Secretion of Estradiol and Progesterone by Bovine Granulosa Cells In Vitro in Presence of Different Concentrations of

Chlorinated Hydrocarbons

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1. Introduction

It is known that chlorinated pesticides, biphenyls and chlorobenzenes may produce different functional disorders in reproductive organs. Sircar and Lahiri[1] found that γ-HCH adversely affected cholesterol side-chain cleavage in the mouse ovaries characterized by decreased conversion of this sterol to pregnenolone and subsequently to progesterone in a dose-dependent manner. Some organochlorine compounds, such as DDT, methoxychlor and chlordecon are well known for their estrogenic effects in animal studies. These compounds are uterotropic and act in a receptor-mediated manner[2]. Furthermore, methoxychlor increases lipid accumulation in interstitial and theca cells of mouse ovaries. This suggests that these cells are unable to synthesize steroids and that methoxychlor mimics the effects induced by estrogen on the ovary[3]. These are results of experimental animal models in which the tested compounds were in toxic concentrations. Recently, investigations were performed to asses the degree of accumulation of chlorinated hydrocarbons in genital tract tissues and subsequent morphological and functional effects after long term low-dose exposure[4]. The role of this group of compounds in the reproductive disorders in ruminants is not well documented. In the present study we have examined the effect of a mixture of chlorinated hydrocarbons and their metabolites on the secretion of estradiol (E) and progesterone (P) by bovine granulosa cells in vitro.

2. Material and methods

Experiment 1. The aim of this experiment was to estimate the degree of accumulation of chlorinated hydrocarbons in ovaries of cows and heifers under natural environmental conditions. Ovaries were collected from animals just after slaughtering. The extraction of organic chlorine pesticides including HCB and PCBs was carried out according to the generally accepted procedures[5]. PCBs were separated from organic chlorine pesticides in the purified extracts by means of HPLC (Varian Model 1638 with a UV detector). All standars were obtained from Poly Science Co. USA and the Institute of Organic Industry in Warsaw, Poland. The whole procedure was checked in the international interlaboratory of the

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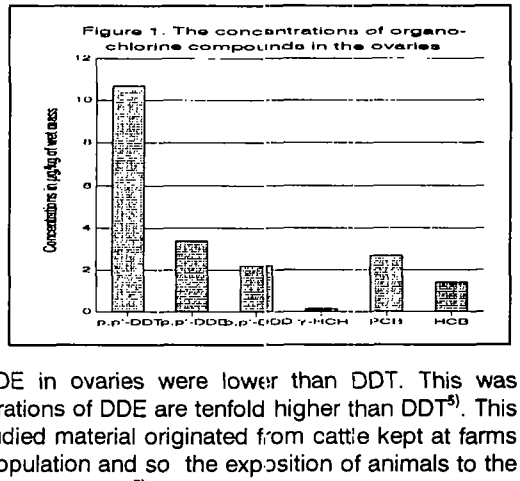
Analytical Quality Assurance Program for OC pesticides and PCBs organized by The National Food Administration in Sweden. Results were expressed as the mean ± SEM in μg/kg wet mass.

Experiment 2. Using the mean concentrations of the compounds found in ovary as reference values, a mixture of chlorinated hydrocarbons in DMSO was prepared. The concentration 10^{-3} contained the mean concentrations of the tested compounds at same proportions as found in the ovaries in experiment 1. Granulosa cells were isolated from preovulatory follicles and were cultured to form monolayer according to the procedure of McNatty et al.[6]. After the monolayer had formed two experimental protocols were established: I. granulosa cells were cultured for 96 hrs in the presence of different concentrations (10^{-1} to 10^{-4}) of the mixture mentioned above.

The medium of the cultured cells were collected each 24 hrs for determination of E and P. These hormones were determined by using kits for EIA (Serono, Switzerland). After collection fresh medium containing the mixture was added to the cell cultures. The experimental protocol II consisted of a continuous culture of granulosa cells in presence of the tested compounds. Concentrations of E and P were measured after 96 hrs of culture. A two-tailed Student's t-test was used to evaluate the significance of the difference between means of different studied groups.

3. Results and discussion

Experiment 1. The levels of organochlorine pesticides residues found in bovine ovaries are shown in Fig. 1. After analyzing the frequency of appearance and concentration of DDT and its metabolites, HCH isomers, HCB and PCB in the samples of ovaries, we confirmed the presence of p,p'-DDE and PCBs in 83 %, p,p'-DDT in 55 %, HCB in 38 % and p,p'-DDD in 33 % of examined samples. The results were expressed as means of concentrations in μg/kg of wet mass. These were: p,p'-DDT 10.73±2.7, p,p'-DDD 2.25±1,5, p,p'-DDE 3.83±0,8, PCB 2.69±2,1, HCB 1.39±0,7, and γ-HCH 0.14±0.1. In the present study the concentrations of DDE in ovaries were lower than DDT. This was unexpected because usually the concentrations of DDE are tenfold higher than DDT[5]. This may be explained by the fact that the studied material originated from cattle kept at farms situated near towns with a high human population and so the exposition of animals to the studied compounds is steadily high. Sitarska et al.[7] showed that accumulation of the studied compounds, in mammary gland and ovaries, even in relatively low concentrations may contribute to the appearance of pathological changes in the reproductive system in cattle.



Experiment 2. Tables 1 and 2 show the results from experimental protocol I, where granulosa cells were cultured with different concentrations of organochlorine compounds in the medium. We found a significant decrease in the secretions of E by the granulosa cells cultured in the presence of 10^{-1} ng/ml of organochlorine compound mixture. A marked decrease in the secretion of E at 48 hrs of culture in all tested groups was also observed.

TABLE1 Content of estradiol (pg/ml) in culture medium of granulosa cells containing different concentrations of organochlorine compound mixture (ng/ml)

Time	Content of estradiol in pg/ml				
(hours)	Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}
24	170,8±22,5	105,2±14,7	154,6±30,3	170,7±21,8	153,6±31,4
48	50,3±6,9	29,2±6,8**	50,7±10,5	58,2±13,4	74,3±27,5
72	62,3±26,2	21,7±6,5**	54,9±24,8	64,9±28,3	66,5±29,7
96	82,1±39,6	19,7±9,6**	42,6±17,3**	47,0±12,1*	41,9±15,9**

** = $p \leq 0.01$, * = $p \leq 0.05$

TABLE 2 Content of progesterone (ng/ml) in culture medium of granulosa cells containing different concentrations of organochlorine compound mixture (ng/ml)

Time	Content of progesterone in ng/ml				
(hours)	Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}
24	239,0±26,8	9,5±2,9**	192,7±67,3	219,5±37,8	225,3±46,8
48	364,6±74,3	19,2±4,3**	337,1±43,9	468,8±64,1	340,0±62,9
72	516,3±71,4	44,9±14,5**	282,7±98,0	307,0±74,9	258,1±50,3
96	366,7±43,0	34,2±9,2**	291,4±92,5	343,6±54,4	314,5±92,4

** = $p \leq 0.01$, * = $p \leq 0.05$

This may be associated with the natural process of luteinization in vitro of the granulosa cells. This was confirmed by the results of the secretion of P, which increased at the same time. A dramatic decrease in the secretion of P was observed in the experimental group containing 10^{-1} ng/ml of organochlorine compound mixture. This effect may be produced by an effective blockade of the luteinization process of granulosa cells. At lower concentrations of the tested mixture the decrease of secretion of P was not significant in comparison with controls. Both in control and experimental groups containing 10^{-1} to 10^{-4} ng/ml of organochlorine compounds, we observed a steady increase in the secretion of P. After continuous culture of granulosa cells, the final concentration of E in control and groups containing 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ng/ml of the tested mixture was: 898.9±122.0, 455.1±92.3, 509.5±91.9, 662.7±103.3, 977.3±132.4 pg/ml. For P the mean values were respectively: 305.1±18.0, 168.2±47.0, 218.6±32.5, 224.6±69.7, 282.7±57.7 ng/ml. The values for E were significantly lower than control in groups containing the mixture at concentrations 10^{-1} and 10^{-2} ($p \leq 0.05$). In the case of P, groups containing the mixture at concentration 10^{-1} ($p \leq 0.01$), 10^{-2} and 10^{-3} ($p \leq 0.05$) had significantly lower values after 96 hrs of continuous granulosa culture. These results indicate that in a prolonged culture with the mixture, lower concentrations of organochlorine compounds were more effective, lowering the secretion of P by granulosa cells. Cummings et al.[8] showed that exposure to methoxychlor during early pregnancy in mice reduced serum progesterone at all tested doses of the pesticide. Lindenau et al.[4], in experiments conducted in female rabbits, found

that serum P levels were not influenced by PCB and γ -HCH, however, DDT showed a tendency towards lower serum progesterone. The same compounds did not affect the serum E levels. The same authors have shown luteal cells to have great affinity and storage capacity for chlorinated hydrocarbons. Lipoproteins may play a role in this affinity. Our study suggests that granulosa cells may also possess affinity to the tested organochlorine compounds. The nature of this affinity remains to be investigated. The range of organochlorine compound concentrations used in the test was wide enough and accomodated the highest concentration detected in ovaries. We suggest that high concentrations of the studied compound mixture can lead to ovary functional disorders and subsequent reproductive failure. The data suggest that bovine granulosa cells from preovulatory follicles may be useful in screening toxic effect of pesticides in reproduction.

4. Conclusions

Six organochlorine compounds were detected in bovine ovaries. The highest concentration corresponds to p,p'-DDT and the lowest to the γ -HCH. The exposure of granulosa cells to a mixture of these organochlorine compounds in vitro results in a slight decrease of estradiol secretion only at the highest studied concentration of the mixture. However, the secretion of progesterone by these cells was seriously decreased, even by concentrations found in ovaries from animals kept under natural environmental conditions. In vitro system of granulosa cells from preovulatory follicles may be useful in screening toxic effect of pesticides in reproduction.

5. References

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