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Impairment of uterine smooth muscle contractions and prostaglandin secretion from cattle myometrium and corpus luteum in vitro is influenced by DDT, DDE and HCH

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ABSTRACT

The aim of this study was to investigate the effect of dichlorodiphenyltrichloroethane(DDT), dichlorodiphenyldichloroethylene (DDE) and γ-hexachlorocyclohexane (HCH) (10 ng/ml) on myometrial motility and the secretory function of the myometrium and corpus luteum (CL) collected from cows on days 8–12 of the estrous cycle. All of the xenobiotics increased ($P<0.05$) myometrial contractility. Moreover, the xenobiotics stimulated the secretion of the following prostaglandins (PGs) from myometrial strips: PGF2α, PGE2 and PGI2. DDT and DDE also increased ($P<0.05$) the release of PGF2α from CL strips, and HCH had the same effect ($P<0.05$) on the secretion of PGE2 and PGI2. The studied xenobiotics did not affect ($P>0.05$) PG synthesis, but DDT and DDE increased the mRNA expression levels of leukemia inhibitor factor (LIF), which can stimulate PG production. In summary, the xenobiotics affected PG secretion from cow myometrium and CL, which may contribute to the mechanism of uterine contraction disturbance.

1. Introduction

1,1,1-Trichloro-2,2,-bis-4-chlorophenyl-ethane (DDT) and γ-hexachlorocyclohexane (HCH), which is commonly referred to as lindane, are recognized as representative organochlorine pesticides from the group of endocrine disruptors. DDT and HCH are currently used in agriculture and forestry in developing countries of Africa and Asia to control pests of crops and to overcome typhoid, malaria (Gunasekaran et al., 2005; Rogan and Chen, 2005; Nweke and Sanders III, 2009; Hlongwana et al., 2013) or scabies (Ngueleu et al., 2013). Because of their widespread distribution, lipophilic properties and high resistance to biodegradation (Rogan and Chen, 2005; Tsai, 2010), DDT and HCH are still detected in fodder plants (Pazou et al., 2013) and in the bodies of farm animals (Kamarianos et al., 2003) and humans (Toft et al., 2004; Llop et al., 2010). The production and use of DDT and HCH are banned in the USA, Japan and many European countries (Fromberg et al., 1999; Glynn et al., 2000; Li et al., 2006). However, due to the ability of DDT and HCH to spread by air and water, these pesticides are also a problem in places far from the sources of application (Glynn et al., 2000; Ayotte et al., 2001; Burns et al., 2013). In the environment, DDT is degraded to bis 4-chlorophenyl-1,1-dichloroethene (DDE), which is a stable and ubiquitous component and is also found in living tissues (Turusov et al., 2002; Kamarianos et al., 2003; Meeker et al., 2009). When these pesticides enter organisms, they can act as endocrine disruptors and compete as agonists or antagonists with more than one type of nuclear steroid receptors (Kuiper et al., 1998; Turusov et al., 2002; Li et al., 2008). Therefore, there is increasing concern that pesticides may cause an impairment in the endocrine system, followed by alterations in reproductive physiology.

The unaffected regulation of uterine motility is one of the crucial factors for the initiation and maintenance of pregnancy. Prostaglandins (PGs) are a group of important regulators of the reproductive process, including contractile activity of the myometrium. PGF2α mediates the stimulation of myometrial contractions, but PGE2 and PGI2 can cause the excitation or relaxation of the myometrium (Senior et al., 1993; Coleman et al., 1994). However, the initiation of uterine contractions and/or labor after PGE2 induction may be a result of cervical ripening rather than its direct myometrial effect (Chiossi et al., 2012). The positive feedback loop between luteal oxytocin (OT) and uterine PGF2α in cow is well documented (Skarzynski et al., 1997; Kotwica et al., 1999). However, it has also been reported that LIF, which plays an important role in mammalian implantation, enhances PGE2 production and expression for its uterine receptors (Horita et al., 2007).

Epidemiological observations in humans show a correlation between the presence of DDT, its metabolite (DDE) or HCH in the blood of mothers and an increased risk of miscarriages

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(Gerhard et al., 1998; Korrick et al., 2001; Longnecker et al., 2005; Venners et al., 2005) and preterm deliveries (Saxena et al., 1980; Longnecker et al., 2001; Torres-Arreola et al., 2003; Pathak et al. 2010; Mustafa et al., 2013). However, data from other studies did not confirm these observations (Berkowitz et al., 1996; Farhang et al., 2005; Fenster et al., 2006; Wood et al., 2007; Cioroiu et al., 2010). In our in vitro study, we showed that DDT and DDE affect the endometrial secretion of PGF2α and PGE2 (M. Wrobel et al., 2009), which are natural regulators of uterine contractions (Senior et al., 1993; Coleman et al., 1994). Because PG secretion could also be followed by the stimulation of myometrial contractions in pregnant cows by these compounds (Mlynarczuk et al., 2010) and because the endometrium is a main source of PGs in the reproductive tract, the involvement of the secreted PGs from the myometrium and corpus luteum (CL) in the adverse effects of organochlorined pesticides on myometrial contractions should be investigated.

Therefore, the aim of this study was to determine the effect of DDT, DDE and HCH on (a) uterine smooth muscle contractions and (b) the synthesis and secretion of PGF2α, PGE2 and PGI2 from myometrial and luteal cells.

2. Materials and methods

2.1. Animals and tissues collection

Uterine horns and CL from healthy cows and mature heifers on days 8–12 of the estrous cycle (Ireland et al., 1980; Fields and Fields, 1996) were collected in a commercial slaughterhouse within 20 min after slaughter. These tissues were placed in ice-cold saline and transported to the laboratory within 1 h. Each medium used was supplemented with gentamycin (20 μl/ml). All materials used in these studies were purchased from Sigma-Aldrich (PL) unless otherwise stated.

2.2. Uterine strip preparation and incubation

To measure the uterine contractions, four strips (3–4 mm wide and 6–7 mm long) of the myometrium were dissected from each animal, and they were cut in the direction of the longitudinal muscle. The slices were immediately immersed in 4 ml of aerated (95% air and 5% CO_2) physiological buffer (PSS; pH=7.4), which was composed of NaCl (116 mM), KCL (4.6 mM), $NaH_2PO_4 \cdot H_2O$ (1.16 mM), $MgSO_4 \cdot 7\ H_2O$ (1.16 mM), $NaHCO_3$ (21.9 mM), $CaCl_2 \cdot 2\ H_2O$ (1.8 mM), dextrose (11.6 mM) and CaNaEDTA (0.03 mM) (Tsai et al., 1996). The strips were then incubated at 4 °C with the treatments for 48 h, as described previously (Wrobel et al., 2005). The medium and all treatments were changed after 24 h. After incubation, the contractility of the strips was measured.

To measure the prostaglandin secretion, 15 slices (40–60 mg) from both the myometrium and CL were obtained from each animal. The strips were pre-incubated for 24 h (38 °C; 95% O_2 and 5% CO_2) in 2 ml of DMEM/Ham's F-12 supplemented with 5% FCS. The strips were then incubated in DMEM/Ham's F-12 medium with 0.1% BSA and studied treatments. All myometrial and CL strips used in one experiment were obtained from the same animal.

2.3. Preparation and incubation of myometrial and luteal cells

The myometrium was separated from the endometrium and perimetrium. The tissue (7 g per uterus) was minced with scissors and placed (2 h) in warmed (38 °C) and oxygenated (95% O_2 and 5% CO_2) digestion mixture containing medium (20 ml of M199 with 0.1% BSA) supplemented with collagenase IA (1.5 mg/ml) and dispase (0.2 mg/ml; Gibco, GB), as previously described (Wrobel and Kotwica, 2005). Luteal cells were obtained by perfusion of the CL with collagenase IA (1 mg/ml) through one of the branches of the ovarian artery (Okuda et al., 1992). Cells obtained from four CL tissues were pooled and used in one experiment. Both myometrial and luteal cells were collected by centrifugation (3 times for 10 min; 1800g and 1000g, respectively). After each centrifugation, the cells were washed with medium (10 ml of M199 with 0.1% BSA). The collected cells were counted, and their viability was estimated using the exclusion of 0.04% trypan blue dye. Only cells with viability greater than 85% were used. Finally, the cells were suspended (2.5×10^5/ml) in DMEM/Ham's F-12 with 5% FCS and sieved (0.5 ml/well) into 48-well plates (Nunclon, NUNC, DE) to measure the cytotoxic effect of HCH. The cells used to measure mRNA expression were suspended in the same medium (5×10^5/ml) and placed (4 ml/well) into 6-well plates (Nunclon, NUNC, DE). The myometrial and luteal cells were cultured for 96 and 24 h, respectively, in a controlled atmosphere (air balanced with 5% CO_2) at 100% humidity and 38 °C (Memmert INC 108, Germany) to allow the cells to attach to the bottom of the plate. The cells were then washed twice with M-199 supplemented with 0.1% BSA. After the washes, the cells were incubated in DMEM/Ham's F-12 culture medium with 0.1% BSA and were subjected to xenobiotic treatment. When the incubation was longer than 24 h, the medium was supplemented with the following antioxidants: ascorbic acid (20 μg/ml; Merck, USA), sodium selenite (5 ng/ml; INC, USA) and transferrin (5 μg/ml), to prevent the free radicals accumulation.

2.4. Treatments.

DDT, DDE (≥ 98% purity) and HCH (97% purity) were dissolved in DMSO, and its final concentrations in culture medium did not exceed 0.1%. Therefore 0.1% of DMSO was added to the control samples.

There have been found 0.2–0.3 ng/ml of DDT, 0.8–3.7 ng/ml of DDE and 0.7–6.1 ng/ml of HCH in the follicular fluid of cattle (Kamarianos et al., 2003). In our previous studies on the effect of DDT and DDE on the function of bovine reproductive tract, these xenobiotics were used in the dose of 0.1–10 ng/ml and the highest of them was the most effective (M. Wrobel et al., 2009; Wrobel et al., 2012). It should be also emphasized that during pregnancy, uterus can accumulate a 3–10-fold higher amount of HCH or other chlorinated compounds than that found in female fat or blood (Polishuk et al., 1977). Therefore, we used all xenobiotics at a dose of 10 ng/ml in the present study.

2.5. The effect of xenobiotics on myometrial and luteal cell viability

It was shown that DDT and DDE (10 ng/ml) did not affect the viability of uterine, ovarian (M. Wrobel et al., 2009) and oviductal cells (Wrobel et al., 2012). However, to avoid the criticism that HCH could affect the viability of myometrial ($n=4$) and luteal ($n=6$) cells, they were incubated (72 h) with this compound (10 ng/ml). Actinomycin D (Act D; 500 ng/ml) is an inhibitor of RNA synthesis and stimulator of apoptosis. Since it greatly reduces the survival of cells, it was used as a negative control to verify the method of cells viability measurement.

2.6. The effect of xenobiotics on contractions of myometrial strips

Myometrial strips from six cows ($n=6$) were treated separately with DDT, DDE or HCH (10 ng/ml) for 48 h. The force of the contractions was then measured.

2.7. The effect of xenobiotics on PG secretion from myometrial and CL slices

Slices of the myometrium and CL from six cows were incubated (24 h) with DDT, DDE, or HCH (10 ng/ml). Arachidonic acid (AA; 20 μg/ml), which is a substrate to PGs synthesis (Goff, 2004), was used as a positive control. Each treatment was performed in triplicate. After the incubation, medium was collected into tubes containing 10 μl of 0.3 M EDTA in 1% acetylsalicylic acid (Meyer et al., 1989) and stored at −20 °C to determine the PG concentration.

2.8. The effect of xenobiotics on COX-2, PGFS, PGES and PGIS mRNA expression in myometrial and luteal cells as well as LIF mRNA expression in myometrial cells

Myometrial and luteal cells were incubated (24 h) with DDT, DDE or HCH (10 ng/ml). Each experiment was repeated four times. After incubation, the medium was removed, and the cells were covered with Phenozol (300 μl for each well; A&A Biotechnology, PL). The plates were stored (at −70 °C) for further real-time PCR analysis of COX-2, PGFS, PGES and PGIS mRNA expression in myometrial and luteal cells as well as LIF mRNA expression in myometrial cells.

2.9. Determination of cell viability

Viability of cells after treatment with HCH was measured by a TOX-1 test (in vitro toxicology assay kit, MTT based) according to the manufacturer's instructions. This method is based on the ability of mitochondrial dehydrogenase in living cells to convert a tetrazolium salt (MTT; yellow color) into formazan (blue color). The myometrial and luteal cells were incubated with MTT (20 μl/well) for 4 and 2 h, respectively. The absorbance of the reaction product was measured at $\lambda=570$ nm (ELISA, Multiscan EX, Labsystem, FI).

2.10. Measurement of smooth muscle contractions

Myometrial strips were individually placed into the chambers of a HSE Schuler Organbath apparatus (March-Hugstetten, DE) connected with computer. Each chamber containing Krebs–Ringer's solution (KRS; pH=7.4; 10 ml), which was composed of NaCl (120.3 mM), KCl (5.9 mM), $CaCl_2$ (2.5 mM), $MgCl_2$ (1.2 mM), NaH_2PO_4 (1.2 mM), $NaHCO_3$ (15.5 mM) and glucose (11.5 mM) (Kotwica et al., 2003). Each strip was tied to both the base and the isometric contraction transducer (HSE Type 372) with a stationary hook and surgical silk, respectively.