Neonatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin increases the mRNA expression of prostatic proteins in C57BL mice

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ABSTRACT — The effects of neonatal exposure to low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on prostatic secretory protein expression were investigated. Male C57BL mice were treated with TCDD at 10, 100, or 1,000 ng/kg body weight at postnatal day (PND) 6. At PND42, the ventral, dorsolateral, and anterior prostatic lobes were dissected and the mRNA expression of prostatic proteins including spermine-binding protein, serine protease inhibitor Kazal type 3, prostate secretory protein 94 (PSP94), immunoglobulin binding protein-like protein (IgGBPLP), experimental autoimmune prostatitis antigen proteins, and peroxiredoxin-6 (Prdx6) was measured by quantitative PCR. There was no significant difference in the weight of the prostatic lobes between the control and TCDD-treated groups. The expression of PSP94 and Prdx6 in the ventral prostate and IgGBPLP in the dorsolateral prostate at PND42 was significantly increased by neonatal TCDD treatment in a dose-dependent manner, while no changes were noted in other prostatic secretions. These data suggest that neonatal exposure to TCDD may have effects on the neonatal differentiation of the prostate and results in the hyper-expression of some prostatic proteins later in life.

Key words: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), Prostatic secretion, Mouse prostate, Neonatal effects

INTRODUCTION

The developing male reproductive system of laboratory rodents is highly sensitive to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Mably et al., 1992; Roman and Peterson, 1998; Theobald et al., 2000). Its toxic effects include a decrease in the weight of the testis and accessory sex organs, degeneration of germ cells, and decreased spermatogenesis. The adverse effects of maternal exposure to TCDD on the development of the prostate gland have been studied extensively in rats and mice. In Holtzman rats, a single maternal dose of 64 ng/kg body weight (bw) of TCDD caused a significant decrease in ventral prostate (VP) weight (Mably et al., 1992). More recently, it was reported that androgen receptor (AR) mRNA expression was reduced in the VP of Holtzman rats following maternal treatment with as low as 12.5 ng/kg bw TCDD (Ohsako et al., 2001). In the mouse, the C57BL/6J strain appears to be sensitive to TCDD, in which the maternal administration of 5 μg/kg bw TCDD suppressed the development of the VP in the offspring, while the weight of the dorsolateral prostate (DLP) and anterior prostate (AP) decreased by approximately 50% (Lin et al., 2002a). Exposure to TCDD during only the lactational period also resulted in offspring with lower prostate weights, but with less severe changes (Lin et al., 2002b).

Although the previous studies have been clearly demonstrated that TCDD affect the development of the prostate morphologically, it is important to examine the effect on the prostatic function, production of prostatic proteins. We recently reported the identification of the major proteins secreted from the mouse prostate (Fujimoto et al., 2006). The secreted proteins included spermine-binding protein (SBP), serine protease inhibitor Kazal type 3,
(SPI-KT3), prostate secretory protein 94 (PSP94), glucose-regulated protein, 78kDa (GRP78), peroxiredoxin-6 (Prdx6), probasin, experimental autoimmune prostatitis antigen protein (EAPA2), and immunoglobulin binding protein-like protein (IgGBPLP). The expression profile of these proteins would be useful for studying prostatic function and may also provide markers for evaluating the effects of environmental chemicals on the prostate. In the present study, we investigated the effects of neonatal exposure to low doses of TCDD on the mRNA expression of prostatic proteins as well as AR in the prostate.

MATERIALS AND METHODS

Animal experiments
The animal experiments were conducted under the approval of the Animal Experiment Committee of the National Institute of Health Sciences (NIHS). All experiments involving TCDD-treated animals were carried out following the rules for the use of TCDD set by NIHS. Five-day-old male C57BL mice were purchased from Charles River Japan Co. and maintained with free access to a basal diet and tap water. At postnatal day (PND) 6, the animals were divided into 4 groups (n = 6, each group): control and 3 TCDD-treated groups. TCDD (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) in corn oil (50 μl) was injected intraperitoneally (ip) at doses of 0, 10, 100, or 1,000 ng/kg bw). At PND42, the animals were killed under ether anesthesia, since our previous study indicated that the mRNA expression of prostatic proteins is matured at PND42 (Fujimoto et al., 2006). The prostatic lobes were dissected under a microscope, then immediately fixed in RNAlater Solution (Life technologies, Grand Island, NY, USA).

Quantification of mRNA by real-time RT-PCR
Total RNA was prepared from prostatic tissues using an RNA isolation kit (NucleoSpin RNA II; Machery-Nagel GmbH & Co. KG, Düren, Germany). An ABI Prism 7500 (Applied Biosystems/Life Technologies Co., Carlsbad, CA, USA) was employed for the RT-PCR based quantification of prostatic protein mRNAs as described previously (Fujimoto et al., 2006). All mRNA levels were normalized with reference to β-actin mRNA.

Statistical analysis
Statistical comparisons were made by Dunnett’s multiple comparison test.

RESULTS

Body and prostate lobe weights
There was no significant difference in body weight between the control and 3 TCDD-treated groups at PND42 (Table 1). There was no significant change in the weight of either the VP, DLP, or AP.

Expression of prostatic protein and AR mRNAs
SBP and SPI-KT3 were preferentially expressed in the VP, while probasin, EAPA2, and IgGBPLP expression was localized in the DLP and AP (Table 2). PSP94 was expressed in both the VP and DLP, while GRP78 and Prdx6 were expressed in all prostatic lobes. The effects of neonatal treatment of TCDD on mRNA expression were evident for PSP94, Prdx6, and IgGBPLP. The effects were lobe specific; that is, neonatal TCDD increased the expression of PSP94 and Prdx6 mRNA in the VP as well as IgGBPLP mRNA in the DLP in a dose-dependent manner. Neonatal TCDD exposure did not change the expression of AR mRNA in the VP or DLP, but decreased its expression in the AP.

DISCUSSION
Maternal exposure to TCDD reportedly causes irreversible changes to the reproductive systems of offspring, including reduced sperm count and reduced size of the reproductive organs. The development of the male reproductive organs in rodents, in particular the prostate gland, has been recognized as a sensitive target to

Table 1. Weight of body and prostatic lobes at PND42

<table>
<thead>
<tr>
<th>Treatment</th>
<th>body weight (g)</th>
<th>VP (mg/g bw)</th>
<th>DLP (mg/g bw)</th>
<th>AP (mg/g bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>16.8 ± 0.28</td>
<td>0.20 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>TCDD 10</td>
<td>16.9 ± 0.37</td>
<td>0.19 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>TCDD 100</td>
<td>17.7 ± 0.25</td>
<td>0.30 ± 0.08</td>
<td>0.20 ± 0.02</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>TCDD 1000</td>
<td>18.9 ± 0.38</td>
<td>0.24 ± 0.08</td>
<td>0.24 ± 0.01</td>
<td>0.31 ± 0.03</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. (n = 6). Male C57BL mice were treated with TCDD (10, 100, or 1,000 ng/kg bw) at postnatal day (PND) 6 and sacrificed at PND42.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>SBP</th>
<th>SPI-KT3</th>
<th>PSP94</th>
<th>GRP78</th>
<th>Prdx6</th>
<th>Probasin</th>
<th>EPA2</th>
<th>IgGBPLP</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>control</td>
<td>315 ± 64.4</td>
<td>280 ± 85.7</td>
<td>12.4 ± 4.2</td>
<td>5.7 ± 0.36</td>
<td>1.5 ± 0.21</td>
<td>7.3 ± 1.24</td>
<td></td>
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</tr>
<tr>
<td>TCDD 10</td>
<td>293 ± 131.2</td>
<td>495 ± 95.7</td>
<td>40.0 ± 11.9</td>
<td>7.6 ± 0.92</td>
<td>2.4 ± 0.38</td>
<td>8.6 ± 1.08</td>
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</tr>
<tr>
<td>TCDD 100</td>
<td>322 ± 63.6</td>
<td>278 ± 44.1</td>
<td>65.7 ± 21.1*</td>
<td>8.3 ± 1.89</td>
<td>4.5 ± 0.79*</td>
<td>4.8 ± 0.27</td>
<td></td>
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</tr>
<tr>
<td>TCDD 1000</td>
<td>450 ± 84.7</td>
<td>308 ± 46.5</td>
<td>110 ± 16.6**</td>
<td>7.1 ± 1.68</td>
<td>5.6 ± 1.0*</td>
<td>7.5 ± 0.71</td>
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<tr>
<td>DLP</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>control</td>
<td>92.3 ± 20.2</td>
<td>11.2 ± 1.23</td>
<td>27.2 ± 4.7</td>
<td>7.8 ± 0.65</td>
<td>2.5 ± 0.44</td>
<td>0.80 ± 0.09</td>
<td>4.5 ± 0.96</td>
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</tr>
<tr>
<td>TCDD 10</td>
<td>59.0 ± 24.3</td>
<td>9.5 ± 1.21</td>
<td>31.7 ± 1.93</td>
<td>9.1 ± 1.36</td>
<td>2.8 ± 0.44</td>
<td>2.2 ± 0.59</td>
<td>3.9 ± 0.45</td>
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<tr>
<td>TCDD 100</td>
<td>82.3 ± 20.7</td>
<td>8.9 ± 1.07</td>
<td>31.6 ± 3.02</td>
<td>8.5 ± 0.57</td>
<td>2.4 ± 0.29</td>
<td>2.7 ± 0.75*</td>
<td>3.5 ± 0.22</td>
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<tr>
<td>TCDD 1000</td>
<td>56.9 ± 15.9</td>
<td>7.3 ± 0.71</td>
<td>31.3 ± 2.46</td>
<td>7.9 ± 0.07</td>
<td>2.5 ± 0.28</td>
<td>3.3 ± 0.47*</td>
<td>3.2 ± 0.50</td>
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<tr>
<td>AP</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>control</td>
<td>13.7 ± 3.23</td>
<td>38.1 ± 5.4</td>
<td>4.5 ± 0.66</td>
<td>3.4 ± 0.29</td>
<td>19.5 ± 1.95</td>
<td>3.6 ± 0.35</td>
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</tr>
<tr>
<td>TCDD 10</td>
<td>18.0 ± 1.59</td>
<td>67.1 ± 4.1</td>
<td>5.8 ± 0.21</td>
<td>3.0 ± 0.33</td>
<td>27.8 ± 5.63</td>
<td>3.0 ± 0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD 100</td>
<td>9.8 ± 0.91</td>
<td>40.9 ± 6.13</td>
<td>3.9 ± 0.52</td>
<td>3.0 ± 0.57</td>
<td>11.2 ± 1.90</td>
<td>1.9 ± 0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD 1000</td>
<td>14.6 ± 1.90</td>
<td>73.6 ± 15.4</td>
<td>5.9 ± 0.75</td>
<td>3.3 ± 0.37</td>
<td>28.3 ± 6.19</td>
<td>2.1 ± 0.32</td>
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</tbody>
</table>

Mean ± S.E.M. (n = 5 or 6). Values are mRNA levels divided by beta actin mRNA levels (*p < 0.05 and **p < 0.01 vs. control).

Male C57BL mice were treated with TCDD (10, 100, or 1,000 ng/kg body weight) at postnatal day (PND) 6 and sacrificed at PND42.
TCDD, especially when it is administered maternally (Bjerke and Peterson, 1994; Lin et al., 2002b; Mably et al., 1992; Theobald et al., 2000). In the present study, we examined the effects of neonatal TCDD exposure on the expression of prostatic proteins and demonstrated that the administration of low doses of TCDD at PND6 resulted in the abnormal hyper-expression of PSP94, Prdx6, and IgGBPLP mRNAs at PND42 in C57BL mice. Although the expression of all the prostatic proteins is regulated by androgen as we previously reported (Fujimoto et al., 2006), only three of them were hyper-expressed, that may suggest the neonatal TCDD did not change androgen levels.

There is a difference in the acute lethality of TCDD among different mouse strains, with an LD50 of approximately 100 μg/kg bw in the “sensitive” C57BL/6J strain, while it is more than 3 mg/kg bw in “non-sensitive” DBA mice (Weber et al., 1995). The C57BL/6J strain demonstrated a higher susceptibility to developmental disruption of the male reproductive system by maternal exposure to TCDD (Theobald et al., 2000). In the rat, there are also great differences in the acute lethality of TCDD among strains, but the effects of TCDD on the development of the prostate seem to be similar among strains (Simanainen et al., 2004).

The development of the prostatic gland begins with the formation of epithelial buds from the urogenital sinus at gestational day (GD) 17; these then develop into the prostatic main ducts (Cunha et al., 1987). After birth, extensive branching and growth from the duct takes place to generate the mature prostate. Approximately 70-80% of ductal tips and 50-70% of branching points are formed during the first 15 days after birth, while ductal branching continues throughout adolescence (Sugimura et al., 1986). Vulnerability to the effects of TCDD on the development of the prostate has been studied extensively in C57BL/6J mice, in which the oral administration of 5 μg/kg bw TCDD on GD 13 reduced VP weight by 84%. Lactational exposure alone to TCDD also significantly suppressed VP weight by 41%. For the DLP and AP, the effects were less severe, with lactational exposure alone reducing their weight by approximately 20%, while in utero exposure caused a 50% reduction (Lin et al., 2002b). Our data may suggest the prostate at PND 6 may be less susceptible for TCDD suppressing the prostatic growth but sensitive for the functional alteration. Further studies are needed to understand what timing of TCDD exposure is critical to lead to changes in expression of prostatic proteins.

Although prostatic secreted proteins are found in the seminal fluid, it is not clear what their physiological roles are. PSP94 is one of the major proteins secreted by the human prostate and is also abundantly secreted by the rodent prostate. PSP94 is known to be expressed mainly in the VP and DLP in mice. This protein may function as an immunoglobulin-binding protein and is involved in the regulation of the immune response in the female reproductive tract (Kamada et al., 1998). It also functions as an inhibitor of sperm motility and of the acrosome reaction. IgGBPLP is abundantly expressed in the DLP and AP and may have a similar function to PSP94 (Kumar et al., 2010). Prdx6 is an antioxidant enzyme that reduces peroxide and alkyl hydroperoxide to water and alcohol, respectively, and it may have a seminal plasma antioxidant capability (Wang et al., 2004). The changes in the composition of prostatic secretions caused by the hyper-expression of these proteins might eventually affect normal fertility.

The present study demonstrated that neonatal exposure to low levels of TCDD changes the normal expression pattern of prostatic protein mRNAs later on in life, although it is not known whether these changes are physiologically detrimental. Previous studies have emphasized the suppressive effects of TCDD on the size of the prostate. However, the present study suggested that exposure to low doses of TCDD in the neonatal period may affect the expression patterns of prostatic proteins.

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