ENDOCRINE DISRUPTORS

Time-dependent action of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) and its metabolite DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) on human chorionic gonadotropin and progesterone secretion

ANNA K. WÓJTOWICZ¹, TOMASZ MILEWICZ², & EWA Ł. GREGORASZCZUK¹

¹Department of Physiology and Toxicology of Reproduction, Chair of Animal Physiology, Institute of Zoology, Jagiellonian University, Krakow, Poland, and ²Department of Gynecological Endocrinology, Jagiellonian University, Krakow, Poland

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Abstract
Explants of human placenta were used to study the effects of two isomers of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) [p,p'-DDT and o,p'-DDT] and their DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) metabolites [p,p'-DDE and o,p'-DDE] on the secretion of progesterone and human chorionic gonadotropin (hCG). Explants were treated with 1, 10, 100 or 1000 ng/ml of each compound for 24 h or 72 h. We found opposite effects (stimulatory after short-term and inhibitory after long-term exposure) of all compounds on progesterone secretion. However, both short- and long-term exposure to all investigated compounds caused decreased hCG secretion. In conclusion, we suggest the existence of a local axis between steroid hormones and hCG in placenta. DDT (which has estrogenic properties) increases progesterone secretion and consequently decreases hCG secretion. After long-term exposure, the low level of hCG is insufficient to stimulate progesterone.

Keywords: Human placenta, explants, DDT, DDE, progesterone, human chorionic gonadotropin

Introduction
The maintenance of pregnancy is dependent on many endocrinological events. Human placenta is responsible for the production of hormones, such as human chorionic gonadotropin (hCG) and progesterone, necessary for normal fetal development and pregnancy maintenance. Progesterone, a major steroid hormone produced by the ovarian corpus luteum and from the second trimester also by the placental syncytiotrophoblast, is considered essential for the successful maintenance of pregnancy. The protein hormone hCG maintains progesterone production in early pregnancy [1]. Disturbance to both progesterone and hCG secretion is one reason for abortion or preterm birth [1,2].

The organochlorine pesticide DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane), and its metabolite DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), still persistent in the environment, are known to influence the endocrine systems of animals and humans. Analysis of maternal adipose tissue, maternal blood serum, umbilical cord serum, mature milk and amniotic fluid indicates circulation of these compounds through all compartments of the maternal body [3–5]. DDT and DDE are able to cross the placenta, when they are accumulated [6,7]. There are also epidemiological studies reporting associations between the levels of these compounds in maternal blood and miscarriage rate, premature rupture of fetal membranes, preterm birth and fetal development [8–10].

We used placental explants as a physiological model to study the action of DDT and its metabolite DDE on hCG and progesterone secretion.

Materials and methods
Reagents
Dulbecco’s modified Eagle’s medium (DMEM), heat-inactivated fetal bovine serum (FBS) and...
dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DDT compounds (p,p'-DDT, o,o'-DDT, p,p'-DDE and o,o'-DDE) were reference standards purchased from EPA (Research Triangle Park, NC, USA). Stock solutions of these test compounds in DMSO were prepared and added to DMEM supplemented with 5% charcoal-stripped FBS. The final concentration of DMSO in the medium was always 0.1%.

Placental explant cultures

Placentas were collected in a gynecological hospital in Krakow, Poland, where clinical information on pregnancy outcomes was gathered. Collection of placentas and recording of clinical histories followed previously established protocols that had ethical approval by the local institutional review board. Patients gave their informed consent to the study. Clinical information recorded on each pregnancy included: smoking history, neonatal mortality and pregnancy outcome. Normal term placentas were obtained from non-smoking women undergoing elective Cesarean section with normal pregnancies at term (37–41 weeks of gestation). Placental cotyledons were harvested, placed immediately in ice-cold phosphate-buffered saline (PBS) and transported to the laboratory within 30 min of delivery.

Experimental procedure

The experiment was designed to study the dose- and time-dependent effect of the test compounds on progesterone and hCG secretion.

Placental tissues were rinsed three times with PBS containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). Decidual tissue and blood vessels were removed from villous placenta by blunt dissection and the tissue was finally minced into 2–3 mm pieces. Explants with a total wet weight of approximately 20–30 mg were cultured in 12-well plates (NUNC, Roskilde, Denmark) containing 1.5 ml of DMEM supplemented with 5% charcoal-stripped FBS and penicillin/streptomycin. The explants were incubated in triplicate for 24 h or 72 h at 37°C in a humidified atmosphere of 95% air and 5% CO₂ in the presence of p,p'-DDT, p,p'-DDE, o,o'-DDT or o,o'-DDE (reference standards; EPA) at concentrations of 1, 10, 100 or 1000 ng/ml. These concentrations cover the range of concentrations of DDT and DDE reported to be present in the serum of pregnant woman [11–13]. After 24 h or 72 h of culture, at the end of experiment, media were collected for hormone analysis and tissue was weighed so that the hormone values could be calculated per mg wet weight.

Hormone analysis

The concentration of progesterone and ß-hCG was determined in the media by enzyme immunoassay using kits (DiaMetra, Milan, Italy) according to the manufacturer’s instructions. All samples were run in duplicate in the same assay.

Inter- and intra-assay coefficients of variation for the hCG kit were 4.32% and 6.25%, respectively. For the progesterone kit, the inter- and intra-run coefficients of variation were 2.9% and 4.8%, respectively.

Statistical analyses

Data are presented as mean ± standard error of the mean of three independent experiments. Each treatment was repeated three times (n = 3) in quadruplicate, and thus the total number of replicates was 12. The average of the quadruplets was used for statistical calculation. Data were analyzed by one-way analysis of variance followed by the Tukey multiple comparison procedure.

Results

Secretion of progesterone

A significant increase in progesterone secretion during 24 h of exposure was noted after the addition of p,p'-DDE, o,p'-DDT and o,p'-DDE at concentrations of 100 and 1000 ng/ml (p < 0.01) (Figure 1A).

Exposure for 72 h to all concentrations of both isomers of DDT inhibited progesterone secretion in a dose-dependent manner (Figure 1B). Both isomers of DDE inhibited progesterone secretion at doses of 10, 100 and 1000 ng/ml (p < 0.01).

Secretion of human chorionic gonadotropin

All concentrations of p,p'-DDT, o,o'-DDT and p,p'-DDE caused a significant decrease in hCG secretion during 24 h of exposure (p < 0.01) (Figure 2A). In the case of treatment with o,p'-DDE, only the higher concentrations of this compound (100 and 1000 ng/ml) decreased hCG secretion (p < 0.01) (Figure 2A).

Exposure for 72 h to all concentrations of both isomers of DDT and their metabolite DDE decreased hCG secretion (Figure 2B).

Discussion

To our knowledge, the present data are the first to show a direct action of DDT and its metabolites on progesterone and hCG secretion by term placental explants. The results of this study showed that the action of DDT and DDE on progesterone
secretion was time-dependent while the influence on hCG secretion was independent of time of exposure.

Although there are no data concerning DDT action on progesterone secretion, there are studies showing the ability of estradiol to stimulate progesterone production by cultured human trophoblast cells [14]. Shanker and Rao [15] found that ex vivo addition of estradiol to first-trimester or term human placental explants caused a significant increase in the quantity of produced progesterone. Moreover, the addition of an aromatase inhibitor, CGS 16949A, or the estrogen receptor antagonist, ICI 182780, markedly inhibited progesterone production, thereby confirming the role of estradiol in the regulation of progesterone synthesis [15]. A similar synergistic mechanism could possibly operate between DDT and progesterone because DDT is reported to have both estrogenic properties and act as an estrogen receptor antagonist [16,17].

There are also some data concerning DDT action on progesterone secretion by ovarian cells. Nejaty and colleagues [18] found increased basal progesterone production in rat granulosa luteal cells after 48 h of treatment with DDT at the concentrations of $10^{-6}$ to $10^{-7}$ M. Crellin and associates [19] showed the stimulation of progesterone production by DDE in 8-bromo-cAMP-stimulated granulosa cells from pigs as well as in the stable porcine granulosa cell line JC-410. They found also that the changes in progesterone synthesis corresponded with the changes in the level of mRNA for cytochrome P450scc, the enzyme important for the first step of progesterone synthesis. Thus, stimulation of P450scc gene expression could also be the possible mechanism of action of DDT and its metabolite in placental

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**Figure 1.** Effect of increasing concentrations of two isomers of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) [$p,p'$-DDT and $o,o'$-DDT] and their DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) metabolites [$p,p'$-DDE and $o,o'$-DDE] on the secretion of progesterone in placental explants cultures after (A) 24 h and (B) 72 h of exposure. Each point represents the mean of four independent experiments, each of which consisted of three replicates per treatment group, with the standard error of the mean shown by vertical bars. *indicates statistically significant ($p < 0.05$) differences between control and experimental groups.

**Figure 2.** Effect of increasing concentrations of two isomers of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) [$p,p'$-DDT and $o,o'$-DDT] and their DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) metabolites [$p,p'$-DDE and $o,o'$-DDE] on the secretion of human chorionic gonadotropin (hCG) in placental explants cultures after (A) 24 h and (B) 72 h of exposure. Each point represents the mean of four independent experiments, each of which consisted of three replicates per treatment group, with the standard error of the mean shown by vertical bars. *indicates statistically significant ($p < 0.05$) differences between control and experimental groups.
cells. However, this conclusion should be confirmed by additional studies.

In the present study, we found that both DDT isomers and their metabolites caused the decrease in hCG secretion after exposure for both 24 h and 72 h. The inhibition of hCG secretion by DDT and DDE observed in our experiments could be explained by the data of Barnea [20] and Sharma [21] and co-workers, who showed the inhibition of hCG secretion by estrogens. A similar effect was observed by Jeschke and collaborators [22] with phytoestrogens (genistein and daidzein). Both phytoestrogens decreased hCG production by trophoblast cells isolated from term placentas. A dose-dependent decrease in the secretion of immunoreactive hCG as well as bioactive hCG induced by bromodichloromethane was also noted in cultured human term placent al trophoblast cells [23].

The second possible mechanism of hCG inhibition may involve the elevation of progesterone level observed after 24 h of exposure to DDT and DDE isomers. This is in accordance with many data showing that hCG secretion is markedly suppressed in the presence of progesterone [20,24–26].

In contrast to exposure for 24 h, we found the opposite effect of DDT and DDE isomers on progesterone secretion after 72 h of exposure. It is well known that hCG stimulates progesterone production both in the early phase of pregnancy, when the progesterone is produced by the corpus luteum, and in the late phase of gestation when this hormone is synthesized mainly by placenta [27]. We suggest that the decrease in progesterone secretion by placental explants after long-term exposure, observed in our study, could be due to low levels of hCG noted in cells exposed to DDT.

In conclusion, we suggest the existence of a local feedback between steroid hormones and hCG in placenta. DDT as exogenous estrogen stimulates progesterone secretion, which suppresses hCG secretion. The low hCG level is insufficient to stimulate progesterone secretion. This results in the decrease in progesterone and hCG secretion during long-term exposure to DDT.

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References


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