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The adverse effects of aldrin and dieldrin on both myometrial contractions and the secretory functions of bovine ovaries and uterus in vitro

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ABSTRACT

Aldrin and dieldrin are chloroorganic insecticides which are recognised as endocrine disruptors. The aim of the study was to investigate their effect on the secretory functions of the uterus and ovary and on myometrial contractions.

Myometrial strips and uterine and ovarian cells from nonpregnant cows were incubated with the xenobiotics (0.1, 1 or 10 ng/ml) for 24 or 72 h. Next, their effect on viability of myometrial, endometrial, granulosa and luteal cells, myometrial strip contractions, the synthesis and secretion of prostaglandins (PGs: PGF2α and PGE2) from uterine cells, the secretion of oestradiol (E2), testosterone (T) and oxytocin (OT) from granulosa cells and the secretion of progesterone (P4) and OT from luteal cells were determined.

Neither of the xenobiotics (10 ng/ml) affected ($P > 0.05$) the viability of the ovarian and uterine cells, while both (0.1–10 ng/ml) decreased ($P < 0.05$) the basal and OT-stimulated myometrial contractions. In spite of these effects, neither of the insecticides affected ($P > 0.05$) the synthesis and the secretion of PGs from the myometrial cells. Although they also did not impair the secretion of the PGs from the endometrial cells, they abolished ($P < 0.05$) the stimulatory effect of OT ($P < 0.05$) on the secretion of the PGs and stimulated ($P < 0.05$) the secretion of OT from the granulosa and luteal cells. Moreover, aldrin and dieldrin stimulated secretion of E2 and T from the granulosa cells, while only dieldrin increased ($P < 0.05$) the secretion of P4 from luteal cells.

The data show that aldrin and dieldrin stimulated the secretory function of the cultured granulosa and luteal cells and inhibited the myometrial contractions of cows in vitro, which may affect on natural parturition.

Introduction

Use of a wide variety of synthetic insecticides is an indispensable part of modern agriculture and farming practice. However, unwanted exposure to these synthetic chemicals is still widespread among human and domestic animal populations and presents a potential risk to their health. Aldrin ($C_{12}H_8Cl_6$—1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene) and the more stable but also more expensive, product of its transformation, dieldrin ($C_{12}H_8Cl_6O$—1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene), belong to the family of cyclodiene organochlorine insecticides. Aldrin and dieldrin are very stable compounds that resist degradation and, because of their long half-lives, both are recognised as persistent environmental pollutants (Jorgenson, 2001; Tully et al., 2000). Both were widely used in agriculture in the USA and Europe from the 1950s up to the early 1970s (WHO, 1989) to preserve seeds, crops, forage, and wood and to control populations of termites, with dieldrin being more extensively used for this purpose (Jorgenson, 2001; Tully et al., 2000; Van Amelsvoort et al., 2009).

In addition to the intended effects, these insecticides may also have adverse health effects for livestock (Casteel et al., 1993) and humans (Stern, 2014; Stevenson et al., 1999; Tomar et al., 2013; Van Amelsvoort et al., 2009). Therefore, their use became progressively more restricted, and they were gradually withdrawn from use in Western countries (Van Amelsvoort et al., 2009). However, they were still widely used in agriculture and public health programmes in India and some African countries into the beginning of the 21st century (Mustafa et al., 2010). After they penetrated into air and water, they can spread in environment far from sources of their emission. Therefore they were found at levels above the permissible limits in ground water in India (Thakur et al., 2010) as well as in waste sites in USA (Stern, 2014). Moreover, dieldrin was measured in follicular fluid of cows in Greece (Kamarianos et al., 2003) and both are still detected in bovine

Abbreviations: Act D, actinomycin D; AA, arachidonic acid; COX-2, cyclooxygenase 2; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; E2, oestradiol; KRS, Krebs–Ringer's solution; MTT, tetrazolium salt; OT, oxytocin; P4, progesterone; PCBs, polychlorinated biphenyls; PG(s), prostaglandin(s); PGFM, 13,14-dihydro-15-keto-PGF2α; PGES, prostaglandin E synthase; PGFS, prostaglandin F synthase; T, testosterone; TBP, TATA box binding protein.

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meat (Letta and Attah, 2013) and milk samples (Deti et al., 2014) obtained in Africa as well as in the blood of humans in Brazil (Freire et al., 2013) and India (Chillar et al., 2013).

Milk-producing animals accumulate insecticides primarily after ingestion of contaminated feed and by inhaling contaminated air (Waliszewski et al., 2003) while in the occupational setting, the most important route for aldrin and dieldrin exposure was skin contact (Van Amelsvoort et al., 2009). After uptake, aldrin is rapidly converted to dieldrin, primarily by the *P*-450 system of the liver (Van Amelsvoort et al., 2009). Thus, aldrin cannot be detected in animals or humans unless there is current exposure (Stevenson et al., 1999). Dieldrin, by virtue of its lipophilic properties, is initially stored in fat-rich tissues and is subsequently translocated and excreted in milk. Therefore, the consumption of dairy products together with other contaminated food may expose consumers to unexpected doses of organochlorine insecticides (Armendariz et al., 2004; Botella et al., 2004; Waliszewski et al., 2003). After penetration of aldrin and dieldrin into the living bodies, they can disrupt the function of endocrine system by binding to oestrogen receptor and inhibition of the androgen signaling pathway (Aube et al., 2011; Lemaire et al., 2006). This can be followed by alterations in reproductive physiology of human, wildlife, as well as farm animals (Majdic, 2010; McKinlay et al., 2008). Indeed, aldrin belongs to the group of insecticides that have been detected in the blood and placentas of women experiencing spontaneous abortion (Saxena et al., 1981). However, its effect on myometrial contractions and the mechanism for this process has not been described as clearly as for other chlorinated xenobiotics, such as dichlorodiphenyltrichloroethane (DDT) or polychlorinated biphenyls (PCBs; Tsai et al., 1996; Wrobel et al., 2005, 2009b, 2014).

Normal regulation of uterine motility is one of the crucial factors for the maintenance of pregnancy and initiation of labour at term. Oestrogens are thought to augment uterine contraction and thereby promote labour (Pepe and Albrecht, 1995; Pinto et al., 1967) by increasing the myometrial expression of the oxytocin (OT) receptor (Welsh et al., 2012) and the output of uterine prostaglandin (PG) F2α (Nakayama et al., 1991). Both PGF2α and OT are major factors that evoke myometrial contractions (Dray and Frydman, 1976; Fuchs et al., 1984; Senior et al., 1993), and the positive feedback loop between them in cows is well documented (Kotwica et al., 1999; Skarzynski et al., 1997). In contrast, increases in the concentration of progesterone (P4) in the pregnant uterus block myometrial activity until parturition (Wood, 1999). Therefore, the aim of this study was to investigate whether aldrin and dieldrin interfere with (a) myometrial contractions, (b) the synthesis and secretion of PGF2α and PGE2 from the uterus and (c) secretion of OT, testosterone (T), oestradiol (E2) and P4 from the granulosa and luteal cells.

Material and methods

Animals and organ preparation. On days 8–12 of the oestrous cycle (Fields and Fields, 1996), uteri and ovaries from healthy cows and mature heifers were collected in a commercial slaughterhouse within 20 min after slaughter. The organs were placed in ice-cold saline (0.9% NaCl) and transported to the laboratory within 1 h. All materials used in these studies were purchased from Sigma-Aldrich (PL) unless otherwise stated. Each medium used was supplemented with gentamycin (20 μg/ml) and amphotericin (2 μg/ml).

Myometrial strips preparation and incubation. Four strips of longitudinal smooth muscle from each myometrium, 6–7 mm long and 3–4 mm wide, were dissected as previously described (Wrobel et al., 2005). The strips were immediately immersed in 2 ml of aerated (95% air and 5% CO_2) physiologic salt solution (116 mM NaCl, 4.6 mM KCl, 1.16 mM $NaH_2PO_4 \cdot H_2O$, 1.16 mM $MgSO_4 \cdot 7H_2O$, 21.9 mM $NaHCO_3$, 1.8 mM $CaCl_2 \cdot 2H_2O$, 11.6 mM dextrose, 0.03 mM CaNaEDTA; pH 7.4), as described by Tsai et al. (1996). They were then incubated with the treatments (24 h, 4 °C) according to Wrobel et al. (2005). After incubation, the contractility of the myometrial strips was measured.

Isolation and culture of cells. Myometrial cells were obtained by enzymatic dispersion after the separation of the myometrium from the perimetrium and endometrium. The tissue (7 g from each uterus) was minced with scissors and then digested (2 h at 38 °C) in oxygenated (95% O_2 + 5% CO_2) medium (20 ml of M199 supplemented with 0.1% BSA) with collagenase IA (1.5 mg/ml) and dispase (0.2 mg/ml Gibco, GB). Endometrial cells were also isolated by enzymatic dispersion. The isolated uterine horns were filled with the digestion mixture of collagenase IA (1.5 mg/ml) and medium (M199 supplemented with 0.1% BSA), and placed in a water bath (38 °C) for 1 h. To obtain pooled luteal cells, corpora lutea (four for each experiment) were perfused with collagenase IA (1 mg/ml) through an ovarian artery branch. Pools of granulosa cells were obtained by vigorous aspiration with the follicular fluid from follicles (10–15 follicles) >1 cm in diameter. Next, all types of cells were collected and partially purified by centrifugation (1800 ×*g* for the myometrium, 1200 ×*g* for the endometrium and granulosa, or 1000 ×*g* for the luteal cells, 3 times for 10 min, at 4 °C). After each centrifugation, the cells were washed with 10 ml of M199 supplemented with 0.1% BSA. Cell viability was estimated by exclusion of 0.04% trypan blue dye. Only preparations of cells showing viability greater than 80% were used for further studies. Finally, all types of cells were suspended in DMEM/Ham's F-12 culture medium with 5% FCS. The cells were suspended at 2×10^5/ml for use in the studies of the cytotoxic effects of the insecticides and for hormone determinations or at 5×10^5/ml for the measurement of mRNA expression. The suspensions were transferred into plates (48-well plates for the studies of the cytotoxic effect of the insecticides and hormone determinations and 6-well plates for the measurement of mRNA expression; Nunclon Δ-Surface, NUNC, NL) and cultured in a controlled atmosphere (95% air and 5% CO_2, 100% humidity, 38 °C; Memmert INCO 180, D). The cells were preincubated for 24 h (granulosa and luteal), 72 h (endometrium) and 96 h (myometrium) to allow them to attach to the bottom of the well. Next, they were washed twice with M199 and the medium was replaced with DMEM/HAM-12 supplemented with 0.1% BSA. For incubations exceeding 24 h, the medium was supplemented with antioxidants: ascorbic acid (20 μg/ml; Merck, USA), sodium selenite (5 ng/ml; INC, USA) and transferrin (5 μg/ml).

Table 1
Primer sequences used to analysis of gene expression in bovine myometrial cells.

Gene	Accession no.	Sequence (5′-3′)	Product size (bp)
COX-2	AF004944.1	Forward: GCCTGATGACTGCCCAACA Reverse: GCAAAGAATGCAAACATCAGATTT	140
PGES	NM_001166554.1	Forward: CCGAGATCAAGTTCTCCTCCTACA Reverse: CGCCTTCATGGGTGGATAGT	131
PGFS	S54973	Forward: TGTGGTGCACGTATCACGACA Reverse: AATCACGTTGCCGTCCTCATC	160
TBP	NM_001075742.1	Forward: CAGAGAGCTCCGGGATCGT Reverse: ACACCATCTTCCCAGAACTGAATAT	194