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The role of retinoic acid receptors and their cognate ligands in reproduction in a context of triorganotin based endocrine disrupting chemicals

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Retinoic acid (RA), an active form of vitamin A, regulates the embryonic development, male and female reproduction and induces important effects on the cell development, proliferation, and differentiation. These effects are mediated by the retinoid (RAR) and rexinoid nuclear receptors (RXR), which are considered to be a ligand-activated, DNA-binding, trans-acting, and transcription-modulating proteins, involved in a general molecular mechanism responsible for the transcriptional responses in target genes. Organotin compounds are typical environmental contaminants and suspected endocrine disrupting substances. They may affect processes of reproductive system in mammals, predominantly via nuclear receptor signaling pathways. Triorganotins, such as tributyltin chloride (TBTCl) and triphenyltin chloride (TPTCl), are capable to bind to RXR molecules, and thus represent potent agonists of RXR subtypes of nuclear receptors not sharing any structural characteristics with endogenous ligands of nuclear receptors. This article summarizes selected effects of biologically active retinoids and rexinoids on both male and female reproduction and also deals with the effects of organotin compounds evoking endocrine disrupting actions in reproduction.

Key words: retinoic acid, retinoic acid receptors, retinoid X receptors, reproduction, organotin compounds, endocrine disruptor

Retinoic acids are vitamin A (retinol)-derived, nonpeptidic, small lipophilic molecules that serve as ligands for two families of nuclear receptors, the retinoic acid (RA) receptors (RARs) and the retinoid X receptors (RXRs). Retinoids are involved in the complex arrangements of physiological and developmental responses in many tissues of higher vertebrates that include embryonic development, vision, reproduction, bone formation, haematopoiesis, metabolism, growth and differentiation of a variety of cell types, apoptosis, and processes of carcinogenesis. It is well known that retinoids are also teratogens and their therapeutic doses are contraindicated during pregnancy (Brtko 2007). In mammals, excess of vitamin A leads to a loss of germ cells (Lamano Carvalho et al. 1978), and vitamin A deficiency leads to an arrest in spermatogenesis at early meiosis (Akmal et al. 1998). Experimental studies have suggested that RA is critical for the initiation of meiosis (Bowles et al. 2006; Koubova et al. 2006; Doyle et al. 2007) and spermiogenesis (Chung et al. 2005; Doyle et al. 2007). Some tissues contain small cytosolic proteins that specifically bind RA (rat brain, skin, testis, adrenals) (Bailey and Siu 1990). These are cellular retinoic acid-binding protein (CRABP) and cellular retinoic acid-binding protein II [CRABP(II)], well described members of a large family that includes a number of fatty acid-binding proteins (Bass 1993). The roles for this family of proteins are thought to help in the solubilization of their hydrophobic ligands and in some

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cases, particularly for the retinoid-binding proteins, to direct metabolism of the ligand. Zheng et al. (1999) have shown that the pattern of CRABP(II) messenger RNA and protein expression correlated with the appearance of corpora lutea and the rise in progesterone production in rat as the corpora lutea get developed (Zheng et al. 1999). The level of RA present in a given tissue is finely tuned by the balance between its synthesis by RALDHs and its oxidative degradation by the following cytochrome P450 enzymes: cytochrome P450, family 26, subfamily a, polypeptide 1 (CYP26A1); cytochrome P450, family 26, subfamily b, polypeptide 1 (CYP26B1) – an enzyme that degrades the potent morphogen RA); and cytochrome P450, family 26, subfamily c, polypeptide 1 (CYP26C1) (Duester 2008; Griswold et al. 2012).

The origin of RA in the urogenital system differs in various species. In humans, RA is produced directly in fetal gonads (Childs et al. 2011). In mouse, RA is produced in mesonephroi and diffuses to the gonads. However, the meiotic entry is triggered exclusively in ovaries. In testes, RA is degraded, most probably in Sertoli and Leydig cells, and this action prevents meiotic entry (Bowles et al. 2006; Koubova et al. 2006; Kashimada et al. 2011). Also in birds, RA is synthesized in the gonads (in the ovarian cortex as well as the testis cords) and it is degraded by Sertoli cells (Smith et al. 2008; Piprek et al. 2013).

The RA:RAR/RXR complex binds to RA response elements (RAREs) in target genes, recruiting corepressors or coactivators and thereby inducing or repressing the transcriptional activity. Huang et al. (2015) have identified the genes involved in the regulation of RAR signaling pathway (ESR1, CYP26A1, TRIM16) and retinol metabolism-related enzyme genes (DHRS3, CYP2C9, CYP26A1) that were highly expressed in porcine endometrium during pregnancy. It has been found that CYP26A1 might block the adverse effect of the RA in order to promote the successful mouse embryo implantation (Ma et al. 2012). These results have shown that, except the progesterone and estrogen, the interaction between the RA and estrogen signaling may be also important for the embryo-maternal communications and endometrium remodeling during the early pregnancy.

Sertoli cells: the role of retinoic acid and retinoic acid receptors

Testicular function is influenced by both the endocrine (extra-testicular) and paracrine (intratesticular) factors. Maintenance of normal spermatogenesis is dependent on the anterior pituitary hormones, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which are synthesized and secreted under control of the hypothalamic gonadotropin-releasing hormone (GnRH). Signals from the gonadotropins exert directly on the Sertoli cells, which regulate spermatogenesis. LH and FSH signal through the luteinizing hormone receptor (LHR) and follicle-stimulating hormone receptor (FSHR), which are expressed by the Leydig and Sertoli cells, respectively. The paracrine regulation of spermatogenesis is provided by steroids, such as testosterone and estradiol, secreted by Leydig cells and by proteins such as inhibin and activin, a member of the TGFβ superfamily, synthesized by Sertoli cells (Cooke and Saunders 2002). Interactions between Sertoli cells and germ cells through physical interaction and the secretion of signaling molecules are essential for the healthy progression of spermatogenesis (Sylvester and Griswold 1994). Inhibin primary negatively regulates the FSH secretion from the pituitary gland, whereas the activin has been proposed to affect germ cell maturation at the step when gonocytes differentiate into the spermatogonia (de Kretser et al. 2001).

It has been shown that vitamin A deficient (VAD) animals are infertile (McCarthy and Cerecedo 1952) and this condition can be reversed by retinol (Griswold et al. 1989). Basal serum FSH and LH levels in VAD rats were higher than those of controls (Huang et al. 1985). Moreover, a synergistic effect of vitamin A and FSH on differentiation of the testicular germ cell has been observed in the adult cryptorchid testis, which only consists of an undifferentiated spermatogonia and Sertoli cells (Haneji et al. 1984). RA, biologically active form of vitamin A, is primarily synthesized by the Sertoli cells in the testis (Deltour et al. 1997). RA is required for the differentiation of Sertoli cells, proliferation of spermatogonia, the initiation of meiosis, and maturation of spermatids (reviewed in Santos and Kim 2010). Nourashrafeddin (2015) has suggested that gonadotropins may trigger the differentiation of spermatogonia and their meiotic entry through regulation of RA signaling in the seminiferous tubules within the testis and provide a novel working hypothesis on the mechanisms of gonadotropins to control spermatogenesis via RA, which is considered to be responsible for the cyclic differentiation of germ cells in the adult testis and the continual production of sperm.

In the Sertoli cells, all retinoid receptors are expressed, whereas RARγ and RXRβ are not expressed in the germ cells (Dufour and Kim 1999). The biological effect of RA is mediated through RARα partnering with RXRγ in germ cells and RXRα (Dufour and

Kim 1999). In adult mouse testes, RARα is localized in the nuclei of Sertoli cells, spermatogonia, preleptotene and pachytene spermatocytes, and round and elongating spermatids.

RARα plays an essential role in the regulation of germ cell development during the spermatogenesis (Akmal et al. 1998; Law 2013). RARAα-null animals have high neonatal mortality and exhibit male infertility phenotype. The surviving males have depleted germ cells and vacuolization in the testis (Doyle et al. 2007), whereas RARβ and RARγ gene KO mice did not show any testicular phenotypes (Luo et al. 1995). Transplantation studies have shown that RARα in germ cells is responsible for the colonization and proliferation of germ cells in the basal area of the seminiferous tubules (Doyle et al. 2007). On the other hand, RARα in Sertoli cells were needed for the progression of germ cells through meiosis (Doyle et al. 2007). RARα has been shown to be important for Sertoli cell differentiation (Walker 2003) and in the synchronization of the stages of the spermatogenic cycle (Chung et al. 2005). RARα was positively regulated by protein kinase C and MAPK (Braun et al. 2002). RA and FSH are important proliferation and differentiation factors for Sertoli cells. FSH inhibited the nuclear localization of RARα, leading to down-regulation of RARα transcriptional function via cAMP and protein kinase A (Santos and Kim 2010) as RARα has two PKA consensus sites in the ligand-binding domain (Braun et al. 2000). Therefore, FSH can stimulate Sertoli cell mitosis before puberty via controlling RARα. Thereafter, as FSH levels decrease around puberty (Eskola et al. 1993), RARα is able to translocate to the nucleus (Dufour and Kim 1999) and may participate in the switch from Sertoli cell proliferation to differentiation (Santos and Kim 2010).

Granulosa cells: the role of retinoic acid and retinoic acid receptors

Early studies have shown the presence of vitamin A in the ovary and its fluctuation in serum during the menstrual cycle, indicating that vitamin A may play a role in the ovarian function (Laurence and Sobel 1953). In humans, the role of RA in meiosis has been demonstrated only in the ovary (Childs et al. 2011; Griswold et al. 2012). Moreover, recent studies have shown the presence of all RXR isoforms in the mammalian ovary (Tatone et al. 2016).

Human ovarian granulosa cells undergo a complex differentiation process during the growth and maturation of the ovarian follicles (Richards 1980). This process depends on the sequential effects of the two principal gonadotropins, FSH and LH. FSH acts on early antral follicles to stimulate growth, steroidogenesis, and the expression of cell surface LH receptors, which mediate the granulosa cell ability to respond to circulating LH. Subsequently, LH, in synergy with FSH, acts on the FSH-stimulated follicles to maintain growth and estradiol production and leads to full development of the dominant follicle, the only follicle reaching the preovulatory stage. Finally, the LH triggers ovulation and conversion of the residual follicle into a corpus luteum that, in turn, produces progesterone preparing the endometrium for a possible implantation (Tatone et al. 2016). It has been shown that the ability of FSH to stimulate the induction of LHR in rat granulosa cells is mediated, at least in part, by cAMP, since exogenously added cAMP or other agents that increase intracellular levels of cAMP mimic the action of FSH (Minegishi et al. 2000). The presence of RARs in ovarian cells, including granulosa and some luteal cells, indicate that these cells would also be targets for RA (Zhuang et al. 1994). These data have suggested that RA may regulate the ovarian function by autocrine and/or paracrine action. In rat granulosa cells, RA (0.1 nM) and retinol (10 nM) each synergistically enhance the ability of FSH to induce LHR and stimulate the formation of cAMP and progesterone. At higher concentrations, both retinoids inhibited these effects of FSH in rat granulosa cells. Minegishi et al. (2000) have shown that the receptor depletion by RA is concentration-dependent and RA (1 nM) abolished the effect of FSH on LHR mRNA in rat granulosa cells. This study has provided evidence for a down-regulation of the LHR when RA is added to granulosa cells in the presence of FSH. The response of LH-R protein and LH-R mRNA to cAMP analogs was inhibited by RA in granulosa cells in this experiment, suggesting that RA diminished the action of FSH at sites distal to cAMP generation in the granulosa cells. The observed inhibition of mRNA levels of LHR by RA may be the result of decreased LHR gene transcription and/or mRNA stability.

In mice, RA and a CYP26 inhibitor stimulated granulosa cell proliferation in a dose-response manner. It has been shown that RAR-mediated signaling is involved in both RA- and activin-induced granulosa cell proliferation (Kipp et al. 2011). Kipp et al. (2011) have provided a new insight into the mechanisms of activin action in the ovary and have suggested CYP26B1 and RA to be novel candidates for regulating postnatal follicle formation and development. To examine signaling mechanisms involved in the stimu-

latory effect of RA, Kipp et al. (2011) have treated the mouse granulosa cells with RA in the presence of the pan-RAR inhibitor AGN193109 (Johnson et al. 1999). AGN193109 completely blocked the stimulatory effect of RA on granulosa cell proliferation, suggesting that the effect was mediated through RARs. Because activin stimulates mouse granulosa cell proliferation and also suppresses Cyp26B1 expression, some of the proliferative effects of the activin may be mediated by decreased Cyp26B1, leading to increased RA levels.

Retinoic acid and initialization of meiosis

It has been shown that RA can stimulate the expression of the premeiotic marker gene Stra8 (stimulated by RA gene 8) and meiosis in mice in both sexes (Griswold et al. 1989). Stra8 was first described as one of the group of RA-responsive genes (Oulad-Abdelghani et al. 1996) and deletion of Stra8 resulted in the prevention of the meiosis in the germ cells of both sexes (Baltus et al. 2006). RA appears to be present in both male and female embryonic urogenital ridges. In the mouse ovary, induction of Stra8 in fetal germ cells expressing Dazl, an intrinsic factor, is required for meiotic DNA replication and the subsequent events of meiotic prophase (Baltus et al. 2006; Lin et al. 2008). In mouse testes, RA action and the subsequent entry of gonocytes into meiosis in the embryonic male is inhibited by the presence of the enzyme cytochrome P450, family 26, subfamily b, polypeptide 1 (CYP26B1) (Bowles et al. 2006; Koubova et al. 2006). CYP26B1 degrades RA into metabolites, some of which are inactive. If CYP26B1 is inhibited in mouse embryonic testes in culture or if the gene encoding CYP26B1 is ablated, Stra8 mRNA is synthesized in male mouse germ cells and meiosis is initiated (Bowles et al. 2006; Koubova et al. 2006; MacLean et al. 2007). After birth, RA induces Stra8 in testicular germ cells, leading to meiotic initiation (Koubova et al. 2006; Anderson et al. 2008). Induction of Stra8 in the embryonic male germ cells is sufficient to induce the synthesis of downstream markers of meiosis, such as synaptonemal complex protein 3 (SCP3) and the meiosis-specific recombinase DMC1 (Bowles et al. 2006). Therefore, the expression of Stra8 is necessary for germ cells to enter into meiosis and is an excellent marker for the action of RA.

However, Koubova et al. (2014) have discovered that RA activates meiosis in two independent ways, both requiring Dazl expression in the germ cells. It has been shown that germ cells in Stra8-deficient murine fetal ovaries express Rec8 (Baltus et al. 2006), encoding a meiosis-specific component of the cohesin complex. Rec8 is required for completion of sister chromatid cohesion, proper synapsis, and chiasmata formation (Bannister et al. 2004). A chromatin immunoprecipitation-sequencing (ChIP-Seq) study in embryonic stem cells identified RAR binding sites in both Stra8 and Rec8 promoter regions, suggesting that Stra8 and Rec8 may be regulated by RA directly (Oulad-Abdelghani et al. 1996; Mahony et al. 2011). However, in the same study, Dmc1, which is dependent on Stra8, does not show RAR binding sites, consistent with Stra8 and Rec8 being regulated directly, unlike Stra8's downstream targets (Koubova et al. 2014).

It has also been suggested that the actual physiological role of RAR antagonists may be different from their reported functions because these compounds may exert nonspecific effects on other receptors (Kumar et al. 2011), based on a known case involving the RAR antagonist Ro 41-5253 (Schupp et al. 2007). This is a fair point, although the two pan-RAR antagonists that have been shown to inhibit meiosis are BMS-204493 and AGN193109, not Ro 41-5253 (Bowles et al. 2006; Koubova et al. 2006).

Retinoic acid and primordial germ cells

In mouse, germ cell formation begins *in utero* with primordial germ cells (PGCs), precursor cells developing into ova and spermatozoa that form around embryonic day 6.25 (E6.25) and migrate from the proximal epiblast through the hindgut towards the genital ridge around E10.5 (Saitou and Yamaji 2012). From here, female (XX) and male (XY) PGCs enter two distinct pathways. While XX PGCs continue to proliferate until E13.5 and subsequently enter meiosis, XY PGCs are enclosed by testicular cords, become prospermatogonia or gonocytes around E12.5 (McLaren 1984) and are then arrested at G0-like state around E13.5. Gonocytes remain quiescent around E16.5 until shortly after birth (Vergouwen et al. 1991).

Koshimizu et al. (1995) have found that RA is a potent growth activator of mouse PGCs *in vitro* and promotes the proliferation of PGCs and slows down the degeneration of colonizing PGCs in culture as a mitogen both *in vitro* and *in vivo* (Koshimizu et al. 1995). It has been also found that RA acts as both a mitogen and a survival factor for germ cells during fetal mouse oogenesis *in vitro* and *in vivo* (Morita and Tilly 1999).

Mouse embryonic stem cells can be induced into primordial germ cell-like cells (PGCLCs) by RA and promote the self-renewal of PGCs *in vitro* (Geijsen et al. 2004; Eguizabal et al. 2009). It has been found

that cultures with the presence of RA attain PGC-like identity and continue to proliferate. Furthermore, the expression patterns of Prmt5 and H3K27me3 in newly formed PGCs are similar to those of 11.5-dpc PGCs *in vivo* (Eguizabal et al. 2009). Tan et al. (2016) have found that RA induced the expressions of cell cycle-related genes. CCND1 is an important regulator of G1-to-S phase progression (Dalton 1999), and CDK2 can interact with cyclin E to drive cells through the G1-to-S transition and combine with cyclin A through the S-phase (Jirawatnotai et al. 2012). The increasing expressions of cell cycle-related genes suggested that cell cycle was affected after RA exposure. Meanwhile, the increase in the percentage of SSEA-1-positive PGCs suggested that RA could promote the proliferation of PGCs derived from mouse embryos *in vitro*, and CCND1 and CDK2 were also up-regulated after RA treatment, which was similar to PGCLCs cultured *in vitro* (Tan et al. 2016).

In chickens, Yu et al. (2011) have further verified that RA promotes the proliferation of chicken PGCs via the protein kinase C and PI3K/Akt signaling pathways. RA treatment increased the expressions of CCND1, CCNE1, CDK6, and CDK2, genes critical for G1-to-S phase progression in the cell cycle (Yu et al. 2011). Moreover, it has been confirmed that RA-treated chicken PGC populations have significantly increased proportion of S-phase cells (Yu et al. 2012; Tan et al. 2016).

Organotins derivates – RXR ligands – and reproduction

Organotin compounds are typical environmental contaminants and suspected endocrine disrupting substances (Brtko and Dvorak 2015). Humans are exposed to tributyltin (TBT), previously used as an antifouling paint in ships, mainly through fish consumption. A remarkable breakthrough in the field came out with the recent findings that triorganotin compounds are agonists of RXR subtypes of NRs (Figure 1), not sharing any structural characteristics with any endogenous ligands of NRs. It has been shown that RXRα with its NR cysteine residue (C432) of helix H11 at the entrance to the ligand binding pocket was found to covalently interact with trialkyltin or triaryltin (Grun 2014; Brtko and Dvorak 2015). After crossing the cell membrane, triorganotins could bind to NRs, which reside in the cytoplasm (e.g. glucocorticoid receptors) or in the nucleus (e.g. RXRs, PPARs) (Toporova et al. 2016). Upon ligand binding, triorganotin-NRs translocate to the nucleus where they form a complex triorganotin-NRs and co-activators, which binds to the response elements on the DNA and induces transcription of target genes, changes in the expression of some proteins, as well as mitochondrial and cell dysfunctions. TBT has been largely released into water from special paintings. At very low concentrations (pM or nM), TBT induced an irreversible sexual abnormality "imposex" in marine gastropods (Nakanishi 2008). Triorganotins have been suggested to have teratogenic and pathologic effects of on endocrine and reproductive system of mammals in both genders (Delgado Filho et al. 2011), and endocrine disrupting effects, such as induction of progesterone biosynthesis, effects on aromatase activity, and capability to induce transcriptional activity of thyroid hormone receptor (Brtko a Dvorak 2015; Illes et al. 2015; Hiromori et al. 2016). There are many reports regarding the biological effects of organotin compounds, which vary in their toxic effects on eukaryotes (reviewed in Delgado Filho et al. 2011). *In vitro* exposure of the human choriocarcinoma cell lines to TBT or triphenyltin (TPT) (300 nM) markedly decreased DNA and protein synthesis (Nakanishi et al. 2002). In the same concentration ranges, TPT

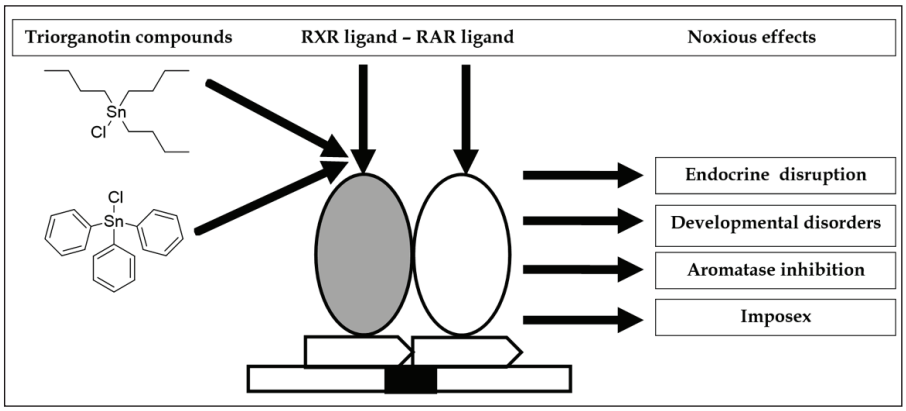

Figure 1. The effect of trioganiotin-based endocrine disrupting chemicals on nuclear retinoid X receptor (RXR) and retinoic acid receptor (RAR) pathways.

also inhibited the catalytic activity of human aromatase (Lo et al. 2003) and other steroidogenic enzymes, affecting sexual development in male and female rats (Delgado Filho et al. 2011).

Organotin compounds can cross the placental barrier and accumulate in large quantities in the placenta and fetal tissues inducing morphological changes (Cooke et al. 2008; Delgado Filho et al. 2011). Kishta et al. (2007) have reported reduced number of gonocytes, Sertoli and Leydig cells of fetal testis after *in utero* exposure to TBTCl in pregnant rats. Growth retardation, delayed ossification of the fetal skeleton and reduced body weight were also detected in male offspring. Additionally, a reduced testosterone concentration as well as a significant delay in the age at preputial separation (Grote et al. 2009) has been found after *in utero* exposure to TBT of rats, without affecting the male reproductive system (Adeeko et al. 2003).

The placenta plays a vital role in the maintaining pregnancy. The production of steroid hormones, such as progesterone and estrogens, is a crucial function of the primate placenta. In human placenta, steroid biosynthesis is regulated by various steroidogenic enzymes. The enzyme 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD) catalyzes the conversion of 3-hydroxy-5-ene-steroids (dehydroepiandrosterone and pregnenolone) to 3-oxo-4-ene-steroids (androstenedione and progesterone) (Simard et al. 2005). Placental production of progesterone is required to protect the conceptus during gestation (Malassine et al. 2003), the ingestion of progestins (i.e., natural and synthetic progesterone and testosterone derivatives that produce biologic effects similar to those of progesterone) during pregnancy is associated with an increased risk of hypospadias (Carmichael et al. 2005). Therefore, the developmental and reproductive toxicities of environmental contaminants known to have endocrine disrupting effects plausibly might involve placental 3β-HSD I in humans. It has been shown that TBT inhibits the catalytic activity of human 5α-reductase I and II, rat 3β-HSD, and porcine 17β-hydroxysteroid dehydrogenase (17β-HSD). TPT has been found to inhibit the catalytic activity of human aromatase, human 5α-reductase II, 17β-HSD I and III (Doering et al. 2002; McVey and Cooke 2003; Ohno et al. 2005).

It has been shown that exposure of human choriocarcinoma JAr cells to nontoxic concentrations of both TBT and TPT enhanced 17β-HSD I mRNA transcription and enzyme activity in a dose-dependent fashion and enhanced aromatase activity (Nakanishi et al. 2006). Moreover, TBT and TPT act as nanomolar agonists for both the RXR and peroxisome proliferator-activated receptor gamma (PPARγ) (Hiromori et al. 2009). PPARγ regulates the transcription of genes by heterodimerizing with RXR and by binding to the PPAR response elements in the target gene promoter (Kliewer et al. 1992). The promotion of estrogen biosynthesis by the organotin compounds involves the activation of RXR rather than PPARγ (Nakanishi et al. 2006).

Hiromori et al. (2009) have found that some organotin compounds, including TBT and TPT, promote human chorionic gonadotropin (hCG) production (Hiromori et al. 2009). hCG is a luteotropic factor and its stimulation by hCG governs not only progesterone production in the corpus luteum during the first trimester, but also the testosterone production within the fetal testes. hCG is a crucial target gene of PPARγ in human placenta and its production and mRNA transcription is ligand-dependently controlled by PPARγ (Tarrade et al. 2001). To investigate the effects of RXR and PPARγ agonists on progesterone production and 3β-HSD I mRNA transcription, Hiromori et al. (2016) have treated JAr cells with LG (RXR agonist) or Rosi (PPARγ agonist). Both LG and Rosi enhanced progesterone production and 3β-HSD I mRNA transcription. These data suggest that the RXR and PPARγ signaling pathways may be involved in organotin-induced progesterone production in human placental cells.

Moreover, Hiromori et al. (2016) have show that triorganotins, TPrTCl, TBTCl, TChTOH, and TPTCl, significantly enhanced the progesterone production in a concentration-dependent manner in human choriocarcinoma JAr cells. Among metabolites of both TBTCl and TPTCl, DBTCl2, MPTCl3, and DPTCl2 altered progesterone production, and the level of stimulation increased proportionally with the alkylation or arylation of these organotin compounds (tri- > di- > mono-). However, the presence of a fourth alkyl group on the tin atom decreased the potency of the organotin compounds, inducing the progesterone production, because tertbutyltin (TeBT) failed to stimulate this placental function at doses <100 nM. The organotin compounds that enhanced progesterone production also significantly increased the mRNA transcription of 3β-HSD I. All active organotins increased the mRNA transcription of 3β-HSD I in a concentration-dependent manner.

Several studies addressing the effect of TBT on male reproductive functions have been reported (Omura et al. 2001; Grote et al. 2004; Delgado Filho et al. 2011). Omura et al. (2001) have shown that dietary treatment with TBTCl resulted in decreased testis,

epididymis, ventral prostate, and body weight during two generation study in rats. In other studies, a significant decrease in the weight of the seminal vesicle and the weights of all reproductive organs has been reported in rodents (Grote et al. 2004). Prostate atrophy, as a consequence of aromatase inhibition, is also well known. Moreover, TBT induces morphological-functional changes in the testes, including vacuolization of seminiferous epithelium, delayed spermiation, spermatid retention into the germinative epithelium, and germ cell degeneration near the basement membrane. The frequencies of these impairments in male sex organs were greater and considered to be abnormal in the TBT-treated F2 generation in rats, although there was a dose-dependent increase in the serum testosterone levels of the rats fed by TBTCl diets and a decrease in serum estrogen levels in the F1 generation (Omura et al. 2001). In rats, the count of caudal epididymal and testicular sperm (Yu et al. 2003) and of homogenization-resistant spermatids decreased (Omura et al. 2001), and the motility, mean angular displacement, lateral head displacement, and dance of sperm from the vas deferens, were also reduced (Yu et al. 2003). Decreased serum concentrations of thyroxine and triiodothyronine were observed in another study, in association with extensive damage to the thyroid gland, and low expression of thyroid hormone receptor alpha in marine fish testes (Adeeko et al. 2003; Zhang et al. 2009).

A large number of studies have shown that exposure to organotins can cause reproductive disruption in the female reproductive system of mammals (reviewed in Delgado Filho et al. 2011). Treatment with organotins in pseudopregnant rats resulted in a decrease in uterine weight and serum progesterone levels, but ovarian weight, number of corpora lutea and estrogen levels remained at average levels. *In utero* exposure to high doses of TBT led to a decrease in the maternal weight gain and fetal weight, induced pre- and post-implantation losses (Nakanishi et al. 2005), provoked fetal toxicity (Itami et al. 1990), altered the anogenital distance of female pups on postnatal day 1 (Ogata et al. 2001), caused precocious completion of vaginal opening in postnatal females (Grote et al. 2009), reduced by about 45% the number of germ cells, and induced morphological-functional changes in the ovaries of fetal female rats (a large number of germ cells with pyknotic nuclei) (Kishta et al. 2007). In human choriocarcinoma cell lines, TBT and TPT increased levels of hCG secretion and aromatase activity in a dose- and time-dependent fashion following exposure to nontoxic concentration ranges (Nakanishi et al. 2002). In human choriocarcinoma JAr cells, trialkyltins and TPT enhanced 17β-HSD I mRNA transcription and enzyme activity in a dose-dependent fashion at nontoxic concentrations. (Nakanishi et al. 2006). However, in human granulosa-like cell line, TBT and TPT suppressed both aromatase activity and gene expression (Saitoh et al. 2001). Based on these results, it has been suggested that organotin compounds function as a powerful agonist for nuclear receptors rather than an aromatase inhibitor (Nakanishi 2008).

In conclusion, the effects of triorganotins have been associated with gender-specific morphological-functional changes in mammalian reproductive organs. Organotin compounds have been shown to act potentially as inhibitors of steroidogenic enzymes (Delgado Filho et al. 2011) and proteasome (Saitoh et al. 2001), or enhancers of histone acetyltransferase (Nakanishi et al. 2006). Moreover, effects of triorganotins have been shown on epigenetic regulation of gene expression (Stel and Legler 2015). Since recent studies have also shown that triorganotin compounds can exhibit anti-tumor activity (Tabassum and Pettinary 2006; Hunakova et al. 2015; Macejova et al. 2015), the further studies on triorganotin compound characteristics and action are essential.

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