Expression of AhR and ARNT mRNA in Cultured Human Endometrial Explants Exposed to TCDD

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Received January 9, 2001; accepted May 15, 2001

**Endometriosis is a debilitating disease found in 10–15% of reproductive-age women and is characterized by the presence of endometrial tissue outside of the uterus. The present study characterizes the expression of AhR and ARNT mRNA in a human endometrial explant culture model in the absence and presence of TCDD exposure. In a parallel, companion study using this model, TCDD exposure was shown to induce CYP1A1 mRNA, CYP1B1 mRNA, EROD (7-ethoxyresorufin-O-deethylase) activity, and CYP1B1 protein in human endometrial explants. Explants were prepared from specimens obtained at laparoscopy or laparotomy from women undergoing surgery for tubal ligation, endometriosis, or pelvic pain unrelated to endometriosis. These specimens were a subset of the specimens used in the parallel study. The explants were cultured in medium containing 10 nM estradiol (E2) or 1 nM estradiol plus 500 nM progesterone (E2 + P4) with or without TCDD (first 24 h). After culture, AhR and ARNT mRNA expression were quantified by RT-PCR. TCDD treatment significantly increased the expression of AhR mRNA, but not ARNT mRNA. The expression of both genes was similar for all individual explants and the ratio of AhR:ARNT mRNA expression across all samples was 1.7 to 1.8. Constitutive AhR mRNA expression was donor age dependent (increasing with age), while ARNT mRNA expression was donor age and tissue phase dependent (increased in older and proliferative phase specimens). Similar to results in the parallel study on expression of CYP1A1 mRNA, CYP1B1 mRNA, EROD activity, and CYP1B1 protein, the presence of endometriosis did not affect the expression of AhR or ARNT mRNA, either constitutively or following TCDD exposure. However, the detection of disease-specific change was limited by small sample size and variability in tissue cycle phase. The human endometrial explant culture model will be useful for future studies of the effects of dioxin-like compounds on human endometrium in relationship to cycle phase, hormonal exposure, and donor age.**

**Key Words: endometrium; endometriosis; TCDD; AhR; ARNT.**

Endometriosis is found in 10–15% of reproductive-age women and produces deleterious consequences to the individual’s quality of life, health, and reproductive potential (Goldman and Cramer, 1990; Haney, 1990). While endometriosis may not be considered a malignant condition, it is expressed as an outgrowth of endometrial tissue usually limited to the peritoneal cavity (Jenkins et al., 1986; Luciano and Pitkin, 1984), but also found at other sites such as the lung and skin (Foster et al., 1981; Steck and Helwig, 1966). Retrograde menstruation, which has been observed in 90% of women undergoing laparoscopy during menstruation (Halme et al., 1984), is believed to account for most cases of endometriosis (D’Hooghe et al., 1994; Haney, 1990).

Concurrent factors are also believed to play a role in the development of endometriosis in susceptible populations. First, multiple abnormalities of the immune system have been associated with endometriosis. These include complement deposition in the uterine endometrium (Weed and Argueboum, 1980), humoral immune dysfunction and autoimmunity (Dmowski, 1995; Gleicher et al., 1987), decreased cell-mediated immune function (Oosterlynck et al., 1991; Steele et al., 1984), and diminished proliferative T-cell response to endometrial antigens (Gilmore et al., 1992). Second, hormonal factors participate in the development of endometriosis. This is supported by the regression of endometriosis in postmenopausal women (Cramer et al., 1986; Dizerenga et al., 1980). It is also supported by the regression of endometriosis in patients on drugs that block estrogen production (Kiesel and Rennebaum, 1990) or on birth control medications high in progesterone (Sensky and Liu, 1980) that down-regulate estrogen receptors (Luciano et al., 1988). Lastly, exposure to environ-
mental factors such as radiation (Wood et al., 1983; Yochmowitz et al., 1985), polychlorinated biphenyls (reviewed by Gerhard and Runnebaum, 1992), and TCDD (Rier et al., 1993, 1995) has been associated with endometriosis.

A link between dioxin exposure and endometriosis was first demonstrated in rhesus monkeys. In a colony of rhesus monkeys, Rier et al. (1993) showed that chronic dietary exposure to TCDD increased the prevalence and severity of endometriosis in a dose dependent manner. However, in the mouse model for endometriosis, the role of TCDD exposure in mediating endometriosis is less clear with some reports indicating an inhibitory effect (Foster et al., 1997; Yang and Foster, 1997) and others stimulatory (Cummings et al., 1996; Johnson et al., 1997).

Nevertheless, TCDD exposure is clearly linked with endometriosis in rhesus monkeys (Rier et al., 1993, 1995) and, in humans, evidence is beginning to accumulate. Koninckx et al. (1994) noted the parallel, but not direct association, between the high incidence of endometriosis and elevated levels of TCDD in breast milk in women in Belgium. In addition, Mayani et al. (1997) demonstrated that infertile women with endometriosis had significantly higher blood levels of TCDD than a comparable endometriosis-free infertile control group. Also, Gerhard and Runnebaum (1992) reported elevated serum concentrations of PCBs in women with endometriosis, and more specifically (Gerhard et al., 1999), significantly higher serum concentrations of the abundant and persistent PCB congeners 138, 153, and 180 (2,2',3,4,4',5'-, 2,2',4,4',5,5'-, and 2,2',3,4,4',5,5'-PCB, respectively). The results of the Seveso Women’s Health Study should be available soon (Eskenazi et al., 2000). This comprehensive study on the reproductive health of a human population exposed to TCDD in a major industrial accident should provide a more definitive picture of the relationship between TCDD exposure and the development of endometriosis in humans.

TCDD is a widespread environmental contaminant produced during the incineration of trash, bleaching of paper pulp, synthesis of chlorinated phenoxy herbicides, and as a by-product of combustion in some industrial processes (Beatty et al., 1978; Crummett and Stelh, 1973; Olson et al., 1980; Schaum et al., 1994; Schwetz et al., 1973; Vanden Heuvel and Lucier, 1993). These sources of TCDD, as well as the redistribution of high levels released prior to current measures to control release of these chemicals (e.g., widespread use of TCDD contaminated herbicides), have led to a concern over potential human health hazards (Bumb et al., 1980; Miller and Zep, 1987; Reggiani, 1981; Swanson et al., 1988). Most people harbor traces of TCDD from dietary or airborne exposure (Furst, 1993; Kimberough et al., 1984).

Along with various toxic responses such as immunotoxicity (Holsapple et al., 1991), carcinogenicity (Kogevas et al., 1997), and hepatotoxicity (Mann, 1997), TCDD has been shown to alter various hormonal milieus in laboratory animals. These include the following hormone systems: luteinizing hormone and follicle stimulating hormone (Li et al., 1997); thyroxine, triiodothyronine, and thyroid stimulating hormone (Henry and Gasiewicz, 1987; Sewall et al., 1995); adrenocorticotropic (Bestervelt et al., 1993); corticosterone (Balk and Piper, 1984); and β-endorphin (Bestervelt et al., 1991). The estrogenic, antiestrogenic, androgenic, and antiandrogenic capabilities of TCDD, which depend upon specific cell and tissue types, have been well reviewed (Gray and Kelce, 1996; Peterson et al., 1993; Safe et al., 1998).

Recently, Bruner et al. (1997) developed a human model of endometriosis in the nude mouse to study the hormone-disrupting effects of TCDD exposure on the development of endometriosis (Bruner-Tran et al., 1999). Proliferative phase human endometrial explants were cultured in vitro for 24 h with or without TCDD and hormones, as described by Osteen et al. (1994), then injected intraperitoneally into ovariectomized athymic (nude) mice treated 24 h before with sc estradiol-releasing pellets. Treatment of cultured human endometrial explants with estradiol (E2) maintained both stromal and epithelial-specific matrix metalloproteinase (MMP) secretion in vitro and spontaneously promoted the establishment of ectopic peritoneal lesions in vivo when the explants were injected into recipient animals. In contrast, treatment with progesterone (P4) in conjunction with E2 suppressed both in vitro metalloproteinase secretion and in vivo lesion formation (Bruner et al., 1997, 1999; Bruner-Tran et al., 1999). This suppression was coordinately regulated by both P4 and TGF-β (Bruner et al., 1999). Treatment with TCDD along with E2 increased both the number and size of the lesions compared to treatment with E2 alone, and treatment with TCDD in the presence of E2 and P4 disrupted the ability of P4 to block lesion formation and metalloproteinase expression (Bruner-Tran et al., 1999). These results provide mechanistic evidence linking the steroidal regulation of MMP secretion in human endometrial tissue with the establishment of an endometriosis-like disease in the nude mouse (Bruner et al., 1997, 1999) and demonstrate the ability of TCDD to disrupt this regulation (Bruner-Tran et al., 1999).

The myriad of biological effects of TCDD are believed to be mediated essentially via the aryl hydrocarbon receptor (AhR; Whitlock, 1993), which must form a complex with the AhR nuclear translocator (ARNT) to activate TCDD responses (Greenlee et al., 1994; Hoffman et al., 1991; Landers and Bunce, 1991; Reys et al., 1992). TCDD exposure has been shown to induce both CYP1A1 (Charles and Shiverick, 1997; Kress and Greenlee, 1997) and CYP1B1 (Hakkola et al., 1997) in various tissues. Both CYP1A1 and CYP1B1 are 17β-estradiol hydroxylases (Hayes et al., 1996; Spink et al., 1992). Since endometriosis is an estrogen-dependent disease (Cramer et al., 1986; Dizerega et al., 1980; Kiesel and Runnebaum, 1990; Sensky and Liu, 1980), altered metabolism of estradiol by TCDD or other dioxin-like halogenated aromatic hydrocarbons may be involved in the pathogenesis of endometriosis.

In a companion study, performed in parallel with the present study, Bofinger et al. (2001) demonstrated the induction of
CYP1A1 mRNA, CYP1B1 mRNA, EROD (7-ethoxyresorufin-O-deethylase) activity, and CYP1B1 protein in the same human endometrial explant culture model (Osteen et al., 1994) used by Bruner-Tran et al. (1999) as part of the human model of endometriosis in the nude mouse. The expression of AhR and ARNT has not been investigated in the human endometrial explant culture model and the purpose of the present study was to evaluate and quantitate the expression of AhR and ARNT mRNA in human endometrial explants cultured with and without TCDD.

**MATERIALS AND METHODS**

**Hormone and TCDD solution preparation.** TCDD (> 95% purity), generously donated by Dow Chemical Company (Midland, MI), was first dissolved in dioxane to provide a 0.52 M solution, then diluted in DMSO (Sigma, St. Louis, MO) generating final stock concentrations of 25, 2.5, 0.25, 0.025, and 0.0025 μM. A 17β-estradiol (E2) solution and a 17β-estradiol + progesterone (E2 + P; Sigma, St. Louis, MO) mixture were prepared in DMSO to give 25 μM and 2.5 μM + 1.25 μM stock solutions, respectively. Aliquots were stored at −20°C.

**Human subjects.** Human subjects (n = 16) were recruited who were having gynecological surgery (laparoscopy or laparotomy) at a major university affiliated hospital. These subjects were a subset of the subjects used by Bofinger et al. (2001). All patients signed a consent form that was approved by the Human Subjects Institutional Review Boards of the hospital and SUNY at Buffalo. Prior to surgery, the last menstrual period (LMP) was recorded and the cycle day at the time of surgery was calculated. Hormonal medications were noted, as well as the patient’s age and history of endometriosis. During surgery, all patients were thoroughly examined for the absence or presence of endometriosis using a standardized 3-step abdominal pelvic examination (Batt, 1999). This technique was specially developed for clinical research to ensure accurate and complete diagnosis of endometriosis involving the appendix, small and large bowel, ureters, reproductive organs, and diaphragm. Most patients had endometriosis (n = 9) or a history of endometriosis without current disease (n = 3), and a smaller population (n = 4) had no disease or known disease history. Patients without endometriosis had surgery for sterilization (tubal ligation) or pelvic pain unrelated to endometriosis.

**Surgical specimens.** A specimen of endometrial tissue was collected by pipelle or curet at the beginning of the surgical procedure. The specimen was divided into 2 portions. One half of the specimen was placed in sterile, phenol red free, D-MEM/F-12 medium (a 50:50 mix of Dulbecco’s Modified Eagle’s Medium and Ham’s F-12 with 15 mM HEPES buffer, L-glutamine, and pyridoxine HCl; Life Technologies, Grand Island, NY). This portion was transported to the laboratory on ice for culture preparation. The second half of the specimen was sent to the pathology laboratory of the hospital for routine histological dating as described by Mazur and Kerman (1995). The histological phase of the endometrium was recorded as proliferative or secretory.

**Tissue culture media.** D-MEM/F-12 medium (phenol red free with 15 mM HEPES buffer, L-glutamine, and pyridoxine HCl; Life Technologies, Grand Island, NY) was used in all stages of tissue preparation and explant culture as described by Osteen et al. (1994). An initial culture medium (3% FBS-D-MEM/F-12) was used to establish the cultures prior to the start of the experiments. This medium consisted of D-MEM/F-12 with 3% charcoal-stripped fetal bovine serum (FBS; Life Technologies, Grand Island, NY), 1% antibiotic/antimycotic solution (penicillin/streptomycin/amphotericin; Life Technologies, Grand Island, NY), and either E2 (10 nM) or E2 + P (1 nM + 500 nM) with DMSO (hormone vehicle) at a final concentration of 0.08%. Charcoal-stripped FBS was prepared by mixing and stirring FBS with washed and dried charcoal (30 g charcoal / 1 L FBS) at 4°C overnight, then removing the charcoal with 1.22, 0.45, and 0.22 mm filters and performing heat inactivation at 56°C for 30 min. After the cultures were established, the experiments were performed in a serum-free D-MEM/F-12 medium containing 1% ITS + Premix™ (insulin, transferrin, selenious acid, bovine serum albumin, linoleic acid; Becton Dickinson Labware, Bedford, MA), 0.1% Excyte III (Bovine Lipoprotein Solution; Bayer Corp., Kanakakee, IL), and 1% antibiotic/antimycotic solution. The cultures were treated with E2 (10 nM) or E2 + P (1 nM + 500 nM) with either TCDD or an equivalent volume of DMSO (TCDD vehicle) such that the final concentration of DMSO was 0.08%. The final concentration of TCDD in the medium was 10 nM for all studies except the dose response study in which the final TCDD concentrations were 0.001, 0.01, 0.1, 1.0, or 10 nM.

**Explant culture.** The surgical specimens were prepared and cultured under sterile conditions similar to the method of Osteen et al. (1994). Each specimen was washed 2–3 times with D-MEM/F-12 medium and blood clots were dissected away as much as possible. The endometrial sample was cut into 1 x 2 mm uniform explants with a sterile scalpel blade and the pieces were washed once more with D-MEM/F-12 and transferred to 3% FBS-D-MEM/F-12 medium. The 3% FBS-D-MEM/F-12 medium contained either E2 (specimens from women at cycle days 1–13 presumed to be in the proliferative phase) or E2 + P (specimens from women at cycle days 14 or higher presumed to be in the secretory phase); the phase of the endometrial samples could not be confirmed until the pathology report was available 2 weeks later. If the explants contained excessive blood, the pieces were incubated in the 3% FBS-D-MEM/F-12 medium for 3 h at 37°C until the blood had dissipated. Otherwise, the pieces were immediately transferred to 0.4 mm culture plate inserts (Millicell-CM, Fisher Scientific Co., Springfield, NJ) at a concentration of 8–10 pieces per insert in a 24-well plate (Costar Corporation, Cambridge, MA) with 1 mL 3% FBS-D-MEM/F-12 medium containing the appropriate hormone(s). The inserts were modified prior to use by punching 3 holes around the wall of the insert, 3 mm above the membrane surface, to allow equilibration of the medium across the membrane. The explants were incubated for 20–30 h at 37°C in a humidified 5% CO2 air environment to establish the cultures. At the start of an experiment (Time 0), the 3% FBS-D-MEM/F-12 medium was replaced with serum-free D-MEM/F-12 medium containing the appropriate hormone(s) and either TCDD or the vehicle control (DMSO). After 24 h incubation, the TCDD and control media were removed, and the explants were either harvested or incubated in media containing hormones, but no TCDD, for another 24–48 h (Time 48–72 h), changing the medium every 24 h. At the end of the incubation, the explants were harvested for total RNA isolation.

**Experimental design.** Tissue samples were used for 3 types of experiments. Not all specimens were used for all experiments because the amount of tissue was limited. The 3 types of experiments were as follows: (1) dose response to 0, 0.001, 0.01, 0.1, 1.0, or 10 nM TCDD for 24 h (n = 2 specimens); (2) time course with 10 nM TCDD for 0, 24, 48, or 72 h (n = 3 specimens); and (3) response to 24-h culture with (n = 13) or without (n = 11) 10 nM TCDD for explants from subjects with or without a history of endometriosis. All of the data analyzed were derived from explants cultured with the medium containing E2 + P, with the following exceptions: The correlation analysis of the expression of AhR, ARNT, CYP1A1, and CYP1B1 mRNA and the analysis to evaluate the relative expression of AhR/ARNT mRNA included data from explants cultured with E2 (n = 2) in the medium, as well as explants cultured with E2 + P (n = 14). Covariates in the study included donor’s age, phase of the tissue, and donor’s hormonal medications (birth control).

**Isolation of total RNA and quantitative RT-PCR.** Total RNA was isolated from the explants by the single-step acid guanidinium thiocyanate-phenol-chloroform (Reagent D) extraction method (Chomczynski and Sacchi, 1987). Extracts were pooled from wells cultured under the same conditions (3 wells per data point) and collected by centrifugation (1500 x g, 5 min). The conditions for preparing the internal standards, the RT-PCR reaction, and the primers used for Quantitative RT-PCR in this laboratory have been previously described (Abbott et al., 1999). Briefly, quantitative RT-PCR was performed using an RNA internal standard (IS) specifically synthesized for each RNA under study. Primers were chosen using gene sequences for human AhR and ARNT, and IS were prepared from primers that included a T7 polymerase
sequence, the gene primer sequence and sequences homologous to regions of the pUC-19 plasmid (Promega, Madison, WI). Gene and IS primers were synthesized by Genosys Biotechnologies (The Woodlands, TX). Human AhR primers were selected based on the GenBank sequence L19872, which is the complete AhR mRNA codon sequence from cultured human HeLa cells (Dolwick et al., 1993). Human ARNT primers were selected based on the GenBank sequence M69238, which is an ARNT mRNA clone from human liver (Hoffman et al., 1991).

**Statistical analysis.** Except where noted, all analyses were performed on log (base 10) transformed gene product concentrations, and the means and confidence intervals were back-transformed to the original scale. Statistical analyses were performed in SAS (SAS 1989, 1990, 1996). Mixed-effects linear models (SAS Proc Mixed) were used to estimate group means and test for significant effects of treatment or time point. All data is presented as arithmetic means and their standard errors calculated by SAS Proc Means. Three data sets were analyzed: the main body of the data which included control and 10 nM TCDD samples collected at 24 h, the dose-response data, and the time-course data. For each of these, the initial model for each gene product included the predictors, PCR-DATE as a fixed effect, and SUBJECT as a random effect, to adjust for differences associated with PCR runs on different days and correlation among endometrial samples from the same subject. The model for the main data set and the dose response data also included dose as a fixed effect, while the time course included h after treatment as a fixed effect. Differences between dose groups or time points were tested with pairwise contrasts. For the time course data, the slope across time was also tested for difference from 0. The main data set was further analyzed to see if any of the potential covariates: donor’s age (<30, 30–39, 40+), tissue phase (proliferative or secretory), history of endometriosis (yes or no), and hormone medications (hormonal birth control—yes or no) were associated with the gene product concentrations. A forward stepwise selection strategy was used to add these covariates and any associated interaction terms to the model, using the inclusion criteria of $p < 0.10$.

The random effects estimates for each subject for the 24 h data are displayed from the models both with and without the added covariates. In this multiplicative model, the random effects estimates represent the proportionate change in the level of AhR or ARNT due to Subject after adjusting for all other variables.

Pearson product-moment correlations were calculated of AhR with ARNT mRNA and of CYP1A1 and CYP1B1 mRNA with AhR and ARNT mRNA. The values used here were the means of data points for each sample. Expression of CYP1A1 and 1B1 mRNA was determined by Northern blot analysis (Bofinger et al., 2001). Partial correlations among the gene products were examined by adjusting for dose group, h, the covariate’s age, endometriosis, tissue phase and hormone medications, other gene product(s) (AhR or ARNT), and combinations of these.

The average ratio of AhR/ARNT mRNA was calculated as the average of the AhR/ARNT mRNA values for each sample. All ratios were calculated on the original data scale (molecules/100 fg).

**RESULTS**

**TCDD and Covariate Effects on AhR and ARNT mRNA**

In human endometrial tissue cultured with E$_2$ + P$_4$ at 24 h, AhR mRNA levels were significantly increased ($p < 0.01$) after treatment with 10 nM TCDD (Fig. 1). The AhR mRNA levels (constitutive and TCDD-induced together) were not correlated with donor endometriosis status or birth control use, or with phase of the tissue (secretory or proliferative). Both constitutive and TCDD-induced AhR mRNA levels increased with age across 3 age groups (Table 1). No interaction existed between age and TCDD exposure; thus, TCDD exposure increased AhR mRNA similarly across age groups. When TCDD exposure and age were considered together, the $p$-values for the TCDD effect and age group effect were 0.03 and 0.022, respectively.

In contrast to AhR, ARNT mRNA levels were only weakly affected by 10 nM TCDD treatment ($p = 0.072$) in human endometrial explants cultured with E$_2$ + P$_4$ (Fig. 1). Similar to AhR, when subject level covariates were added to the model, ARNT mRNA levels were correlated with the age of the donor.

**TABLE 1**

<table>
<thead>
<tr>
<th>TCDD (nM)</th>
<th>Donor’s age</th>
<th>AhR</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;30</td>
<td>1.05 ± 0.21</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>&lt;30</td>
<td>1.43 ± 0.14</td>
<td>6</td>
</tr>
<tr>
<td>0</td>
<td>30–39</td>
<td>1.23 ± 0.14</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>30–39</td>
<td>1.94 ± 0.30</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>40+</td>
<td>1.93 ± 0.21</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>40+</td>
<td>2.32 ± 0.24</td>
<td>2</td>
</tr>
</tbody>
</table>

**Note.** AhR values are mean ± SE. Endometrial explants were from donors aged <30, 30–39, or 40+ years and were cultured with or without TCDD. AhR increased with age ($p = 0.022$) and TCDD exposure ($p = 0.003$). No significant interaction between age and treatment.
endometrial expression of AhR and ARNT

increasing with age (p < 0.03; Table 2). Unlike AhR, ARNT mRNA levels were weakly correlated with the phase of the tissue, being higher in proliferative phase specimens (p = 0.068; Table 2). When donor age and cycle phase were taken into consideration, ARNT mRNA levels were no longer associated with TCDD treatment (p = 0.71). A significant interaction was detected between TCDD treatment and the phase of the tissue (p < 0.024) and the age of the donor (p = 0.054). Thus, it appeared that the faint relationship between ARNT mRNA and TCDD treatment could be partially ascribed to these covariates, although small sample sizes make firm conclusions difficult.

range of AhR and ARNT expression

The level of expression of AhR and ARNT mRNA may differ between individual subjects (Larsen et al., 1998; Masten and Shiverick, 1996) and this could affect the potential to respond to TCDD. Therefore it was of interest to examine the distribution of AhR and ARNT mRNA expression and their response across individuals in the E2 and E2 + P4 data set. This was done using random effects estimates for the control and 10 nM TCDD samples at the 24-h endpoint. These estimates represent the change in the levels of AhR and ARNT mRNA due to individual subject variation after adjusting for covariates that significantly affect outcomes such as tissue phase, donor age, and their interactions with TCDD treatment (Fig. 2). Donor age was the only statistically significant covariate for AhR mRNA levels (see above) and with or without the covariates, the samples had a continuous distribution. Similarly, the distribution of ARNT mRNA was continuous whether or not the covariates (tissue phase, donor age, and their interactions with TCDD treatment) were added to the analysis.

Dose Response

The response of human endometrial explants cultured with E2 + P4 to increasing concentrations of TCDD is shown in Figure 3. Neither AhR mRNA nor ARNT mRNA levels demonstrated a noticeable dose-response relationship to TCDD exposure (0.001 nM to 10 nM) after 24 h in culture. However, small sample sizes affect the ability to detect dose-dependent effects.

Time Course

The time courses for the induction of AhR and ARNT mRNA in human endometrial explants exposed to 10 nM

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**Table 2**

<table>
<thead>
<tr>
<th>Tissue phase</th>
<th>TCDD (nM)</th>
<th>Donor’s age</th>
<th>ARNT</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>0</td>
<td>&lt;30</td>
<td>0.57</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&lt;30</td>
<td>0.42</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>30–39</td>
<td>1.66</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30–39</td>
<td>1.78 ± 0.34</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>40+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Secretory</td>
<td>0</td>
<td>&lt;30</td>
<td>0.82 ± 0.09</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>&lt;30</td>
<td>1.14 ± 0.26</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>30–39</td>
<td>1.21 ± 0.70</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30–39</td>
<td>1.16 ± 0.79</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>40+</td>
<td>1.06 ± 0.32</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40+</td>
<td>1.12 ± 0.29</td>
<td>2</td>
</tr>
</tbody>
</table>

**Note.** ARNT values are mean ± SEM. ND = No data, no samples within that category. ARNT expression significantly correlated with age of donor (p = 0.03), phase of tissue (p = 0.068), and significant interactions occurred between treatment × phase (p = 0.024) and treatment × age (p = 0.054).
TCDD during the first 24 h are shown in Figure 4. Maximal induction occurred at 48–72 h for AhR mRNA and at 48 h for ARNT mRNA. The slope for the induction of AhR mRNA was significantly increased over time (\(p = 0.032\)), but not for ARNT mRNA (\(p = 0.172\); Fig. 4).

**Correlation of AhR, ARNT, and CYP1A1/1B1 mRNA**

These analyses included the 2 specimens cultured with \(E_2\) as well as the specimens cultured with \(E_2 + P_4\). The levels of AhR and ARNT mRNA were significantly correlated over the entire dataset (\(p < 0.01\)) and in the 24-h control vs. 10 nM TCDD subset (\(p < 0.05\)) with correlation coefficient (\(r\)) values between 0.42–0.44. A significant partial correlation between AhR and ARNT was also present after adjusting for dose, h, donor endometriosis status, age and hormonal medication (birth control), and tissue cycle phase (\(p < 0.05, r = 0.426\)).

The expression of AhR mRNA was 1.7-fold greater than that of ARNT mRNA based on all samples and 1.8-fold greater based on the 24-h \(E_2 + P_4\) subset. These values were calculated by determining the ratio of AhR:ARNT mRNA for tissue samples across time and treatment groups and calculating the average across the appropriate grouping.

As reported in the collaborating report (Bofinger et al., 2001), mRNA for CYP1A1 and CYP1B1 was evaluated by Northern blot. Statistical analysis to correlate these data with AhR and ARNT mRNA expression are reported here. AhR mRNA correlated with both CYP1A1 mRNA (\(p = 0.013, r = 0.5\)) and CYP1B1 mRNA (\(p = 0.047, r = 0.409\)), while ARNT mRNA was weakly correlated to CYP1A1 mRNA (\(p = 0.07, r = 0.374\)), but not at all with CYP1B1 mRNA (\(p = 0.241, r = 0.256\)). After adjusting for ARNT, AhR mRNA was still correlated with CYP1A1 mRNA, but not with CYP1B1 mRNA. ARNT mRNA, after adjusting for AhR mRNA, was not correlated with either CYP mRNA. Thus, AhR and ARNT mRNA are correlated both across and within treatment and time groups.

**DISCUSSION**

This study examined the constitutive and TCDD-induced expression of AhR and ARNT mRNA in explant cultures of human endometrium obtained from women with and without endometriosis. It used an explant culture model similar to that developed by Osteen et al. (1994) and used by Bofinger et al. (2001) in a parallel study demonstrating the viability of this model and its responsiveness to TCDD as measured by the induction of CYP1A1 mRNA, CYP1B1 mRNA, EROD (7-ethoxyresorufin-O-deethylase) activity, and CYP1B1 protein by 10 nM TCDD.

To our knowledge, this is the first report quantitating the expression of either AhR or ARNT mRNA in human endome-
trium. Constitutively, mean AhR and ARNT mRNA expression ranged from 1.0 to 1.3 molecules/100 fg total RNA. These levels are similar to those measured by Abbott et al. (1999) in a study in which the same RT-PCR primers and conditions were used to determine embryonic palatal AhR and ARNT mRNA expression levels of 1 to 4 molecules/100 fg total RNA. The AhR:ARNT mRNA ratio of 1.7–1.8 is within the range of AhR:ARNT protein ratios of 0.3 to 10 found in various cell types (Holmes and Pollenz, 1997).

In response to TCDD treatment, AhR mRNA expression increased. While increased AhR mRNA expression was dependent upon the age of the donor, TCDD treatment still increased AhR expression across age groups. Unlike donor age, donor endometriosis status or hormonal medication (birth control), or phase of the tissue (proliferative or secretory) had no statistically significant effect on AhR mRNA expression in the endometrial samples.

The increase in AhR mRNA with TCDD exposure differs from previous reports. In these reports, human cells/tissues, such as immortalized human endometrial stromal cells, IM-9 B lymphoblast, hepatoma (HepG2), peripheral blood lymphocytes, primary mammary tissue, and embryonic palates, that were exposed to TCDD had decreased AhR mRNA (Abbott et al., 1999; Larsen et al., 1998; Masten and Shiverick, 1996; Yang, 1999) or had no effect on AhR mRNA (Giannone et al., 1998; Li et al., 1998; Masten and Shiverick, 1996). Also, in human mammary stromal fibroblasts and epithelial cells, TCDD treatment decreased AhR protein (Eltom et al., 1998; Larsen et al., 1998). This wide variety of responses in AhR mRNA expression in different cell types, after TCDD treatment, suggests a dependence of the overall response to TCDD on the type of cell or tissue or possibly the treatment of the cultured cell/tissue.

Variations between tissues in responses to TCDD are also seen for CYP1A1 and CYP1B1 mRNA and protein expression. Similar to the companion parallel study by Bofinger et al. (2001), both CYP1A1 and CYP1B1 protein and mRNA levels were shown to increase in response to TCDD exposure in human embryonic palates, mammary epithelium, and B lymphocytes (Abbott et al., 1999; Charles and Shiverick, 1997; Li et al., 1998; Masten and Shiverick, 1996). Yet in other human cell/tissues, effects on CYP1A1 or CYP1B1 mRNA were not detected following TCDD exposure (Eltom et al., 1998; Hakkola et al., 1997; Yang, 1999).

Despite the variations between tissues in responses to TCDD, it is interesting to note that in human endometrial cells in the present study and in human embryonic palatal cells (Abbott et al., 1999) there does not appear to be much variation across individuals in ability to respond to TCDD (high responders vs. low responders). Also, the range of expression of mRNA for AhR and members of the CYP family can be very similar in laboratory animal cells/tissues and human cells exposed to TCDD (Abbott et al., 1999; Pollenz et al., 1998; Roman et al., 1998; Vanden Heuvel et al., 1994; Walker et al., 1999).

In contrast to the induction of AhR mRNA by TCDD treatment, the expression of ARNT mRNA in human endometrial explants was not affected by TCDD treatment. The lack of induction of ARNT mRNA expression by TCDD was also reported by Hakkola et al. (1997), Li et al. (1998), and Yang (1999) who studied various cultured human cell types, including immortalized human endometrial stromal cells. On the other hand, significantly increased ARNT mRNA levels were observed over time in human embryonic palates (Abbott et al., 1999), but control values were also significantly decreased. Again, this variation in the overall responsiveness of ARNT mRNA to TCDD exposure further supports its dependence on the cell or tissue type, or possibly the treatment of the cultured cell/tissue. Yet, similar to constitutive AhR mRNA expression, there does not appear to be a cluster of high or low expressers of ARNT mRNA in response to TCDD treatment.

For both AhR and ARNT mRNA, expression increased significantly with increased age of the donor. In addition, the expression of ARNT mRNA, but not AhR mRNA, was affected by the phase of the endometrial tissue with ARNT mRNA expression being greater in the proliferative phase. Although a paucity of published data exists on both human and laboratory animal AhR and ARNT mRNA expression associated with age, AhR and ARNT mRNA expression in human keratinocytes has been reported to increase as differentiation progresses (Wanner et al., 1996). Similar to the present study, Küchenhoff et al. (1999), with confirmation by Yang (1999), qualitatively established that AhR mRNA is expressed constitutively (mRNA was not quantified) in normal human endometrium and immortalized endometrial stromal cells. In that study, AhR protein was inversely correlated with the age of the donor and expression was detected in less than half of the individuals (43%).

Bofinger et al. (2001) also reported a relationship between TCDD-induced EROD activity and age and cycle phase in human endometrial explants cultured for 72 h in the presence of E2 or E2 + P4 with or without TCDD exposure during the first 24 h. In this companion parallel study, both younger age and proliferative phase were generally associated with higher TCDD-induced EROD activities. Only 5 data points overlapped between the EROD activity data set and the AhR and ARNT mRNA data set of the present study. With this limited data, TCDD-induced EROD activity correlated positively and significantly with the constitutive expression of AhR ($r = 0.941; p = 0.017$, data not shown). Of the common data points, 4/5 were from donors under the age of 30, 2/5 were from proliferative phase specimens, and 3/5 were from secretory phase specimens, all treated with E2 + P4.

Other correlation studies demonstrated that the levels of AhR and ARNT mRNA were significantly correlated with each other, and that AhR mRNA was correlated with both CYP1A1 mRNA and CYP1B1 mRNA, but ARNT mRNA was only
weakly associated with CYP1A1 mRNA, an effect that can be attributed mainly to the relationship between AhR and ARNT mRNA. In a previous report (Abbott et al., 1999), human embryonic palatal AhR expression was also shown to be correlated with CYP1A1 mRNA. The correlation of AhR, ARNT, and CYP1A1 mRNA expression has also been demonstrated in human peripheral blood samples (Hayashi et al., 1994). As CYP1A1 is one of the AhR-activated genes, it is readily apparent that AhR and CYP1A1 mRNA expression should be associated. Although it has been shown that CYP1B1 mRNA expression in human cells is induced by TCDD (Charles and Shiverick, 1997; Eltom et al., 1998), this appears to be the first paper demonstrating a correlation between AhR and CYP1B1 mRNA expression in a human tissue.

In summary, AhR and ARNT mRNA have been quantified in human endometrial explant cultures. The expression of both of these mRNAs increases with increasing donor age, and the expression of ARNT mRNA is correlated with the phase of the endometrial tissue. Also, TCDD treatment induced the expression of AhR, but not ARNT mRNA, in a time-dependent, but not dose-dependent, manner. The expression of AhR and ARNT mRNA in response to TCDD treatment was continuously distributed without high or low responder/expressers. The expression of AhR mRNA in control and TCDD-exposed specimens correlated with ARNT, CYP1A1, and CYP1B1 mRNA, but ARNT mRNA did not correlate with either CYP mRNA.

Although no endometriosis-related differences were observed in the constitutive or TCDD-induced expression of AhR or ARNT mRNA, the power of the study to detect diseasespecific change was limited by small sample size and variability in the cycle phase of the tissues. The real value of this study is that it characterizes AhR and ARNT mRNA expression in the human endometrial explant culture model and supports the companion parallel study of Bofinger et al. (2001) in demonstrating the usefulness of this model for future studies of the effects of dioxin-like compounds on human endometrium in relationship to cycle phase, hormonal exposure, and donor age.

ACKNOWLEDGMENTS

This research project was supported in part by grant number R03ES0853901 from the National Institute of Environmental Health Sciences, NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, NIH. The project was also supported by the Multidisciplinary Research Pilot Project Program of the State University of New York at Buffalo.

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