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Effects of 2,2′,4,4′-tetrabromodiphenyl ether (BDE47) on the enzymes of phase I (CYP2B1/2) and phase II (SULT1A and COMT) metabolism, and differences in the action of parent BDE-47 and its hydroxylated metabolites, 5-OH-BDE-47 and 6-OH-BDE47, on steroid secretion by luteal cells

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ABSTRACT

In this study we determined the effects of BDE-47 on the expression and activity of phase I (CYP2B1/2) and phase II (SULT1A and COMT) enzymes, and assessed the actions of BDE-47 and its metabolites on luteal steroidogenesis. Luteal cells collected during early (ELP), middle (MLP) and late (LLP) luteal phase were exposed to BDE-47 (0.5, 25, and 50 ng/ml) or metabolites (2.5, 5 and 25 ng/ml). BDE-47 decreased CYP2B1/2 activity and expression but had no effect on SULT1A or COMT. BDE-47 exerted a stimulatory action on estrogen secretion in MLP and an inhibitory in LLP, but had no effect on progesterone secretion. 5-OH-BDE-47 and 6-OH-BDE-47 decreased progesterone, but had no effect on estrogen secretion.
Conclusions: The inhibitory effect of BDE-47 on CYP2B1/2 suggests the possibility of BDE-47 accumulation in the corpus luteum; by affecting steroid secretion and steroidogenesis enzymes, BDE-47 and its metabolites can be responsible for shortening luteal phase.

1. Introduction

The ovary was previously shown to be a target tissue for polybrominated diphenyl ethers (PBDEs) (Gregoraszczuk et al., 2008a,b). The corpus luteum, a mini-endocrine gland, plays a central role in the regulation of the estrous cycle and in the maintenance of pregnancy. This function is carried out largely by progesterone, which is the main steroid synthesized by this transient endocrine gland. We previously reported the in vitro effects of endocrine disruptors, such as polychlorinated biphenyls (PCB-126 and PCB-153) and 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD), on luteal cell function (Gregoraszczuk et al., 2000; Augustowska et al., 2001). Available information in the literature indicates that, apart from our previous study (Gregoraszczuk et al., 2012), there are no reports on the effect of brominated diphenyl ethers on luteal cell function. In this previous study, we showed that despite an initial stimulatory effect of PBDEs on the secretion of progesterone by corpus luteal cells (reflecting the fact that the biochemical apparatus responsible for the conversion of cholesterol into pregnenolone remains uninterrupted), PBDEs are also a key executor of apoptosis (by activating both the extrinsic and intrinsic pathways of apoptosis after longer exposure periods), which can lead to premature dysfunction of the corpus luteum (Gregoraszczuk et al., 2012).

It has been shown experimentally that PBDEs may be metabolized to more polar compounds in living organisms. PBDEs are transformed into hydroxylated and methoxylated metabolites (OH- and MeO-PBDEs) in exposed mice and rats (Malmberg et al., 2005; Qiu et al., 2007) via phase I and phase II metabolic enzymes. The most important phase I transformation is oxidation conducted by cytochrome P450 enzymes (CYPs). In general, cytochrome P450 introduces oxygen on carbon atoms, mostly by hydroxylation.

CYP2B, a member of the cytochrome P450 superfamily, is expressed both constitutively and inducible (Pustylnyak et al., 2009), mainly in the liver, but it is also present in many other tissues, such as the lungs, prostate gland, uterus, adrenal glands, placenta, kidney, brain, and testis (Nishimura et al., 2003). We have reported that CYP2B and CYP1A1 are expressed in porcine follicles in the absence of any external stimulus (Ptak et al., 2006, 2008), but

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found that BDE-47 stimulates CYP2B1/2 activity but not CYP1A1 (Karpeta et al., 2012). This may indicate possible local formation and action of hydroxylated metabolites prior to their detoxification during phase II metabolism (Karpeta et al., 2012). Swine express cytochrome P450 forms similar to those identified in humans and rodents (Myers et al., 2001).

There are six possible mono-hydroxylated metabolites of BDE-47 produced by cytochrome P450 enzymes: 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4-OH-BDE-42, 4′-OH-BDE-49, and 2′-OH-BDE-66 (Qiu et al., 2007). In human blood, 5-OH-BDE-47 is the most abundant BDE-47 metabolite, followed by 6-OH-BDE-47 (Qiu et al., 2009).

The first phase of metabolism does not significantly eliminate PBDEs; OH-PBDEs are still relatively hydrophobic and remain in the organism (Canton et al., 2008). They are present in blood samples from rats and mice after exposure to PBDE mixtures (Malmberg et al., 2005; Qiu et al., 2007) and are observed in blood samples from wild animals such as fish, birds, and mammals (Marsh et al., 2006; Valters et al., 2005; Verreault et al., 2005). OH-PBDEs are also present in human plasma (Athanasiadou et al., 2008; Qiu et al., 2009) and breast milk (Lacorte and Ikonomou, 2009).

Phase II enzymes subsequently inactivate and remove phase I metabolites from the organism. Reactions performed by these enzymes generally result in a decrease in biological activity and an increase in the hydrophilicity of xenobiotics or endogenous compounds, thereby facilitating their excretion. Phase II transformation generally produces polar derivatives, known as conjugates, of the functional group. Phase II enzymes are expressed mainly in the liver, but are also found in the ovary. These enzymes include catechol-O-methyltransferase (COMT), and ubiquitously expressed enzyme that catalyzes the S-adenosyl-l-methionine-dependent methyl conjugation of hydroxyl groups and, among other things, converts 2- and 4-hydroxyestrogen to their methoxyestrogen counterparts (Lachman et al., 1996). Salih et al. showed that COMT is expressed in both porcine and human granulosa cells in two forms: membrane-bound COMT (MB-COMT) and soluble COMT (S-COMT). Another phase II biotransformation enzyme present in ovaries is sulfotransferase (SULT), which catalyzes the sulfation of many xenobiotics, steroids, and neurotransmitters (Nagata and Yamazoe, 2000; Blanchard et al., 2004). Lin et al. (2004) showed that SULT1A mRNA is expressed in porcine ovaries, and our previous studies demonstrated for the first time that SULT1A protein is expressed in porcine follicular cells (Karpeta et al., 2012). Additionally, we showed that BDE-47 increases SULT1A and COMT activity in follicular cells.

Despite our previous data showing an impact of BDE-47, -99 and -100 on steroid secretion by corpora lutea collected from MLP, to our knowledge there are no data on PBDE metabolism in the corpus luteum or possible actions of hydroxylated metabolites. The current study was designed to determine (1) the basal expression of enzymes involved in the metabolism of steroids and xenobiotics in different stages of the corpus luteum; (2) the effects of BDE-47 on phase I (CYP2B1/2) and phase II (SULT1A and COMT) enzyme activity and expression; and (3) the effects of BDE-47, 5-OH-BDE-47, and 6-OH-BDE-47 on luteal steroidogenesis (steroid secretion and expression and activity of steroidogenic enzymes).

2. Materials and methods

2.1. Reagents

Parker medium (M199) phenol red free, trypan blue, antibiotic/antimycotic solution (10,000 units/mL penicillin, 10 mg/mL streptomycin, and 25 mg/mL amphotericin B), trypsin–EDTA, 4-nitrocatechol, S-adenosyl-L-methionine (SAM), Na-deoxycholate, Nonidet NP-40, protease inhibitor (EDTA-free), dithiothreitol (DTT), adenosine 3′-phosphate 5′-phosphosulfate (PAPS), p-nitrophenyl sulfate, 2-naphthol, pregnen-3β-ol-20-one (P5), testosterone and anti-β-actin antibody were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tris, SDS, Tween-20 were purchased from Lab Empire S.C. (Rzeszów, Poland). Antibodies against 3β-hydroxysteroid dehydrogenase (3β-HSD) (sc-30820), aromatase(CYP19) (sc-14244), CYP2B1/2 (sc-53242), COMT (sc-25844), SULT1 (sc-27980), horseradish peroxidase-conjugated secondary antibody and Western blotting luminol reagent were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyvinylidene difluoride (PVDF) membrane was purchased from Merck Millipore (Darmstadt, Germany). The congener 2,2′,4,4′-tetrabromodiphenylether (BDE-47; Chiron AS, Trondheim, Norway) was dissolved in isooctane (Aventor Performance Materials Poland S.A., Gliwice, Poland). The metabolites of BDE-47, 5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether, and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE-47 and 6-OH-BDE-47) were purchased from AccuStandard Inc. (New Haven, CT, USA). The purities of BDE-47, 5-OH-BDE-47 and 6-OH-BDE-47 were 99.5%. Stock solutions of hydroxylated metabolites BDE-47 were prepared in acetonitrile (Aventor Performance Materials Poland S.A., Gliwice, Poland). The final concentration of isooctane and acetonitrile in the medium was always 0.1%.

2.2. Sample collection

Porcine ovaries were collected from estrus cycling adult cross-bred gilts (7–9 months of age; Large White and Polish Landrace) at a local abattoir. Approximately 15 min elapsed from the time of slaughter to ovary collection.

Corpora lutea were staged on the basis of morphologic criteria as early luteal phase (ELP: 1–2 days after ovulation; $n = 6$), middle luteal phase (MLP: 7–10 days after ovulation; $n = 6$), and late luteal phase (LLP: 13–15 days after ovulation; $n = 6$), according to previously published criteria (Gregoraszczuk and Obłonczyk, 1996). Luteal tissue, obtained from pools of freshly excised corpora lutea of three animals in the same phase of the estrus cycle was enzymatically dissociated according to our own technique (Gregoraszczuk, 1983). Finally, cells were spun and suspended in M199 medium supplemented with 5% FBS to yield a suspension of 1.6×10^5 cells/ml medium. Cell viability was measured using the trypan blue exclusion test and was determined to be 80–85%. In each experiment, six ovaries from three different animals in the same phase of the estrus cycle were selected.

2.3. Experimental procedure

Exp. 1. For determination of the activity of biotransformation enzymes, luteal cells obtained from ELL, MLP, and LLP were seeded in 96-well tissue culture plates at 1.6×10^5 viable cells/well in M199 medium supplemented with 5% FBS (control) or were incubated with different concentrations of BDE-47 (0.5, 25, and 50 ng/ml) for 24 h for CYP2B1/2 assays, or for 48 h for SULT1A and COMT assays. Concentrations of BDE-47 were chosen based on concentrations determined in human blood (Guvenius et al., 2003; Mazdai et al., 2003).

Exp. 2. For determination of CYP2B1/2expression, luteal cells obtained from ELL, MLP, and LLP were cultured in 24-well plates in M199 medium supplemented with 5% FBS (control) or with 50 ng/ml of BDE-47. After 24 h of incubation with BDE-47, luteal tissues were collected and stored at −20 °C for Western blot analysis.

Exp. 3. For determination of the concentrations of progesterone and estradiol in media, cells were seeded in 96-well tissue culture plates at 1.6×10^5 viable cells/well in M199 medium and exposed to