PCB 153 ACTION ON STEROID SECRETION BY CULTURED IN VITRO PORCINE THECA AND GRANULOSA CELLS

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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 (1). High concentrations of PCB were found especially in fat tissue, ovaries, oviductal and uterine tissues, in follicular fluid and in uterine secretions (2). PCB has the potential to interfere with the endocrine system of animals and humans. High levels of PCB and hexachlorobenzene (HCB) were found follicular fluid from women in Germany and Austria (3). The direct effect of PCB on ovarian steroidogenesis is unknown. However, there is some evidence that PCB can interfere directly in the ovarian steroidogenesis. In the present study the steroid production in follicular cells was assessed after exposure to PCB 153 because it is one of the most commonly detected PCB congeners in biological tissues (5,6,7).

Material and Methods.

Reagents

Parker medium M199, trypsin, and calf serum was purchased from Laboratory of Sera and Vaccines, Lublin Poland. Antibiotic antimycotic solution (100x) and testosterone was obtained from Sigma Chemical Co. St. Louis, MO, USA. Stock solutions PCB 153 (2,2',4,4',5,5'-CB; 25 μg/ml) and PCB 126 (3,3',4,4',5-CB; 25 ng/ml) were prepared by dissolution of pure powder in ethanol (Prochem GmbH, Wesel, Germany; purity 0.997.

Cell cultures

Porcine ovaries were obtained from a local abattoir. Large follicles were obtained from ovaries collected at day 16-18 of oestrus cycle. Granulosa cells (Gc) and theca interna layers (Tc) subsequently prepared according to the technique described by Stoklosowa et al. (8). After collection, granulosa and theca cells were suspended in M-199 medium supplemented with 5% calf serum. Then the cells were plated in one well in 1ml 24 well plastic cell-culture plates (Nunc). The cultures were maintained at 37° C in humidified atmosphere of 5% CO_2.

Experimental procedure

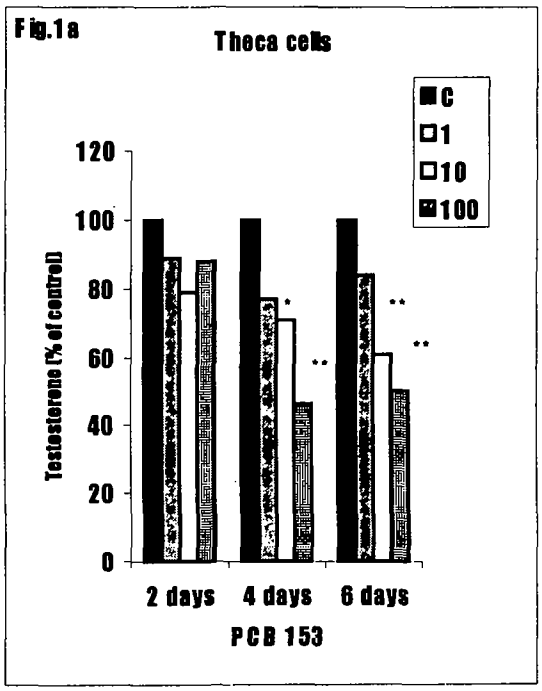
In order to show the time-dependent effect of PCB congeners on steroid secretion by particular types of follicular cells harvested from large preovulatory follicles the granulosa and theca cells were treated with PCB 153 at the concentration 1.0, 10.0, 100ng. The cells were cultured for 48, 96 and 144 hrs and the medium was frozen (-20°C) prior to steroid analysis.

Progesterone, estradiol and testosterone were determined radioimmunologically using Spectria RIA kits (Orion, Diagnostica, Finland), supplied by Polatom (wierk, Poland).

Results

Effects on steroids secretion by theca interna cells

A significant inhibitory effect on estradiol secretion by theca cells was found after treatment with 10 and 100ng/ml PCB 153 for 4 days and at all doses in 6 days cultures. Inhibitory effect on testosterone secretion was found after treatment with all doses for 4 days and after 6 days of culture at dose of 10 and 100 ng/ml. (Fig1a,b).



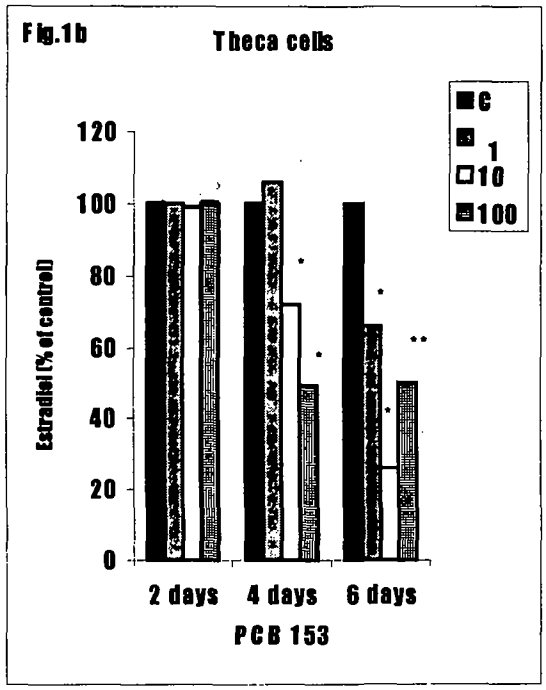


Fig. 1 Dose- and time-dependent effect of PCB153 on (a) testosterone and (b) estradiol secretion by theca cells

Effect on steroids secretion by granulosa cells

Except from the short-term culture (2 days) all doses of PCB 153 caused a significant increase in progesterone secretion. A negative effect of PCB 153 on estradiol production was indicated by the fact that the higher doses significantly reduced estradiol concentration after 4 and 6 days in culture (Fig. 2a,b).

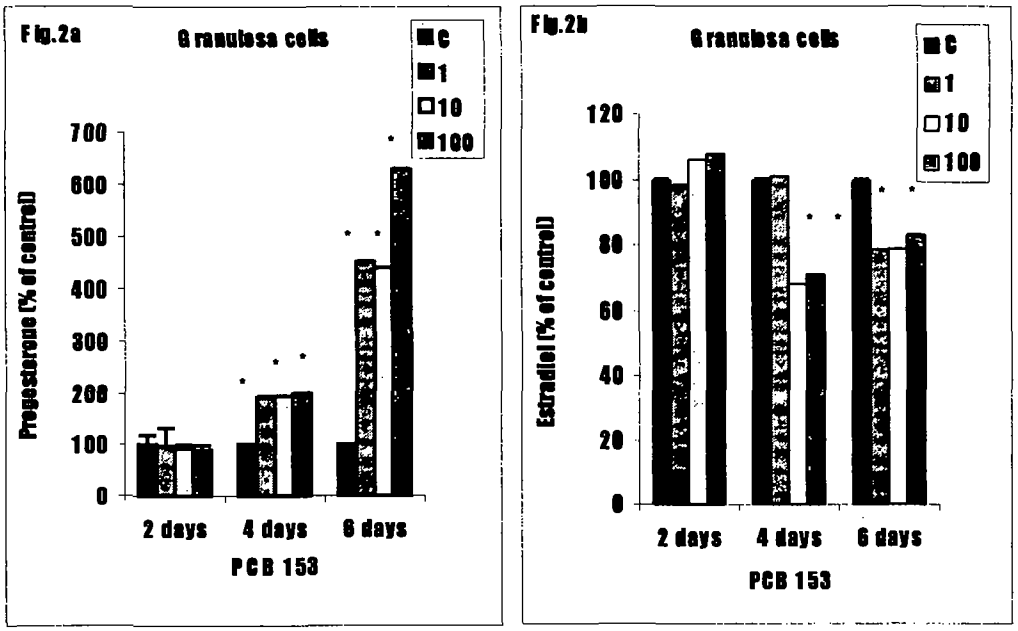


Fig. 2 Dose- and time-dependent effect of PCB153 on (a) progesterone and (b) estradiol secretion by granulosa cells.

Discussion

The current study demonstrated that the exposure of porcine follicular cells to PCB 153 significantly influenced steroidogenesis. To our knowledge there are no other reports in the literature in which the effects of single PCB congeners on follicular cells have been studied. The presented data that PCB 153 in long term cultures decreased the concentration of the hormone in the culture medium. However, there are reports in the literature on the effects of PCB on various aspects of reproduction (for review: Safe, 1994), some of which are anti-estrogenic. Considering that the theca cells are responsible for androgen production in situ, the decreased estradiol secretion in theca cells caused by PCB 153 probably involve intermediate steps after synthesis of P4, most likely the provision of androgens for aromatization, or more specifically, cytochrome P450-17 Hydroxylase/17-20 lyase (P450c17) in the production of androgen precursors for estrogen synthesis. Another possibility is that the endogenous testosterone produced by thecal cells during long term culture is decreased resulting in lack of substrate for estradiol production by theca cells. Previously estrogenicity of PCB congeners has been be related to ortho-substitution (9, 10). The coplanar non-ortho-substituted PCB126 was found to be antiestrogenic (4,5), whereas the non-coplanar diortho-substituted PCB153 was demonstrated to exert estrogenic activity (11).

The high stimulatory effect of PCB 153 on progesterone secretion and concomitant decrease of estradiol secretion by granulosa cells observed in the present study, could be explained by a luteinizing effect of this congener. It has been shown previously that a high level of progesterone might have an inhibitory effect on aromatase activity (12, 13, 14).

Conclusion: This data suggests inhibition of aromatase activity by depletion of substrate for aromatization or/ and increasing luteinization process in granulosa cells under the influence of PCB 153.

SINCE INFORMATION ABOUT MECHANISM OF PCB ACTION ON GONADAL CELLS IS SCARE SO THESE EXPERIMENTS ARE OF PIONIER CHARACTER.

Acknowledgements

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