Effect of polychlorinated biphenyls (Aroclor-1248) on the secretory function of bovine luteal cells affected by LH, noradrenaline and high density lipoproteins

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ABSTRACT: The corpus luteum (CL), formed from the ruptured follicle, is required for the course of normal cyclicity and the duration of pregnancy in females. The influence of a mixture of polychlorinated biphenyls – PCBs (Aroclor-1248) – on the secretory function of CL (dispersed bovine luteal cells) during different stages of the estrous cycle was studied. The cells (1.2×10^5/ml) were pre-incubated for 24 h and were then treated with 10, 100 or 500 ng/ml of PCBs. After 24, 48, 72, 96 or 144 h luteinizing hormone (LH; 100 ng/ml; positive control) was added to the medium. The most evident impaired secretion of progesterone was measured after 72 h of incubation with PCBs and this time was selected for the further experiments. In Exp. 2 high density lipoproteins (HDL), as a source of cholesterol (25 μg), increased progesterone secretion from luteal cells; PCBs enhanced this effect in mid and late stage of the estrous cycle. PCBs had no effect on the stimulatory influence of LH, which itself stimulated progesterone secretion. In Exp. 3 PCBs (500 ng/ml) decreased progesterone secretion from the early CL and increased stimulatory effect of noradrenaline (NA) on progesterone secretion from mid CL. Aroclor-1248 stimulated oxytocin (OT) secretion from all stages of CL development. NA alone increased OT secretion from mid and late CL and moreover, it amplified effect by Aroclor on CL from all studied stages of their development. We conclude that the mixture of PCBs, commercially available as Aroclor-1248, can directly impair the function of bovine CL and thus it can affect the estrous cycle duration or embryo development.

Keywords: bovine; PCBs; corpus luteum; progesterone; oxytocin; HDL

The corpus luteum (CL) formed from the cells of ovarian follicle following its ovulation is required for the duration of normal pregnancy in mammals. The main function of CL is synthesis and secretion of progesterone, which is necessary for implantation of the blastocyst and further pregnancy development. About 30–40% of embryonic losses in cow occur by day 17 of pregnancy and this depends on many factors (Thatcher *et al.*, 1994). In this paper we studied the mechanism of action of polychlorinated biphenyls (PCBs) as potential factors affecting impairment of the CL function.

The usage of PCBs, resistant for the degradation, was widely employed from the beginning of 1930s until the later half of 1970s (Safe, 1994). Nowadays, their production and usage is forbidden in developed countries; nevertheless, they are still widely present throughout the environment and in the food chain. PCBs can enter the bodies of people and animals and be accumulated within (Clarkson, 1995; Thomas *et al.*, 1999). In biological material, 2,2′,3,3′,4-pentachlorobiphenyl (PCB-138), 2,2′,4,4′,5,5-hexachlorobiphenyl (PCB-153) and 2,2′,3,4,4′,5,5-heptachlorobiphenyl (PCB-180) (Safe, 1994; Cogliano, 1998) are the PCBs most often detected. Polychlorinated biphenyls and their secondary metabolites or derivatives can impair many processes in organisms of animals and man (Orberg and Kilhstrom, 1973; Muller *et al.*, 1978). In reproductive processes they can affect the length of the estrous cycle, evoke estrus symptoms or their lack of, affect the development of the ovum, and

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impair the rate of egg fertilization (Johnsson *et al.*, 1976; Pocar *et al.*, 2001). High concentrations of PCBs have been detected in adipose tissue (Kannan *et al.*, 1994; Mes *et al.*, 1995), ovaries, oviduct, uterus (Lindenau *et al.*, 1994), follicular fluid (Trapp *et al.*, 1984; Pauwels, 1999) and in placenta (Pasanen, 1999; Chen *et al.*, 2002) in different species and they affect the function of luteal cells as proved in swine (Wojtowicz *et al.*, 2000; Augustowska *et al.*, 2001).

The aim of the present investigation was to study the prolonged effect of different doses of a PCBs mixture on the secretion of progesterone and oxytocin from luteal cells during different stages of CL development in cows. Since PCBs comprise a family of 209 molecules present in the environment as a mixture, we used Aroclor-1248 and assumed that this to some extend is representative of PCBs in the environment. Moreover, we wanted to study the influence of PCBs on: (i) utilizing HDL as a source of cholesterol by luteal cells (Pate and Condon, 1989), (ii) stimulatory influence of LH (Carlson *et al.*, 1971) and (iii) noradrenaline (NA) (Kotwica *et al.*, 1991; Kotwica and Skarzynski, 1993) on the secretion of progesterone and OT by bovine luteal cells.

MATERIAL AND METHODS

Luteal cells preparation

Ovaries with corpus luteum (CL) were collected in a commercial slaughterhouse within minutes after the cows were stunned. The stage of the estrous cycle was estimated on the basis given earlier (Ireland *et al.*, 1980). Ovaries were transported to the laboratory in a container with saline on an ice-bath. Luteal cells were obtained by CL perfusion with a mixture of collagenase IA (Sigma; 1 mg/ml) and DNase I (Sigma; 5 μg/ml) as described by Okuda *et al.* (1992). Cells obtained from CL on day 4–7; 8–12 and 13–17 of the eastrous cycle were pooled and their viability was estimated by means of 0.04% Trypan Blue. Only cells with viability greater than 80% were used for further studies. Luteal cells (1.2×10^5/ml), suspended in 0.5 ml of DMEM (Sigma) and supplemented with 10% FCS, were placed into 48-well plates (Corning Inc., USA) and then pre-incubated for 24 hours. Thereafter, the medium was changed and cells were washed twice with M-199 (Sigma) containing 0.1% BSA (Sigma). Then 0.5 ml of DMEM that had been supplemented with 0.1% BSA, ascorbic acid (20 μg/ml, Sigma), transferrin (5 μg/ml, Sigma), and sodium selenite (5 μg/ml, ICN) were added. The cells were cultured at 38°C in an atmosphere of air containing 5% CO_2 at 100% humidity (Heraus BB-6060, Germany). All media were enriched with gentamycin (20 μg/ml; ICN). Luteal cells from each CL was prepared in 3 or 4 repetitions for each treatment.

Experiment 1

To define the time after which PCBs affect luteal cells function, cells from the CL ($n = 4$) on day 8–12 of the estrous cycle were incubated for 24, 48, 72, 96 and 144 h with 10, 100 or 500 ng/ml of Aroclor (Chem Service, West Chester, UK); Aroclor-1248 is a mixture of less than 2% of mono- and di-, 21% of tri-, 55% of tetra-, 21% penta- and less than 2% of hexa-chlorobiphenyls (Krogenaes *et al.*, 1998). After 48 h of incubation LH (100 ng/ml; AFP 9360-B) was added to medium as positive control. The cells were incubated further for 24 h after which the medium was collected, frozen and stored until hormone measurement.

Experiment 2

In this experiment we studied whether PCBs impair stimulatory effect of HDL and LH on the luteal cells function. Therefore cells from the CL of early (day 4–6), mid (day 8–12) and late (day 14–18) ($n = 6$ each) stages of the oestrous cycle were pre-incubated for 24 h and attached to the plates. Then, cells were washed twice and then treated with different doses (10, 100, 500 ng/ml) of Aroclor. After 48 h the medium was supplemented with HDL (Sigma), containing 25 μg of cholesterol as established earlier (Skarzynski *et al.*, 2003), or LH as given in Exp. 1.

Experiment 3

In this study we investigated whether or not PCBs can affect the stimulatory effect of noradrenaline (NA; Jelfa, Poland) on progesterone secretion from luteal cells. Therefore, luteal cells from 3 stages of the cycle were incubated with Aroclor as described in Exp. 2. Thereafter medium was supplemented with NA (10^{-5}M) as established by Bogacki and Kotwica (1999) for further 24 hours.

Experiment 4

In this trial we studied the influence of PCBs on NA stimulating ovarian oxytocin (OT) secretion from luteal cells. The experimental design was similar to that outlined in Exp. 3, but the days representing the stages of the cycle were different. Luteal cells from the early cycle (days 6–7) of the CL, *i.e.* the end of OT precursor synthesis in CL (Fehr *et al.*, 1987), the mid-cycle (day 10–14) of the CL, *i.e.* the highest concentrations of OT in CL (Schams *et al.*, 1985) and the late cycle (days 16–18) of the CL were harvested; note that luteolysis depletes the CL of all OT (Schams *et al.*, 1985). Incubation of PCBs with NA was shorter and it lasted for only 12 hours.

Hormone analysis

Progesterone concentrations in media were determined by EIA as described by Okuda *et al*. (1997) using a Multiscan EX (Labsystem, Finland). Progesterone antiserum (IFP4) used at a final dilution of 1 : 100 000 was characterised in a prior work (Kotwica and Skarzynski, 1993). The range of the standard curve was 0.1–25 ng/ml and the sensitivity of the procedure was 0.15 ng/ml. Intra- and inter-assay coefficients of variation were on average 8.7% and 11.0%, respectively. The relationship between the added and measured amounts (n = 7) of hormone was r = 0.97.

The concentrations of OT in the culture medium were also determined by EIA as reported earlier (Skarzynski and Okuda, 1999). Antioxytocin serum (R-1), which has been previously characterised (Kotwica and Skarzynski, 1993), was used at a final dilution of 1 : 25 000. The range of the curve was 3.9–1 000 pg/ml and the sensitivity of the procedure was 16–20 pg/ml. Intra- and inter-assay coefficients of variation were on average 10.9% and 13.0%, respectively. Accuracy and reliability of the procedure was expressed by a coefficient of regression of r = 0.86.

Statistical analysis

Mean (± SEM) progesterone concentrations within groups were compared by two-way or one-way factorial ANOVA, using treatment group as treatments and the individual animals as block structures. Analysis of variances was followed by Newman-Keuls as a post-test (PRISM, GraphPad Software). Furthermore, mean progesterone and oxytocin concentrations (Experiment 4) were compared to the control values by the Student t-test.

RESULTS

Aroclor-1248 did not affect progesterone concentrations in medium after either 24 or 48 hours. However, a significant ($P < 0.05$) decrease of progesterone was noted after 72 h (Figure 1) of incubation. The progesterone content in the medium also decreased ($P < 0.05$) after 144 h of incubation by all tested doses of Aroclor but response of these cells on LH stimulation also decreased by 50% (data not showed).

Added individually, LH and HDL significantly ($P < 0.01$) increased the release of progesterone from luteal cells (Figure 2) compared to that of the control. Aroclor enhanced the effect of HDL on progesterone secretion during the mid- and late-luteal phases (Figures 2B, 2C). The stimulatoriest effect of LH alone on progesterone secretion was evident in a fully developed and late CL (Figures 2A, 2B). Aroclor did not affect LH on stimulating progesterone secretion.

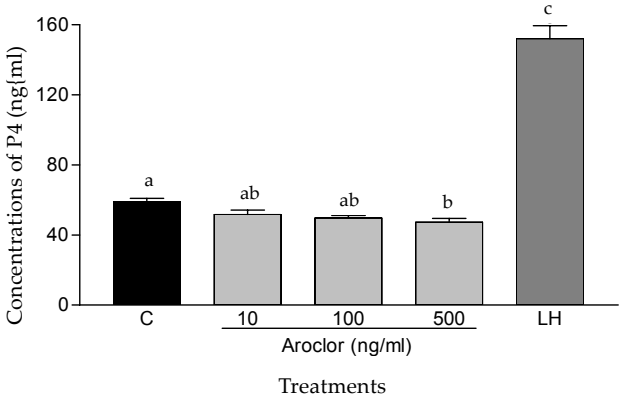

Figure 1. Influence of different doses of Aroclor-1248 for 72 h and LH (positive control) for the last 24 h of incubation on progesterone (P_4) secretion (mean ± SEM) from luteal cells on day 8–12 of the estrous cycle (n = 4)

^{a,b,c} values with different superscripts are different (at least $P < 0.05$)

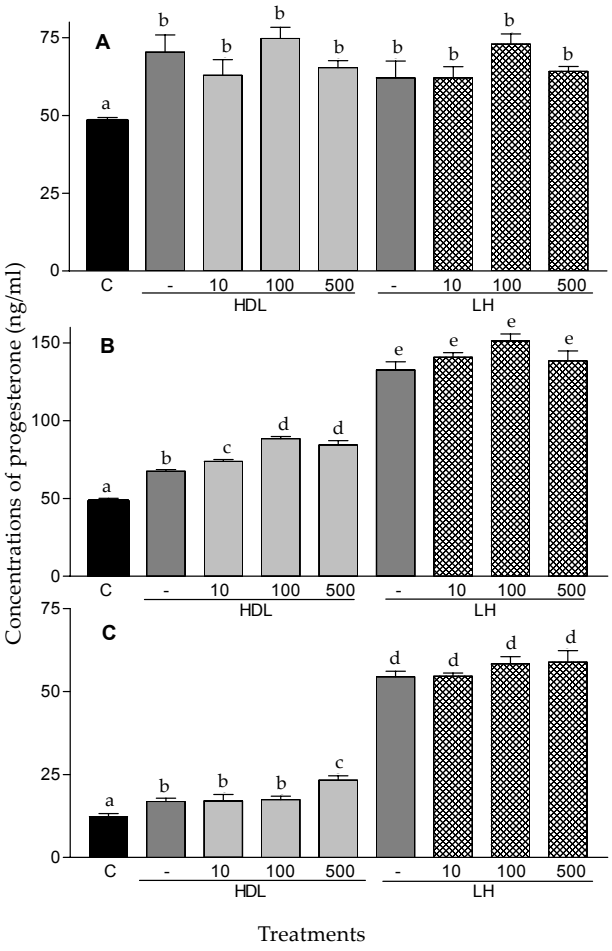

Figure 2. Effect of Aroclor-1248 (10, 100, 500 ng/ml) on HDL and LH stimulating progesterone content (mean ± SEM) in culture medium; (A) early, (B) mid, (C) late luteal phase (n = 6 CL each) of the estrous cycle

[a,b,c,d,e] values with different superscripts are different (at least $P < 0.05$)

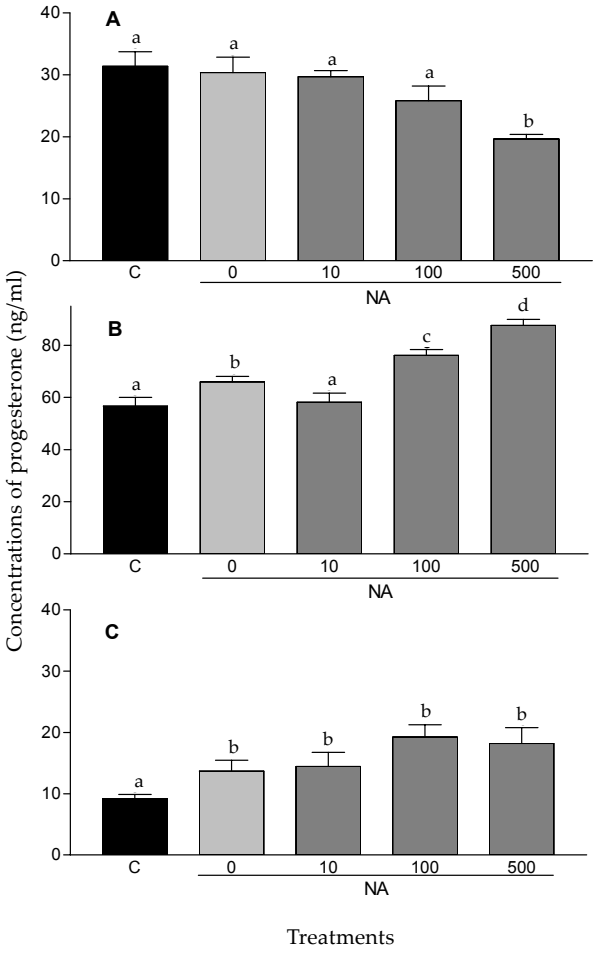

Figure 3. Effect of Aroclor-1248 (0, 10, 100, 500 ng/ml) on noradenaline (NA) stimulating progesterone medium concentrations (mean ± SEM) from luteal cells of (A) early, (B) mid and (C) late corpora lutea (n = 6 each)

[a,b,c,d] values with different superscripts are different (at least $P < 0.05$)

Addition of 500 ng/ml of Aroclor to the medium decreased progesterone release from luteal cells of the early CL (Figure 3A) compared to control and NA treated cells. However, treatment of luteal cells from the mid CL with NA and Aroclor-1248 (100 and 500 ng/ml) stimulate ($P < 0.05$) progesterone secretion compared to NA alone (Figure 3B). But Aroclor-1248 did not affect NA influence on progesterone secretion by the luteal cells from the late CL

All doses of Aroclor, but 10 ng/ml in early CL, stimulated ($P < 0.05$–0.001) OT secretion from luteal cells during all stages of the CL development (Figure 4). NA added individually also affected OT secretion from the luteal cells of mid and late CL. Moreover, NA increased the stimulatory effect of Aroclor on OT secretion from luteal cells of all stages of CL development examined (Figures 4A, 4B, 4C).

DISCUSSION

Aroclor-1248, as a mixture of PCBs, was applied over a wide concentration range (10–500 ng/ml), but similar to levels used in studies on oocyte maturation and fertilisation (Frame *et al.*, 1996; Pocar *et al.*, 2001) on granulosa (Roselli *et al.*, 2000; Wojtowicz *et al.*, 2000) or on intersticial cells (Muller *et al.*, 1978).

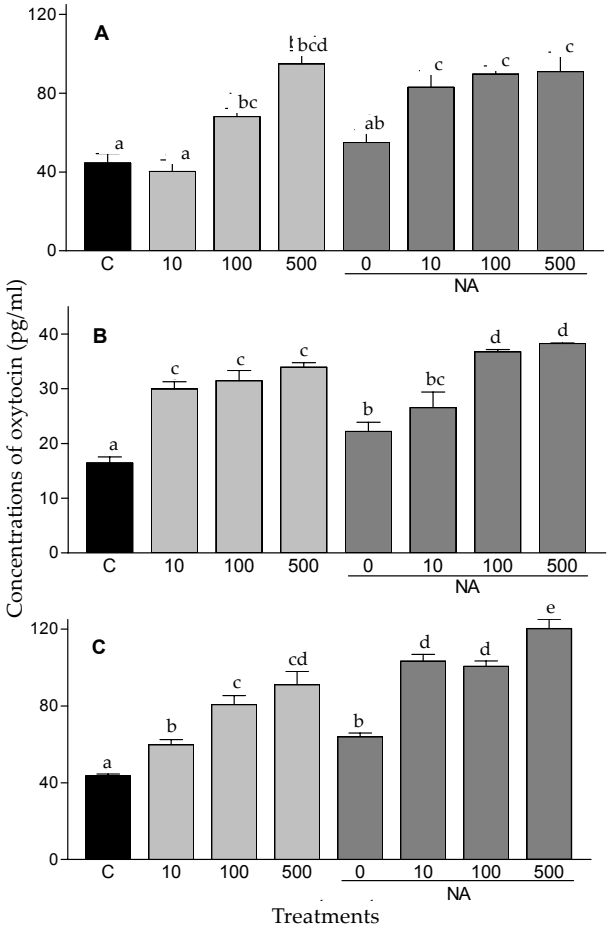

Figure 4. Effect of Aroclor-1248 (10, 100, 500 ng/ml) on noradenaline (NA) stimulating oxytocin medium concentrations (mean ± SEM) from luteal cells; (A) early, (B) mid and (C) late stage of the oestrous cycle (n = 6 CL each)

[a,b,c,d,e] values with different superscripts are different (at least $P < 0.05$)

Moreover used doses of PCBs were within the range of PCBs amount measured in tissue or fluids of living bodies (Trapp *et al.*, 1984). The mixture of PCBs used at the dose 500 ng/ml reduced the basal secretion of progesterone from luteal cells within 72 h of incubation (Figure 1). The doses of PCBs we used did not impair stimulatory effect of LH (Figure 2) on progesterone secretion. Thus we assume PCBs doses were not toxic to the cells after 72 h of incubation, although data by Augustowska *et al.* (2002) indicate PCBs an affect cell membrane permeability and hormone outflow. Other studies have suggested that PCBs can either decrease (Johnsson *et al.*, 1976) and augment LH-induced progesterone synthesis (Fuller *et al.*, 1980) or not affect progesterone concentrations at all (Lundqvist and Kindahl, 1989) in laboratory females. However, there is much to be learned regarding the physiological consquences of PCBs found in the environment. For example, PCB-153 is commonly present in milk at nanograms concentrations (Stewart and Jones, 1996), whereas PCB-126 is present in the human body at pg/g levels, but this compound is much more toxic (Liljegren *et al.*, 1998).

HDL increased progesterone secretion (Figure 2) by supply the luteal cells with cholesterol as a substrate for progesterone synthesis (Pate and Condon, 1989; Skarzynski *et al.*, 2003), whereas LH also increased progesterone release to activate enzymes involved in CL steroidogenesis (Carlson *et al.*, 1971). Interestingly, Aroclor enhanced the effect of HDL during the mid- and late-stage of the oestrous cycle. An explanation for this observation is that a single or a number of the PCBs can affect the activity of steroidogenic enzymes (Kannan *et al.*, 1994; Machala *et al.*, 1998; Andric *et al.*, 2000) or to impair lipids metabolism (Bell *et al.*, 1994).

Noradrenaline can stimulate progesterone secretion from the CL by affecting the activities of cytochrome P-450$_{scc}$ and 3β-HSD (Miszkiel and Kotwica, 2001). In Experiment 3, PCBs were able to enhance the stimulatory effect of NA but only in luteal cells of the mid-stage of the oestrous cycle. It is assumed that 72 h of treatment with PCBs can inhibit steroidogenesis in luteal cells of the CL as shown in Experiment 1, but this can also affect the accumulation of progesterone precursors. Supposedly the activation of aforementioned steroidogenic enzymes

by NA increases progesterone release. The same tendency can be observed in luteal cells of the late CL. However in luteal cells of the early CL, NA did not prevent a significant ($P < 0.01$) decrease of progesterone levels in the medium by the highest dose of Aroclor (Figure 3A) applied.

Noradrenaline stimulates ovarian OT secretion and synthesis during all stages of development of the CL (Kotwica and Skarzynski, 1993; Kotwica *et al.*, 2002), and this tendency is seen in these studies in spite of long incubation of cells. Unexpectedly, PCBs stimulated a cellular release of OT, and NA significantly enhanced this process in all days of the oestrous cycle examined (Figure 4). The experimental model used in these studies does not offer an explanation of the mechanism of PCBs and action of NA. Nevertheless, it is known that NA can release OT from a releasable pool of luteal cells and can also affect the post-translational process of OT synthesis in the CL (Bogacki and Kotwica, 1999). So, it can only be assumed that PCBs enhance or mimic the action of NA. However, ovarian secretion of OT depends also on $PGF_{2\alpha}$. It was found that luteal estradiol affects the release of $PGF_{2\alpha}$ (Okuda *et al.*, 2001) and it can further release OT from luteal cells. It is assumed PCBs can act as both estrogens and their antagonists (Neseretnam *et al.*, 1996; Neseretnam and Darbre, 1997; Krogenaes *et al.*, 1998). It was also shown that PCBs could activate AhR (Safe, 1994), which then affect a number of estradiol receptors (Kharat and Saatcioglu, 1996). Furthermore, PCBs can react with the sex hormone binding globulin, thereby affecting the function of native steroid hormones (Hodgert *et al.*, 2000). Therefore it cannot be excluded that PCBs mimic the effect of estradiol, can also influence the secretion of OT in this manner.

The data so obtained indicates that PCBs (Aroclor-1248) can inhibit as well as stimulate progesterone secretion from luteal cells. This effect can depend upon the developmental stage of the CL and type of cells (small and large) which constitute the CL (O'Shea *et al.*, 1989) on the hormonal milieu affecting the physiological status of the female. Furthermore, PCBs affect OT secretion/synthesis (Figure 4). It is known that OT supports the function of the CL during the early stage of the estrous cycle (Miyamoto and Schams, 1991) on one hand, but it can also hasten and strengthen luteolysis in the end of the luteal phase (Kotwica *et al.*, 1999) on the other hand. Just therefore PCBs are so dangerous; they can either extend or shorten the life span of the CL and thus to impair pregnancy or to affect the course of the estrous cycle. It is difficult to know whether these observations will be confirmed in studies *in vivo*. PCBs taken *per os* are partly metabolised to their derivatives and then their effect can be either more or less pronounced, but this required further observations.

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