CONGENER-SPECIFIC MECHANISM OF POLICHLORINATED BIPHENYLS (PCB 126 AND PCB 153) ACTION ON THE CORPUS LUTEUM.

Katarzyna Augustowska, Ewa Lucja Gregoraszczuk,

Laboratory of Physiology and Toxicology of Reproduction, Dept of Animal Physiology, Institute of Zoology, Jagiellonian University, Krakow, Poland

Introduction

Polychlorinated biphenyls (PCB) are fat-soluble compounds belonging to a large group of persistent environmental contaminants known to produce adverse effects on female reproduction (Fuller GB and Hobson WC, 1986; Wójtowicz et al., 2000a; Wójtowicz et al., 2000b). The high incidence of embryonic loss observed in the pig in early pregnancy has in the past decade resulted in numerous studies designed to elucidate mechanisms contributing to the prenatal mortality.

Recently, using a system of porcine luteal cells we have shown concentration dependent decrease in progesterone (P4) secretion by luteal cells under the influence of both PCB 126 and PCB 153 after 24h and 48h exposition. This effect was not due to their action on cell viability as measured using the LDH cytotoxicity test (Augustowska et al., 2001). However, the mechanism of this action is still unclear.

The aim of the present study was to show first, the influence of PCBs on specific enzymatic steps in the biosynthetic pathway of progesterone and second to investigate the involvement of the aryl hydrocarbon receptor (AhR) or estradiol receptor (ER) in this process.

Material and Methods

Cell culture: Pig ovaries in the midluteal phase (8-10 days after ovulation) were collected at slaughter. Luteal cells obtained according to the technique of Gregoraszczuk (1983) from pools of freshly excised mature corpora lutea from three animals were cultured for 48 h in M199 medium supplemented with 5 % of calf serum as a control medium or incubated with various concentration of PCB 126 or PCB 153 (Prochem GmbH, Wqesel, Germany). The cultures were maintained at 37 ^{o}C in a humidified atmosphere of 5 % CO_2.

Experimental procedure

Exp.1 Was conducted to show if the action of PCB 126 and PCB 153 on progesterone secretion by luteal cells is dose dependent. Cells were plated into 24 well plates for 48 h incubation with M199 supplemented with 5% of calf serum as a control medium or incubated with PCB 126 (5, 10, 50 and 100 pg/ml) or PCB 153 (5,10,50 and 100 ng/ml).

Exp.2 was conducted to show PCB 126 and PCB 153 action on:

1) The activity of cholesterol side chain cleavage (CYP11A) as measured by the conversion of hydroxylated cholesterol derivative (25-hydroxylated cholesterol; 10 ug/ml) to progesterone. PCB 126 in a dose of 50 pg/ml or PCB 153 in a dose of 50 ng/ml were added to the control and 25-OH treated cells. 48 hrs later cultures were terminated and the media were frozen until P4 analysis.

2) The activity of 3â-HSD activity as measured by the conversion of pregnenolone (P5) to progesterone. PCB 126 in a dose of 50 pg/ml or PCB 153 in a dose of 50 ng/ml were added to the

control and P5 treated cells. 48 hrs later cultures were terminated and the media were frozen until P4 analysis.

Exp. 3. This experiment was performed to show the involvement of the aryl hydrocarbon receptor (AhR) and/or estradiol receptor (ER) in PCBs action on luteal cells. Luteal cells were cultured in M199 medium supplemented with 5 % of calf serum as a control medium or with the addition of PCB 126 in a dose of 50 pg/ml or PCB 153 in a dose of 50 ng/ml. In this experiment, luteal cells were cultured in the absence or presence of α-naphtophlavone (an inhibitor of AhR receptor; 10ìM) or tamoxifen (an inhibitor of estradiol receptor; 10^{-7} M) with or without PCBs. 48 hrs later cultures were terminated and the media were frozen until P4 analysis.

Progesterone levels were determined using Spectra kits (Orion, Diagnica, Finland), supplied by Polatom (Œwierk, Poland).

All data points are expressed as means ± SEM from at least three different experiments (n=3) each in triplicates. Significance of differences between the concentrations of progesterone in the control and corresponding experimental cultures were compared by analysis of variance and by using Dunce's new multiple range test.

Results

The effect of PCB 126 and PCB 153 on progesterone production by cultured in vitro luteal cells collected from mature corpora lutea.

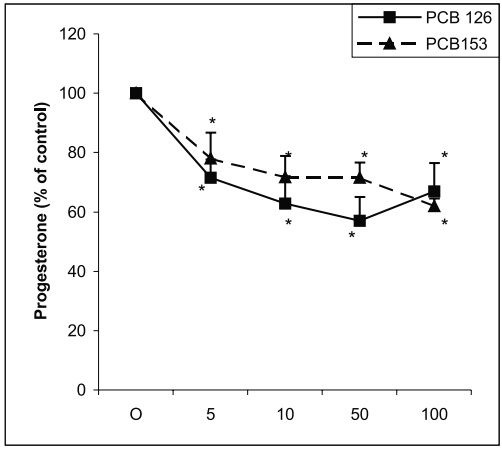

Figure 1. Dose-concentration dependent effect of PCB 126 and PCB 153 on progesterone secretion by luteal cells. * P<0.05.

*PCB 126and PCB 153 action on the activity of mitochondrial CYP11A, and microsomal 3*b*-hydroxysteroid dehydrogenase $_{in}$ luteal cells.*

Both PCBs added to the medium decreased basal progesterone secretion. In 25-hydroxylated cholesterol (25-OH) supplemented cultures no influence on progesterone secretion was observed under the influence of PCB 153, while the same degree of inhibition of progesterone secretion as in the

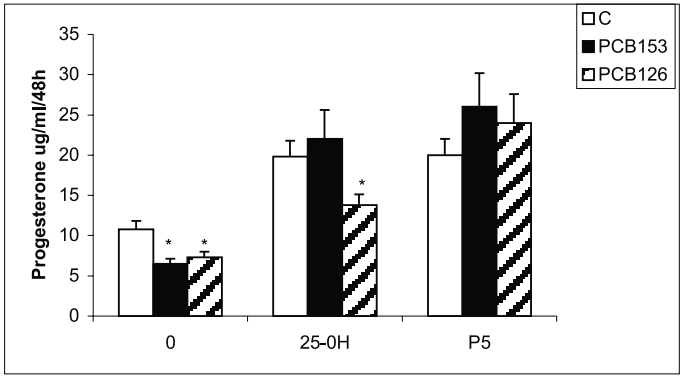

Figure 2. The influence of PCB126 and PCB 153 on basal (C), 25-hydroxycholestero (25-0H) and pregnenolone (P5) -stimulated progesterone secretion by luteal cells. * P<0.05.

control was noted in PCB 126 treated cultures. Both studied congeners had no influence on progesterone secretion in P5 supplemented cultures

The involvement of AhR and ER in PCB 126 and PCB 153 action in luteal cells

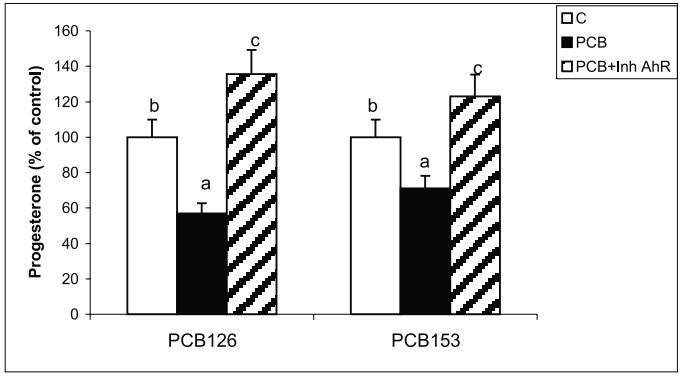

Figure 3. The influence of á-naphtophlavone, the AhR-inhibitor (InhAR) on progesterone secretion. Bars with different letter are significantly different (p<0.05).

Discussion.

The differentiation of follicular cells into luteal cells capable of producing progesterone is accomplished by an increased expression of enzymes necessary for the conversion of cholesterol to progesterone. In the present study, we have shown a dose dependent decrease in progesterone secretion by luteal cells after exposure to both studied PCBs for 48 h. Preimplantation phenomena are highly dependent on progesterone action (Roblero *et al.*, 1987). In this period of pregnancy the corpus luteum is the main source of progesterone. It is possible that PCBs acting as progesterone antagonists cause an inhibition of implantation and as a consequence termination of pregnancy.

The de novo synthesis of progesterone starts with the conversion of cholesterol derivatives to pregnenolone by CYP11A and then by 3ß-HSD. The present studies provide evidence that PCB 153 can reduce progesterone secretion through a direct action on the activity of both CYP11A and 3ß-HSD. The effect of PCB 153 was reversed by the addition of both 25-OH and P5 to the culture medium. The situation was different in the case of PCB 126. Decrease in progesterone secretion has been observed not only in control but also in 25-OH supplemented cells suggesting that PCB 126 inhibits luteal steroidogenesis predominantly by the inhibition the mobilization of cholesterol to CYP11A. Moor *et al.*, 1991 suggested this mechanism of action for TCDD in testicular cells. However, reversed PCB 126 action on progesterone secretion in P5 treated cells suggested possible action on the activity of 3ß-HSD.

Additionally, results of the present study suggested the involvement of AhR receptor in action of both PCBs congeners. AhR dependent but not ER-dependent mechanism was suggested for TCDD action in luteal cells (Gregoraszczuk *et al.*, 2001)

Conclusion.

A reduction in the activity of mitochondrial by PCB 153 and microsomal by PCB 126 and PCB 153 enzymes causes disruption of progesterone secretion by both congeners. Moreover, in the case of both congeners this reduction in steroid secretion is dependent on the AH locus.

Acknowledgements

The authors with to thank Dr M Mika from Department of Physiology, Academy of Agriculture, Kraków for radioimmunological determinations of steroid hormones. KBN Grant State Committee supported the work for Scientific Research Number 5PO6d 01918

References

1. Fuller GB, Hobson WC, (1986) IN Waid IS (ed) PCB'S and the environmental. CRC Press, Boca Raton, PP 101-125.
2. Wójtowicz AK, Gregoraszczuk EL, Lychte JL, Ropstad E, (2000a) J Physiol Pharmacol 51: 555-568.
3. Wójtowicz AK, Gregoraszczuk EL, Mika M, (2000b) PCB 153 action on steroid secretion by cultured in vitro porcine theca and granulosa cells. Organochloric Compounds 49: 363-366.
4. Gregoraszczuk EL, (1983) Endocr Exp 17: 59-68.
5. Augustowska K, Wójtowicz A, Kajta M, Ropstad E, Gregoraszczulk EL (2001) Exp Clin Endocrinol Diabetes 109: 416-418.
6. Roblero LS, Fernandez O, Croxatto HB, (1987) Toxicology 26: 113-124.
7. Moore RW, Jefcoate CR, Peterson RE, (1991) Toxicol Appl Pharmacol 109: 85-97.
8. Gregoraszczuk EL, Zabielny E, Ohwat D, (2001) J Physiol Pharmacol 52: 303-311.