Cell specific effects of PCB 126 on aryl hydrocarbone receptors in follicular cells of porcine ovaries

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Introduction

Polychlorinated biphenyles (PCBs) like other endocrine disrupters could interfere with natural hormones by binding to their receptors and thus mimicking the cellular response to them. They are known to possess either estrogenic or antiestrogenic properties. [1] In our previous papers we demonstrated that PCBs are able to disrupt ovarian steroidogenesis. [2,3] We found that the coplanar PCB 126 caused the decrease in estradiol secretion in whole cultured pig ovarian follicles. [3] PCB 126 congener is structurally related to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Since TCDD effects are known to be mediated by aryl hydrocarbone receptors (AhRs), we decided to determine if PCB 126 affects signal transduction pathway activated by these receptors.

It has been reported that the functional AhR is present in ovary including oocytes, granulosa and theca cells of rat, mouse, rhesus monkey and human ovary. [4,5,6] Moreover, the expression of AhR in the rat ovary appeared to be estrous cycle-dependent, thus suggesting that AhR expression may be regulated by fluctuating hormone levels. [7] This study was designed to investigate the effects of the non-ortho-substituted 3,3',4,4',5-pentachlorobiphenyl (PCB126) on the AhR activation, localization and protein level in pig ovarian follicle cells.

Material and Methods

Tissue Preparation and Cell Cultures

Granulosa and theca cells were isolated from small (3-5 mm) pig ovarian follicles according to Stoklosowa et al. [8] Following 24 hrs of incubation the culture medium was changed and replaced with medium supplemented with 5% calf serum and PCB 126 100 pg/ml.

AhR immunocytochemistry

For immunocytochemical labelling of AhR the fixed granulosa and theca cells were labelled the primary antibody specific against the Ah receptor protein (goat polyclonal antibody (N-19; sc-8088, Santa Cruz Biotechnology Inc, Santa Cruz, CA) and then with secondary donkey anti-goat IgG –horseradish peroxides conjugated antibody; Santa Cruz Biotechnol. sc-2020). Cells were then washed and incubated 1 hour with goat peroxidase-anti-peroxidase, PAP, DAKO A/S, Denmark). The labelling was developed in 0.02% diaminobenzidine (DAB) in PBS. Finally, the cells were counterstained with haematoxylin dehydrated in ethanol and mounted in DEPEX. The cells were observed and photographed using Leica DMLB Microscope and Digital Camera Leica DC 200.

Immunobloting

The 20 µg of protein samples were separated on 7.5% SDS-polyacrylamide gel electrophoresis in BIO-RAD Mini-Protean II Electrophoresis Cell. After electrophoretic separation proteins were transferred to nitro-cellulose membranes using Bio Rad Mini Trans-Blott apparatus. Following transfer non-specific binding sites were blocked with 5% milk and 0.2%, Tween 20 in 0.02M TBS and the membranes were incubated with anti-AhR antibody (for specification see immunocytochemistry). After incubation with the primary antibody the membranes were washed and incubated 1 hour with horseradish peroxidase-conjucted antibody (donkey anti-goat IgG-HRP, sc-2020, Santa Cruz Biotechnology). Signals were developed by chemiluminescence (ECL) using Western Blotting Luminol Reagent; sc-2048, Santa Cruz Biotechnology) and visualised with the use of PhosphoImager FujiLas 1000.

Results and discussion

Our previous experiments indicated that PCB 126 exhibited antiestrogenic activity both in whole follicles and in separately cultivated theca and granulosa cells [3,2]. However, the mechanism of its action was not specified. Hydrocarbon receptors are crucial for mediating effects of dioxin. Since non-orto substituted PCB 126 congener has a coplanar structure similar to TCDD and elicits similar toxicity, it seems possible that PCB 126 binds to the AhR in the same TCCD-like way.

Using immunocytochemical labelling we localized AhR protein in both granulosa and theca cells. As we observed, the diffuse cytoplasmic pattern of AhR staining was localized in granulosa cells cultured for 24 hours in basal conditions (data non shown). AhR immunocytochemical labelling in theca cells was less evident and only few cells displayed cytoplasmic staining. More intensive cytoplasmic and nuclear labelling in granulosa cells was noted in PCB 126 treated cells. Western Blot analysis showed decreased AhR expression after 1 h treatment with PCB 126 (Fig. 1).
Both, AhR immunopositivity in nucleus and decreased AhR expression in PCB 126-treated granulosa cells suggested the activation of AhR as a mechanism of PCB 126 action. Unliganded AhR is predominantly localized in the cytoplasm, as a complex with hsp90 and AhR –interacting protein (AIP).[9] After ligand binding the associated proteins dissociate and AhR is translocated into nucleus, where heterodimerization with another trancription factor Arnt takes place. It has been established[10, 11] that following ligand binding AhR protein are rapidly degraded both in vivo and in vitro.

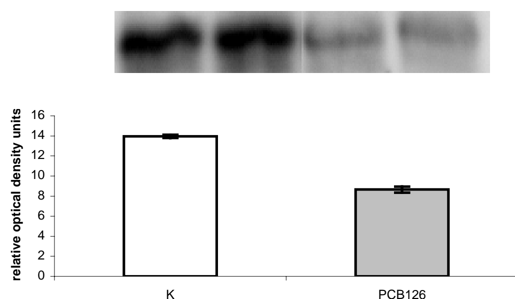

Fig.1 The expression of AhR protein in granulosa cell from small ovarian follicles.

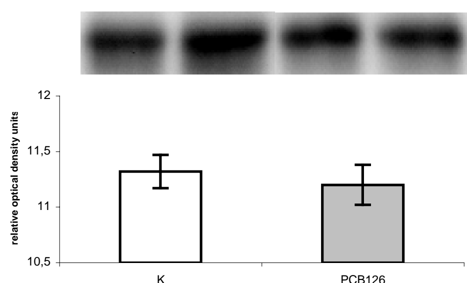

Fig.2 The expression of AhR protein in theca cell from small ovarian follicles.

Treatment of theca cells with PCB 126 resulted only in small increase in diffuse cytoplasmic staining of AhR (data non shown), but did not cause any nuclear staining. Western Blot analyses confirmed this observation (Fig. 2).

Conclusion: The presented results suggested that toxic effect of PCB 126 in granulosa cells is mediated by the AhR activation, while in theca cells the mechanism of its action is not receptor-mediated. These preliminary experiments reported here indicate that in order to understand the differences in the action of PCBs in theca and granulosa cells further studies on the induction of CYP isozymes in ovarian follicular cells are needed.

References:

Safe S, Krishnan V. (1995) Toxicol Lett. 1995 Dec; 82-83:731-6. Review
Wójtowicz AK, Gregraszczuk EL, Lyche JL, Ropstad E. (2000) Journal of Physiol. and Pharmacol. 51:555-568
Gregoraszczuk EL, Grochowalski A, Chrzaszcz R, Wegiel M (2003) Chemosphere 50: 481-488
Chaffin CL, Hutz RJ. J (1997) Reprod Develop 43:47-51
Davis BJ, Mccurdy EA, Miller BD, Lucier GW, Tritscher AM. (2000) Cancer Res 60:5414-9
Robles R., Morita Y., Mann KK, Perez GI, Yang S, Matikainen T, Sherr DH, Tilly JL (2000) Endocrinology 141:450-3.
Chaffin CL, Trewin AL, Hutz RJ (2000) Chem Biol Interact. 124: 205-16
Stoklosowa Stoklosowa S., Bahr J., Gregoraszczuk. (1978) Biol Reprod. 19: 712-719
Ma and Whitlock (1997) J Biol. Chem. 274:2502-2510
Giannone J, Li W, Probst M, Okey A (1998) Biochem Pharmacol 55:489-497
Davarinos NA and Pollenz RS (1999) The Journal of Biological Chemistryn 274:28708-28715