ENDOCRINE DISRUPTION INDUCED BY ORGANOTIN COMPOUNDS: IMPACTS IN THE REPRODUCTIVE FUNCTION

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RESUMO

Compostos organoestânicos, como tributilestanho (TBT) e o trifenilestanho (TPT), são típicos contaminantes ambientais e produtos químicos ativos que causam o desenvolvimento de uma desregulação endócrina irreversível (masculinização) em moluscos do sexo feminino, conhecido de "imposex". Cabe lembrar, que ainda não está claro se os compostos organoestânicos podem causar toxicidade essencial em mamíferos, incluindo humanos e roedores, no seu desenvolvimento sexual e as funções reprodutivas. Ademais, estes compostos podem atuar como potenciais inibidores competitivos da enzima aromatase ou outras enzimas da esteroidogênese, afetando a capacidade reprodutiva dos ambos os gêneros de mamíferos. Nesta revisão, provemos uma discussão da bioquímica, celular e mecanismos moleculares pelos quais os compostos organoestânicos podem causar efeitos adversos nos genes envolvidos na modulação da função reprodutiva.

Palavras chave: organoestânicos, desregulador endócrino, aromatase, função reprodutiva nos mamíferos

ABSTRACT

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are typical environmental contaminants and suspected endocrine-disrupting chemicals because they cause irreversible sexual abnormality (masculinization) in female mollusks, called "imposex". However, it remains unclear whether organotin compounds also cause crucial toxicities in mammalian, including in human and rodents, in their sexual development and reproductive functions. Moreover, these compounds can act as potential competitive inhibitors of aromatase enzyme or others steroidogenic enzymes, affecting the reproductive capacity of male and female mammals. In this review, we provide a discussion of the cellular, biochemical, and molecular mechanisms by which organotin compounds may cause adverse effects in the modulated genes involved in reproductive function.

Keywords: organotin, endocrine disruptor; aromatase, mammalian reproductive function.

1. Introduction

Organotin compounds (OT), 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 (FENT, 1996; SWENNEN *et al*., 2009). Due to its widespread use as an antifouling agent in boat paints, OT are a common contaminant of marine and freshwater ecosystems exceeding acute and chronic toxicity levels (NAKANISHI, 2008). OT are one of the most significant pesticides in marine and freshwaters and consequently its environmental level, fate and toxicity are of current concern (FENT, 1996; NAKANISHI, 2008).

There are many reports of the biological effects of OT, which vary in their toxic effects on eukaryotes (FENT, 1996; TAKAHASHI et al., 1999; LAHBIB et al., 2008; MENG et al., 2009; DELGADO et al., 2010). These compounds are potent endocrine disrupters in marine invertebrates (COSTA, 2008a, b; LIMAVERDE et al., 2007), mainly, but not exclusively, in gastropod mollusks. For example, very low concentrations of TBT and TPT induce irreversible sex-organ alterations in females, a phenomenon known as “imposex” (MATHIESSEN AND GIBBS, 1998). These endocrine abnormalities are the result of a masculinization process by which male sex organs are developed, notably a penis and a vas deferens, in female animals, that could lead to sterility and death of affected females (SHI et al., 2005). In certain species, growth of a vas deferens disrupts the structure and function of oviducts, impairing normal breeding activity and causing population decline (NAKANISHI, 2008). In addition, it had been reported in more than 190 marine species (SHI et al., 2005; NAKANISHI, 2008) and has been considered as the most important endocrine disruption effect derived from a specific class of compounds (SNOEIJ et al., 1987; MATHIESSEN AND GIBBS, 1998). This high specificity makes this syndrome a useful biomarker of organotin pollution (SNOEIJ et al., 1987; FERNANDEZ et al., 2002; FERNANDEZ et al., 2005a, b). Besides gastropods, organotins have been implicated in inducing hormonal alterations in bivalve mollusks (ALZIEU, 1986; MORCILLO AND PORTE, 2000; SIAH et al., 2003), in crustaceans (TANG et al., 2009; AONO and TAKEUCHI, 2008) and fish (MORTENSEN and ARUKWE, 2007).

Unfortunately, these OT are also suspected to cause endocrine-disrupting effects in mammals (KONSTANZE et al., 2006), including humans (KANNAN, 1995; KEITHLY, et al., 1999) and rodents (GROTE et al., 2004; GROTE et al., 2006; DELGADO et al., 2010), due in part the possibility of transferring through marine food chains by consumption of contaminated seafood (TANABE, 1999; DORNELES et al, 2008).

Based on studies *in vitro,* the human choriocarcinoma cell lines exposure to 300 nM TBT or TPT markedly decreased DNA and protein synthesis (NAKANISHI et al., 2002). At the same concentration ranges, TPT also inhibit the catalytic activity of human aromatase (TAKAYANAGI AND NAWATA, 2001; LO et al., 2003) and others steroidogenic enzymes, affecting sexual development in male (OMURA et al.,

2001; GROTE et al., 2004) and female rats (OGATA et al., 2001). Therefore, OT clearly has many complex actions in endocrine system at both genders, which explains the information on their relative dangers.

2. ORGANOTINS AS ENDOCRINE-DISRUPTING CHEMICALS

The production of sex hormones steroids from cholesterol requires trafficking between mitochondria and smooth endoplasmic reticulum, and involves many enzymatic steps (WHITEHEAD AND RICE, 2006). Most of these pathway use cytochrome P450 (CYP) haem-containing enzymes are abbreviated to CYP (see Fig. 1). Some OT are known as encoding-disrupting chemicals to change steroid hormone biosynthesis (BETTIN et al., 1996; MATTHIESSEN AND GIBBS, 1998). As mentioned above, these OT have been suspected to masculinize reproductive organs in vertebrates because, in some gastropods, very low concentrations of these organotins induce "imposex" (HORIGUCHI et al., 1997; MATTHIESSEN AND GIBBS, 1998). Some evidences have theorized that these OT act as a specific inhibitor of aromatase enzyme which converts androgen to estrogen (BETTIN et al., 1996; MATTHIESSEN AND GIBBS, 1998; DELGADO et al., 2010). For example, exposure to OT increase testosterone levels in female gastropods and organotin-induced imposex can be mimicked by specific inhibitor of aromatase (BETTIN et al., 1996). In addition, TBT was reported to be catalyzed to dibutytin, which is a metabolite of TBT, by aromatase enzyme (LEE, 1985). However, it remained unclear whether OT especially inhibits catalytic activity of aromatase in vertebrates.

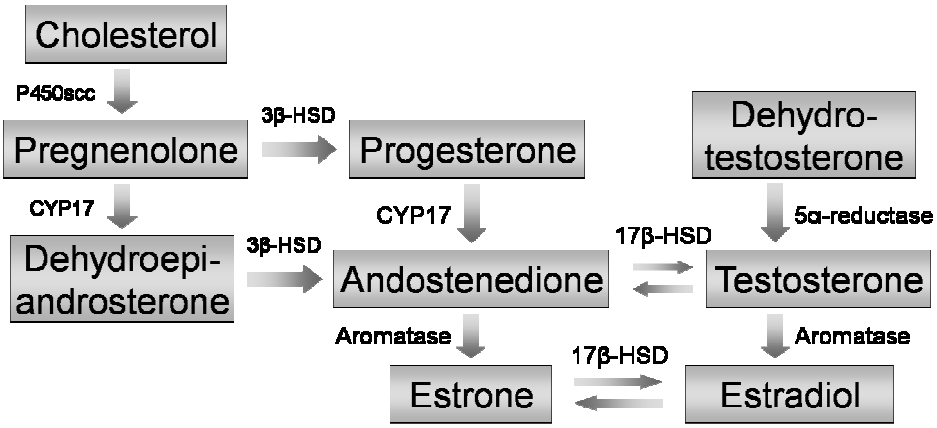

Figure 1. Pathway of steroid hormone biosynthesis whose enzymatic actions can be altered by organotin compounds. Cholesterol side-chain cleavage enzyme complex (P450scc); Cytochrome P450 (CYP, as 17α-hydroxylase and 17,20-lyase, respectivaly); 3β-hydroxysteroid dehydrogenase (3β-HSD); 17β-hydroxysteroid dehydrogenase (17β-HSD). Figure modified from Ref. Nakashima et al., 2008.

In others experiments, butyltins were demonstrated to exhibit structure-related inhibition of the catalytic human aromatase protein from human placenta cell line (HEIDRICH et al., 2001) or transfected cells (COOKE, 2002). However, at effective concentrations (micromolar level) for the inhibition of aromatase, TBT and TPT are generally toxic to mammalian cells because they cause apoptosis or necrosis (SAITOH et al., 2001; NAKANISHI et al., 2006). In human choriocarcinoma cell lines, Jar, JEG-3 and BeWo, exposure to greater than 300 nM TBT or TPT markedly decreases DNA and protein synthesis (NAKANISHI et al., 2002; NAKANISHI et al., 2006). Concentrations under 1 μM of either OT did not significantly affect aromatase activity in microsomes isolated from human choriocarcinoma cells (NAKANISHI et al., 2002). In addition to aromatase, at above 1 μM, TBT inhibit the catalytic activity of human 5*α*-reductase I and II (5*α*-R I and II) (DOERING et al., 2002), rat 3*β*-hydroxysteroid dehydrogenase (3*β*-HSD) (McVEY AND COOKE, 2003) and pig 17*β*-hydroxysteroid dehydrogenase I(17*β*-HSD I) (OHNO et al., 2005). At same concentrations ranges, TPT also inhibit the catalytic activity of human aromatase, 5*α*-R II, 17*β*-HSD I and III (LO et al., 2003). These observations suggest that these OT at micromolar level not specifically inhibit the catalytic activity of aromatase and we have to consider the toxicity of OT in distinguishing between nonspecific toxicity to cells and the specific inhibition of steroidogenic enzymes.

In addition, gonadal steroid receptors and steroidogenic enzymes for sex steroid hormones have not yet been identified in gastropods, and it remains unclear whether sex steroid hormones are critical factors for sexual maturation in gastropods. Furthermore, homologues of both the estrogen receptor (ER) and androgen receptor (AR) have not been found in invertebrates (ESCRIVA et al., 2000) and the composition of nuclear receptor family members is very different between vertebrates and invertebrates (ESCRIVA el al., 1997). Therefore, there is some doubt as to whether OT function as inhibitors of enzyme that metabolize androgens in gastropods, and this doubt led us to suspect that organotin compounds affect other target molecule in mammals.

3. ORGANOTINS AFFECT ENDOCRINE FUNCTIONS IN MAMMALIAN GONADAL SYSTEM

Sexual differentiation is a sequential process beginning with the establishment of chromosomal sex at fertilization, followed by the development of gonadal sex, and culminating in the development of secondary characteristics, collectively termed the male and female phenotypes (FILICORE et al., 1986) The endocrine system reflects deeply at the reproductive morphophysiology most likely due to specifics genes and gonadal steroids actions and endocrine/paracrine pathways (see Fig. 2) on the gonadal system (LIM AND HAWKINS, 1998; PIPREK, 2009; DELGADO et al., 2010). Moreover, it also been shown that exerts different effects on either males or females (PIPREK, 2009), that are time and dose dependents of exposure to toxic effect of OT. Studies suggested that potential toxicity of OT in mammalian, that include human and rodents, are endocrinophatic, as well as potential toxicity reproductive, teratogenic and developmental (Nakanishi, 2008), in both genders (OMURA et al., 2001; GROTE et al., 2004, GROTE et al., 2006).

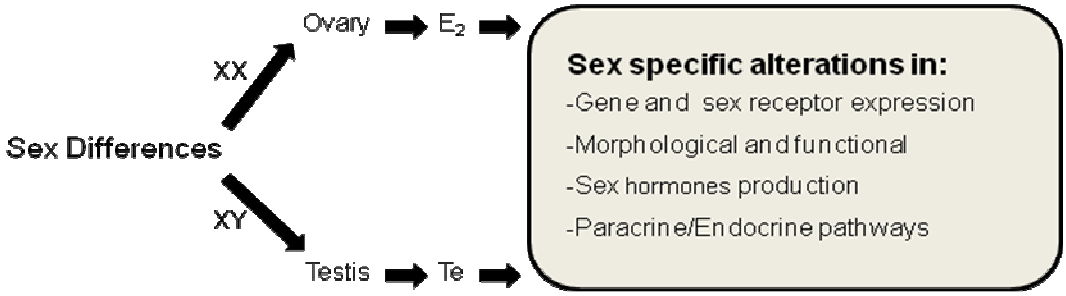

Figure 2. Mechanisms underlying sexual dimorphism by genetic, gonadal sex and differences actions the sex hormones. The sex steroids (estradiol and testosterone) can be alterations directly in gene expression, endocrine/paracrine pathways, morphologic development and functional, and ultimately phenotype, specifically; whereas changes directly influence physiological function, with sex development. X, Y: sex chromosomes; E_2: estradiol; Te: testosterone.

3.1 MALE REPRODUCTIVE SYSTEM

Several studies addressing the effect of TBT on male reproductive functions have been reported (ADEEKO et al., 2003; CHEN et al., 2008; GROTE et al., 2004; KONSTANZE et al., 2004; KIM et al., 2008; KISHTA et al., 2007; MAKITA AND OMURA, 2006; OMURA et al., 2001; WANG et al., 2006; YU et al., 2003a,b; ZHANG et al., 2009a,b). A study conducted during two generations showed that weight of the testis, epididymis and ventral prostate weights decreased in all groups, but mainly in the 125 ppm. However, no reduction was observed in weight of the seminal vesicle in F1 generation. Unfortunately, the effects on F2 generation compared with those in the F1 generation are greater (OMURA et al., 2001). Despite this effect, some studies showed significant decreased in weight of the seminal vesicle (YU et al. in 2003b; GROTE et al., 2004) and in all weights of reproductive organs at 15mg TBT/Kg bw (GROTE et al., 2004). The caudal epididymal and testicular sperm (YU et al., 2003a), and homonization-resistent spermatid (OMURA et al., 2001) counts were decreased, and some of motion kinematic parameters of sperms from vasa deference were reduced too (YU et al., 2003a).

Based on histopathological analysis, TBT causes changes in testes, included vacuolization of seminiferous epithelium, delayed spermiation, spermatide retention in the epithelium and germ cell degeneration near the basement membrane Frequencies were low in F1 generation, but in F2 generation

these are greater and considered abnormal (OMURA et al., 2001). Increase of detached debris and sloughed cells were observed in the tubules of epididymis, and seminal vesicle was narrowed and become occupied with epithelial cells (YU et al., 2003b).

A large number of evidences indicate that *in utero* exposure to OT (ADEEKO et al., 2003; MAKITA AND OMURA, 2006; KISHTA et al., 2007) has a different pattern of response by pre- and postnatal offspring. There was reduce in number of Sertoli cells and gonocytes, a large intracellular space between those cells and an increased abundance of lipid droplets in the Sertoli cells (KISHTA et al., 2007). Furthermore, in the intertubular region between adjacent interstitial cells, this study revealed abnormally dilation on endoplasmatic reticulum in Sertoli cells and gonocytes; immunostaining for connexin 43, the gap junctional protein, was reduced or absent in treated rats (KISHTA et al., 2007).

Additionally, the body weights of the male offspring were decreased, and growth retardation and delayed ossification on the fetal skeleton were found after TBT *in utero* exposition of rats, without direct effects on male reproductive system (ADEEKO et al., 2003; MAKITA AND OMURA, 2006). However, decreased concentrations of thyroxine and triiodothyronine (ADEEKO et al. 2003) in serum, was also observed in another study (ZHANG et al., 2009a), and associated with large damage on thyroid gland, and low expression of thyroid hormone receptor alpha in marine fishes' testes. Also, exposure has caused interstitial fibrosis and pyknotic nuclei. Results implied that inhibition of thyroidal status induced by TBT possibly affect testicular development. Similarly, after contact of TBT, the gonadosomatic index had decreased in a dose-dependent manner (ZHANG et al., 2009b). Furthermore the level of 17β-estradiol was decreased and result in a down-regulation of estrogen receptor alpha mRNA, which in addiction with enlargement of lipid droplets, may contribute for Sertoli cells dysfunction, leading to disrupted spermatogenesis (ZHANG et al., 2009b).

In the same line of investigation, *in vitro* analysis demonstrated that TBT enhanced the chance of occur apoptosis on Leydig cells, in a time- and dose-dependent manner (WANG et al., 2006). It´s probably mediated by increase of Ca^{2+} cytoplasmic concentration. Immature male mice given a simple administration of TBT presented lumen formation in seminiferous tubule delayed, and increased number of apoptotic germ cells inside tubules, whereas was not signal of apoptotic Leydig cells (WANG et al., 2006). Reduced serum testosterone concentration (WANG et al., 2006; ZHANG et al., 2009a,b; GROTE et al., 2004) and down-regulated expressions of the mRNAs for P450scc (cholesterol side-chain cleavage enzyme complex), P450 (for example, 17α-hydroxylase), 3β-HSD and 17β-HSD were also observed (KIM et al., 2008; CHEN et al., 2008). Indeed, others works had related increase of serum testosterone (OMURA et al., 2001).

3.2 FEMALE REPRODUCTIVE SYSTEM

The production of germ cells is essential for the continuation of a species. This function, in the female, is accomplished by the ovaries. In addition, the ovaries secrete steroidal (main are progesterone and estradiol) and nonsteroidal hormones, as relaxin, that not only stimulate the secretion of anterior pituitary hormones, but also act on various targets on female reproductive system´s organs (FILICORE et al., 1986).

A number of studies have showed that exposure to OT and sea food contaminated by organotin cause reproductive disrupting in mammalian reproductive female system (HARAZONO et al., 1996; EMA et al., 1997; EMA et al., 1999; EMA, 2000; OMURA et al., 2001; OGATA et al., 2001; NAKANISHI et al., 2002; GROTE et al., 2004; NAKANISHI et al., 2005; GROTE et al., 2006; KONSTANZE et al., 2006; DELGADO et al., 2010). After treatment with organotin in pseudopregnant rats, decreased in uterine weight and serum progesterone levels were shown. It is correlated with decreased of pregnancies rate and number of embryologic implantations (EMA et al., 1997; EMA, 2000; EMA and MIYAWAKI , 2002).

In mammalian, others works have shown that *in utero* exposure to high doses of TBT led to decreased on maternal weight gain and fetal weights, induced pre- or post-implantation losses (ADEEKO et al., 2003; EMA et al., 1995; HARAZONO et al., 1998), and caused fetal toxicity (ITAMI et al., 1990), altered anogenital distance in both postnatal day 1 female pups (OGATA et al., 2001), gestational day 20 male rat fetuses (EMA et al., 1997) and reduced, in the ovaries of fetal female rats (20 mg/kg, days 0–19; 10 mg/kg, days 8–19), the number of germ cells by 44% and 46%, respectively (KISHTA et al., 2007).

Based on genetic analysis, the TBT and the TPT increased the catalytic activity of aromatase and 17β-HSD I enzymes, which converts low-activity estrone to high-activity estradiol in human choriocarcinoma cells (NAKANISHI et al., 2006) along with their mRNA expression in a dose-dependent fashion following

exposure to non-toxic concentration ranges (NAKANISHI, 2008), indicating the regulation of mRNA levels of both steroidogenic enzymes, not of enzyme complex. However, the TBT and the TPT suppressed both activity and gene expression of aromatase enzyme in the human ovarian granulose-like cell line (SAITOH et al., 2001). This discrepancy in the action of OT on the gene expression of human aromatase is due to the tissue-specific expression of aromatase, which is strictly regulated in each type cell (NAKANISHI, 2008).

4. CONCLUSION

Several studies revealed that exposure to OT alters development and sexual parameters of reproductive system in gastropods, acting as endocrine disruptors, influencing the steroidal metabolism, mainly inhibiting the enzymatic activity of aromatase. These endocrine abnormalities are known as imposex. OT induced toxic effects in endocrine system of mammals changing the gene expression of aromatase in different cell lines and animals models or changing the activity of other glands, as thyroid. Moreover, there are others steroidogenic enzymatic pathways that can be impaired by organotin. These organotins´ effects were associated a both gender-specific alterations in mammalian reproductive organs. Despite those several evidences, further epidemiological, clinical and molecular investigations in different human cells and experimental animal should be developed to clarify the toxic effects of organotins on the mammals' endocrine system.

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