EFFECT OF PCB3 AND E2 ON CYP1A1, 1A2 AND 2B ACTIVITY IN OVARIAN FOLLICULAR CELLS.

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Introduction

Lower chlorinated PCBs, especially these with one or two free para-positions, are rapidly metabolized and they are called "episodic congeners" (Hansen 1998). The cytochrome P450 monooxygenases (CYP) are responsible for the biosynthesis and metabolism of endogenous compounds such as steroid hormones. [1] Pig and human ovaries contain CYP1A1 and CYP1B1 isoforms. [2,3,4] Leighton et al. [5] have also demonstrated CYP1A1 RNA in porcine ovarian granulosa cells and porcine ovarian granulosa cell line (MDG2.1). For this reason, ovary may play an important role in the metabolism of endogenous and exogenous compounds.

Polychlorinated biphenyls (PCBs), are inducers of CYP1A1/2 and CYP2B1/2 isoforms. [6,7] These enzymes are also involved in the metabolism of estradiol to catechol estrogens in the ovary. [2,4] Thus, it is possible that PCB is metabolized by this enzymes and/or interferes with estrogen metabolism in this extra-hepatic tissues.
The aim of this studies was to support the hypothesis that the porcine ovary contains CYP1A1, CYP1A2 and CYP2B monooxygenases and to compare the action of PCB3 and E2 on its activity.

Material and Methods

Cell cultures

Porcine prepubertal ovaries obtained from a local abattoir were collected into a bottle filled with sterilized saline and transported to the laboratory. Granulosa cells (Gc) and theca interna cells (Tc) were isolated from antral follicles (4-6 mm in diameter) according to the technique described by Stokłosowa et at., [8]. Cells were cultured in M199/FBS for 24hrs to allow for attachment. Then medium was replaced and cells were cultured for another 48 hours to investigate the action of PCB3 (6ng/ml) or 17β-estradiol (20nM) At the end of incubation, the medium was removed and cells were stored in –70°C.

EROD (CYP1A1), MROD (CYP1A2) and PROD (CYP2B) assay.

Cells were lysed by removal from freezer and allowed to thaw for 10 min. To each reaction well the following reagents were added: 50µl of BSA (1.33mg/ml in 50mM Tris, pH 7.2; final concentration in the reaction mixture) and 100 µl of ethoxyresorufin (10µM in 50mM Tris, pH 7,2; final reaction concentration) for EROD or 100 µ of methoxyresorufin (5µM in 50mM Tris, pH 7,2; final concentration) for MROD or pentoxyresorufin (5µM in 50mM Tris, pH 7.2; final concentration) for MROD. Plates were incubated for 15 minutes at 37°C with gentle shaking. Then, to start the reaction, 50 µl of NADPH (1.67 mM in 50mM Tris, pH 7.2; final concentration) was added to each reaction well (except for blank). Samples were incubated at room temperature without shaking, and fluorescence was read at 15-minute intervals for 2 hours in fluorescence plate reader (FLx 800, Bio-Tek, USA) using 530nm excitation filter and 590nm emission filter. After 2 hours, 100 µl of fluorescamine with acetonitryle (90 µg of fluorescamine/well, final concentration) was added to each well to stop the reaction and determine protein concentration (400nm excitation filter and 460nm emission filter). Results were calibrated against respective standard curve (0-100nM, 200µl final volume) prepared in 48-well plate and BSA standard curve (0-1000µg, 200µl final volume) prepared in 48-well plate.

Results and Discussion

Basal CYP1A1, 1A2 and 2B activity

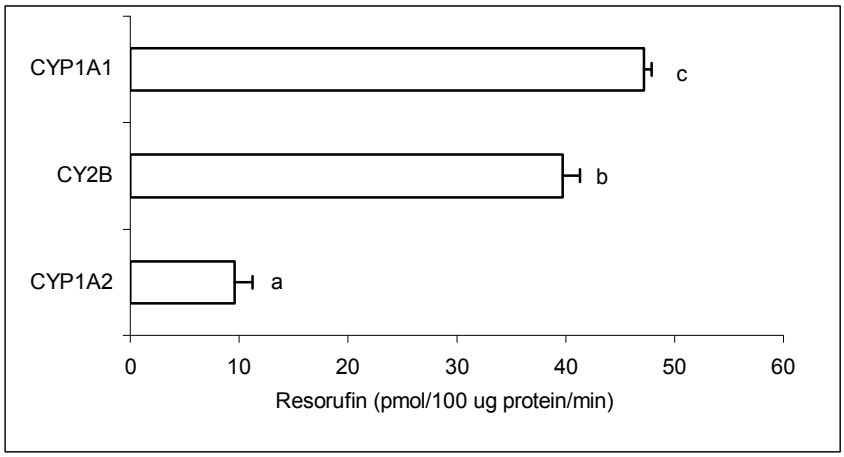

Figure. 1. Basal CYP1A1, 1A2 and 2B activity in porcine ovary, measured by EROD, MROD and PROD assay.

Effect of PCB3 and E2 on CYP1A1, 1A2 and 2B activity

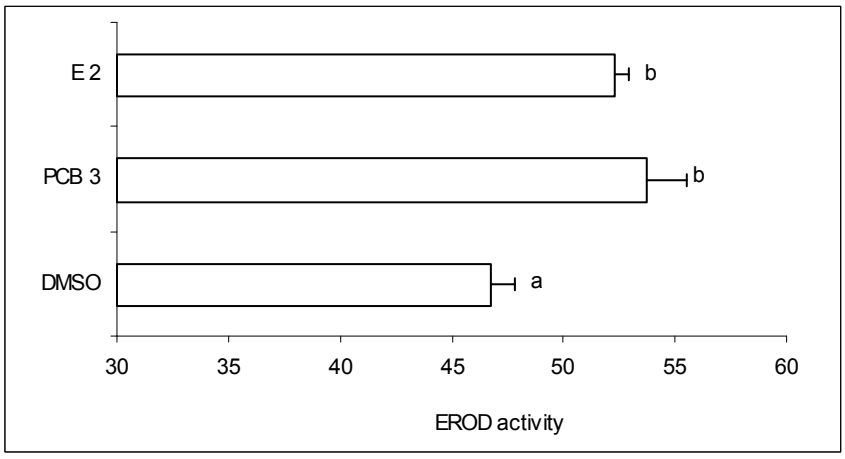

Figure. 2. Effect of PCB3 and 17β-estradiol (E2) on CYP1A1 (in pmol resorufin/ 100μg protein/min).

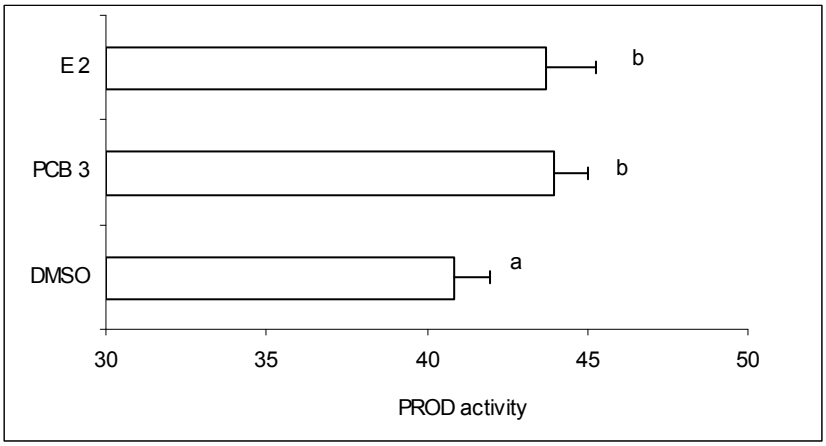

Figure. 3. Effect of PCB3 and 17β-estradiol (E2) on CYP2B (in pmol resorufin/ 100µg protein/min).

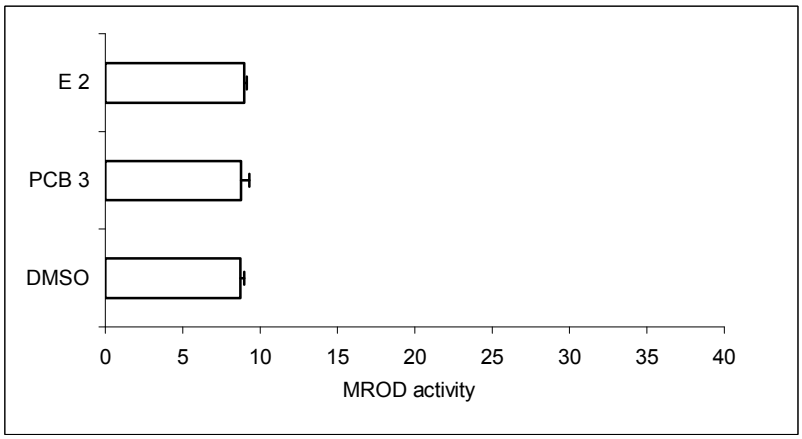

Figure 4. Effect of PCB3 and 17β-estradiol (E2) on CYP1A2 (in pmol resorufin/ 100µg protein/min).

We report here that granulosa-theca co-cultures express xenobiotic metabolizing cytochrome P450 activities, with CYP1A1 > CYP2B >> CYP1A2. A significant increase in CYP1A1 and 2B, but not CYP1A2, activity was seen in cells that were exposed to 6 ng/ml PCB3 or 20 nM 17-β-estradiol.

Very few studies describe xenobiotic metabolizing enzyme activities in ovaries. The expression of isoforms 1A1, 2B, and 3A was determined immunohistochemically in ovary tissue from carp and tench [9]. Cannady et al. [10] showed that exposure to 4-vinylocyclohexene and its metabolite increased mRNA and protein for CYP2E1, CYP2A and CYP2B in mouse ovary. Moreover, catalytic assay in ovarian homogenates revealed that CYP2E1 and CYP2B were functional. Swine express cytochrome P450 forms similar to those identified in humans and rodents in the liver [11] , and Leighton and coworkers [12] identified CYP1A1 mRNA in porcine ovarian granulosa cells. To our knowledge we show here for the first time the activity of three P450 isoforms, 1A1, 1A2, and 2B in porcine prepubertal ovary cells. The rank order of activity level was CYP1A1>CYP2B>CYP1A2, determined with the EROD, PROD and MROD assays, respectively. The activity level of CYP1A1 and CYP2B was 5 times higher than the level CYP1A2 activity and ranged around 40-50 pmole/100µg protein/min. This activity is similar to the basal CYP2B activity reported by Cannady and coworkers [10] in mouse ovary. These results support the hypothesis that ovarian cells are capable of metabolic activation of xenobiotics.

We previously reported that PCB3 and its mono- and dihydroxylated metabolites 4OH- and 3,4diOH-PCB3 significantly increased estradiol levels in the porcine ovarian cells, and that this effect is in part due to increased aromatase

activity [13]. Moreover, the presented data clearly show that both, PCB3 and E2, can acts as inducers of CYP1A1 and CYP2B. Although PCB3 is not known to be an efficient CYP inducer, exposure to only 6 ng/ml PCB3 produced a significant increase in both activities, slightly above the increase produced by 20 nM to17β-estradiol.

In conclusion, CYP-induction may be another mechanism by which PCB3 changes steroid secretion by these follicle cells. In addition, both estradiol and PCB3 are metabolized by these CYP isoforms and it therefore can not be excluded that the competition of PCB and the steroid hormone for interaction with CYPs may be a mechanism by which PCBs could inhibit the inactivation of estradiol.

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Acknowledgements

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