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Dithioerythritol (DTE) prevents inhibitory effects of triphenyltin (TPT) on the key enzymes of the human sex steroid hormone metabolism

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This work is dedicated to Prof. Dr. Frank Bidlingmaier on the occasion of his 65th birthday.

Abstract

Organotins are known to induce imposex (pseudohermaphroditism) in marine neogastropods and are suggested to act as specific endocrine disruptors, inhibiting the enzyme-mediated conversion of steroid hormones. Therefore, we investigated the in vitro effects of triphenyltin (TPT) on human 5α-reductase type 2 (5α-Re 2), cytochrome P450 aromatase (P450arom), 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD 3), 3β-HSD type 2 and 17β-HSD type 1 activity. First, the present study demonstrates that significant amounts of TPT occurred in the blood of eight human volunteers (0.17–0.67 μg organotin cation/l, i.e. 0.49–1.92 nmol cation/l). Second, TPT showed variable inhibitory effects on all the enzymes investigated. The mean IC_{50} values were 0.95 μM for 5α-Re 2 (mean of $n = 4$ experiments), 1.5 μM for P450arom ($n = 5$), 4.0 μM for 3β-HSD 2 ($n = 1$), 4.2 μM for 17β-HSD 3 ($n = 3$) and 10.5 μM for 17β-HSD 1 ($n = 3$). To exclude the possibility that the impacts of TPT are mediated by oxidizing essential thiol residues of the enzymes, the putative compensatory effects of the reducing agent dithioerythritol (DTE) were investigated. Co-incubation with DTE ($n = 3$) resulted in dose-response prevention of the inhibitory effects of 100 μM deleterious TPT concentrations on 17β-HSD 3 (EC_{50} value of 12.9 mM; mean of $n = 3$ experiments), 3β-HSD 2 (0.90 mM; $n = 3$), P450arom (0.91 mM; $n = 3$) and 17β-HSD 1 (0.21 mM; $n = 3$) activity. With these enzymes, the use of 10 mM DTE resulted in an at least 80% antagonistic effect, whereas, the effect of TPT on 5α-Re 2 was not compensated. In conclusion, the present study shows that TPT acts as an unspecific, but significant inhibitor of human sex steroid hormone metabolism and suggests that the inhibitory effects are mediated by the interaction of TPT with critical cysteine residues of the enzymes.

Keywords: Triphenyltin; Dithioerythritol; Plasticizers

1. Introduction

Organotin compounds are widely used as unselective biocides for pest control. Applications include a variety of industrial products like preservatives, antifouling paints and stabilisers but also everyday materials like sports wear, plastic gloves and silicon baking parchment [1–3]. Tributyltin (TBT) and triphenyltin (TPT) additives in coatings for marine vessels prevent sessile animals, which need to adhere to a substrate during their life cycle, to settle down. These substances are permanently leaching into the aquatic environment [4]. Photochemical and biochemical influences lead to successive degradation of organotins and TPT disintegrates into diphenyltin, monophenyltin and inorganic tin [4]. However, organotins stored in sediments are stable up to several years. This is a potential threat to aquatic life as a consequence of natural resuspension and particulate consumption by benthic organisms which live in seabeds and riverbeds [5,6]. TPT compounds are rather selective in their action against fungal species, demonstrating a low risk for fungal resistance, a low volatility and a relatively rapid disintegration to “non-toxic” compounds by sunlight. Consequently, they were utilised for pest and fungal plant pathogen control [4]. Since August 2002, the use of TPT acetate and TPT hydroxide has been banned within the European Union.

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The extensive use of organotins as biocides leads to an ongoing contamination of aquatic and terrestic environment. In the aquatic environment, a strong food chain accumulation of organotins has been noticed [7]. Examination of marine vertebrates showed considerable concentrations in liver and kidney, as well as in hair, nails and feathers [8]. Terrestic animals and humans incorporate organotins by consumption of contaminated food and surface water, or by direct contact with contaminated products. In previous studies, reasonable butyltin concentrations were detected in all of the human liver and blood samples investigated [9].

Both TBT and TPT are reported to affect the immune system, the nervous system and the hormone system as well as embryogenesis [10–12]. TBT is known to act as an endocrine disruptor causing imposex in various female prosobranch snails. Investigations showed that incorporated TBT induced an increased testosterone/estradiol ratio in the snails suggesting that TBT inhibits cytochrome P450 aromatase (P450arom) [1]. Previously, TBT and TPT were shown to act as inhibitors of human P450arom activity [13,14]. As a consequence, most developed nations have imposed a ban on TBT-based antifouling paints for vessels under 25 m in length since the late 1980s [15]. Other triorganic tin compounds, such as TPT, have recently been found to likewise cause imposex [16]. Hence, TPT should similarly be considered as an endocrine disruptor. The release of environmental chemicals, such as pesticides, detergents and plasticizers, are suggested to play a role in the observed increased incidence of male reproductive disorders.

The enzymes examined in the present study maintain the proper balance of androgens and estrogens in the human body. 3β-HSD converts Δ^5-3β-hydroxysteroids into the corresponding Δ^4-3-ketosteroids [17]. 17β-HSD 3 catalyses the testicular conversion of the weak androgen Δ_4-androstendione into the strong androgen testosterone. Testosterone is the most abundant androgen in the male sex steroid hormone system [18]. Dihydrotestosterone is synthesized from testosterone via 5α-Re 2 activity [18]. It represents the most potent androgen naturally occurring [18] and is indispensable for the normal virilization of the male external genitalia and prostate [19]. P450arom is responsible for the conversion of C_{19} androgens into the corresponding C_{18} estrogens in a variety of tissues, including the ovary, testis, placenta, brain and adipose tissue [20,21]. 17β-HSD 1 predominantly catalyses the conversion of the weak estrogen estrone into the strong estrogen 17β-estradiol [22].

Organotins possess both lipophilic and ionic properties. The former encourages their accumulation in lipids and their membrane toxicity, while the latter enables their binding to macromolecules [8,23]. The biochemical effects of organotins on human sex steroid hormone metabolism remain to be elucidated. Therefore, we studied the inhibitory effects of TPT on the in vitro activity of the key enzymes of human sex steroid hormone metabolism using human tissue samples. In several experiments, it was demonstrated that sulfhydryl compounds antagonise the harmful effects of organotins [24–27]. It is suggested that organotins interact with any thiol residues accessible, thus, the sulfhydryl antagonist prevents modification of the tertiary structure of the proteins [24–27]. Therefore, we also investigated the in vitro effects of the reducing agent dithioerythritol (DTE) on TPT inhibited enzyme activities. To elucidate the potential risk of TPT evoked endocrine disruption in man, the content of a variety of organotin compounds was determined in blood samples of eight healthy adult human volunteers.

2. Materials and methods

2.1. Steroids and other chemicals

[1β-^{3}H]-androstenedione (25.9 Ci/mmol), [4-^{14}C]-estrone (51.3 mCi/mmol) and [4-^{14}C]-dehydroepiandrosterone (53.8 mCi/mmol) were obtained from New England Nuclear Co. (Dreieich, Germany) and purified by thin layer chromatography (TLC) prior to use. Non-radioactive reference steroids (5α-androstane-3,17-dione (androstanedione), androst-4-ene-3,17-dione (androstenedione), 5α-androstane-17β-ol-3-one (dihydrotestosterone), 5α-androstane-3α-ol-17-one (androsterone), androst-4-ene-17β-ol-3-one (testosterone) and 5α-androstane-3α,17β-diol (3α-androstanediol), [10]-estratriene-3α-ol-17-one (estrone), [10]-estratriene-3α,17β-diol (17β-estradiol), androst-5-ene-3β-ol-17-one (DHEA), androst-5-ene-3β,17β-diol (androstenediol)), EDTA, Folin & Ciocalteu's phenol reagent, TRIZMA^{TM} (a, a,a-tris-(hydroxymethyl)-methylamin), TRIZMA^{TM}–HCl, citric acid, sodium potassium tartrat, activated charcoal (Niorit A) and dithioerythritol were purchased from Sigma^{TM} Chemical Company (Deisenhofen, Germany). Atamestane was provided by Schering (Berlin, Germany). Finasteride was purchased from MSD Sharp & Dohme (Haar, Germany). Dextran T-710 was obtained from Pharmacia Biotech (Uppsala, Sweden). The liquid scintillation cocktail, Ultima Gold^{TM}, was obtained from Packard-Instrument, B.V., Chemical Operations (Groningen, The Netherlands). NADPH was purchased from Roche (Mannheim, Germany). Triphenyltin chloride and all other chemicals were purchased from Merck A.G. (Darmstadt, Germany). All chemicals were purchased at the highest grade commercially available.

2.2. Buffers

Homogenisation buffer contained 10 mmol/l TRIZMA^{TM}–HCl and 1 mM EDTA at pH 7.4. Assay buffer consisted of 160 mmol/l TRIZMA^{TM}–citrate and 10 mM $MgCl_2$ and was adjusted to the indicated pH values.

2.3. Source and preparation of tissues

Human term placenta (cytosolic 17β-HSD 1 activity; microsomal P450arom activity) was obtained following

caesarean section. Macroscopically normal testicular tissue (microsomal 17β-HSD 3 activity) was obtained from patients with testicular germ cell tumour undergoing orchiectomy, macroscopically normal prostate tissue (microsomal 5α-Re 2 activity) was obtained from patients with bladder cancer undergoing cystectomy and prostatectomy. Macroscopically normal adrenal tissue (microsomal 3β-HSD 2 activity) was obtained from patients with kidney cancer undergoing nephrectomy. All utilized human tissue samples were immediately frozen in liquid nitrogen after removal and stored at −80 °C until further processing. The study was approved by the local ethics committee and informed consent from all tissue donors or their family members had been obtained.

All steps of tissue preparation were carried out at 4 °C. According to the enzyme content, 25 to 200 mg tissue wet weight were homogenized and centrifuged as described previously [21]. The cell-free supernatants were used as microsomal preparations and stored in liquid nitrogen until utilization in the experiments.

To prepare a cytosolic fraction for the 17β-HSD 1 assay the cell-free placental homogenate was further centrifuged at 100,000 × *g* for 60 min. The obtained soluble supernatant was stored in liquid nitrogen until utilisation in the experiments. Aliquots of all tissue preparations were removed for protein determination [28]. Experiments were conducted in the linear range of protein content versus enzyme activity.

2.4. Incubation procedures

The in vitro activities of P450arom, 3β-HSD 2, 5α-Re 2, 17β-HSD 1 and 17β-HSD 3 were determined with some modifications according to methods described previously [29–31]. Briefly, solutions of the substrates were prepared in assay buffer. The measurement of enzyme activity was conducted with 1 μM final concentration of [4-^{14}C]-DHEA (3β-HSD 2), with 0.5 μM final concentration of [4-^{14}C]-estrone (17β-HSD 1) and 0.1 μM final concentration of [1β-^{3}H]-androstenedione (17β-HSD 3, 5α-Re 2 and P450arom). Stock solutions of TPT were prepared in ethanol. The assay buffer was adjusted to pH 7.5 for the measurement of the activity of P450arom, 3β-HSD 2, 17β-HSD 1 and 17β-HSD 3 and to pH 5.5 for the measurement of 5α-Re 2 activity.

The reaction mixture contained 50 μl assay buffer (containing DTE concentrations of 0–10 mM in the DTE experiments), 50 μl of the tissue preparation (or 50 μl homogenisation buffer for tissue-less control incubations) and 3 μl ethanol containing TPT at the indicated concentrations. To achieve equilibrium of the substances in the tissue preparation, the preliminary reaction mixture was preincubated for 5 min with constant shaking at 37 °C. Preincubation was stopped by chilling. Then, 50 μl assay buffer containing the substrate was added to the reaction mixture. The reactions were started by the addition of another 50 μl homogenisation buffer containing 3 mM final concentration of the required cofactor (NAD for measurement of 3β-HSD 2 activity and NADPH for measurement of P450arom, 17β-HSD 1, 17β-HSD 3 and 5α-Re 2 activity). All incubations were performed in duplicate. Reaction tubes were capped, vortexed and incubated for 30 min with constant shaking at 37 °C. Reactions were stopped by chilling.

2.5. Product isolation assay

Measurement of 3β-HSD 2, 5α-Re 2, 17β-HSD 1 and 17β-HSD 3 in vitro activity based on product isolation post incubation according to methods described previously [29–31]. Briefly, steroids were extracted from the incubation mixtures, 100 μl of the tritium containing organic phase or 300 μl of the ^{14}C containing organic phase were evaporated to dryness and redissolved in a mixture of 35 μl chloroform and 15 μl ethanol containing 25 μg each of non-radioactive reference steroids.

Metabolites were separated by TLC. The silica sheets were dried and stained by spraying with a mixture of acetic acid:H_2SO_4:4-methoxybenzaldehyde (100:2:1, v/v/v) and charred at 135 °C. Within each lane, the zones corresponding to the stained reference steroids were cut out and transferred into counting vials containing 15 ml liquid scintillation cocktail. Radioactivity was counted as automatically quench-corrected dpm with a Wallac 1409 liquid scintillation counter (Turku, Finland).

The relative amount of each corresponding radioactive steroid was calculated, in percentage, with the total radioactivity recovered from a single TLC lane set as 100%. Blank values were subtracted from tissue metabolism rates. Enzyme activity was assessed by quantifying the formation of radioactive labelled products.

2.6. Tritiated water-release assay

Measurement of P450arom in vitro activity based on the proportional release of 1β-^{3}H from [1β-^{3}H]-androstenedione into $^{3}H_2O$ during the P450arom catalysed reaction [21]. In brief, organic compounds were extracted from incubation mixtures by adding ice-cold chloroform. 350 μl of the aqueous phase was stripped from remaining steroids with 5% dextran-coated charcoal and 1.5 ml chloroform. 250 μl of the highly purified aqueous phase containing $^{3}H_2O$ was quantified as quench-corrected dpm by counting for 15 min using a Wallac 1409 liquid scintillation counter (Turku, Finland). The amount of $^{3}H_2O$ was corrected for dilution and the blank values were subtracted.

2.7. Determination of different organotin compounds in human blood

Determination of the concentration of monobutyltin, dibutyltin, tributyltin, tetrabutyltin, monooctyltin, dioctyltin and triphenyltin in the blood of eight human volunteers (Table 1) was conducted by GALAB (Geesthacht,

Table 1
The serum of eight human volunteers was analysed

	Gender							
	Female	Female	Male	Male	Male	Female	Female	Male
Profession	Student	Teacher	Civil engineer	Physician	Reporter	Physician	Student	Camera-man
Age (years)	18	50	54	53	54	31	27	41
BMI	25	24	27	22	25	21	20	24
Monobutyltin	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Dibutyltin	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Tributyltin	0.04	0.02	<0.02	0.02	<0.02	0.05	<0.02	<0.02
Tetrabutyltin	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Monooctyltin	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Dioctyltin	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Triphenyltin	0.32	0.67	0.17	0.32	0.23	0.35	0.18	0.23

The gender, age, profession and body mass index (BMI) of the subjects are listed. Organotincations were determined by GC–MIP–AED. The content is expressed as μg organotincation/l.

Germany) according to a "one unique extraction-derivatisation step" method described previously [32]. Blood was sampled in organotin-free glassware. The organotin compounds were extracted with tetra-methyl-ammonium-hydroxide and methanol, alkylated with sodium tetraethylborate and transferred by extraction with hexane into the organic phase. An aliquot of the hexane layer containing the tetrasubstituted organotincompounds was separated using capillary gas-chromatography (Perkin-Elmer GC 8400, Überlingen, Germany), and detecting and quantifying the organotins via atomic emission spectrometry (GC-AED from Hewlett-Packard, Agilent). According to GALAB, the recovery of the procedure was 75–100%. The chromatographic data were processed with a Perkin-Elmer Nelson 2600 software package. A column from ICT (Frankfurt, Germany) was used (DB 1701, length 30 m, i.d. 0.32 mm, 0.24 μm film thickness). The temperature programme of the GC was 80–280 °C at 30 °C/min. The flow rate of the helium carrier gas was 1.8 ml/min at 80 °C. For atomisation, a heated quartz furnace was used, with furnace gases hydrogen (120 ml/min) and air (35 ml/min). The temperature of the transfer line was constant at 250 °C; the pre-furnace temperature was 280 °C and the atomisation temperature 700 °C. The 286.3 nm tin line was generated by an electrode-less discharge lamp operated at 7 W.

2.8. Data analysis

Dose-response analyses were performed with a computer-assisted non-linear curve-fitting method using the linear dose versus effect model (FigP 2.7, Biosoft, Cambridge, UK). To calculate inhibitor concentration resulting in 50% inhibition (IC_{50} value) or the concentration of enhancer provoking a response halfway between baseline and maximum (EC_{50} value) the following equation was used.

$$V = V_{min} + \frac{V_{max} - V_{min}}{1 + (C/C_{50})^{-p}}$$

with V representing the initial velocity of enzyme activity, V_{min} and V_{max} representing minimum and maximum velocity, C representing the concentration of TPT or DTE and C_{50} resembling the IC_{50} value and the EC_{50} value, respectively.

3. Results

3.1. Determination of different organotin species in human blood

The blood of eight healthy human volunteers was analysed for the presence of different organotin compounds. As shown in Table 1, TPT is the major organotin compound found in human blood (0.17–0.67 μg organotin cation/l, i.e. 0.49–1.92 nmol cation/l). Furthermore, we were able to demonstrate the presence of minor concentrations of TBT in human blood, whereas the concentrations of monobutyltin, dibutyltin, tetrabutyltin, monooctyltin as well as dioctyltin were below the detection limit of 0.02 μg organotin cation/l.

3.2. Dose-response analyses of the inhibitory effects of TPT on the key enzymes of human sex steroid hormone metabolism

The putative inhibitory effects of TPT on the activity of key enzymes of human sex steroid hormone metabolism were investigated. For this purpose, incubations with TPT at various concentrations were carried out. Product formation in the absence of the inhibitor represented 100% enzyme activity. TPT demonstrated dose-response inhibitory effects on all the enzymes investigated. In Fig. 1A, the inhibition of 17β-HSD 1 activity is shown as an example. The average IC_{50} values for the different enzymes, calculated according to data analysis, are given in Table 2.

The investigation of the inhibition of 17β-HSD 1 activity by TPT revealed an IC_{50} value of 10.5 μM ($n = 3$ experiments; Fig. 1A). As shown in Table 2, TPT showed similar dose-responsive inhibitory effects on all the enzymes investigated with the lowest IC_{50} value of 0.95 μM for 5α-Re 2 (Fig. 2A). To compare the inhibitory effects of TPT on steroidal enzymes with those of specific inhibitors,

Table 2
Activity of the enzymes investigated corresponding to 100% non-inhibited enzyme activity in the absence or the presence of DTE

	3β-HSD 2	17β-HSD 3	17β-HSD 1	P450arom	5α-Re 2
Activity (nmol/h/mg protein)	2.876–28.994	0.013–0.020	3.393–11.455	0.202–0.318	0.028–0.131
Activity with 10 mM DTE (nmol/h/mg protein)	3.476–34.389	0.0451–0.278	2.327–21.435	0.294–0.420	0.013–0.034
IC_{50} (TPT, μM)	4.0 ($n = 1$ experiment)	4.2 ($n = 3$)	10.5 ($n = 3$)	1.5 ($n = 5$)	0.95 ($n = 4$)
EC_{50} (DTE, mM)	0.90 ($n = 3$)	12.9 ($n = 3$)	0.21 ($n = 3$)	0.91 ($n = 3$)	ND[a] ($n = 3$)

Moreover, the detected mean IC_{50} values of TPT and mean EC_{50} values of DTE are listed.
[a] ND: not detectable.

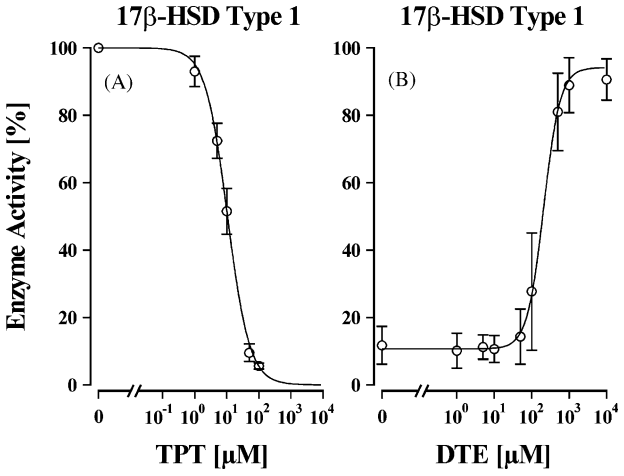

Fig. 1. The inhibitory effect of increasing TPT concentrations on human 17β-HSD 1 activity (A) were investigated using a cytosolic placental tissue preparations with 0.5 μM estrone and 3 mM NADPH at pH 7.5. The results represent mean ± S.D. values of $n = 3$ assays performed in duplicate as described in Section 2.3. The compensatory effect of increasing DTE concentrations (B) were determined at 100 μM deleterious TPT concentrations with 0.5 μM estrone as well as with 3 mM NADPH.

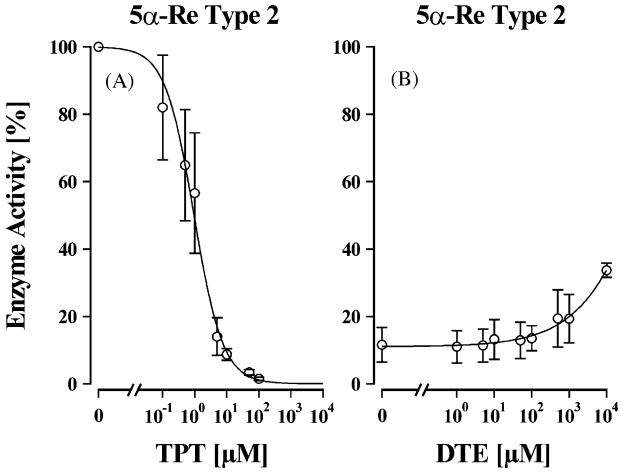

Fig. 2. The inhibitory effects of increasing TPT concentrations on human 5α-Re 2 activity (A) were investigated using a microsomal prostate tissue preparations with 0.1 μM androstenedione and 3 mM NADPH at pH 5.5. The results represent mean ± S.D. values of $n = 4$ (A) and $n = 3$ (B) assays performed in duplicate as described in the experimental section. The compensatory effects of increasing DTE concentrations (B) were determined at 100 μM deleterious TPT concentrations with 0.1 μM androstenedione and 3 mM NADPH.

we investigated the influence of atamestane on P450arom (Fig. 3A) and of MK906 (finasteride) on 5α-Re 2 activity (Fig. 3B). Atamestane and MK906 were used in a concentration range from 0 to 10 μM and computer-assisted non-linear curve-fitting analysis revealed an IC_{50} value of 0.11 μM for the inhibition of placental P450arom, whereas an IC_{50} value of 1.6 nM was observed for the inhibition of prostatic 5α-Re 2.

3.3. Compensatory effects of DTE on the TPT inhibited enzyme activity

Further experiments were conducted to examine the putative compensatory effects of DTE on the inhibitory effects of TPT. For this purpose, the reaction mixtures contained 100 μM TPT final concentration and varying DTE concentrations from 0 to 10 mM. Product formation in the absence of TPT and the presence of 10 mM DTE final concentration represented 100% enzyme activity. DTE concentrations above 10 mM resulted in decreasing enzyme activities (data not shown). All experiments were conducted thrice.

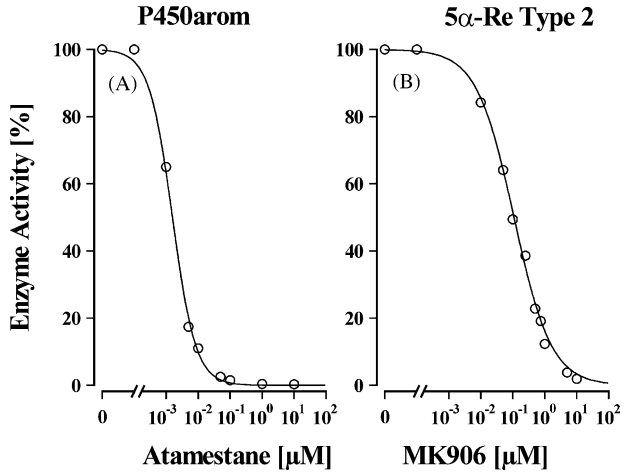

Fig. 3. The inhibitory effects of increasing atamestane concentrations on human P450arom activity (A) were investigated using microsomal placental tissue preparations with 0.1 μM androstenedione and 3 mM NADPH at pH 7.5. The results represent mean values of one assay performed in duplicate as described in the experimental section. The inhibitory effects of increasing MK 906 concentrations on human 5α-Re 2 activity (B) were investigated using microsomal prostate tissue preparations with 0.1 μM androstenedione and 3 mM NADPH at pH 5.5. The results represent mean values of one assay performed in duplicate.

With the exception of 5α-Re 2, DTE demonstrated a strong dose-responsive compensatory effect on the TPT-inhibited activity of the enzymes under debate. 17β-HSD 1 showed the lowest EC_{50} value (0.21 mM) observed (Fig. 1B). The EC_{50} values of the other enzymes investigated are listed in Table 2. DTE resulted in an approximately 80% antagonistic effect with 17β-HSD 1, P450arom, 3β-HSD 2 and 17β-HSD 3. In contrast to the other enzymes, DTE resulted in only very weak compensatory effects on TPT inhibited 5α Re 2 activity (Fig. 2B). An EC_{50} value for this enzyme was not detectable.

4. Discussion

In the present study, investigations regarding the organotin blood load demonstrated the presence of significant amounts of TPT in the human blood, while the other organotin compounds were close to the detection limit (Table 1). Women showed a slightly higher average blood concentration of TPT and TBT. This possibly reflects the higher percentages of body fat in women [33] and the accumulation of tinorganic compounds in lipids [8].

One might suggest that humans incorporate organotins mainly by consumption of contaminated food. Marine fishery products contain high TBT concentrations (14–455 ng/g wet weight fish muscle [8]), whereas agriculture products are more likely to be contaminated with TPT caused by its use as pesticide [4]. Different diets are expected to result in different organotin loads in human tissues and blood. The information available on butyltin deposition in humans is limited. Two studies verify hepatic TBT deposition [2,8]. Japanese people, who certainly consume a considerably higher dietary intake of fish, showed significantly higher liver TBT loads than Polish people. To the authors' best knowledge, TPT deposition in human tissues has not been studied to date. According to our results, reasonable TPT levels are generally detectable in the blood of German adults.

Moreover, our experiments clearly document a dose-dependent complete inhibition of all the steroidogenic enzymes investigated. These results indicate that TPT is an unspecific inhibitor of human sex steroid hormone metabolism. Several mechanisms could account for the loss of the enzymes activity caused by TPT: (i) binding of TPT to cell membrane components, indirectly leading to inhibition of the catalytic activity of the enzymes [23]; (ii) inhibition of components of the electron transport chain affecting the availability of the coenzymes necessary for full enzyme activity; and (iii) binding of the organotin to the proteins itself, resulting in direct destruction of the catalytic activity of the enzymes. It is very unlikely that binding of TPT to cell membrane components is the cause of the inhibitory potency of TPT, since TPT affects not only microsomal enzymes, but also the activity of the cytosolic 17β-HSD 1. The specific inhibition of components of the electron transport chain can also be excluded, since TPT inhibits both NADPH- and NAD-dependent enzyme activities. Previously, it was suggested that TPT binds to specific amino acids, such as cysteine and histidine, leading to an impeded enzyme activity [5]. Thiol compounds abolish TBT mediated haemolysis [23] and the protective effect of the thiol compounds were attributed to a chemical interaction of the tinorganic Lewis acid, with the thiol Lewis base indicating a putative reaction of organotins with cysteine residues of proteins [23–25].

Treatment with DTE partly resulted in a substantial compensation of the adverse effects of TPT on the key enzymes of human sex steroid hormone metabolism. The EC_{50} values of DTE varied in a wide range indicating that the effectiveness of the compensatory activity of DTE differs among the enzymes investigated. DTE resulted in an approximately 80% antagonistic effect with 17β-HSD 1, P450arom, 3β-HSD 2 and 17β-HSD 3. Interestingly, DTE demonstrated hardly any compensatory effect on TPT inhibited 5α-Re 2 (Fig. 2B, Table 2). Equally, this enzyme showed the highest sensibility (lowest IC_{50} value) towards TPT inhibition (Fig. 2A, Table 2). Accordingly, 17β-HSD 1 shows the highest IC_{50} value for TPT at the lowest EC_{50} value for DTE.

In order to identify a relation between the aminoacic sequence of the enzyme and its interaction with the inhibitor or the antagonist, the ratio between the number of amino acids and the number of cysteine residues of the enzyme was calculated. Noteworthy is that 5α-Re 2 features the highest frequency of cysteine residues (3.41 cysteine residues per 100 amino acids), whereas the other enzymes demonstrate reasonably lower cysteine contents (2.26 cysteine residues per 100 amino acids in 17β-HSD 3, 1.83 in 17β-HSD 1, 1.39 in P450arom and 1.35 in 3β-HSD 2). The present data recommend that critical (in terms of accessibility) cysteine residues are responsible for the inhibitory effects of TPT. Consequently, one might suggest that 5α-Re 2 is characterized by the occurrence of a relatively high number of critical cysteine residues compared to the other enzymes. In proteins, the proper pairing of cysteine residues and maintenance of disulfide bonds is essential for normal structure and activity. In eukaryotic cells, the naturally occurring thiol compound glutathione prevents the formation of disulfide bonds in the cytosol and catalyses their formation in the endoplasmic reticulum [34]. The inhibitory in vitro effects of TPT could possibly be attenuated by glutathione in vivo. On the other hand, an adverse effect of TPT loads on glutathione functions might also be suggested, since a distinct proper ratio of the reduced and oxidized form of the thiol compound is required in the cytosol and the endoplasmic reticulum, respectively [34].

Endocrine disrupting or interfering effects of organotins observed in non-mammalian species can only cautiously be extrapolated to humans, but effects targeting the endocrine system will potentially occur at lower exposure levels than most other toxic effects. The in vitro effects of TPT on the key enzymes of human sex steroid hormone metabolism might point to possible risks for the endocrine system in

vivo. Hormonal imbalance, caused by endocrine modulators, has been associated with negative outcomes such as cancer in hormone sensitive tissues, declining reproductive health, congenital anomalies and even brain diseases [35–39]. The IC_{50} values of TPT detected are relatively high (0.95–10.5 μM), compared to the average TPT content found in human blood samples (0.49–1.92 nM organotin cation). On the other hand, it was shown that the lipophilic substances TPT and TBT are accumulated at high concentrations in specific organs and tissues, such as liver, fat and brain tissue [40]. Interestingly, fat tissue expresses reasonable P450arom activity and is the main source for estrogens in postmenopausal women [41]. Moreover, steroid hormone imbalance is observed in patients with liver diseases, indicating the importance of steroid hormone metabolism in this organ [42,43]. A study on the neurotoxicity of organotins proved that TPT crosses the blood–brain barrier and causes deficits in the learning ability in rats [44]. Noteworthy, steroid hormones play a crucial role for the functions of the central nervous system [45] and a complex system of enzymes catalysing the metabolism of steroid hormones exists in the brain [46]. Altogether, even relatively low TPT loads might affect the endocrine system due to putative enrichment of the compound in fat and membrane-rich brain tissue.

In conclusion, the present study demonstrates a reasonable TPT blood load in humans and indicates that TPT acts as an unspecific inhibitor of the key enzymes of human sex steroid hormone metabolism. Moreover, the experiments show that DTE is able to compensate the adverse effects of TPT and that the effectiveness of the compensatory activity of DTE differs among the enzymes investigated. Conceivably, critical cysteine residues are responsible for the inhibitory effects of TPT. Explicit studies concerning human load of organotin compounds are still lacking. Consequently, we emphasize the importance of further studies to evaluate patho-physiological effects of organotin compounds on human sex steroid hormone metabolism.

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