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Organotin compounds enhance 17β-hydroxysteroid dehydrogenase type I activity in human choriocarcinoma JAr cells: Potential promotion of 17β-estradiol biosynthesis in human placenta

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Abbreviations:
TBT, tributyltin
TPT, triphenyltin
17β-HSD I, 17β-hydroxysteroid dehydrogenase type I
17β-HSDs, 17β-hydroxysteroid dehydrogenases
E1, estrone
E2, 17β-estradiol
9cRA, 9-*cis* retinoic acid
atRA, all-*trans* retinoic acid

ABSTRACT

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are typical environmental contaminants and suspected endocrine-disrupting chemicals because they cause masculinization in female mollusks. However, it remains unclear whether organotin compounds also cause crucial toxicities in human sexual development and reproductive functions. We investigated the effects of 17 tin compounds on the catalytic activity and mRNA expression of 17β-hydroxysteroid dehydrogenase type I (17β-HSD I) in human choriocarcinoma JAr cells. At nontoxic concentrations, both trialkyltins with propyl, butyl or cyclohexyl substituents on the tin atom and triphenyltin (TPT) enhanced 17β-HSD I mRNA transcription and enzyme activity in a dose-dependent fashion. Although tetraalkyltin compounds such as tetrabutyltin and tributylvinyltin also increased the mRNA expression and enzyme activity of 17β-HSD I, the concentrations necessary for activation were >30–100 times greater than those for trialkyltins. Inorganic tin had no effect on the catalytic activity and mRNA expression of 17β-HSD I. Interestingly, diphenyltin and monophenyltin, which are metabolites of TPT, enhanced 17β-HSD I activity with a concomitant increase in mRNA expression, whereas dibutyltin and monobutyltin, which are metabolites of tributyltin, enhanced 17β-HSD I activity without a concomitant increase in mRNA expression. These results suggest that organotin compounds are potent stimulators of 17β-estradiol biosynthesis to enhance 17β-HSD I activity in the human placenta in vitro; the placenta represents a potential target organ for these compounds, whose endocrine-disrupting effects might be the result of local changes in 17β-estradiol concentrations in pregnant women.

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FCS, fetal calf serum
MEM, minimal essential medium
RXR, retinoid X receptor
LG, LG100268
RAR, retinoic acid receptor

1. Introduction

The placenta plays a vital role in maintaining pregnancy by delivering oxygen and nutrients from the maternal circulation to the fetus and by returning fetal metabolites to the mother. In addition, the placenta performs many crucial endocrine functions. For example, the human placenta is the main source of estrogenic steroids during human pregnancy [1].

17β-Hydroxysteroid dehydrogenases (17β-HSDs) catalyze the interconversion of 17-ketosteroids and 17β-hydroxysteroids, such as estrone (E1) to 17β-estradiol (E2), and androstenedione and testosterone. Thus, these members of the short-chain alcohol dehydrogenase protein family catalyze the conversion of low-activity steroids to high activity forms and vice versa. So far, multiple different types of 17β-HSDs have been cloned [2], and these isoenzymes have been found to differ from each other in substrate specificity as well as in tissue distribution and subcellular localization. In the human placenta and ovarian granulosa cells, the type I enzyme (17β-HSD I) is highly expressed [3–5] and catalyzes primarily the reaction from low-activity E1 to the biologically more active form E2. In addition to being found in steroidogenic tissues, 17β-HSD I is present in some estrogen target cells, such as breast [6] and endometrial epithelial cells [7], which suggest its involvement in the regulation of intracellular E2 supplies for estrogen receptors. Given the pivotal functional roles of 17β-HSD I, the developmental and reproductive toxicity of environmental contaminants known to have endocrine-disrupting effects plausibly might involve 17β-HSD I.

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been widely used as biocides, agriculture fungicides, wood preservatives, and disinfecting agents in circulating industrial cooling waters, as well as antifouling paints for marine vessels [8,9]. There are many reports of the biological effects of organotin compounds, which vary in their toxic effects on eukaryotes. One of the most notable toxicities in sexual development and reproduction is that of TBT- and TPT-mediated endocrine disruption in some species of gastropods [10,11]. This phenomenon is known as ''imposex''—the superimposition of male genitalia on female animals. Therefore, these organotin compounds are suspected to cause endocrine-disrupting effects in mammals, including humans. Human exposure to organotin compounds may result from consumption of organotin-contaminated meat and fish products or occupational exposure during the manufacture and formulation of organotin compounds or the application and removal of organotin-containing paints [12,13]. The possible exposure of humans to organotins therefore has prompted great concern about potential toxicities.

To facilitate the application of current knowledge of the toxicity of organotin compounds to sexual development and reproduction in humans, we assessed the possible effects of 17 tin compounds on E2 production and mRNA expression of 17β-HSD I in human placental cells by using human choriocarcinoma JAr cells. We discuss the potential toxicity of organotin compounds as endocrine disruptors in humans.

2. Materials and methods

2.1. Chemicals and cell culture

Tin compounds tested in this study are listed in Table 1. 9-*cis* retinoic acid (9cRA) and all-*trans* retinoic acid (atRA) were from Wako Pure Chemicals (Osaka, Japan). LG100268 (LG, >95% pure) was obtained from Astellas Pharma (Tokyo, Japan). All chemicals were dissolved in DMSO (Wako Pure Chemicals). The human choriocarcinoma cell line JAr was obtained from American Type Culture Collection (ATCC; Rockville, MD). JAr cells (ATCC No. HTB-144) were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1 mM pyruvate, 4.5 g/l glucose, and 10% fetal calf serum (FCS). JEG-3 cells (ATCC No. HTB-36) were cultured in minimal essential medium (MEM) supplemented with 2 mM L-glutamine, 0.1 mM MEM nonessential amino acid solution (Invitrogen, Carlsbad, CA), and 10% FCS. To determine the effect of tin compounds on [^{3}H]thymidine incorporation and mRNA expression of JAr cells, cells were seeded, precultured for 24 h, and then treated with either various concentrations of tin compounds in 0.1% DMSO or vehicle alone (0.1% DMSO) for another 24 or 48 h. In control experiments, 0.1% DMSO did not affect the [^{3}H]thymidine incorporation, catalytic activity, and mRNA expression of 17β-HSD I.

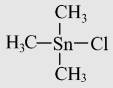
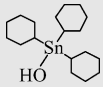
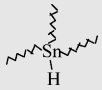
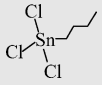
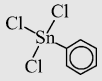
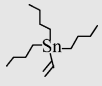
2.2. [^{3}H]thymidine uptake assay

To determine the cytotoxicity of tin compounds, JAr cells (10^3 cells/well) were seeded in 96-well plates. After 24 h, cells were treated with various concentrations of these compounds for another 48 h. Each culture was pulsed with 20 kBq of [^{3}H]thymidine (Amersham Biosciences, Piscataway, NJ) for 2 h before harvesting, and the radioactivity incorporated into cells was determined by liquid scintillation. A nontoxic concentration of a tin compound was defined as a concentration at which the uptake of [^{3}H]thymidine was ≥80% that seen with the vehicle alone.

2.3. 17β-HSD I activity measurements

JAr cells (3×10^4 cells/well) were plated in 24-well plates. After 24 h of culture, JAr cells were treated with various concentrations of tin compounds for a further 48 h. At the end point of

Table 1 – Tin compounds tested in this study

Tin compounds	Abbreviation	Structure	Purify (%)	CAS No.	Maximum nontoxic concentration[a]	Source
Trimethyltin chloride	TMTCl		>98	1066-45-1	1 μM	Aldrich Chemicals
Triethyltin bromide	TETBr		>97	2767-54-6	100 nM	Aldrich Chemicals
Tripropyltin chloride	TPrTCl		>98	2279-76-7	30 nM	Merck
Tributyltin chloride	TBTCl		>95	1416-22-0	100 nM	Tokyo Kasei Kogyo
Triphenyltin chloride	TPTCl		>95	639-58-7	100 nM	Aldrich Chemicals
Tricyclohexyltin hydroxide	TChTOH		>99	13121-70-5	30 nM	Aldrich Chemicals
Trioctyltin hydride	TOTH		>95	869-59-0	>10 μM	Tokyo Kasei Kogyo
Butyltin trichloride	$MBTCl_3$		>95	1118-46-3	>10 μM	Aldrich Chemicals
Dibutyltin dichloride	$DBTCl_2$		>97	683-18-1	30 nM	Tokyo Kasei Kogyo
Tetrabutyltin	TeBT		>93	1461-25-2	3 μM	Aldrich Chemicals
Phenyltin trichloride	$MPTCl_3$		>98	1124-19-2	3 μM	Aldrich Chemicals
Diphenyltin dichloride	$DPTCl_2$		>96	1135-99-5	300 nM	Aldrich Chemicals
Tributyltin hydride	TBTH		>98	688-73-3	100 nM	Aldrich Chemicals
Tributyltin bromide	TBTBr		>90	1461-23-0	100 nM	Aldrich Chemicals
Triphenyltin hydroxide	TPTOH		>95	76-87-9	100 nM	Aldrich Chemicals
Tributylvinyltin	TBVT		>97	7486-35-3	>10 μM	Tokyo Kasei Kogyo
Tin chloride	$SnCl_4$		>98	7646-78-8	>10 μM	Wako Pure Chemicals

[a] Maximum nontoxic concentration of each tin compound was defined as the maximum concentration at which the uptake of [^{3}H]thymidine was ≥80% of that of the vehicle alone.

each treatment, cells were rinsed with fresh serum-free culture medium and then added 0.5 ml of fresh serum-free culture medium supplemented with 1 μM E1 (Sigma, St. Louis, MO). After incubation for 4 h at 37 °C in an atmosphere of 5% CO_2, culture media were collected, and the total E2 content was determined by assay with a Correlate-EIA 17β-estradiol Enzyme Immunoassay kit (Assay Designs, Ann Arbor, MI).

2.4. *Quantitative reverse transcription-PCR (RT-PCR)*

JAr cells were treated with various tin compounds in regular culture medium supplemented with 5% charcoal-stripped FCS instead of 10% normal FCS, and then total RNA was extracted from the cells by using TRIzol reagent (Invitrogen). mRNA expression of 17β-HSD I in JAr cells was determined by quantitative RT-PCR. We reverse-transcribed 5 μg total RNA extracted from JAr cells in a total volume of 20 μl by using SuperScript III reagent (Invitrogen) and oligo-(dT) as primer and incubating for 1 h at 42 °C. After termination of cDNA synthesis, each reaction mixture was diluted with the addition of 80 μl TE buffer. Aliquots (2 μl) of diluted reverse-transcription products were amplified in a reaction mixture containing QuantiTect SYBR Green PCR reagent (Qiagen, Valencia, CA) and 0.5 μM of each primer using LightCycler (Roche Diagnostics, Mannheim, Germany). After preincubation of reaction mixtures at 95 °C for 15 min, PCR amplification was performed with 35–40 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 30 s, and elongation at 72 °C for 10 s. Primers used were for human 17β-HSD I (HSD17B1), 5′-GGGCTGCCTTTCAATGACGTTT-3′ and 5′-ATCAGGCTCAAGTGGACCCCAA-3′, and human β-actin, 5′-CTACGAGCTGCCTGACGGC-3′ and 5′-GCCACAGGA-CTCCATGCCC-3′.

2.5. *Statistics*

Data were analyzed with Tukey's multiple comparisons test by using SPSS software (Chicago, IL). Control and treatment group data always were obtained from equal numbers of replicate experiments, and experiments were performed independently at least twice. Values at which P was <0.05 were considered statistically significant.

3. Results

3.1. *Effects of organotin compounds on DNA synthesis in JAr cells*

To confirm the nontoxic concentration ranges of 17 tin compounds (Table 1) and to determine whether treatment with organotin compounds was associated with cytotoxic effects, we performed DNA synthesis assays. JAr cells were treated for 48 h with tin compounds at various concentrations, and DNA synthesis was evaluated by [^{3}H]thymidine incorporation. To help interpret the results, we classified these experiments into three groups as follows: Group I, comparison of different structures of alkyl and aryl chains in trialkylated and triarylated tin compounds; Group II, comparison of different numbers of alkyl or aryl chains in butyltin and phenyltin compounds; and Group III, comparison of different fourth functional groups on the tin of TBT and TPT (Fig. 1).

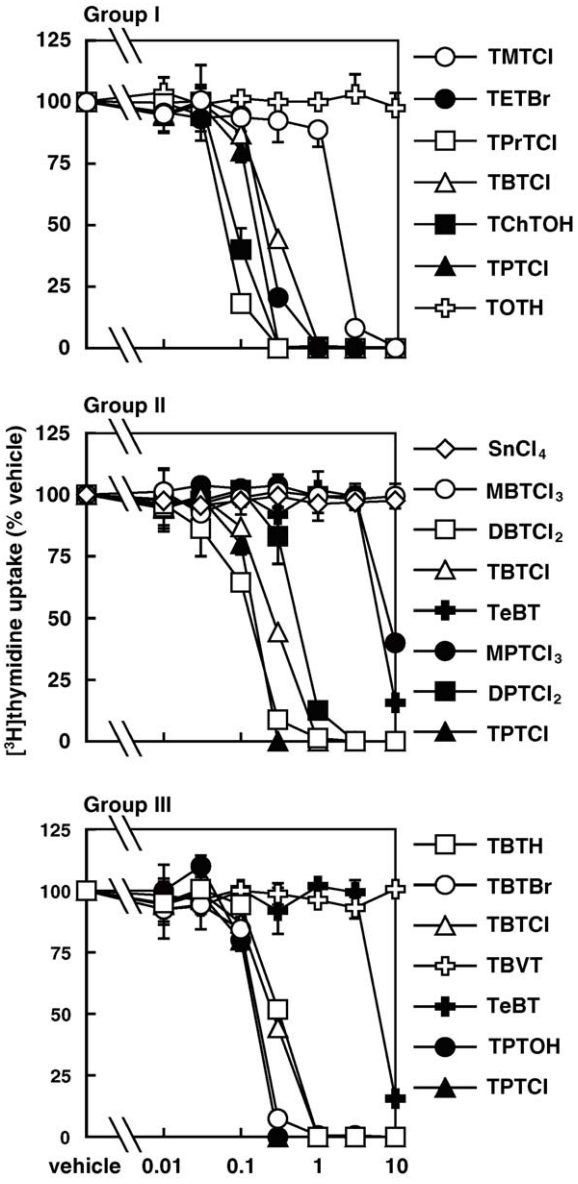


Fig. 1 – Effect of organotin compounds on DNA synthesis in JAr cells. Cells were treated with various concentrations of tin compounds for 48 h. Results are expressed as mean ± 1 S.D. of triplicate cultures. The radioactivity in vehicle-only cells, calculated from all experiments, was 111,709 ± 6182 cpm (n = 15). Group I, comparison of different structures of alkyl and aryl chains in trialkylated and triarylated tin compounds; Group II, comparison of different numbers of alkyl or aryl chains in butyltin and phenyltin compounds; and Group III, comparison of different fourth functional groups on the tin of TBT and TPT. The abbreviation for each compound used are indicated in Table 1.

Many of the Group I trialkylated and triarylated tin compounds, which have ethyl, propyl, butyl, cyclohexyl, or phenyl groups on the tin atom, were highly toxic, and

exposure to >100–300 nM significantly inhibited [^{3}H]thymidine incorporation in JAr cells. Although TMTCl was one of the less toxic chemicals of Group I, a striking reduction of [^{3}H]thymidine incorporation to 5% of the control value occurred after treatment with >1 μM. TOTH had no significant effect on [^{3}H]thymidine incorporation at a concentration range of 10 nM to 10 μM. Among the Group II chemicals, the cytotoxicity of $DBTCl_2$ was nearly as high as that of the most highly toxic trialkyltins. $DPTCl_2$ was also toxic but less so than $DBTCl_2$. Although TeBT and $MPTCl_3$ were less toxic than other Group II compounds, they induced marked reduction of [^{3}H]thymidine incorporation at 10 μM. $SnCl_4$ and $MBTCl_3$ showed no effect, even at concentrations of 10 μM. Among the Group III chemicals, the TBT and TPT derivatives were similar in toxicity, and exposure to doses of 300 nM decreased [^{3}H]thymidine incorporation to <50% of control levels. TBVT showed no significant effect at the concentration range of 10 nM to 10 μM. Using these results as a guide, we established the maximal nontoxic concentration of each compound for use in investigating possible effects on the 17β-HSD I activity of JAr cells (Table 1).

3.2. *Effect of organotin compounds on 17β-HSD I activity in JAr cells*

At lethal concentrations, at which uptake of [^{3}H]thymidine was <10% of control levels, all organotin compounds abolished E2 production because of extinction of the cells. All tested TBT and TPT derivatives (Group III) were active and induced 17β-HSD I activity (Fig. 2): exposure to 100 nM of each of these organotin compounds caused statistically significant increases in 17β-HSD I activity in JAr cells. There were no significant differences in 17β-HSD I activity among the TBT and TPT derivatives (Group III), suggesting that the ligand on the trialkylated and triarylated tin compounds (as long as it is not an alkyl or aryl group) is relatively unimportant to stimulation of endocrine functions. However, the presence of a fourth alkyl group on the tin atom decreased the potency of the organotin compounds in inducing 17β-HSD I activity, because both TeBT and TBVT failed to stimulate this placental function at doses of <100 nM (Fig. 2, Group III). Among the other trialkyltin compounds (Group I), TETBr, TPrTCl, and TChTOH were significantly active. Metabolites of both TBTCl and TPTCl ($MBTCl_3$, $DBTCl_2$, $MPTCl_3$ and $DPTCl_2$; Group II) also altered 17β-HSD I activity, but the level of activation decreased in proportion to the dealkylation or dearylation of these organotin compounds (mono- < di- < tri-). These results suggest that the potency of the effects induced by organotin compounds is related to both the number and structure of the alkyl and aryl groups.

3.3. *Effect of organotin compounds on mRNA expression of 17β-HSD I (HSD17B1) in JAr cells*

We investigated the tin compound-induced mRNA expression of 17β-HSD I (*HSD17B1* gene) in JAr cells at either the concentration that elicited the greatest response in catalytic activity or the maximal nontoxic concentration. Except for

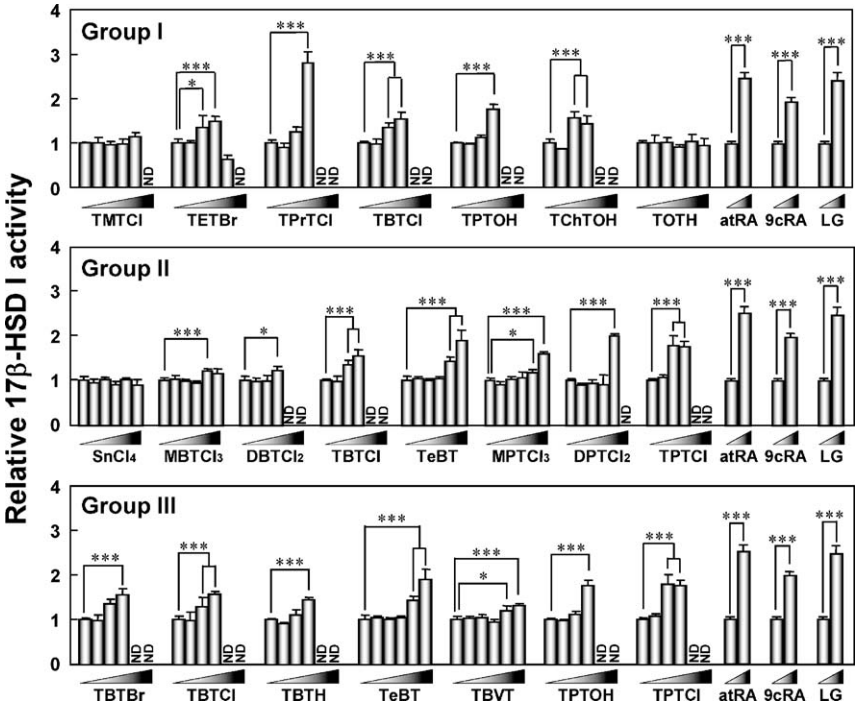


Fig. 2 – Effects of tin compounds on 17β-HSD I activity in JAr cells. Cells were treated for 48 h with tin compounds at various concentrations (0 nM, 1 nM, 10 nM, 100 nM, 1 μM, and 10 μM) or with 0 or 1 μM of atRA, 9cRA, or LG. Results are expressed as mean ± 1 S.D. of triplicate cultures. The 17β-HSD I activity (amount of converted E2) in vehicle-only cells, calculated from all experiments, was 4.44 ± 1.01 ng/well/4 h (n = 18). Groups I, II, and III correspond to the groups described in the legend for Fig. 1. $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.005$ indicate values significantly different from vehicle-control values. ND, not detectable.

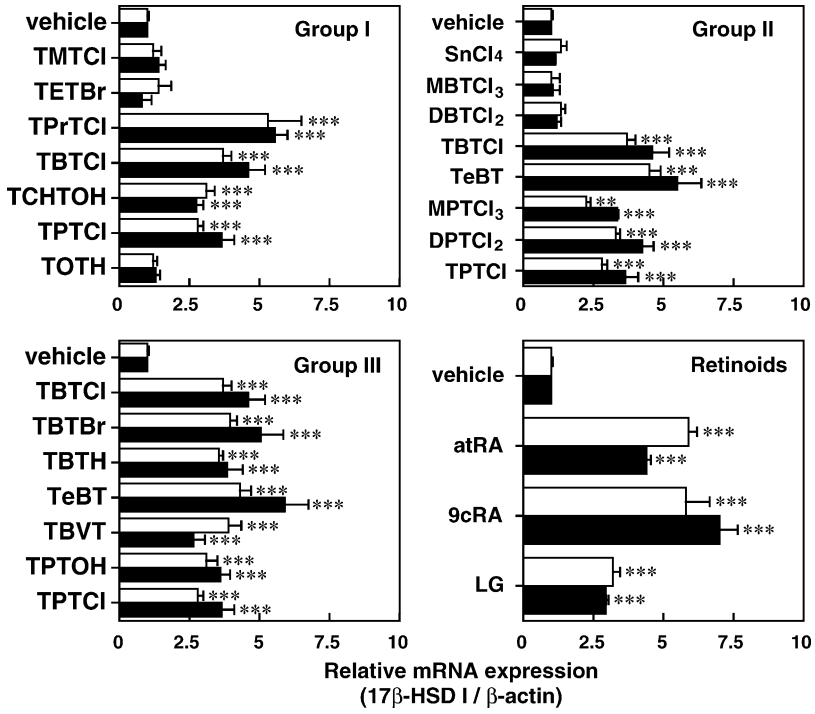


Fig. 3 – Effects of tin compounds on the mRNA expression of 17β-HSD I (HSD17B1) in JAr cells. Total RNA was isolated from JAr cells treated with tin compounds for 24 h (open bars) and 48 h (solid bars). The doses of each compound were: 100 nM of TETBr, TPrTCl, TBTCl, TChTOH, TPTOH, TPTCl, TBTH, TBTBr and $DBTCl_2$; 1 μM of TMTCl, $MBTCl_3$, $DPTCl_2$, 9cRA, atRA and LG; and 10 μM of TOTH, $SnCl_4$, $MPTCl_3$, TeBT and TBVT. The relative mRNA levels for each condition were determined by quantitative RT-PCR assays for each of the 3 independent cultures (see Section 2). Results are expressed as means ± 1 S.D. of three independent cultures. Groups I, II, and III correspond to the groups described in the legend for Fig. 1. $^{**}P < 0.01$; and $^{***}P < 0.005$ represents values significantly different from vehicle-control values.

TETBr, $MBTCl_3$, and $DBTCl_2$, the organotin compounds that significantly enhanced the catalytic activity of 17β-HSD I also significantly increased its mRNA expression. However, the mRNA effects were much more pronounced than the changes in catalytic activity (Figs. 2 and 3). Furthermore, atRA and 9cRA, which are known to enhance 17β-HSD I activity with mRNA expression, also showed their induction of mRNA expression more obviously than that of catalytic activity (Figs. 2 and 3).

In a recent study, we demonstrated that some organotin compounds function as agonists for retinoid X receptor (RXR), a nuclear receptor for retinoids, to stimulate the mRNA expression of human placental aromatase and human chorionic gonadotropin in human choriocarcinoma cells [14]. As shown in Figs. 2 and 3, organotin compounds that induced the transactivation function of RXR in our previous study (TBT and TPT derivatives, TPrTCl, TChTOH, TeBT, and TBVT) also enhanced 17β-HSD I mRNA transcription and enzyme activity. In addition, a synthetic RXR-specific ligand LG also enhanced 17β-HSD I enzyme activity and mRNA transcription (Figs. 2 and 3). These results suggest that these organotin compounds induce the expression of 17β-HSD I mRNA via RXR transactivation.

However, organotin compounds that did not induce the transactivation function of RXR in our previous study (TETBr, $MBTCl_3$, $DBTCl_2$, $MPTCl_3$ and $DPTCl_2$) also significantly enhanced 17β-HSD I enzyme activity. Interestingly, $MPTCl_3$ and $DPTCl_2$ significantly enhanced mRNA expression of 17β-HSD I, whereas TETBr, $MBTCl_3$, and $DBTCl_2$ had little effect on mRNA expression (Fig. 3). These results indicate that the observed organotin-induced alterations in 17β-HSD I activity are due not only to regulation at the mRNA level but also another mechanism.

4. Discussion

Recently, organotin compounds have become recognized as endocrine-disrupting chemicals, because numerous marine organisms have been shown to exhibit sexual abnormalities after exposure to TBT or TPT. In gastropod mollusks, which are among the species most sensitive to organotin compounds, these chemicals have been demonstrated to induce the superimposition of male sex organs, such as a penis and/or a vas deferens, over female sex organs, a phenomenon known as imposex [10,11]. It has been theorized that TBT increases androgen levels through inhibition of aromatase activity or suppression of androgen excretion [15–17]; nevertheless this theory is not well founded. Although these organotin compounds also are reported to inhibit the catalytic activity of human steroidgenic enzymes, including aromatase [18,19], 3β-hydroxysteroid dehydrogenase type II, 5α-reductase type II, and 17β-HSD I and III [20,21], the concentrations effective for the inhibition of these enzymes were relatively high (>1 μM)

and generally toxic to mammalian cells [22–24], including human choriocarcinoma cells (Fig. 1). Therefore, in regard to effects on humans, we have to distinguish between the nonspecific cellular toxicity of organotins and their inhibition of steroidgenic enzymes. We previously demonstrated that nanomolar concentrations (i.e., 3–100 nM) of some organotin compounds, including TBT and TPT, enhance aromatase activity, which catalyzes the conversion androgen to estrogen, and that this increase in enzymatic activity occurs concurrently with increases in mRNA expression and E2 biosynthesis from androstenedione in human choriocarcinoma cells [14,22]. In addition, our current study showed that many of the same organotin compounds also enhance 17β-HSD I activity, which predominantly catalyzes the conversion of the weakly estrogenic chemical E1 into the strongly estrogenic compound E2 in JAr cells (Figs. 2 and 3). Our findings suggest that the endocrine-disrupting action of these organotin compounds in pregnant women may be to promote the biosynthesis of E2 in the placenta, an effect opposite to that theorized in gastropods.

Our current study has demonstrated that organotin compounds alter E2 biosynthesis in human placental cells in vitro. Although several reports have established the in vivo reproductive toxicity of organotin compounds in rodents [25–28], there are no reports on whether organotin-induced production of placental E2 is associated with teratogenetic effects. Accordingly, it remains unclear which endocrine-disrupting effects or malformations result, at least in part, from organtoin-induced local changes in E2 concentrations of the placenta in vivo. Further, the in vivo endocrine effects of environmental contaminants on the human placenta are difficult to estimate from animal studies, particularly those involving rodents, because the endocrine functions of the placenta vary considerably among different species. In particular, estrogen biosynthesis during pregnancy in humans is much different from that in rodents. In humans, ovarian function gradually declines after fertilization, as the placenta becomes the primary site of estrogen biosynthesis during pregnancy [29]. In contrast to the process in humans, the ovary (not the placenta) is the main source of estrogen during pregnancy in rodents, because the placenta of rodents expresses neither aromatase nor 17β-HSD I [30–32]. It has been suggested that rodents are therefore unsuitable for evaluating the effects of environmental contaminants on estrogen biosynthesis in the human placenta. The regulation of estrogen biosynthesis in placenta is very important for human embryo because altering placental function can cause permanent effects in the embryo. For example, the lack of placental aromatase causes female pseudohermaphroditism, as is seen in patients with aromatase deficiency [33]. Consequently, there is an urgent need to establish effective tools to evaluate the endocrine-disrupting effects and teratogenicity of environmental contaminants that induce changes in local estrogen concentrations of the placenta in vivo.

Several stimuli, such as cAMP analogues [34], the natural retinoic acid receptor (RAR) ligand atRA [35], and the natural RAR and RXR ligand 9cRA [36], induce the mRNA expression of 17β-HSD I, thereby increasing its activity. Organotin compounds, such as TBT and TPT, have no effect on intracellular cAMP production [22]. In a recent study, we demonstrated that some organotin compounds function as RXR agonists to stimulate the mRNA expression of human placental aromatase and human chorionic gonadotropin in human choriocarcinoma cells [14]. These chemicals bind directly to the ligand-binding domain of RXR and function as transcriptional activators. The RXR-agonistic organotin compounds also enhanced the expression of 17β-HSD I mRNA in the present study. The level of activation was nearly equal to the level of RXR activation induced by these compounds [14]. The RAR response element is located at −512 to −479 bp in the promoter region of 17β-HSD I [35], but the RXR response element has not yet been identified. However, Ro41, a specific RAR antagonist, fails to abrogate 9cRA-induced expression of 17β-HSD I mRNA [36]. In addition, we found that the RXR-specific ligand LG consistently enhanced 17β-HSD I enzyme activity and mRNA transcription (Figs. 2 and 3). Unlike 9cRA, these organotin compounds are RXR-specific and do not activate the RAR pathway [14]. In light of these findings, although transcriptional regulation in the 17β-HSD I promoter is not yet fully understood, we suggest that the *cis*-elements which have a response to the RXR-dependent signaling pathway may be located in the promoter region and that RXR-agonistic organotin compounds induce the expression of 17β-HSD I mRNA via RXR transactivation.

We assayed 17 tin compounds for their ability to induce both the catalytic activity and mRNA expression of 17β-HSD I in JAr cells. The concentrations needed to induce these two features of 17β-HSD I did not differ significantly among the TBT and TPT derivatives. Compared with those for the TBT and TPT derivatives, approximately 30- to 100-fold higher concentrations of tetraalkyltin compounds (e.g., TeBT and TBVT) were needed to affect 17β-HSD I activity or transcript levels. This observation may indicate that tetraalkyltin compounds are metabolically converted to trialkyltins, which are the active form, in the cells. This hypothesis is supported, in a broad sense, by previous results that show that organotin compounds undergo dealkylation by the microsomal monooxygenase system, which is dependent on cytochrome P450 in the liver and other organs [37–39]. In addition, our previous study suggested that it may be necessary for activation of RXR by these tetraalkyltin compounds to metabolically convert them into the active dealkylated form (e.g., TBT) in cells, because the presence of a fourth alkyl group on the tin atom interferes with the binding of alkyltin compounds to the receptor [14]. These findings support our speculation that these organotin compounds induce the expression of 17β-HSD I mRNA via RXR transactivation.

Tin compounds that failed to act as RXR agonists ($MBTCl_3$, $DBTCl_2$, $MPTCl_3$, $DPTCl_2$ and TETBr) [14] also significantly increased the catalytic activity of 17β-HSD I. Strangely enough, metabolites of TPT ($MPTCl_3$ and $DPTCl_2$) significantly enhanced mRNA expression of 17β-HSD I, whereas metabolites of TBT ($MBTCl_3$ and $DBTCl_2$) and TETBr had little effect on mRNA expression (Figs. 2 and 3). It remains unclear why these organotin compounds enhanced the activity of 17β-HSD I with or without altering its mRNA expression. At least, the induction appears due to a mechanism other than activation of RXRs.

To our knowledge, our study is the first to show that organotin compounds potentially promote estrogenic action to enhance 17β-HSD I activity in human placenta. However, the mRNA changes that the compounds induced were not comparable to the changes in catalytic activity. Consequently, we conclude that the observed organotin-induced alterations in JAr cells are due to other mechanisms in addition to regulation of 17β-HSD I mRNA levels. The toxic mechanisms of organotin compounds appear very intricate. For instance, organotin compounds function as inhibitors of steroidogenic enzymes [18–21] and RXR ligands [14] but also have been shown to enhance histone acetyltransferase activity [40]. Future studies need to clarify the precise mechanism of action of organotin compounds in human endocrine disruption in vitro and in vivo.

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