A POSSIBLE ROLE FOR PCB 153 IN A HORMESIS-LIKE DOSE-RESPONSE CURVE FOR STEROID SECRETION BY OVARIAN FOLLICULAR CELLS EXPOSED TO A REAL-LIFE MIXTURE OF POPs.

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Introduction

Persistent organic pollutants (POPs) such as dioxins, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), dichlorodiphenyldichloroethylenes (DDTs) and brominated diethyl ethers (BDEs) are distributed globally[1]. Exposure to POPs is an important subject, because of the potential hazards to human and animal development and health. Particular attention has been given to xenobiotic compounds that are capable of modulating or disrupting the endocrine system[2,3]. It is critically important that we determine whether environmental concentrations of POPs, such as PCBs and OCPs, may elicit adverse effects in animals and ultimately humans, especially during sensitive stages of development, in, for example, gonadal cells. POPs may have adverse effects on reproductive function. Younglai et al.[4] reported that the contaminants most frequently found in ovarian follicular fluid in a survey of women, were1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE), mirex, hexachloroethane,1,2,4-trichlorobenzene, and PCBs (i.e. PCB 49, PCB 153, and PCB 180). p,p'-DDE was the most frequently detected chemical contaminant in the serum and follicular fluid , had the highest residue levels, and was associated with decreased reproductive success.

Real-life mixtures of POPs are composed of different compounds, at different concentrations in different biological matrices. There are gaps in our knowledge on which agents occur in a mixture, and at which levels they exert toxicity. Moreover, there is also considerable uncertainty about their possible mechanisms of action. The fact that cod livers from the Baltic Sea may be unsuitable for human consumption because of heavy contamination with PCBs, DDTs, and hexachlorobenzene (HCB) has been known for many years[5]. Ten years later Asplund et al.[6] showed that fatty fish species, e.g., salmon and herring, in the Baltic Sea have high levels of (PCBs) and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT), and its main metabolite p,p'-DDE.

The aim of the present study was to investigate the effects on steroid secretion by ovarian follicular cells of exposure to three different compounds of POPs (PCB 153, p,p-DDE and BDE-47), all of which are significantly associated with human exposure. These effects were compared with those of a real-life mixture of POPs harvested from cod liver oil from the Atlantic Ocean, in which the three compounds of interest are known to occur at high concentrations.

Material and Methods

Contaminants were extracted from liver oil from Atlantic cod (*Gadus morhua*). The production of pharmaceutical cod liver oil includes several steps to remove pollutants. In a preliminary step, dioxins and planar PCBs are removed with charcoal. This is followed by a distillation process, which removes fractions of the oil and most of the lipid-soluble pollutants. The waste from this distillation process was used in our study, and enabled us to conduct the study without the presence of components contributing to dioxin-like toxicity.

After removal of dioxins and planar PCB by charcoal, 1 g of the waste from the manufacturing of cod liver oil was treated with 20 ml cyclohexane (CHX) and 50 ml of sulphuric acid (SA). The organic phase was removed the next day and the volume reduced to 5 ml under N_2. Addition of SA was repeated twice (20 and 5 ml, respectively) resulting in 1 ml CHX with contaminants,. In a second step, CHX was replaced by repeated addition of ethanol followed by evaporation under N_2 and adjusted to three ml. From this stock solution contaminants were determined by GC-MS and GC-ECD. Briefly, the measured contaminants in the real-life mixture (marine mixture) were (ng/g): ΣPCBs:21589; ΣBDEs:1949; ΣDDTs:11160; ΣChlordanes:3460; ΣToxaphenes:6990. The measured concentrations in the marine mixture of single compounds used in this study were (ng/g): PCB 153: 4120; p,p'-DDE: 5890 and BDE 47: 1700.

Theca interna and granulosa cells were isolated from prepubertal porcine follicles. For co-culture experiments, granulosa and theca cells were inoculated at concentrations of 4.0 x 10^4 and 1.0 x 10^4 viable cells/well. Cells were initially cultured in M199/CS without test compounds for 24h to allow for cell attachment to the wells. After 24 h, the medium was discarded and 0.3 ml of fresh M199 alone was added to the control culture. In the experimental group, particular reagents were added in the following concentrations: Marine mixture: 1, 5,10, 50, 100, 500, 1000 ng/ml; PCB 153: 50, 100, 500, 5000 ng/ml ; p,p.-DDE- 50, 500, 1000, 5000 ng/ml or BDE-47 ; 10, 100, 500, 1000 ng/ml. The doses of particular congeners were selected with consideration of the lowest and higher concentrations in the marine mixture. For analysis of steroid levels, the media were collected after 24 h of exposure and frozen at - 20ºC. Estradiol and testosterone levels in culture media were determined using the Dia.Metra (Segrate Milano, Italy). Data were analyzed by 1-way analysis of variance (ANOVA) followed by the Tukey (HSD) multiple range test. P-values below 0.05 were considered statistically significant.

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Results and Discussion

Progesterone secretion

Similar to the real-life marine mixture (Mmix), a hormesis-like dose-response curve for progesterone secretion was found upon exposure to PCB 153. p,p'-DDE in all the doses used decreased progesterone secretion while BDE-47 in doses from 100-1000 ng/ml increased progesterone secretion (Fig. 1).

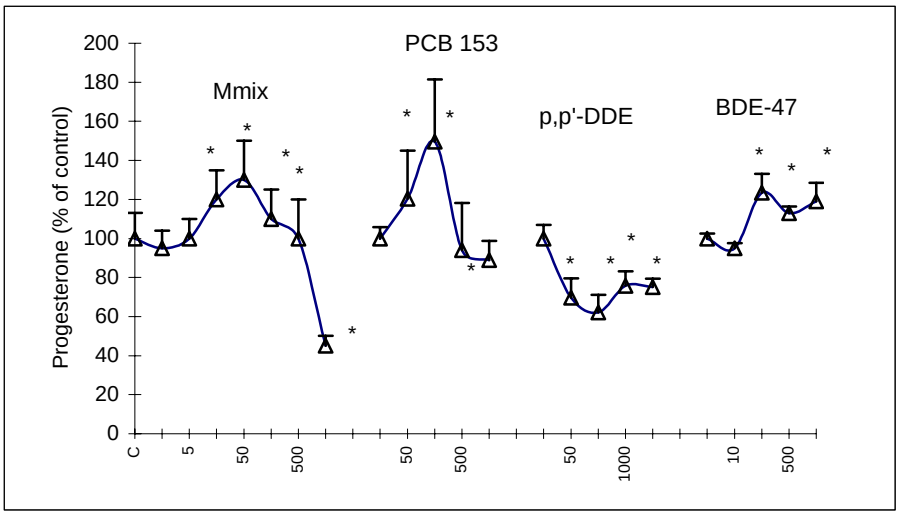

Fig. 1. Mean progesterone concentrations relative to control (%; +SE) in co-cultures of porcine theca and granulosa cells. Cells were exposed to different doses of PCB 153, p,p'-DDE and BDE-47. The dose-response relationship for a real-life mixture of POPs (marine mixture, Mmix) is included for comparison. Doses are given in ng/ml. C= Control. * Exposed group significantly different from control (P< 0.05).

Testosterone secretion

Increased testosterone secretion was found in response to increasing dose levels of PCB 153 and p,p-DDE. A similar dose-response relationship was seen in the real-life mixture of POPs (Mmix). BDE-47 decreased testosterone secretion in all the doses used (Fig. 2)

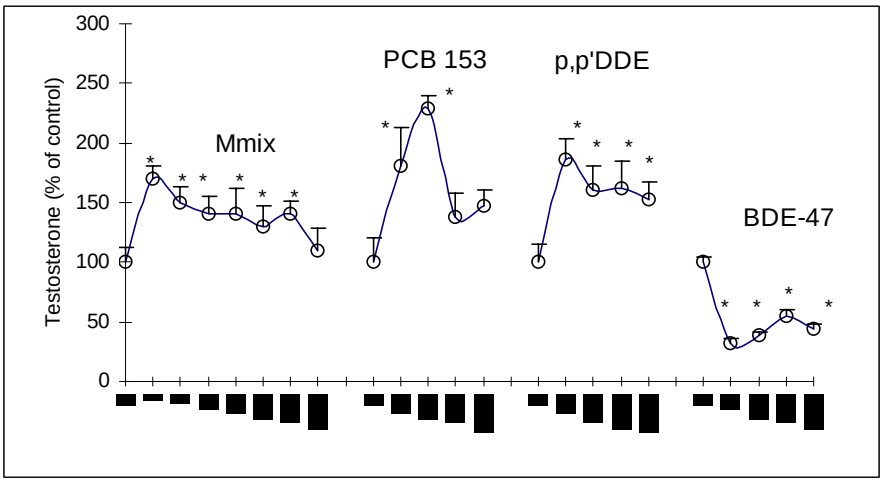

Fig. 2. Mean testosterone concentrations relative to control (%; +SE) in co-cultures of porcine theca and granulosa cells. Cells were exposed to different doses of PCB 153, p,p'-DDE and BDE-47. A dose-response relationship for a real-life mixture of POPs (marine mixture, Mmix) is included for comparison. Doses are given as ng/ml. C= Control. * Exposed group significantly different from control (P< 0.05).

Estradiol secretion

A hormesis-like dose response-curve for estradiol secretion obtained from exposure to PCB 153, was similar to that obtained with the real-life mixture (Mmix). p,p'-DDE in doses from 500-1000 ng/ml, increased estradiol secretion. BDE 47 was anti-estrogenic in dose levels of 10 and 100 ng/ml (Fig. 3).

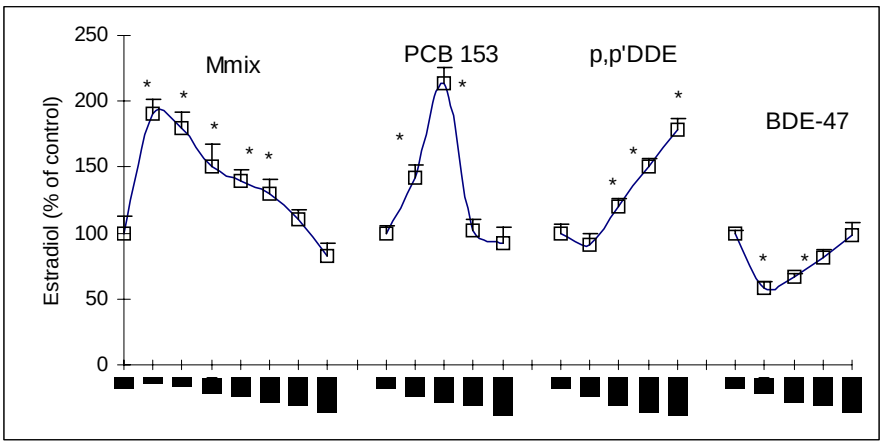

Fig. 3. Mean estradiol concentration relative to control (%; +SE) in co-cultures of porcine theca and granulosa cells. Cells were exposed to different doses of PCB 153, p, p'-DDE and BDE 47. A dose-response relationship for a real-life mixture of POPs (marine mixture, Mmix) is included for comparison. Doses are given as ng/ml. C= Control. * Exposed group significantly different from control (P< 0.05).

It is relevant to question whether our knowledge about single components present at high concentrations can be extrapolated to predict of the likely biological effects due to real-life mixtures of POPs. From a public health point of view, most exposures are to mixtures of potential toxicants, and effects are seldom a simple summation of single mixture component effects.

The present results indicated that a hormesis-like dose response-relationship characterized the steroid secretion by ovarian follicular cells in response to increasing doses of single POP compounds, as well the real-life mixture of POPs. Among the compounds investigated, PCB 153 produced a steroidogenic response most similar to that obtained with the real-life mixture in ovarian follicular cells (Mmix; Figs. 1-3). Small doses were stimulatory, while large doses had no effect or were inhibitory. The hormesis dose-response of PCB153 has been previously described [6]. The observed increase of all investigated hormones when exposed to PCB 153 (Figs. 1-3) suggests that PCB 153 has a stimulatory action on P450scc activity in the first step of steroidogenesis.

The dose-dependent estrogenic effect of p.p'- DDE (Fig. 3), and the concomitant increase in testosterone and decrease in progesterone, would suggest that this compound has a stimulatory effect on both CYP 17 and CYP 19 activity. Our results concur with those from a study by Younglai et al.[7], who reported that concentrations of p,p'-DDE similar to those present in human follicular fluid enhanced basal and FSH-stimulated human granulosa cell aromatase activity. p,p'-DDE has also been demonstrated to increase aromatase expression and activity in hepatic microsomes[8] and endometrial stromal cells[9].

There is little information on the reproductive effects of BDEs. Studies on a commercial mixture of Penta-BDE reported that high doses resulted in a delay in male and female reproductive development[10]. Our results are the first to show a direct action of BDE 47 on follicular steroidogenesis. Inhibitory action on both testosterone and estradiol secretion was found during low dose exposure (10- 500 ng/ml), while a high dose (1000 ng/ml) had no effect on estradiol secretion. The anti-estrogenic effect of BDE-47 is probably due to insufficient testosterone production (Fig. 2). Moreover, the simultaneous increase in progesterone suggests an effect of this congener on CYP17 activity. This would concur with the results of with Canton et al.[11], who showed a strong inhibition of CYP17 when human adrenocortical carcinoma cells (H295R) were treated with the major metabolite of BDE-47 (6OH-BDE-47)[12].

In conclusion, among the compounds investigated, PCB 153 was the compound that produced a steroidogenic response in ovarian follicular cells most equivalent to that seen with a real-life mixture. Further studies are needed to confirm whether this is due to the very high concentrations of this compound in this mixture or to specific mechanisms of action.

Acknowledgements

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