Toxicology, 74 (1992) 161–172
Elsevier Scientific Publishers Ireland Ltd.

Investigation of the role of estrogenic action and prostaglandin E_2 in DDT-stimulated rat uterine contractions ex vivo

Daland R. Juberg and Rita Loch-Caruso

The Toxicology Program, Department of Environmental and Industrial Health, University of Michigan, Ann Arbor, MI 48109-2029 (USA)

(Received December 26th, 1991; accepted May 8th, 1992)

Summary

Previous work in our laboratory showed that *o,p'*-DDT increases the frequency of rat uterine contractions in vitro. The present study investigated whether this response was related to prostaglandin E_2 (PGE_2) release from the uterine strips or to the estrogenicity of *o,p'*-DDT. Contraction frequency was evaluated by recording isometric spontaneous contractions in longitudinal uterine strips from pregnant rats. Assessment of PGE_2 levels in the muscle bath showed no significant differences between control and DDT-treated strips, although significant amounts of PGE_2 were detected in both groups and increased contraction frequency was observed in *o,p'*-DDT-treated strips. Furthermore, a role for direct estrogenic action in the mediation of *o,p'*-DDT-stimulated uterine contraction was not supported by the contractility data, because: (i) unlike *o,p'*-DDT, 17-β-estradiol had no stimulatory effect, but instead exerted a significant inhibitory effect on uterine contraction; (ii) the estrogen antagonist, tamoxifen, failed to block the stimulatory effect of *o,p'*-DDT; and (iii) *p,p'*-DDD, a non-estrogenic DDT analogue, significantly stimulated contraction frequency, similar to *o,p'*-DDT. These results suggest that the stimulatory effect of *o,p'*-DDT on contraction frequency is not dependent on PGE_2 release or direct estrogen receptor-related action.

Key words: DDT; Contractility; Myometrium; Estrogen; Prostaglandins

Introduction

Elevated maternal serum levels of DDT and other organochlorine insecticides have been observed in women [1–3] and California sea lions [4] in association with preterm birth and/or spontaneous abortion. In a study involving the effect of DDT on pregnancy, rabbits treated orally with DDT delivered prematurely in a majority of cases [5]. While a mechanistic basis for this association is not known, our laboratory has recently reported the first description of a direct stimulatory effect of *o,p'*-DDT on spontaneous contractions in pregnant rat uterine strips ex vivo [6].

Correspondence to: Rita Loch-Caruso, Toxicology Program, Department of Environmental and Industrial Health, M6242, SPH II, University of Michigan, 1420 Washington Heights, Ann Arbor, MI 48109-2029, USA.

0300-483X/92/$05.00

More recently we have shown that *o,p'*-DDT increases contraction frequency in a dose- and time-dependent manner [7]. These observations may be relevant to previous associations of preterm birth and organochlorine insecticide exposure, because independent clinical investigations have demonstrated that contraction frequency is significantly elevated in women who subsequently undergo preterm labor compared to women who deliver at term [8,9].

Prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ are potent stimulants of uterine activity in several species, including rats and humans and are likely to be involved in the regulation and onset of parturition. During the later stages of pregnancy in humans, the rise in estrogen levels may also play a role in parturition by influencing synthesis of myometrial contractile proteins and increasing uterine activity [10]. Because *o,p'*-DDT binds to the estrogen receptor [11,12], the present study examined if *o,p'*-DDT increases uterine contraction frequency through a direct estrogen receptor-mediated mechanism. We evaluated this hypothesis in contractility studies by: (i) assessing the effect of a physiologically active estrogen, 17-β-estradiol; (ii) determining the effect of *o,p'*-DDT in the presence of tamoxifen, an estrogen receptor antagonist; and (iii) investigating whether a non-estrogenic DDT isomer, *p,p'*-DDD, is capable of stimulating contraction frequency similar to *o,p'*-DDT. Additional experiments examined whether *o,p'*-DDT was acting indirectly by increasing the uterine release of PGE_2. We found no evidence that DDT-enhanced contraction frequency in explanted rat uterine strips is related to either direct estrogenic stimulation or increased tissue release of PGE_2.

Materials and methods

Chemicals

Tamoxifen citrate, prostaglandin E_2 and 17-β-estradiol were from Sigma Chemical (St. Louis, MO) and *p,p'*-DDD and *o,p'*-DDT were from Crescent Chemical (Hauppauge, NY). Tamoxifen citrate stock solution was 20 mM in methanol. Stock solutions of 40 mM 17-β-estradiol, 56 mM *o,p'*-DDT and 62 mM *p,p'*-DDD were prepared in absolute ethanol and stored at 4°C.

Tissue isolation

Mid-gestation (day 10 of pregnancy) Sprague–Dawley rats were anesthetized with ether and sacrificed by exsanguination in accordance with procedures approved by the University of Michigan Committee on the Use and Care of Animals. The uteri were removed and small strips of longitudinal muscle (3 mm × 10 mm) cut from the fetal side of one of the uterine horns were placed in physiological saline solution (PSS) (116 mM NaCl, 21.9 mM $NaHCO_3$, 11.1 mM dextrose, 4.6 mM KCl, 1.16 mM $MgSO_4(7H_2O)$, 1.16 mM $NaH_2PO_4(H_2O)$, 1.8 mM $CaCl_2(2H_2O)$, pH 7.4). For each experiment, strips were randomly designated as control or test using a systematic coin-toss, to reduce biased selection of strips which may possess intrinsically different contractility characteristics.

Isometric contraction recording

Uterine strips were mounted in 50-ml muscle baths (University of Michigan Glass Shop, Ann Arbor, MI) which contained PSS at 37°C (5% CO_2/95% O_2). There were

two strips per bath. One end of each strip was tied with silk thread to a fixed post and the other end to a force transducer (Grass FT-O3, Quincy, MA). Constant tension of 0.5 g was placed on each strip and all strips were depolarized with 60 mM KCl to measure maximal contractile force and to insure that all preparations were viable. Strips were then maintained at 37°C (5% CO_2/95% O_2) until spontaneous, regular contractions developed (3–4 h). PSS was changed every 30 min during this acclimation period. Following development of phasic activity, contractions were measured in all strips for the same period of time (0.5–1.0 h), designated as the baseline period. A treatment period of 3 h, followed by a 3-h post-treatment period was used in all experiments except those with 17-β-estradiol, in which both periods were shortened due to treatment effect. Contraction frequency was assessed by enumerating spontaneous contractions which exceeded 50% maximal force, for each experimental period (baseline, treatment, post-treatment). Following experimentation, strips were again depolarized with KCl as a check of both tissue viability and maximal contractile force; in no case was degradation observed. Six animals were used for each experiment, except for the studies with 17-β-estradiol in which five animals were employed.

PGE_2 contraction frequency experiment

The testing protocol for these experiments incorporated the same time periods as previously described (1-h baseline, 3-h treatment, 3-h post-treatment). The PSS volume in the muscle baths was reduced to 25 ml to insure that measurable PGE_2 concentrations were available. After equilibration of 3–4 h, the baths were rinsed twice. Following the 1-h baseline period, 15 ml of the PSS from each bath was collected and frozen at -20°C for later analysis. The baths were changed to fresh PSS (25 ml) after each sampling. Test strips were treated with 100 μM *o,p'*-DDT for 3 h, followed by a 3-h post-treatment period. Solvent controls were treated with equivalent levels of the solvent ethanol (0.17% v/v). After each of these periods, 15 ml of PSS was sampled from each bath for analysis. All strips were blotted dry and weighed after experimentation.

PGE_2 analysis

For PGE_2 analysis, an ACE enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI) was used which utilized acetylcholinesterase-linked PGE_2 as tracer, a rabbit anti-PGE_2 antibody and a mouse anti-rabbit, monoclonal second antibody. From the samples obtained during experimentation, a 50-μl aliquot of each was placed in a 96-well microtiter tray which was coated with mouse anti-rabbit monoclonal second antibody. A volume of 50 μl of tracer and 50 μl of the rabbit anti-PGE_2 antibody were added to each sample and these were incubated overnight at room temperature. All wells were washed with 0.05 M phosphate buffer and Tween 20 at pH 7.4. Ellman's reagent (200 μl) was added to each well and the samples were incubated in the dark on a shaker for 4–5 h at room temperature. This incubation allows the bound enzyme tracer to react with Ellman's reagent to yield a compound which may be measured photometrically (based on optical density) using a microplate reader at 405 nm. A standard curve was developed using standards ranging from 2.6–250.0 pg PGE_2/ml sample. All samples were run in duplicate. The assay detected quantifiable levels of PGE_2 in all samples. The PGE_2 values are expressed as ng PGE_2/g tissue/h.

Estrogen experiments: 17-β-estradiol

Because of the inhibitory nature of estradiol on uterine contractility, the treatment and post-treatment periods were shortened considerably. After a 1-h baseline period, test strips were treated with 20 μM 17-β-estradiol for 30 min, while controls were exposed to equivalent concentrations of the solvent, 0.05% (v/v) ethanol. The baths were rinsed twice with PSS and a post-treatment period of 30 min ensued. A 20-μM concentration of 17-β-estradiol represented the optimal concentration based on an initial dose-response characterization of the compound (data not shown).

Tamoxifen

All strips were treated with 1 μM tamoxifen citrate beginning 1 h after placement in the muscle bath and continuing throughout the experiment. This concentration was selected based on tamoxifen's competitive inhibition of the estrogen receptor and its associated dissociation constant [13–15]. In order to ensure that sufficient tamoxifen was present in the muscle bath, we chose a concentration that was 100-fold higher than the predicted dissociation constant [13–15]. Following the equilibration and baseline periods (3–5 h), test strips were treated with 100 μM *o,p'*-DDT while controls received equivalent amounts of the solvent, ethanol (0.17% v/v). We chose a concentration of 100 μM *o,p'*-DDT because this concentration was most effective in stimulating contraction frequency [7] and studies have shown that *o,p'*-DDT competitively inhibits estradiol binding in the 10^{-4} to 10^{-6} M range [16,17]. The baths were rinsed after 3 h and a post-treatment period of 3 h followed, during which only tamoxifen (1 μM) was present. The methanol concentration (tamoxifen vehicle) in the baths was negligible (0.005%, v/v).

p,p'-DDD

After a baseline period of 1 h, test strips were treated with 100 μM *p,p'*-DDD while controls received the equivalent concentration of solvent, 0.16% v/v ethanol. Because this experiment was designed to compare the effectiveness of *p,p'*-DDD and *o,p'*-DDT in stimulating contraction frequency, 100 μM *p,p'*-DDD was the selected concentration. After a 3-h treatment period, the baths were rinsed and a 3-h post-treatment period followed.

Dose-response to PGE_2 and KCl

In order to demonstrate that our uterine preparations were responsive to additional agents besides DDT, we conducted separate cumulative dose-response experiments with PGE_2 and depolarizing concentrations of potassium chloride (KCl). Following establishment of stable contractile activity, uterine strips were treated with increasing concentrations of PGE_2 or KCl for 15 min per dose.

Statistical analyses

For 17-β-estradiol, tamoxifen and *p,p'*-DDD experiments, statistical treatment of the data included two-way analysis of variance (ANOVA) with repeated measures to assess for time and treatment effects between control and test strips. Posthoc comparison of contraction frequency means for each time period utilized the Student-Newman-Keuls method [18]. Differences in PGE_2 production for each time period

were analyzed using both Wilcoxon test for correlated samples and Mann-Whitney *U* for population differences [19]. Contraction frequency data associated with prostaglandin experiments were analyzed using Student-Newman-Keuls for comparison of means. Contraction frequencies for all experiments are represented as mean per min ± SEM. An alpha level of 0.05 was assumed in all analyses.

Results

Similar to previous findings [7], there were no significant changes in contraction frequency among control uterine strips, while test strips exposed to *o,p'*-DDT showed significantly greater contraction frequency during the treatment and post-treatment periods, compared to baseline (Fig. 1a; $P < 0.05$). Unexpectedly, the baseline value for control strips was significantly greater than for test strips (Fig. 1a; $P < 0.05$), which may explain why significant differences between test strips and matched controls were not noted for the treatment or post-treatment periods. To assess whether prostaglandin production and/or release contributed to increased contraction frequency in *o,p'*-DDT treated strips, we sampled and quantified PGE_2 levels during the three time periods of the experiment shown in Fig. 1a. There were no significant differences in PGE_2 concentrations released to the baths between control and test strips during any time period (Fig. 1b), although PGE_2 levels for both control and test strips were substantially higher during the post-treatment period than either baseline or treatment levels.

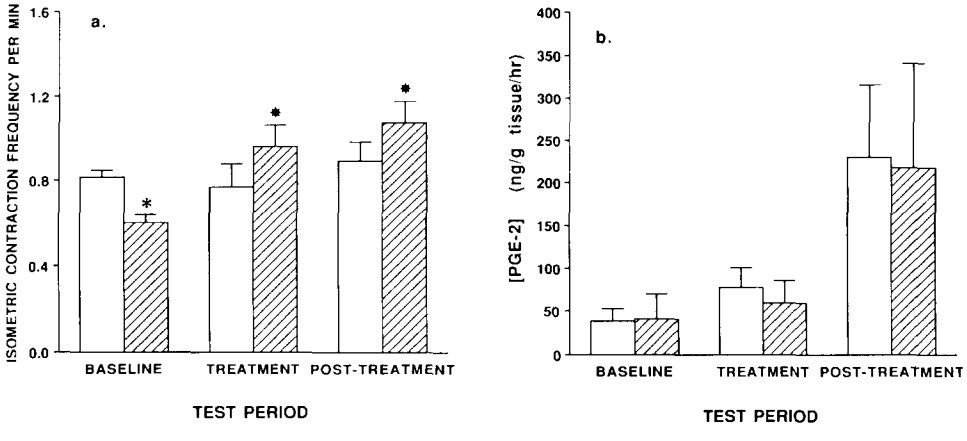

Fig. 1. a, Contraction frequency in 100 μM *o,p'*-DDT-treated rat uterine strips (hatched bars) from which prostaglandin E_2 (PGE-2) samples were analyzed. Each bar represents the mean contraction frequency per min ± SE of six replicate experiments. Asterisks (*) denote values significantly different from matched controls (open bars), while stars denote values significantly greater than the baseline value in *o,p'*-DDT-treated strips ($P < 0.05$). b, Prostaglandin E_2 release from rat uterine strips, treated in vitro with 100 μM *o,p'*-DDT (hatched bars) compared to matched controls (open bars). Each bar represents the mean concentration ± SE of PGE_2 in the bathing medium (PSS) during each experimental period, expressed as ng PGE_2/g tissue/h. Each value is the mean of six replicate experiments.

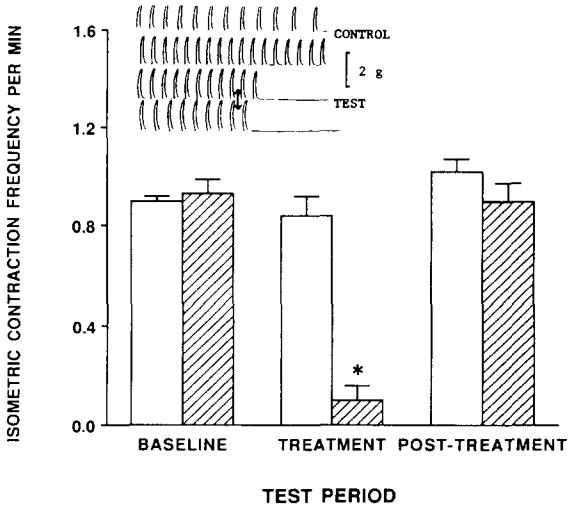

Fig. 2. Contraction frequency in rat uterine strips treated with 20 μM 17-β-estradiol (hatched bars). Each bar represents the mean contraction frequency per min ± SE of five replicate experiments. Asterisks (*) denote values significantly different from matched controls (open bars) at $P < 0.05$. Inset shows the acute inhibitory effect of 17-β-estradiol in the bottom two tracings and the arrow denotes the addition of 17-β-estradiol; time shown is 20 min.

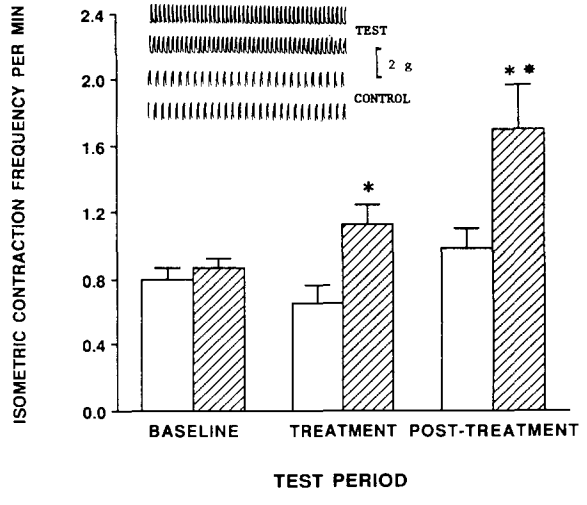

Fig. 3. Contraction frequency in rat uterine strips treated with 1 μM tamoxifen (open bars) or 1 μM tamoxifen + 100 μM *o,p'*-DDT (hatched bars). Each bar represents the mean contraction frequency per min ± SE of six replicate experiments. Asterisks (*) denote values significantly greater than matched controls, while the star denotes that the post-treatment value was significantly greater than either treatment or baseline in tamoxifen + *o,p'*-DDT-treated strips ($P < 0.05$). Inset shows representative tracings of contractions in strips treated with tamoxifen + *o,p'*-DDT (top two tracings) or tamoxifen alone (bottom two tracings); time shown is 20 min.

To investigate the role of estrogenic action, initial experiments evaluated the acute effects of a physiologically relevant estrogen, 17-β-estradiol (20 μM), on uterine contraction frequency. This treatment produced a rapid (within 1 min) and sustained cessation of contractility, until the baths were rinsed free of 17-β-estradiol (inset, Fig. 2). This inhibitory effect was significant in comparison to matched controls (Fig. 2; $P < 0.05$). Following removal of 17-β-estradiol from the muscle baths, spontaneous contractions resumed rapidly in all test strips (see post-treatment, Fig. 2). We observed a similar inhibitory effect at 30 μM, but no acute effect on contraction frequency at 10 μM 17-β-estradiol (data not shown).

In subsequent experiments, uterine strips were treated with the anti-estrogen, tamoxifen, followed by *o,p'*-DDT, to test the hypothesis that *o,p'*-DDT-induced increases in contraction frequency are mediated by the estrogen receptor. Addition of 1 μM tamoxifen failed to block or reduce the stimulatory effect of *o,p'*-DDT, as shown in Fig. 3. There were no remarkable differences in the degree of contraction frequency stimulation between those strips treated with *o,p'*-DDT/tamoxifen in this study and those treated with *o,p'*-DDT alone (Fig. 1a and as reported in our previous characterization [7]). For both treatment and post-treatment periods, strips exposed to 100 μM *o,p'*-DDT had significantly greater contraction frequencies than matched controls exposed to tamoxifen alone (Fig. 3; $P < 0.05$). In addition, the post-treatment value for *o,p'*-DDT-treated strips was significantly greater than either treatment or baseline values (Fig. 3; $P < 0.05$).

To further examine whether *o,p'*-DDT is acting through an estrogenic mechanism, we conducted contractility studies using *p,p'*-DDD, whose binding affinity for the estrogen receptor is negligible compared to *o,p'*-DDT and which has no

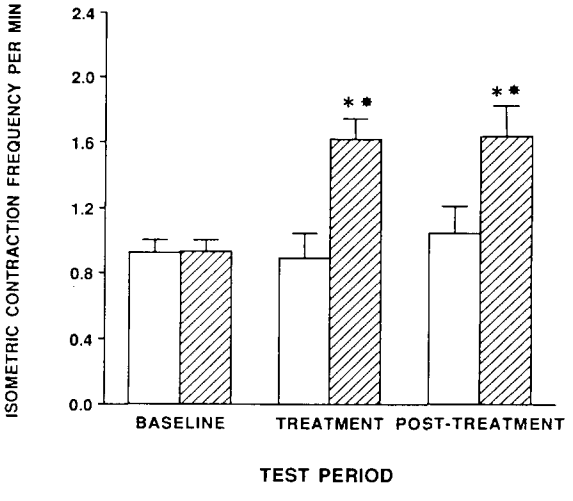

Fig. 4. Contraction frequency in rat uterine strips treated with 100 μM *p,p'*-DDD (hatched bars). Each bar represents the mean contraction frequency per min ± SE of six replicate experiments. Asterisks (*) denote values significantly different from matched controls (open bars), while stars denote values significantly greater than the baseline value in *p,p'*-DDD-treated strips ($P < 0.05$).

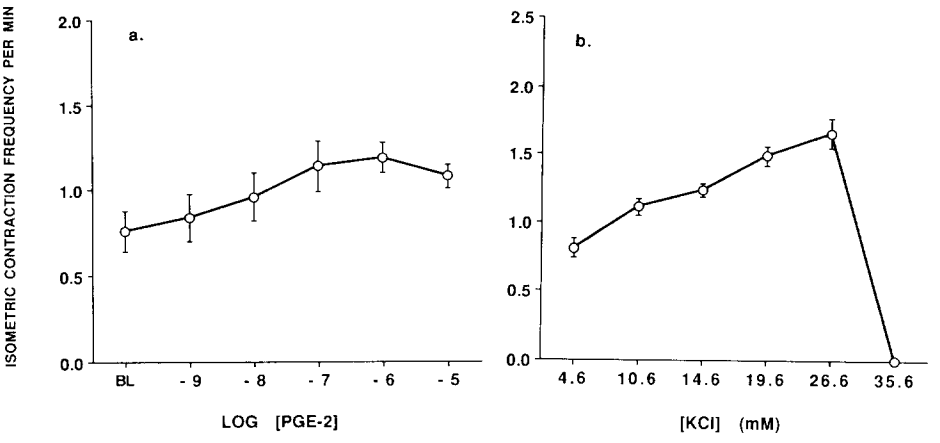

Fig. 5. a, Dose-response curve to prostaglandin E_2 (PGE_2) in longitudinal uterine strips from pregnant rats. Each point is the mean contraction frequency per min ± SE of eight uterine strips. Values for each point were averaged over a 15-min period. The value associated with the first point (BL) represents baseline contraction frequency prior to addition of PGE_2. b, Dose-response curve to potassium chloride (KCl) in longitudinal uterine strips from pregnant rats. Each point is the mean contraction frequency per min ± SE of eight uterine strips. Values for each point were averaged over a 15-min period. The first point associated with 4.6 mM KCl represents the normal physiological KCl concentration in the bathing medium. The highest concentration (35.6 mM KCl) induced a sustained, tonic contracture and thus no phasic contractile activity was associated with this dose.

known effect in eliciting estrogenic responses such as increased uterine wet weight [11]. Treatment of uterine strips with *p,p'*-DDD significantly increased contraction frequency compared to both matched controls and to the baseline value associated with *p,p'*-DDD-treated strips, during both treatment and post-treatment periods (Fig. 4; $P < 0.05$). This response was similar to that seen in our former study with *o,p'*-DDT [7] and to the *o,p'*-DDT response observed in this study (Fig. 1a), except that the stimulatory effect of *p,p'*-DDD was more rapid in its onset than *o,p'*-DDT.

Additional experiments demonstrated that both PGE_2 and KCl increased spontaneous contraction frequency in a dose-dependent manner as shown in Fig. 5a,b. The maximal stimulatory effect on contraction frequency was observed at 10^{-6} M for PGE_2 and 26.6 mM KCl. The maximal stimulatory effects of PGE_2 on contraction frequency were less than the increases normally observed in *o,p'*-DDT or *p'p'*-DDD-treated strips (Fig. 1a and Fig. 4). Additionally, we observed a fused, tonic contracture in rat uterine strips treated with doses of KCl exceeding 26.6 mM KCl, such that enumeration of individual contractions was not possible (Fig. 5b).

Discussion

Both prostaglandins and estrogens are important stimulatory modulators of myometrial activity that may be fundamental to the regulation of parturition [10]. In previous studies, we showed that *o,p'*-DDT exerts a strong stimulatory effect on

the frequency of contractions in explanted rat uterine strips [6,7]. Because PGE_2 is known to stimulate uterine contractions in vitro [20,21] and is produced by uterine tissue [22], we examined whether *o,p'*-DDT may be acting indirectly by increasing release of PGE_2. Additionally, because *o,p'*-DDT competes with estradiol for the estrogen receptor [12] and exhibits estrogenic activity related to this binding [11], the present study was also designed to determine if the *o,p'*-DDT-effect on contraction frequency is mediated through a direct estrogenic mechanism.

Vane and Williams [20] and Wainman et al. [21] have shown that various prostanoids, including PGE_2, stimulate uterine contractility in isolated rat uterus. Furthermore, increased frequency of spontaneous contractions of rat uterus in vitro has been associated with increased prostaglandin release from uterine tissue [22–24], with two studies reporting a specific correlation with PGE release [23,24]. In our study, we found that PGE_2 levels were not significantly altered by *o,p'*-DDT treatment compared to controls, independent of increased frequency of contraction in the *o,p'*-DDT-treated uterine strips during treatment and post-treatment periods. We did observe a pronounced increase in PGE_2 levels during the post-treatment period in both control and *o,p'*-DDT-treated strips; however this response was clearly unrelated to *o,p'*-DDT exposure because PGE_2 levels increased irrespective of treatment. Elevated levels of PGE_2 during the post-treatment period may be related to the long incubation time in the muscle bath. Other investigators have reported a similar trend of increased prostaglandin release with time during prolonged incubation in a muscle bath setting [20,25] and more specifically, Gimeno et al. [23] showed that rat uterine horns released significantly more PGE-like material after 6 h than after 1 h; these reports are in agreement with the results from our study. While it has been suggested that gradual tissue degradation or a mechanical stimulus such as stretching may contribute to increased prostaglandin release [26], we observed no changes in functional activity of the uterine strips. Because we observed no difference in PGE_2 release between control and *o,p'*-DDT-treated strips during any time period, despite the increased contraction frequency among *o,p'*-DDT-treated strips, it seems unlikely that *o,p'*-DDT is exerting its stimulatory influence on contraction frequency via this mechanism.

Estrogenic responses in the rat uterus have been classified as involving genomic, non-genomic and other independent hormonal mechanisms [27]. While *o,p'*-DDT may be involved in uterine responses that are independent of estrogen receptor binding and activation, most investigations have demonstrated that only those DDT isomers that interact with the uterine estrogen receptor are capable of eliciting estrogenic responses [11,28]. Moreover, Robison and Stancel [29] and Robison et al. [30] have shown that biological responses to *o,p'*-DDT are positively correlated with nuclear estrogen receptor translocation, which strongly suggests that *o,p'*-DDT is eliciting estrogenic responses through a genomic mechanism. Galand et al. [31] have concluded that *o,p'*-DDT, in accordance with the action of estradiol, is a purely estrogenic agonist in the rat uterus both in vivo and in vitro as a result of its interaction with the estrogen receptor.

Estrogens are typically regarded as stimulatory agents of uterine contraction in vivo, most likely resulting from a genomic response [10]. However, the acute effects we observed with 17-β-estradiol on uterine motility were inhibitory, suggesting that,

in vitro, this estrogen may exert its inhibitory effects on contractility through a non-genomic mechanism [32,33]. Furthermore, the rapid onset of inhibition observed in our studies (less than 1 min), coupled with rapid reversal, do not support an estrogen receptor-mediated mechanism. This effect of 17-β-estradiol is compatible with other studies demonstrating an inhibitory effect on uterine motility [32,34,35]. It has been suggested that such an inhibitory effect may be attributed to altered cytosolic calcium [32], decreased prostaglandin production [34], or histamine release [35]. Because 17-β-estradiol's inhibitory effect was inconsistent with *o,p'*-DDT's stimulatory effect, these results do not support an estrogenic mechanism for *o,p'*-DDT's stimulation of uterine contraction frequency.

The role of estrogen receptor activity was further examined using a competitive inhibitor of the estrogen receptor, tamoxifen [36]. In these experiments, tamoxifen had no effect when administered alone, nor did it block *o,p'*-DDT stimulation of contraction frequency. Although it is possible that the selected concentration of tamoxifen may have failed to effectively inhibit the estrogen receptor, this is unlikely given the predicted dissociation constant for tamoxifen [13–15]. These data suggest that *o,p'*-DDT stimulation of uterine contractility is not related to estrogen receptor activation. Perhaps the strongest support that the observed increase in contraction frequency in DDT-treated rat uterine strips is not related to an estrogen receptor-mediated mechanism is provided by the studies with *p,p'*-DDD, which binds very poorly to the estrogen receptor yet exerts a stimulatory effect similar to *o,p'*-DDT. Welch et al. [11] have shown that estrogenic compounds such as *o,p'*-DDT, *p,p'*-DDT and technical-grade DDT, significantly reduce the uptake of 17-β-estradiol in rat uterus, while *p,p'*-DDD is without effect, suggesting that certain estrogenic DDT isomers actively compete with 17-β-estradiol for binding sites within the uterus. More relevant to our study is the observation that *p,p'*-DDD, while exhibiting little, if any, estrogen receptor-binding ability, significantly stimulated contraction frequency in rat uterine strips. This observation, coupled with the rapid time of onset of the stimulatory effect (10–20 min), suggests that *p,p'*-DDD is not acting through an estrogen receptor-mediated mechanism. Finally, it was interesting to note that the effect of *p,p'*-DDD was more rapid in onset and stabilized faster than that of *o,p'*-DDT, which suggests that structure/activity and lipid solubility may be relevant with respect to increased contraction frequency.

Since we did not observe a correlation between *o,p'*-DDT stimulation of uterine contraction and PGE_2 release, it was of interest to determine whether the uterine strips in our study were responsive to exogenous PGE_2. The dose-response experiments demonstrated that PGE_2 stimulated contraction frequency in our rat uterine preparations. The fact that we observed a maximal effect at 10^{-6} M PGE_2 suggests that the stimulatory influence on uterine contraction frequency is saturable. Interestingly, increases in contraction frequency due to PGE_2 stimulation were not as great as those due to *o,p'*-DDT or *p,p'*-DDD stimulation. The dose-response to KCl demonstrated that a non-uterotropic, membrane depolarizing agent was also capable of increasing contraction frequency.

Collectively, these studies demonstrate that the stimulatory effect of *o,p'*-DDT on contraction frequency in explanted rat uterine strips does not appear to result from direct estrogen receptor-mediated action or from *o,p'*-DDT-induced PGE_2 release

in vitro. On-going studies continue to investigate other possible mechanisms related to DDT-stimulation of uterine contraction. Because DDT has pronounced effects on ion channels in nerve tissue related to its insecticidal mode of action, recent studies in our laboratory have focused on the plasma membrane as an initial site of perturbation. These studies indicate that *p,p'*-DDD exerts effects on membrane-bound sodium and calcium channels, as well as on membrane depolarization in rat myometrial smooth muscle cells, actions that are consistent with increases in uterine contraction frequency (unpublished data). Since contraction frequency is an important parameter relative to onset of labor and parturition [8,9], we plan to continue the study of rat uterine strips as a model to investigate direct effects of environmental agents on uterine function.

Acknowledgments

We thank Dr. Craig Harris for uterine tissue and Dr. R. Clinton Webb for laboratory use and helpful discussions. This research was conducted as partial fulfillment of DRJ's doctoral dissertation and was supported, in part, with doctoral training grants to DRJ (NIH HD07048 and NIH GM07767) and a research grant to RLC (NIH ES04424). Additional support was provided by the Laboratory Animal and Data Analysis Cores of the P30 Center for the Study of Reproduction (NIH HD18258).

References

1 M.C. Saxena, M.K.J. Siddiqui, A.K. Bhargava, T.D. Seth, C.R. Krishnamurti and D. Kutty, Role of chlorinated hydrocarbon pesticides in abortions and premature labor. Toxicology, 17 (1980) 323.
2 M.C. Saxena, M.K.J. Siddiqui, T.D. Seth, C.R. Krishnamurti, A.K. Bhargava and D. Kutty, Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature, or full-term delivery. J. Anal. Toxicol., 5 (1981) 6.
3 M. Wassermann, M. Ron, R. Bercovici, D. Wassermann, S. Cucos and A. Pines, Premature delivery and organochlorine compounds: polychlorinated biphenyls and some organochlorine insecticides. Environ. Res., 28 (1982) 106.
4 R.L. DeLong, W.G. Gilmartin and J.G. Simpson, Premature births in California sea lions: associations with high organochlorine pollutant residue levels. Science, 181 (1973) 1168.
5 M.M. Hart, R.H. Adamson and S. Fabro, Prematurity and intrauterine growth retardation induced by DDT in the rabbit. Arch. Int. Pharmacodyn., 192 (1971) 286.
6 D.R. Juberg and R. Loch-Caruso, Increased contraction frequency in rat uterine strips treated in vitro with *o,p'*-DDT. Bull. Environ. Contam. Toxicol., 46 (1991) 751.
7 D.R. Juberg, R.C. Webb and R. Loch-Caruso, Characterization of *o,p'*-DDT stimulated contraction frequency in rat uterus in vitro. Fundam. Appl. Toxicol., 17 (1991) 543.
8 M. Katz, R.B. Newman and P.J. Gill, Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery. Am. J. Obstet. Gynecol., 154 (1986) 44.
9 R. Bell, The prediction of preterm labor by recording spontaneous antenatal uterine activity. Br. J. Obstet. Gynecol., 90 (1983) 884.
10 G. Huszar and M.P. Walsh, Biochemistry of the myometrium and cervix, in R.M. Wynn and W.P. Jolle (Eds.), Biology of the Uterus, 2nd Edn., Plenum Publishing Corp., New York, 1989, p. 355.
11 R.M. Welch, W. Levin and A.H. Conney, Estrogenic action of DDT and its analogues. Toxicol. Appl. Pharmacol., 14 (1969) 358.
12 J.A. Nelson, Effects of dichlorodephenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (PCB) mixtures on 17-β-[^{3}H]estradiol binding to rat uterine receptor. Biochem. Pharmacol., 23 (1974) 447.

13 V.C. Jordan and G. Prestwich, Binding of [^{3}H]tamoxifen in rat uterine cytosols: a comparison of swinging bucket and vertical tube rotor sucrose density gradient analysis. Mol. Cell. Endocrinol., 8 (1977) 179.
14 J.C. Faye, B. Lasserre and F. Bayard, Antiestrogen specific, high affinity saturable binding sites in rat uterine cytosol. Biochem. Biophys. Res. Comm., 93 (1980) 1225.
15 L.C. Murphy and R.L. Sutherland, A high-affinity binding site for the antiestrogens, tamoxifen and CI 628, in immature rat uterine cytosol which is distinct from the estrogen receptor. J. Endocrinol., 91 (1981) 155.
16 J.A. Nelson, Effects of dichlorodiphenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (PCB) mixtures on 17β-[^{3}H]estradiol binding to rat uterine receptor. Biochem. Pharmacol., 23 (1974) 447.
17 M.S. Forster, E.L. Wilder and W.L. Heinrichs, Estrogenic behavior of 2(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane and its homologues. Biochem. Pharmacol., 24 (1975) 1777.
18 B.J. Winer, Statistical Principles in Experimental Design, McGraw-Hill, New York, 1971.
19 R.B. McCall, Fundamental Statistics for Psychology, Harcourt, Brace and World, Inc., New York, 1970, p. 293.
20 J.R. Vane and K.I. Williams, The contribution of prostaglandin production to contractions of the isolated uterus of the rat. Br. J. Pharmacol., 48 (1973) 629.
21 B.C. Wainman, I. Burcea and D.J. Crankshaw, The effects of prostanoids on estrogen-dominated rat myometrial longitudinal muscle in vitro. Biol. Reprod., 39 (1988) 221.
22 N.H. Dubin, R.B. Ghodgaonkar and T.M. King, Role of prostaglandin production in spontaneous and oxytocin-induced uterine contractile activity in in vitro pregnant rat uteri. Endocrinology, 105 (1979) 47.
23 M.F. Gimeno, N. Sterin-Speziale, A. Landa, A. Bonacossa and A.L. Gimeno, Is the spontaneous motility of isolated rat uterus controlled by prostaglandin E? Prostaglandins, 17 (1979) 673.
24 A.M. Franchi, M. Chaud, E.S. Borda, M.F. Gimeno, M.A. Lazzari and A.L. Gimeno, Spontaneous motility and prostaglandin generation in rat uterine horns isolated during the estrous cycle. Prostaglandins, 22 (1981) 637.
25 S.R. de Barioglio and J.L. Lacuara, Release of prostaglandin E and F from rat uterine strips incubated in depolarizing and hyperpolarizing solutions. Prostaglandins Leukot. Med., 20 (1985) 129.
26 P.J. Piper and J.R. Vane, The release of prostaglandins from lung and other tissues. Ann. N.Y. Acad. Sci., 180 (1971) 363.
27 A.N. Tchernitchin, Eosinophil-mediated non-genomic parameters of estrogen stimulation: a separate group of responses mediated by an independent mechanism. J. Steroid Biochem., 19 (1983) 95.
28 D. Kupfer and W.H. Bulger, Estrogenic properties of DDT and its analogs, in J.A. McLachlan (Ed.), Estrogens in the Environment, Elsevier, New York, 1980, p. 239.
29 A.K. Robison and G.M. Stancel, The estrogenic activity of DDT: correlation of estrogenic effect with nuclear level of estrogen receptor. Life Sci., 31 (1982) 2479.
30 A.K. Robison, V.R. Mukku, D.M. Spalding and G.M. Stancel, The estrogenic activity of DDT: the in vitro induction of an estrogen inducible protein by *o,p'*-DDT. Toxicol. Appl. Pharmacol., 76 (1984) 537.
31 P. Galand, N. Mairesse, C. Degraef and J. Rooryck, *o,p'*-DDT (1,1,1-trichloro-2 (*p*-chlorophenyl) 2-(*o*-chlorophenyl) ethane is a purely estrogenic agonist in the rat uterus in vivo and in vitro. Biochem. Pharmacol., 36 (1987) 397.
32 T. Osa and T. Ogasawara, Effects in vitro of progesterone and estradiol-17β on the contractile and electrical responses in rat myometrium. Jpn. J. Physiol., 34 (1984) 427.
33 K. Belghmi, E. Badia, J.-C. Nicolas and A.C. de Paulet, Effects of estradiol and indomethacin on the contractile activity of the isolated rat uterus. Prostaglandins Leukot. Med., 24 (1986) 111.
34 N. Sterin-Speziale, M.F. Gimeno, A. Bonacossa and A.L. Gimeno, The effect of estradiol on isolated rat uterine motility and on prostaglandin generation. Prostaglandins, 20 (1980) 233.
35 M. Viggiano, A. Faletti, M.A.F. Gimeno and A.L. Gimeno, Influence of estrogens on in vivo and in vitro rat uterine motility. Relationships with histamine, cimetidine and tissue levels of 3',5' cyclic adenosine monophosphate. Methods Find. Exp. Clin. Pharmacol., 10 (1988) 247.
36 R.C. Winneker and J.H. Clark, Estrogenic stimulation of the antiestrogen specific binding site in rat uterus and liver. Endocrinology, 112 (1983) 1910.