Chemico-Biological Interactions xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Inhibition of human and rat 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances☆

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ARTICLE INFO

Article history:
Received 22 December 2009
Received in revised form 29 June 2010
Accepted 1 July 2010
Available online xxx

Keywords:
PFOS
Perfluoroalkylated substances
Human
Rat
Enzyme inhibition
3β-Hydroxysteroid dehydrogenase
17β-Hydroxysteroid dehydrogenase 3

ABSTRACT

Perfluoroalkylated substances (PFASs) including perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS) have been classified as persistent organic pollutants and are known to cause reduced testosterone production in human males. The objective of the present study was to compare the potencies of five different PFASs including PFOA, PFOS, potassium perfluorooctane sulfonate (PFOSK), potassium perfluorohexane sulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in the human and rat testes. Human and rat microsomal enzymes were exposed to various PFASs. PFOS and PFOSK inhibited rat 3β-HSD activity with IC_{50} of 1.35 ± 0.05 and 1.77 ± 0.04 μM, respectively, whereas PFHxSK and PFBSK had no effect at concentrations up to 250 μM. All chemicals tested weakly inhibited human 3β-HSD activity with IC_{50}s over 250 μM. On the other hand, PFOS, PFOSK and PFOA inhibited human 17β-HSD3 activity with IC_{50}s of 6.02 ± 1.02, 4.39 ± 0.46 and 127.60 ± 28.52 μM, respectively. The potencies for inhibition of 17β-HSD3 activity were determined to be PFOSK > PFOS > PFOA > PFHxSK = PFBSK for human 17β-HSD3 activity. There appears to be a species-dependent sensitivity to PFAS-mediated inhibition of enzyme activity because the IC_{50}s of PFOS(K) for inhibition of rat 17β-HSD3 activity was greater than 250 μM. In conclusion, the present study shows that PFOS and PFOSK are potent inhibitors of rat 3β-HSD and human 17β-HSD3 activity, and implies that inhibition of steroidogenic enzyme activity may be a contributing factor to the effects that PFASs exert on androgen secretion in the testis.

1. Introduction

Perfluoroalkylated substances (PFASs) are polyfluoro compounds that have been widely used in the manufacture of several products since the 1950s for their unique characteristics of extreme stability and surface activity. These chemicals are used as coatings of textiles, paper and upholstery and as reaction additives in various processes [1–3]. Some PFASs, including perfluorooctane acid (PFOA, 8-carbon perfluoroalkyl chain), perfluorooctane sulfonate (PFOS, 8-carbon perfluoroalkyl chain) and perfluorohexane sulfonate (PFHxS, 6-carbon perfluoroalkyl chain) have been classified as persistent organic pollutants in the environment and are present in tissues of the general population [4]. The levels of PFOA, PFOS and PFHxS measured in blood of human subjects are related to the exposure level and duration. For example, serum levels of PFOA, PFOS and PFHxS in the United States in 2006 averaged 3.4, 14.7 and 1.5 ng/ml, respectively [5]. However, blood levels may exceed 100 μg/ml in factory workers [2]. In particular,

☆ Supported in part by Population Council Fellowship (B.Z and G.X.H.) and Natural Science Foundation of China (30871434).
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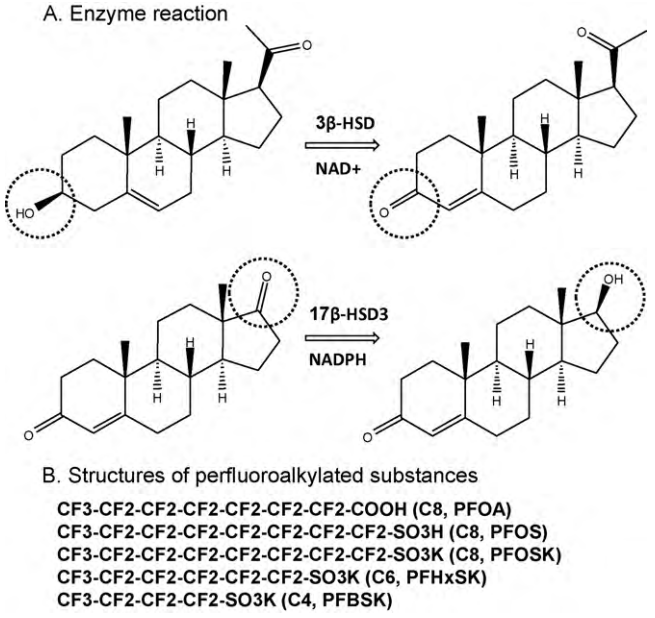
doi:10.1016/j.cbi.2010.07.001



Scheme 1. The reactions of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) and the structures of perfluoroalkylated substances (PFASs). (Panel A) The reactions of 3β-HSD and 17β-HSD3; (Panel B) structures of PFASs.

concerns have been raised regarding DuPont workers in China, whose blood PFOA levels increased to 2.25 μg/ml in just 1 year [6]. Due to long elimination *t*1/2 for these chemicals and potential persistent hazards, the 3M Company automatically phased out the production of PFOA and PFOS in 2000 [5]. Subsequently, another PFAS with short carbon chain perfluorobutane sulfonate (PFBS, 4-carbon perfluoroalkyl chain) was introduced to replace PFOA, PFOS and PFHxS compounds (Scheme 1). The serum elimination of PFBS is expected to be more rapid than that of PFOA or PFOS. It was reported recently that the *t*1/2 of PFBS was 3.1 h in male rats and about 10 h in monkeys [7]. There is growing evidence to show that PFASs may act as endocrine disruptors on reproductive system. Workers in 3M company in Cottage Groove that produced PFOA had higher level of PFOA and decreased serum testosterone concentrations (Minnesota, USA) [8,9]. Laboratory animal studies showed that rats exposed to PFOA and related chemicals had lower testosterone levels [10,11]. The effects of PFASs on androgen secretion in rodents may be associated with their interference with Leydig cell function. In one study, ammonium perfluorooctanoate acted directly in rat Leydig cells to inhibit hCG-stimulated testosterone production [10]. Leydig cells utilize cholesterol as a substrate to produce testosterone. The conversion of the cholesterol substrate into testosterone occurs in a series of reactions catalyzed by four enzymes: cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1), 3β-HSD, cytochrome P450 17α-hydroxylase/17-20 lyase (CYP17A1) and 17β-HSD3. Hydroxysteroid dehydrogenases (3β-HSD and 17β-HSD3) are localized to the smooth endoplasmic reticulum in Leydig cells. 3β-HSD catalyzes conversion of pregnenolone (PREG) into progesterone in the presence of the cofactor NAD^+, whereas 17β-HSD3 catalyzes conversion of androstenedione (DIONE) into testosterone in the presence of cofactor NADPH (Scheme 1). Exposure of adult rats to perfluorododecanoic acid, a PFAS, at 5 or 10 mg/kg body weight/day for 2 weeks inhibited gene expression for several proteins involved in androgen biosynthesis, including the cholesterol transport protein steroidogenic acute regulatory protein (*Star*), scavenger receptor class B member 1 (*Scarb1*), and the steroidogenic enzymes (*Cyp11a1*, *Hsd3b1*, *Cyp17a*1 and *Hsd17b3*) [11]. In our previous study, we determined that PFOA inhibited 3β-HSD and 17β-HSD3 in rat Leydig cells with the half maximal inhibitory concentration (IC_{50}) of 53.2 and 17.7 μM, respectively [12]. In the present study, we have compared the potencies of five PFASs for the inhibition of 3β-HSD and 17β-HSD3 activities in both human and rat testes.

2. Materials and methods

2.1. Materials

[1,2-^{3}H] androstenedione (^{3}H-DIONE), [^{3}H] pregnenolone (^{3}H-PREG), specific activity, 40 Ci/mmol were purchased from Dupont-New England Nuclear (Boston, MA). Unlabeled DIONE, PREG, progesterone and testosterone were purchased from Steraloids (Newport, RI). PFOA, PFOS, and the potassium salts of PFHxS (PFHxSK), PFOS (PFOSK) and PFBS (PFBSK) were purchased from Sigma–Aldrich (St. Louis, MO). PFASs were dissolved in dimethyl sulfoxide (DMSO). 90-day-old male Sprague–Dawley rats (250–300 g) were purchased from Charles River Laboratories (Wilmington, MA). All animal procedures were approved by the Rockefeller University's Animal Care and Use Committee (protocol #07080). Human testis samples were obtained from Wenzhou Medical College Affiliated Hospitals (Wenzhou, China). These testis biopsy samples were mixed testis samples from infertile patients with normal Leydig cell function or testosterone parameters. The use of human testis samples was approved by the Ethics Committee of Wenzhou Medical College.

2.2. Preparation of microsomal protein

Microsomal preparations of human and rat testes were done as described previously [13]. In brief, testes were homogenized in cold 0.01 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 mM sucrose and centrifuged at 700 × *g* for 30 min at 4C to remove cellular fragments. The supernatants were transferred to new tubes and were centrifuged at 10,000 × *g* for 30 min at 4C to remove mitochondria. The supernatants were centrifuged twice at 105,000 × *g* for 1 h at 4 °C to collect microsomal pellets. Pellets were resuspended in order to measure protein contents. The protein concentrations of samples were measured using the Bio-Rad Protein Assay Kit (cat# 500-0006, Bio-Rad, Hercules, CA) according to manufacturer's protocol. Microsomal protein concentrations were adjusted to 4 mg/ml and used for measurement of 3β-HSD and 17β-HSD3 activities.

2.3. 3β-HSD activity assay

3β-HSD activity in testis microsomes was measured as described previously [13]. In brief, 3β-HSD activity assay tubes contained 0.2 μM PREG plus 40,000 cpm [^{3}H] PREG and 0.2 mM NAD^+ in 250 μl 0.5% Tween-20 PBS. PFASs were dissolved in DMSO, and the final DMSO concentration was 0.5%, which had no effects on 3β-HSD activity. We determined the linearity of reactions using different concentrations of rat and human microsomes for 3β-HSD activity. The 30 min reactions were initiated by addition of 20 μg rat or 90 μg human testis microsome in the presence of different concentrations of PFASs to determine IC_{50}. To determine the inhibitory mode of PFASs, different concentrations of PREG (0.002–10 μM) plus 0.2 mM NAD^+ was added into reaction mixtures (0.5% Tween-20 PBS buffer) containing 20 μg rat or 90 μg human testis microsome and PFASs (different concentrations). To determine whether the inhibitory mode of PFASs occurred by competing with NAD^+, different concentrations of NAD^+ concentrations (0.002–100 μM) plus 0.2 μM PREG were added into 0.5% Tween-20 PBS reaction mixture containing 20 μg rat testis microsome and

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PFASs (various concentrations). A preliminary experiment was performed to determine the velocity of 3β-HSD activity within the linear range in the above condition. The reactions were stopped by adding 2 ml ice cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. Steroids were separated chromatographically on thin layer plates in chloroform and methanol (97:3, v/v), and radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of PREG to progesterone was calculated by dividing the radioactive counts identified as progesterone by the total counts associated with PREG plus progesterone.

2.4. 17β-HSD3 assay

17β-HSD3 activity in testicular microsomes was measured using our usual procedures [13]. In brief, 17β-HSD3 activity assay tubes contained 0.2 μM DIONE plus 40,000 cpm [^{3}H] DIONE and 0.2 mM NADPH in 250 μl 0.5 Tween-20 PBS. 17β-HSD3 is a reductase which uses NADPH as cofactor (see Scheme 1). PFASs were dissolved in DMSO, and the final DMSO concentration was 0.5%, which had no effects on 17β-HSD3 activity. We determined the linear reaction using different concentrations of rat and human microsomes for 17β-HSD3 activity. The 90 min reactions were initiated by the addition of 20 μg rat or 90 μg human testis microsomes in presence of varying concentrations of PFASs to determine the IC_{50}. To determine the inhibitory mode of PFASs, different concentrations of DIONE (0.002–10 μM) plus 0.2 mM NADPH was added into reaction mixtures (0.5% Tween-20 PBS buffer) containing 20 μg rat and 90 μg human testis microsomes and PFASs (different concentrations). To determine whether the inhibitory mode of PFASs was through competing with NADPH, different concentrations of NADPH concentrations (0.002–100 μM) plus 0.2 μM DIONE was added into 0.5% Tween-20 PBS reaction mixtures containing 20 μg rat and 90 μg human testis microsomes and PFASs (various concentrations). A preliminary experiment was performed to determine the velocity of 17β-HSD3 activity within the linear range in the above condition. The reactions were stopped by adding 2 ml ice cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (97:3, v/v), and radioactivity was measured using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC). The percentage conversion of DIONE to testosterone was calculated by dividing the radioactive counts identified as testosterone by the total counts associated with DIONE plus testosterone.

2.5. Statistics

Assays were repeated twice to four times. The IC_{50} was calculated using GraphPad version 4.0 (GraphPad Software Inc., San Deigo, CA) using nonlinear regression of curve fit with one-site competition. Lineweaver–Burk plot was used to determine the mode of inhibition. Data were subjected to analysis by one-way ANOVA followed by DUNCAN multiple comparison testing to identify significant differences between groups when three and more groups were calculated. All data are expressed as means ± SEM. Differences were regarded as significant at $P < 0.05$.

3. Results

3.1. Effects of PFASs on 3β-HSD activity

3β-HSD activity, which catalyzes the conversion of PREG to progesterone, was measured in human and rat testis microsomes in the presence of 250 μM PFASs (Fig. 1). This highest concentration was selected based on the report that the highest serum

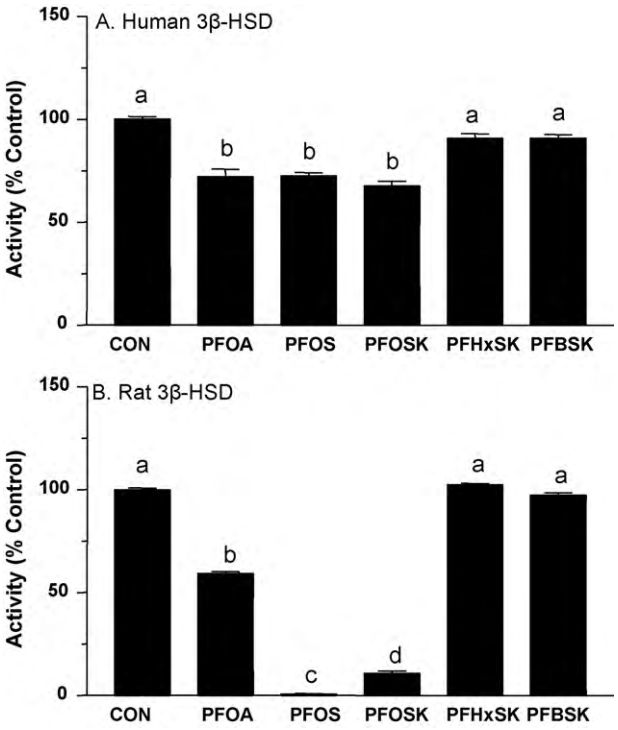

Fig. 1. The inhibition of perfluoroalkylated substances (PFASs) on human and rat 3β-hydroxysteroid dehydrogenase (3β-HSD) activities in testicular microsome. 3β-HSD activity was measured by the conversion of 0.2 μM PREG to progesterone in the presence of 250 μM PFASs. Mean ± SEM ($n = 4$). Similar letters show no significant difference between two groups at $P < 0.05$.

PFOA level was 114.1 mg/l (~250 μM) in occupational workers [14]. The activities for human and rat 3β-HSD without inhibitors were 12.54 ± 0.48 (Mean ± SEM, $n = 4$) and 72.91 ± 0.78 nmol/mg protein min, respectively. However, PFOA, PFOS and PFOSK inhibited human 3β-HSD activity by 25% compared to control but PFHxSK and PFBSK had no effect (Fig. 1A). On the other hand, PFOA, PFOSK and PFOS were more potent inhibitors of rat 3β-HSD activity and inhibited enzyme activity by 40, 75 and 98% compared to control, respectively (Fig. 1B). The IC_{50}s were determined for PFASs for the inhibition of human and rat enzymes. As shown in Table 1 and Fig. 2, the IC_{50}s for all PFASs tested for the inhibition of human 3β-HSD activity were >250 μM, while IC_{50}s of PFOS and PFOSK for the inhibition of enzyme activity were 1.35 ± 0.05 and 1.77 ± 0.04 μM, respectively. Thus, PFOS and PFOSK were more potent than PFOA, which had a previously reported IC_{50} of 53.2 μM for the 3β-HSD activity [12]. There was a clear structure-dependent inhibition by PFASs for rat 3β-HSD activity, which was determined to be PFOS(K) > PFOA > PFHxSK = PFBSK. The PFOS(K) compound, which has 8 carbon atoms plus a sulfur atom, was the most potent

Table 1
IC_{50}s of PFASs inhibiting human and rat 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) in testicular microsome.

IC_{50}s (μM)	Human		Rat	
	3β-HSD	17β-HSD3	3β-HSD	17β-HSD3
PFOA	>250	127.60 ± 28.52	53.2 ± 25.9[a]	17.7 ± 6.8[a]
PFOS	>250	6.02 ± 1.02	1.35 ± 0.05	>250
PFOSK	>250	4.39 ± 0.46	1.77 ± 0.04	>250
PFHxSK	NI[b]	NI[b]	NI[b]	NI[b]
PFBSK	NI[b]	NI[b]	NI[b]	NI[b]

[a] See Ref. [12].
[b] NI, no inhibition at 250 μM of PFASs.

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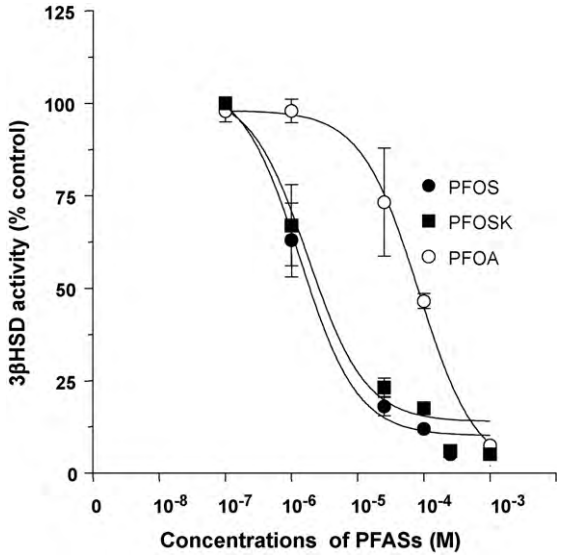

Fig. 2. Dose-dependent inhibition of perfluoroalkylated substances (PFASs) on rat 3β-hydroxysteroid dehydrogenase (3β-HSD) activities in testicular microsome. 3β-HSD activity was measured by the conversion of 0.2 μM PREG to progesterone in the presence of different concentrations of PFASs. Mean ± SEM (n = 4).

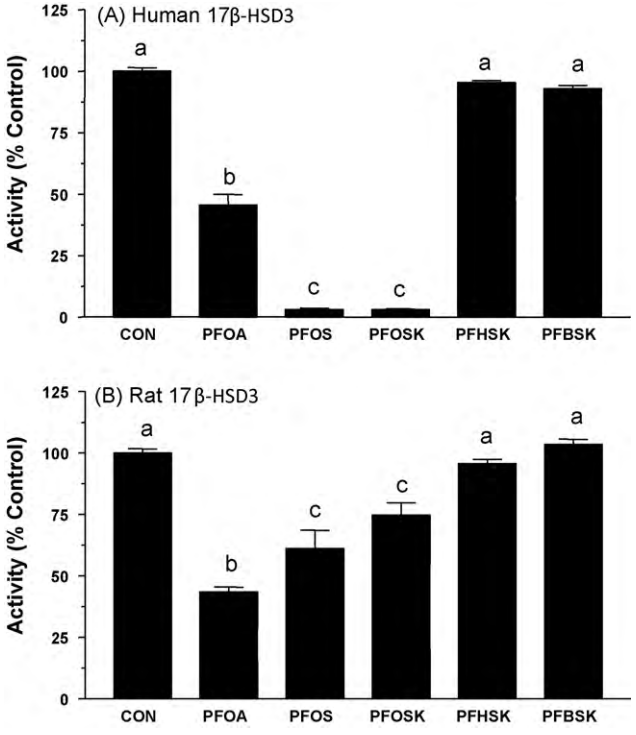

Fig. 3. The inhibition of perfluoroalkylated substances (PFASs) on human and rat 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in testicular microsome. 17β-HSD3 activity was measured by the conversion of 0.2 μM DIONE to testosterone in the presence of 250 μM PFASs. Mean ± SEM (n = 4). Similar letters show no significant difference between two groups at $P < 0.05$.

inhibitor of rat 3β-HSD activity. In contrast, PFASs with shorter carbon chains, including PFHxSK (C6) and PFBSK (C4), showed no inhibitory effect even at concentrations as high as 250 μM. The data show that there are species- and structure-dependent inhibitions of 3β-HSD activity.

3.2. Effects of PFASs on 17β-HSD3 activity

Activity of the 17β-HSD3 enzyme, which catalyzes the conversion of DIONE to testosterone, was measured in human and rat testis microsomes in the presence of 250 μM of each PFAS (Fig. 3). The activities of the human and rat 17β-HSD3 enzymes in the absence of inhibitors were determined to be 6.23 ± 0.09 and 11.16 ± 0.11 nmol/mg protein min, respectively. PFOS and PFOSK each inhibited human 17β-HSD3 activity by 95%, and PFOA inhibited activity by 60% relative to control, but PFHxSK and PFBSK had no effect (Fig. 3A). In addition, PFOA, PFOS and PFOSK inhibited the rat 17β-HSD3 enzyme by 60, 40 and 25% compared to control but PFHxSK and PFBSK showed no effect (Fig. 3B). The IC_{50}s for the inhibition of human and rat 17β-HSD3 activity by PFASs are described in Fig. 4. As shown in Table 1, the IC_{50} for PFOS, PFOSK and PFOA in the inhibition of human 17β-HSD3 activity were determined to be 6.02 ± 1.02, 4.39 ± 0.46 and 127.60 ± 28.52 whereas the IC_{50} of PFOSK and PFOS in the inhibition of rat 17β-HSD3 activity were >250 μM, implying less potency than for PFOA (17 μM) as previously reported [12]. As for the 3β-HSD enzyme, there appeared to be a structure-dependent pattern for the inhibition of human 17β-HSD3 activity by PFASs, which was determined to be PFOS(K) > PFOA > PFHxSK = PFBSK. The PFOS(K) compound with 8 carbon atoms plus a sulfur atom was the most potent inhibitor, whereas compounds with shorter carbon chains, including PFHxSK (C6) and PFBSK (C4), had no effects on 17β-HSD3 activity even at concentrations as high as 250 μM.

3.3. The modes of inhibition of PFASs on 3β-HSD and 17β-HSD3 activities

The modes of PFOS or PFOSK-mediated inhibition of rat 3β-HSD activity were investigated by enzyme kinetic analysis. When different concentrations of PREG were used, the Lineweaver–Burk plot analysis showed that PFOS was a competitive inhibitor for 3β-HSD activity (Fig. 5A). Furthermore, when different concentrations of cofactor NAD^+ were used, data showed that PFOS noncompetitively inhibited cofactor-mediated action (Fig. 5B). PFOSK was also a noncompetitive inhibitor for the cofactor (data not shown). The noncompetitive inhibition of rat 3β-HSD activity by PFOA was previously reported [12].

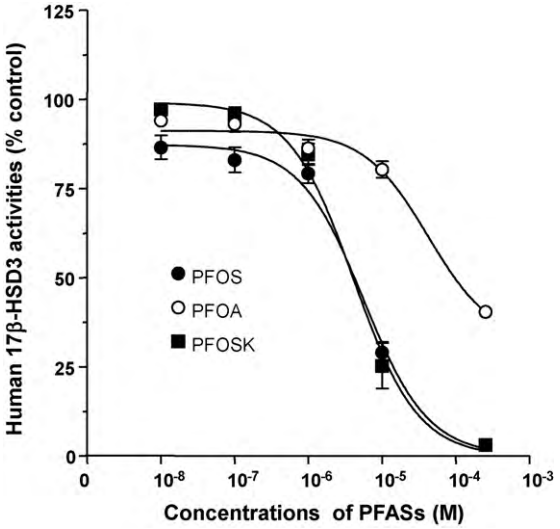

Fig. 4. Dose-dependent inhibition of perfluoroalkylated substances (PFASs) on human 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in testicular microsome. 17β-HSD3 activity was measured by the conversion of 0.2 μM DIONE to testosterone in the presence of different concentrations of PFASs. Mean ± SEM (n = 4).

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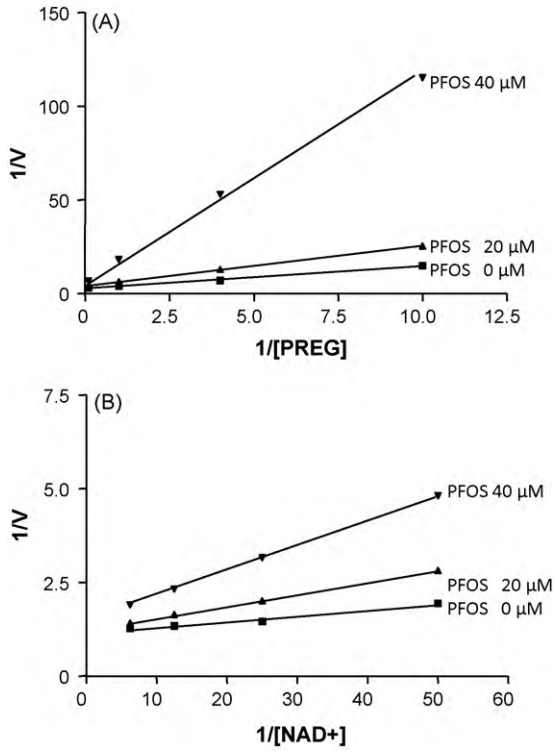

Fig. 5. Lineweaver–Burk plot rat testis microsomal 3β-hydroxysteroid dehydrogenase (3β-HSD) in the presence of PFOS. Panel A shows 1/V versus 1/[PREG], and Panel B shows 1/V versus 1/[NAD+]. V, velocity (nmol/min mg protein); [PREG], concentrations of pregnenolone. 3β-HSD was measured by the conversion of PREG to progesterone. Mean ± SEM ($n = 4$). It showed the competitive inhibition on 3β-HSD for substrate PREG and noncompetitive inhibition for NAD^+ by PFOS.

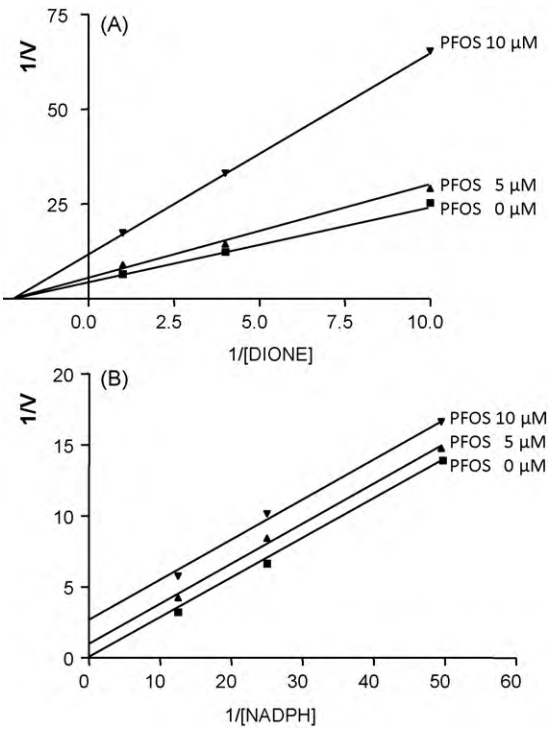

Fig. 6. Lineweaver–Burk plot human testis microsomal 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) in the presence of PFOS. Panel A shows 1/V versus 1/[DIONE], and Panel B shows 1/V versus $1/[NADPH]^+$. V, velocity (nmol/min mg protein); [DIONE], concentrations of androstenedione. 17β-HSD3 was measured by the conversion of DIONE to testosterone. Mean ± SEM ($n = 4$). It showed the noncompetitive inhibition on 17β-HSD3 for substrate DIONE and uncompetitive inhibition for NADPH by PFOS.

The modes of PFOS or PFOSK-mediated inhibition of human 17β-HSD3 activity were also assessed by enzyme kinetic analysis. When different concentrations of DIONE were used, the Lineweaver–Burk plot analysis showed that PFOS was a noncompetitive inhibitor for human 17β-HSD3 activity (Fig. 6A). When different concentrations of cofactor NADPH were used (Fig. 6B), data analysis showed that PFOS uncompetitively inhibited cofactor-mediated action (Fig. 6B). PFOA and PFOSK had similar modes of inhibition with noncompetitive inhibition on substrate DIONE binding site and uncompetitive inhibition on cofactor NADPH binding site of human 17β-HSD3 activity (data not shown). The mode of PFOA inhibition of human 17β-HSD3 activity differs from that previously reported for the rat enzyme in that PFOA inhibited rat 17β-HSD3 activity with competitive inhibition on substrate DIONE binding site and noncompetitive inhibition on cofactor NADPH binding site of the enzyme [12].

4. Discussion

The present study demonstrates that PFASs have the capacity to cause direct inhibition of steroidogenic enzyme activity in the testis. In particular, PFOS and PFOSK were more potent to inhibit human 17β-HSD3 activity (IC_{50}: 4–6 μM) compared to the 3β-HSD activity (IC_{50}: >250 μM). The 3β-HSD and 17β-HSD3 enzymes are almost exclusively expressed in Leydig cells and inhibition of both enzymes potentially adversely affects testosterone production.

The potencies of inhibition of the rat 3β-HSD activity were: PFOSK = PFOS > PFOA > PFHxSK = PFBSK. The PFASs with shorter carbon numbers including PFHxSK and PFBSK were ineffective to inhibit the enzyme even at high concentrations. However, it was interesting to note that although they contain the same carbon numbers, PFOS(K) was 47–62-fold more potent than PFOA for inhibition of rat 3β-HSD activity. This difference could be contributed by the longer chain of PFOS(K), which has eight carbon atoms plus a sulfur atom. Interestingly, none of these chemicals caused any inhibition of human 3β-HSD activity, suggesting that there are species differences in enzyme inhibition capacity. Clearly, further studies to elucidate the structure activity relationships of these chemicals are warranted. Similarly, the potencies of inhibition of the human 17β-HSD3 activity were: PFOSK = PFOS > PFOA > PFHxSK = PFBSK. The PFASs with fewer carbon atoms including PFHxSK and PFBSK showed no inhibitory effect on enzyme activity even at high concentrations Interestingly, PFOS(K) was over 20-fold more potent than PFOA to inhibit human 17β-HSD3. This observation is probably related to the longer chain of PFOS(K). On the other hand, PFOS(K) only marginally inhibited rat 17β-HSD3 activity, while PFOA was a most potent inhibitor. Structure-dependent activity of PFASs has also been reported regarding their transcriptional activation of peroxisome proliferation in the liver [15]. The present study showed that PFASs caused direct inhibition of enzyme activity but it is also possible that PFASs regulate testis function by causing changes in transcriptional activity affecting steroidogenic enzyme gene expression.

The mode of PFOS(K) in the inhibition of rat 3β-HSD activity was competitive. To examine whether PFOS(K) competes with cofactor NAD^+ for 3β-HSD, we assayed different concentrations of NAD^+ in the presence of various concentrations of PFOS(K). The data showed that PFOS(K) was noncompetitive with NAD^+ for the inhibition of rat 3β-HSD activity. This mode of inhibition by PFOS(K) is similar to that by PFOA [12]. Therefore, we speculate that PFOS(K) blocks the binding of PREG to the active site of the substrate on 3β-HSD and does not compete with the NAD^+ or cofactor binding site.

Similarly, the mode of PFOS(K) and PFOA in the inhibition of human 17β-HSD3 activity was noncompetitive. We assayed

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human 17β-HSD3 activity with different concentrations of cofactor NADPH in the presence of various concentrations of PFOS(K). Results showed that PFOS(K) and PFOA showed an uncompetitive mode of inhibition for NADPH (Fig. 6). However, this mode of inhibition by PFOS(K) is dissimilar to that due to PFOA for the rat 17β-HSD3 enzyme [12].

Apparently, the IC_{50}s for the inhibition of 17β-HSD3 activity by PFOS(K) were within the low micromolar range and are higher than average serum PFOS levels, which are in the nanomolar range in the United States have the capacity to interfere with steroidogenic enzyme activity and androgen secretion in the testis. However, PFOSK at 10 nM exerted significant inhibition on human 17β-HSD3 activity (Fig. 4). This observation is also relevant to concerns for occupational workers serum PFOA levels averaging 2.25 μg/ml, which is equivalent to 5.5 μM [16]. Indeed, PFOS(K) and PFOA may reach 250 μM in some occupational workers [14]. The adverse effects of PFASs on males include the effects on the development of male reproductive tract in fetus [17,18], initiation of puberty, and supporting spermatogenesis in the adult [19]. In a recent human study, Joensen et al. [20] analyzed serum samples for the levels of ten different PFASs and reproductive hormones, and assessed semen quality in 105 Danish men from the general population High serum PFOS (14.2–42.1 ng/ml) and PFOA (2.7–7.2 ng/ml) levels were associated with impairment of spermatogenesis. Decreased testosterone concentrations were also measured in highly exposed 3M workers at a plant producing PFOA in Cottage Groove (Minnesota, USA) [8,9]. It has been suggested that these levels may predispose exposed workers to testicular cancers [9]. The data from exposed workers are supported by results of laboratory studies. For example, rats exposed to PFOA and related chemicals had lower testosterone levels [10,11]. The effects of PFASs on androgen secretion in rodents were also associated with Leydig cell hyperplasia and [10] and down-regulation of CYP11A1 [11]. The present study confirmed that some of PFASs can potently inhibit steroidogenic enzymes, 3β-HSD and 17β-HSD3 activities, thus leading to possibly lower testosterone synthesis. However, caution has to be exercised in order to extrapolate data from rat models to the population due to significant differences in species sensitivity to different PFASs.

In conclusion, the present study demonstrated species- and structure-dependent relationships in the capacity for PFASs to disrupt steroidogenic enzyme activity. Suppression of steroidogenic enzyme activity is likely a contributor to adverse effects of PFASs on testis function. Additional studies are required to investigate the consequences of both short- and long-term exposures of factory workers to PFASs.

Conflict of interest statement

None.

Acknowledgements

We thank Chantal M. Sottas for technical support. We also thank Dr. Dianne O. Hardy for critical comments on the draft manuscript.

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