonal regulation of PFCA accumulation

Previous studies have shown that the rate of urinary excretion of PFOA is regulated by sex hormones (36,39). Castration of male rats increased the excretion rate of PFOA to the level of female rats (36,39) and treatment of castrated rats with testosterone reversed the effect of castration (39). Hepatic concentrations of PFOA in male rats that had been hormonally manipulated by castration and testosterone treatment were consistent with urinary excretion rates of those animals (Table 1). In addition to PFOA, hepatic accumulation of PFNA was also regulated by testosterone (Table 1), suggesting that urinary excretion of PFNA is also regulated by sex hormones.

In the present study, we demonstrated that the different potency to induce peroxisomal β-oxidation is due to, in principle, the difference in accumulated amounts between PFCAs in the liver, but not to difference in their chemical structure and that the marked difference observed in the accumulation of PFOA and PFDA produced the striking sex-related difference in the induction of peroxisomal β-oxidation. The mechanism by which PFOA and PFNA cause sex-related difference in their accumulation in the liver is now under investigation.

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Table 1 Effects of castration and testosterone treatment on the accumulation of PFCA in rat liver

Male rats (24-26 day-old) were castrated and the administered with testosterone (10 mg/ kg body weight) or corn oil for 3 weeks. These rats were administered with PFNA or PFDA at a dose of 20 mg/ kg body weight once a day for 5 days. Each value represent the mean ± SD for 4 rats. Differences between experimental groups are statistically significant without a common superscript ($P<0.05$). If no superscript appears, the differences between experimental groups are not statistically significant.

PFCA	sex	treatments	PFCA concentration (μg/ g liver)	peroxisomal β-oxidation (nmol/ min/ mg protein)
PFNA	male	none	358.4 ± 19.2 [a]	45.4 ± 4.2 [a]
		castration	200.9 ± 19.7 [b]	53.6 ± 4.3 [a]
		castration + testosterone	414.3 ± 46.2 [a]	49.2 ± 2.3 [a]
	female	none	101.7 ± 10.5 [c]	31.8 ± 5.2 [b]
PFDA	male	none	453.6 ± 19.3	53.6 ± 4.7 [a]
		castration	535.1 ± 60.0	57.1 ± 2.3 [a]
		castration + testosterone	542.1 ± 84.9	62.3 ± 7.9 [a]
	female	none	412.7 ± 33.1	33.1 ± 9.7 [b]

(Legend to Figures)

Figure 1 Effects of PFCAs and clofibric acid on the activity of peroxisome β-oxidation in the liver

Male (stippled bars) and female (closed bars) rats were intraperitoneally administered with PFHA (30 mg/ kg body weight), PFOA, PFNA, PFDA (20 mg/ kg body weight) or clofibric acid (300 mg/ kg body weight) once a day for 5 days. The activity of peroxisomal β-oxidation was assayed as cyanide-insensitive β-oxidation activity using liver homogenates as an enzyme source. Values represent means ± SD for 4 animals.

*, Significantly different from control (vehicle-treated) rats ($P<0.05$).

#, Differences are statistically significant ($p<0.05$) between male and female rats.

Figure 2 Dose-dependency of the induction of peroxisomal β-oxidation activity by PFCAs in the liver.

Male (closed symbols) and female (open symbols) rats were administered with PFHA (A), PFOA (B), PFNA (C) or PFDA (D) at indicated doses once a day for 5 days. Values represent means ± SD for 4 rats.

*, Differences are statistically significant ($p<0.05$) between male and female rats.

Figure 3 Accumulation of PFCAs in the liver.

Male (closed symbols) and female (open symbols) rats were administered with PFOA (A), PFNA (B) or PFDA (C) at doses ranging from 2.5 -10 mg/kg body

weight once a day for 5 days. Concentrations of PFCAs 24 h after the final injection were determined. Values represent means ± SD for 4 rats.

*, Differences are statistically significant ($p<0.05$) between male and female rats.

Figure 4 Relationship between the activity of peroxisomal β-oxidation and concentrations of PFCAs in the liver of rats.

Data from Figure 2 and 3 were represented for male (open symbols) and female (closed symbols) rats. Control, (circle); PFOA, (square); PFNA, (triangle); PFDA, (diamond). The activity of peroxisomal β-oxidation was expressed as a percent of the maximum activity in male and female rats, respectively.

Regression analyses were performed on the mean data from Figures 2 and 3 ranging from 0 to 500 nmol/ g liver. Regression analysis was performed on all data (twentyone sets of data, solid line); PFCA concentration versus peroxisomal β-oxidation activity, $Y=0.152X + 24.98$, $r=0.850$, $P<0.001$. Regression analysis was performed on the data of PFNA- and PFDA-treated male rats and PFDA-treated female rats (eleven sets of data, dotted line); PFCA concentration versus peroxisomal β-oxidation activity, $Y=0.166X + 14.28$, $r= 0.984$, $P<0.001$.

Figure 5 The induction of peroxisomal β-oxidation activity by PFCAs in cultured rat hepatocytes.

A, hepatocytes were isolated from male rats and cultured for 72 h in the presence of PFOA. *, Statistically significant difference from control ($p<0.05$). B, hepatocytes were isolated from male and female rats and cultured for 72 h in the presence of 100 μM PFOA, 100 μM PFDA or 50 μM Wy 14,643. Open bar, no addition; waved bar, PFHA; closed bar, PFOA; crossed bar, PFNA; hatched bar, PFDA; stippled bar, Wy 14,643. Data represent means ± SD for 3 determinations. Difference between experimental groups are statistically significant without a common superscript ($p<0.05$).

Figure 1

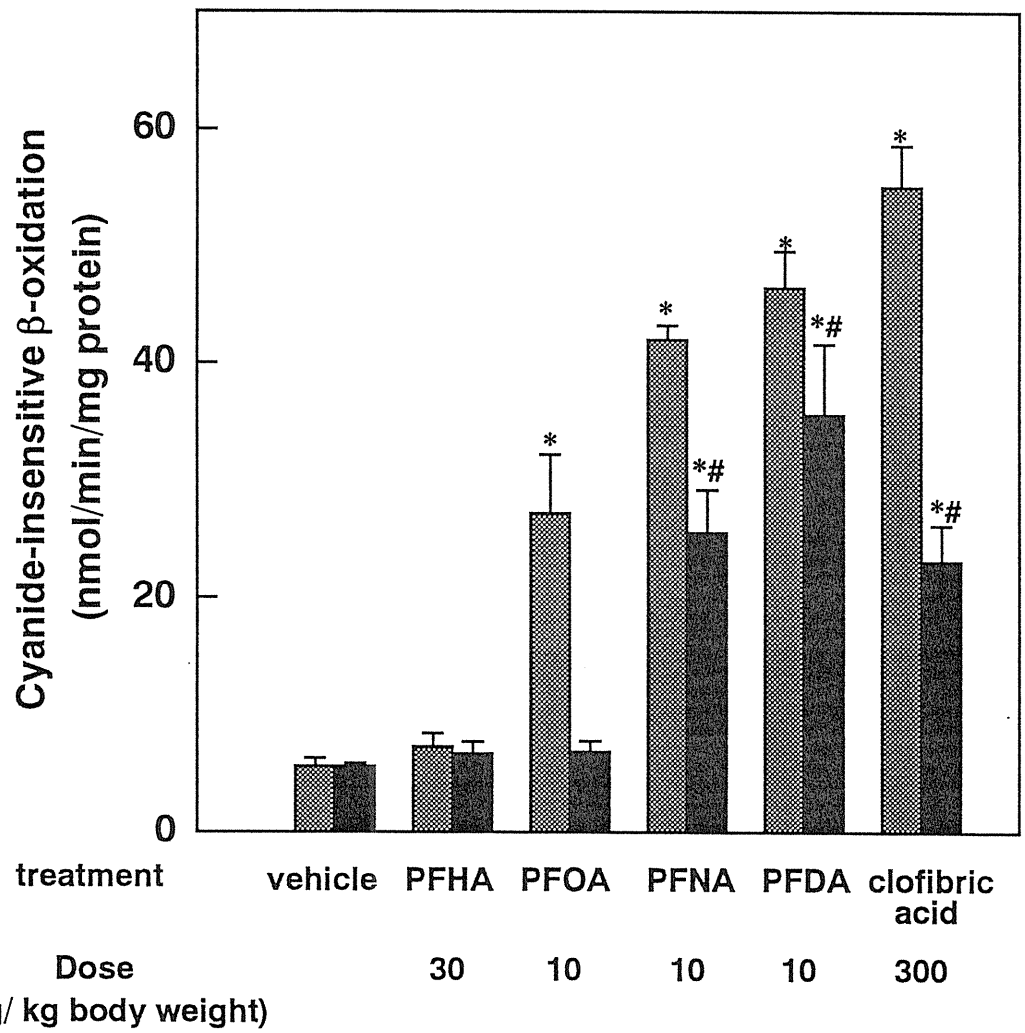

Cyanide-insensitive β-oxidation
(nmol/min/mg protein)
60
40
20
0
*
*#
treatment
vehicle
PFHA
PFOA
PFNA
PFDA
clofibric acid
Dose
(mg/ kg body weight)
30
10
10
10
300

Figure 2

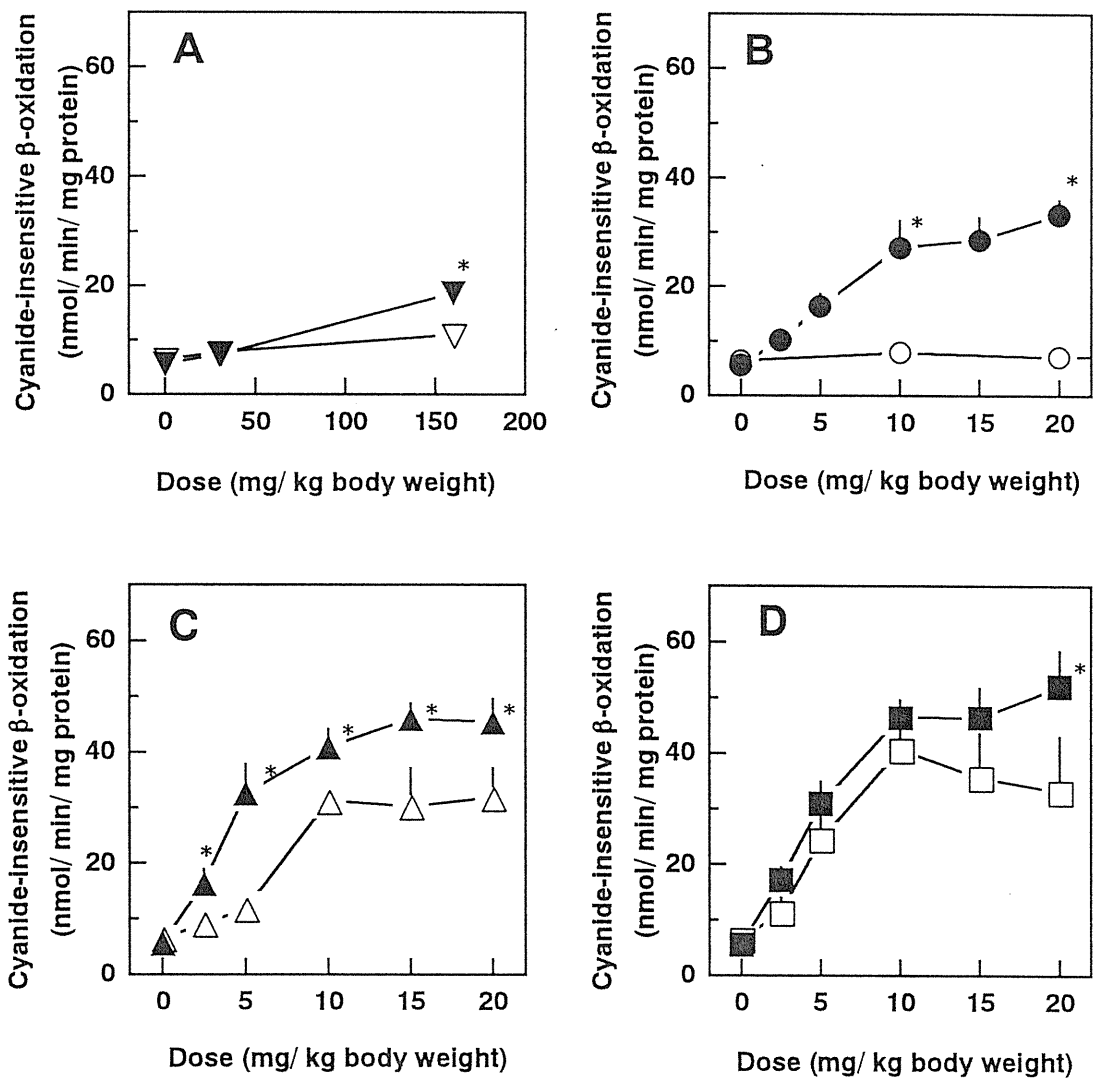

A
B
C
D
Cyanide-insensitive β-oxidation
(nmol/ min/ mg protein)
Dose (mg/ kg body weight)
0
20
40
60
0
50
100
150
200
0
5
10
15
20
*

Figure 3

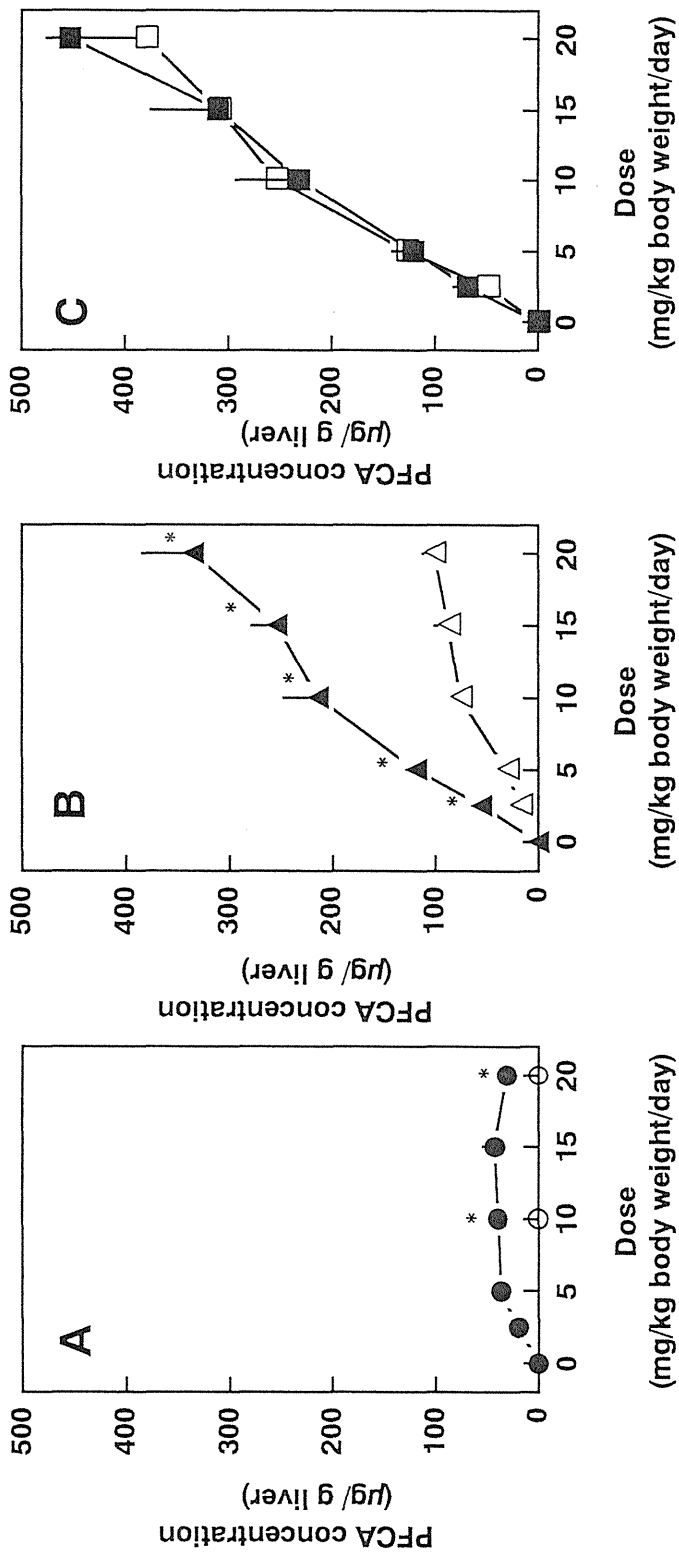

A
B
C
PFCA concentration
(μg/ g liver)
Dose
(mg/kg body weight/day)
0
100
200
300
400
500
0
5
10
15
20
*

Figure 4

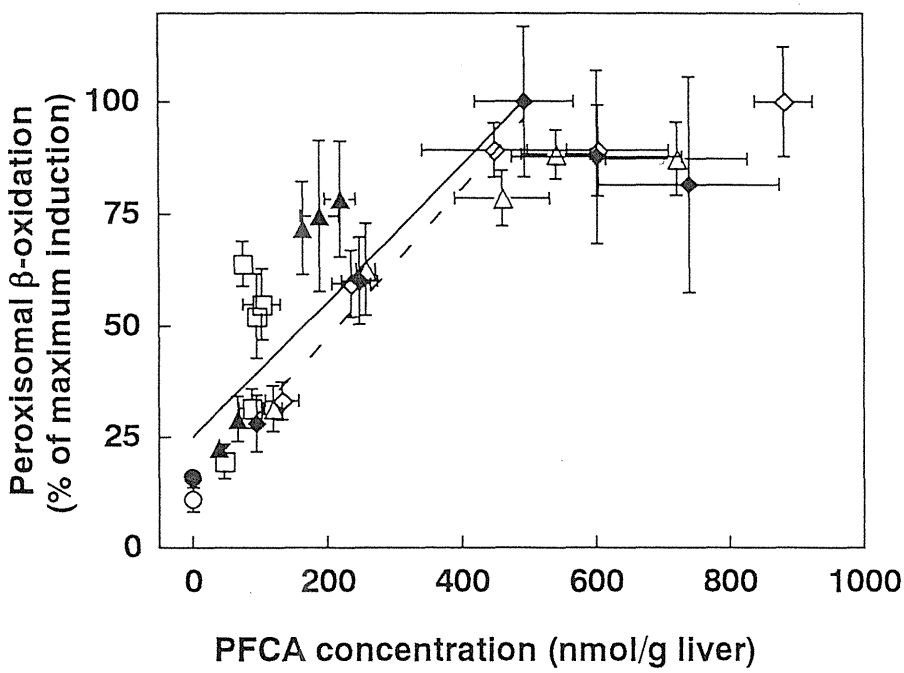
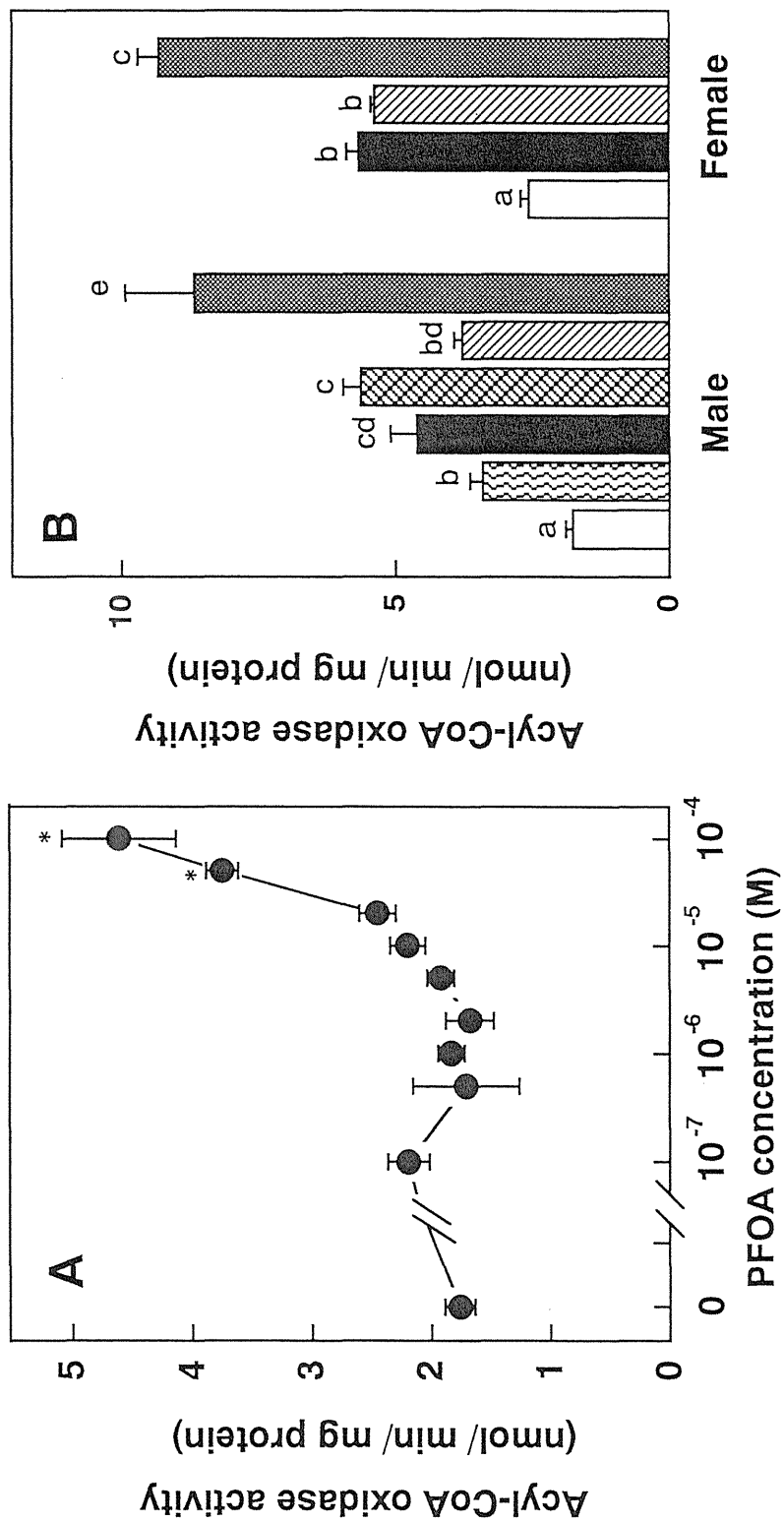

Peroxisomal β-oxidation
(% of maximum induction)
100
75
50
25
0
0
200
400
600
800
1000
PFCA concentration (nmol/g liver)

Figure 5


A
Acyl-CoA oxidase activity
(nmol/ min/ mg protein)
0
1
2
3
4
5
*
*
0
10^-7
10^-6
10^-5
10^-4
PFOA concentration (M)
B
Acyl-CoA oxidase activity
(nmol/ min/ mg protein)
0
5
10
a
b
cd
c
bd
e
Male
a
b
b
c
Female