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Influence of 4-hydroxylated polychlorinated biphenyls on the secretory function of bovine ovarian cells: Role of the steroidogenic factor-1 receptor

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ABSTRACT

The hydroxy-derivatives (OHPCBs) of polychlorinated biphenyls (PCBs) can accumulate in the tissues of the reproductive tract in animals and humans and may still have estrogen-like properties. Moreover, the "orphan" nuclear receptor Steroidogenic Factor-1 (SF-1) can be the target of PCBs.

The aim of the present study was to determine the effect 4OH4CB and 4OH3CB on the secretion of estradiol (E2), progesterone (P4), and oxytocin (OT) from granulosa cells of follicles <1 cm and >1 cm in diameter and from luteal cells collected at four stages of the estrous cycle of cows. Furthermore, the possibility that 4OHPCBs have an effect on OT synthesis and secretion via the SF receptor was studied using receptor blocker (F0160).

Used OHPCBs increased the secretion of P4 from the granulosa cells of follicles of both sizes and increased the secretion of OT from follicles with a diameter of >1 cm. These increases were inhibited by an SF-1 receptor blocker. In luteal cells, 4OH3CB increased the secretion of P4 and OT from luteal cells at all phases of the estrous cycle, while 4OH4CB increased OT secretion during the first half of the estrous cycle. Concomitant with the increase in OT secretion from the cells, an increase in the expression of OT precursor mRNA (NP-I/OT) was observed. This effect was inhibited by SF-1 receptor blocker.

These results indicate that 4OHPCBs impair the secretory function of ovarian steroidogenic cells by disrupting steroidogenesis and increasing OT secretion, and the receptor SF-1 appears to be essentially involved in these processes.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of organic chemicals that permanently pollute the environment. Many of these chemicals can mimic or block the effects of hormones involved in the regulation of reproduction in animals and humans; hence, they are referred to as "endocrine disruptors" (Roselli et al., 2000). Despite their high resistance to biodegradation in the environment, PCBs can be decomposed under the influence of physical factors (mainly light and temperature), via biological dechlorination processes by anaerobic or aerobic bacterial degradation (Fish and Principe, 1994), and by hydroxylation (Furukawa and Fujihara, 2008). Thus, hydroxylated PCBs derivatives, which are relatively stable compounds, may also accumulate in the environment (Berg et al., 2010; Nomiyama et al., 2010). As a result, the amount of PCBs in the environment declines, while the amount of 4OHPCBs, increases (Marek et al., 2013).

Depending on their chemical structure, PCBs biodegrade in different ways. Low chlorinated congeners (1–4 chlorine atoms per molecule), and congeners that have aromatic rings chlorinated at the *meta*-, or *para*-position (Komancová et al., 2003; Borja et al., 2006) are

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hydroxylated particularly easily. Hence, they are the most common 4-hydroxy derivatives of PCBs (Furukawa et al., 2004). Adding an —OH to the PCB molecule has a significant influence on the physicochemical properties and biological activity compared to the basal substance. The presence of hydroxyl groups enhances the solubility of these substances in water and makes them similar to estradiol (Colborn et al., 1993; Kitamura et al., 2005). Hydroxy-derivatives of PCBs enter the bodies of animals and humans as regular PCBs (Fernandez. et al., 2008). Furthermore, PCBs may undergo hydroxylation in the body and be re-accumulated in the tissues (Wu et al., 2011).

The effect of OHPCBs on the reproductive processes of animals and humans is not completely understood, although some studies have indicated that they have adverse effects similar to those of PCBs. It has been shown that OHPCBs can interact with the estrogen receptor (Takeuchi et al., 2011), interfere with the E2 metabolism (Kester et al., 2000), alter prostaglandin synthesis and secretion from the bovine oviduct epithelium (Wrobel et al., 2010), and increase the synthesis of leukemia inhibitor factor in the fallopian tubes of human and cattle (Reinhart et al., 1999). This latter effect can be evoked without mediation by estrogen receptors. However, OHPCBs do not always mimic the action of the parent compound; hence, it is difficult to predict their activity (Antunes-Fernandes et al., 2011). Some xenobiotics such as atrazine, PCBs 77 (3,3′,4,4′-tetrachlorobiphenyl), and PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) may affect reproductive processes via the orphan receptor SF-1 (NR5A1) (Fan et al., 2007; Mlynarczuk et al., 2013), which is recognized as an important factor in the regulation of adrenal and ovarian steroidogenesis (Sadowsky and Crawford, 1998; Mendelson et al., 2005). This receptor affects the expression of the StAR gene (Sugawara et al., 2000) and other genes involved in steroidogenesis (Parker et al., 2002) in cattle; additionally, it increases the expression of the oxytocin precursor gene NP-I/OT (Wehrenberg et al., 1994). It should be noted that ovarian OT alters steroidogenesis such that progesterone is more favorably produced during luteinization of granulosa cells after the preovulatory LH surge (Brendtson et al., 1996; Jo and Fortune, 2002), stimulates the synthesis of progesterone (P4) in the early and middle stages of the cycle (Miyamoto and Schams, 1991), and is involved in a luteolytic process already underway (Flint and Sheldrick, 1983; Kotwica et al., 1997).

Therefore, the purpose of this study was to verify hypothesis that the PCB 4-hydroxy derivatives 2,4′,6′-trichlorobiphenyl-4-ol (4OH3CB) and 2,2′,4′,6′-tetrachlorobiphenyl-4-ol (4OH4CB) (Kunisue and Tanabe, 2009), can affect ovarian steroidogenesis and stimulate the SF-1 receptor, thereby affecting the synthesis and secretion of OT in ovarian steroidogenic cells.

2. Materials and methods

2.1. Collection of ovaries

Ovaries from cows and mature heifers were collected from a local slaughterhouse 15–20 min after the animals were killed and transported to the laboratory within 1 h in an ice bath containing 0.9% NaCl supplemented with penicillin (10 IU/ml), streptomycin (100 μg/ml), amphotericin (2 μg/ml), and L-glutamine (100 μg/ml). The stage of the estrous cycle (days 2–5, 6–10, 11–15, and 16–19) was assessed using the morphological observations described by Ireland et al. (1980) and Fields and Fields (1996). Unless otherwise stated, all of the materials used in this study were purchased from Sigma (Poland).

2.2. Collection of granulosa and luteal cells

Granulosa cells were obtained by vigorous rinsing followed by the aspiration of follicular fluid from two groups of ovarian follicles: <1 cm diameter (small) and >1 cm diameter (large). Harvested follicular fluid was centrifuged at 1200 × *g* at 4 °C for 15 min. Subsequently, the supernatant was discarded, and the sediment was flushed with 10 ml of DMEM/Ham's F12 medium with 0.1% BSA and centrifuged again. The last step was then repeated. The cells obtained were suspended in DMEM/Ham's F12 medium enriched with 10% of new-born calf serum and gentamycin (20 mg/ml). Cell viability was estimated using trypan blue (0.04%) exclusion. Cells with a viability above 75% (for small follicles) and above 85% (for large follicles) were used in experiments. The cells were re-suspended (2.5×10^5 cells/ml) and plated onto culture plates (Nunclon, NUNC, Roskilde, Denmark) as described above. For one experiment ($n = 1$), a mixture of granulosa cells from 10 to 12 follicles <1 cm in diameter and cells obtained from 16 to 20 follicles >1 cm in diameter was used.

Luteal cells from 6 to 10, 11 to 15, and 16 to 19 days of the estrous cycle were obtained by perfusing the corpus luteum (CL) with collagenase II solution (1 mg/ml) through one of the branches of the ovarian artery (Okuda et al., 1992). Cells from days 2 to 5 of the estrous cycle were obtained by finely etching cut luteal tissue using a collagenase II solution (1.5 mg/ml). The viability of the collected cells was estimated by the exclusion of 0.04% trypan blue dye. Cells with a viability of >85% were used. Next, the cells were suspended (2.5×10^5 ml^{-1}) in DMEM/HAM-12 + 10% FCS without phenol red and were transferred into 6-well (2 ml) or 48-well (0.5 ml) plates and cultured (Memmert, Schwabach, Germany) in a controlled atmosphere (air with 5% CO_2) with 100% humidity at 38 °C. After pre-incubation (24 h), the cells were washed twice with M-199 + 0.1% BSA and suspended in 4 ml of DMEM/HAM-12 with 0.1% BSA and 20 mg/ml gentamycin. Sets of luteal cells for one experiment were collected from four corpora lutea.

2.3. The effect of OHPCBs and an SF-1 antagonist on cell viability

An effective but non-cytotoxic dose of SF-1 antagonist (F0160, ethyl 2-[2-[2-(2,3-dihydro-1,4-benzodioxin-7-ylamino)-2-oxoethyl]-1-oxoisoquinolin-5-yl] oxypropanoate, Madoux et al., 2008; LifeChemicals, Lviv, Ukraine) and agonist (HxP, 4-(heptyloxy) phenol, Del Tredici et al., 2008) was established previously (Mlynarczuk et al., 2013, 2014). The cytotoxicity of 4OH3CB and 4OH4CB (Fig. 1) alone (at a dose of 10 ng/ml) and in combination with F0160 (1×10^{-7} M for granulosa