Hydronephrosis induced in the kidney of neonatal mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) via lactation is a sensitive and characteristic hallmark of TCDD teratogenicity. We previously found that cyclooxygenase-2 (COX-2) activity induced in mouse neonate kidneys by lactational TCDD exposure is required for this toxicity. COX-2 is an inducible form of cyclooxygenase and is responsible for producing prostaglandins (PGs) and thromboxane. PGE_2, a prostaglandin, is elevated in TCDD-exposed mouse pups. In this study, we investigated the role of microsomal prostaglandin E synthase-1 (mPGES-1), an inducible form of PGE_2 synthase, in TCDD-induced hydronephrosis. A dose of 10 μg TCDD/kg to dams increased mPGES-1 messenger RNA abundance, urinary PGE_2 levels, and the incidence of hydronephrosis in mPGES-1 wild-type pups. In homozygous mPGES-1 knockout (KO) mice, in contrast, TCDD-induced hydronephrosis was suppressed, demonstrating an essential role of mPGES-1 in the response. Lack of the mPGES-1 gene also suppressed urinary PGE_2 level to near the basal level in TCDD-exposed pups. In conclusion, mPGES-1 upregulation upon lactational TCDD exposure is a causal factor for TCDD-induced hydronephrosis in mouse neonates.

Key Words: dioxin; kidney; developmental toxicity.

Dioxins, a group of halogenated aromatic hydrocarbons, are ubiquitously present in the environment and bioaccumulate in wildlife and humans via the food chain (Schecter and Gasiewicz, 2003). Because of its potent toxicity and persistence in humans, a health risk assessment of dioxins has been performed nationally and internationally (JECFA, 2001; Liem et al., 2000). Dioxin toxicity includes reproductive toxicity, teratogenicity, neurobehavioral disorders, immune dysfunction, and carcinogenicity (Pohjanvirta and Tuomisto, 1994). The majority of toxicity is mediated by aryl hydrocarbon receptor (AhR), a transcription factor. The essential nature of AhR for dioxin toxicity was revealed by experiments using AhR-null mice, in which no such toxicity was produced (Gonzalez and Fernandez-Salgadero, 1998; Mimura et al., 1997; Schmidt et al., 1996). Although some of the genes required to elicit dioxin toxicity have recently been found, the detailed mechanisms of dioxin toxicity are largely unknown (Yoshioka et al., 2011).

AhR binds dioxin-like compounds to be activated, and the liganded AhR translocates from the cytoplasm to the nucleus to transactivate the expression of genes with the xenobiotic responsive element (also named dioxin-responsive element or AhR-responsive element), in their promoter region. AhR genetic polymorphisms have been shown to result in different binding affinities for dioxin-like compounds and dioxin toxicity responsiveness in mice (Poland and Glover, 1980; Poland et al., 1994). The AhR^b1 and AhR^b2 alleles are carried by the C57BL/6 and BALB/c strains, respectively, and have a similar affinity to dioxins. In contrast, the AhR^d allele is found in the DBA/2 and 129/Sv strains, which makes these strains more resistant to dioxin toxicity.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most potent congener among the dioxins, induces hydronephrosis and cleft palate in mice. Hydronephrosis is a more sensitive indicator of TCDD-induced teratogenicity in that it occurs at doses where there is no palatal clefting (Couture et al., 1990; Moore et al., 1973; Thomae et al., 2004). Cross-fostering studies reveal that the kidney is more sensitive to TCDD-induced hydronephrosis caused by postnatal exposure than by prenatal exposure in mice (Moore et al., 1973) and rats (Nishimura et al., 2006), although similar incidence in these two periods of exposure was reported in mice (Couture-Haws et al., 1991a). The critical period of hydronephrosis induced by lactational TCDD exposure is estimated to be the first few days after birth (Couture-Haws et al., 1991b) and thus is a developmental stage–specific toxicity.

We previously found that AhR-dependent upregulation of cyclooxygenase-2 (COX-2) plays a key role in the onset of...
hydrounephrosis induced by lactational TCDD exposure in mice. COX-2 upregulation by TCDD was demonstrated to be AhR-dependent in vivo, and the imperative role of COX-2 was experimentally demonstrated in which TCDD-induced hydrounephrosis was eliminated with the administration of a COX-2 selective inhibitor (Nishimura et al., 2008). The role of COX-2 was further confirmed by the ability of lithium administration, a COX-2 inducer (Rao et al., 2004, 2005), eliciting hydrounephrosis in mouse neonates (Yoshioka et al., 2009). COX-2 is an inducible form of cyclooxygenase, which catalyzes the first step in the conversion of arachidonic acid to prostanooids, consisting of prostaglandins (PGD\(_2\), PGE\(_2\), PGF\(_2\alpha\), and PGL\(_2\)) and thromboxane (TXA\(_2\)). The pivotal role of COX-2 in TCDD-induced hydrounephrosis indicates that at least one of these prostaglandins and TXA\(_2\) should be involved in this toxicity phenotype.

In the present study, we investigated the possible role of microsomal prostaglandin E synthase-1 (mPGES-1), an inducible form of PGE\(_2\) synthase, in the pathogenesis of TCDD-induced hydrounephrosis in mouse neonates using mPGES-1 null mice. We found that the lack of the mPGES-1 gene suppressed an overproduction of PGE\(_2\), the predominant prostaglandin in the kidney (Antonucci et al., 2007), caused by the lactational TCDD exposure. In addition, homozygous mPGES-1 KO mouse neonates treated with TCDD mimicked the pharmacological inhibition of COX-2 (Nishimura et al., 2008), which resulted in the suppression of TCDD-induced hydrounephrosis.

**MATERIALS AND METHODS**

**Animals and treatment.** TCDD (50 µg/ml in n-nonane) was purchased from Cambridge Isotope Laboratory (Andover, MA) and was diluted in corn oil (Wako Pure Chemicals, Osaka, Japan). Corn oil containing 1% n-nonane was used as the vehicle.

The C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan), and mPGES-1 KO mice and their wildtype littermates were genotyped by PCR to amplify the genomic DNA from the tails, with the following primer sequences: CAGTATTACGGAGTGACCGAGTTGACTGCA and CCCCTCGGCCCAGGTGTTTTCAGCAGATGTC for mPGES-1 WT; ATCTGCGTCTATGCTGCTGAG and GAGAAAACCTCCTCCCGGAGATTCCTCAGCAGTGTGAGGACTTGTACAG and CACAGGATGATGATGGCAAGCCGAGTTCAG for AhR\(^b1\) (C57BL/6J); and ATATAAAAAATCTGACGTGTACAA and GCTGCCTGCTGCAAGGAGGTTCAT for AhR\(^b2\) (BALB/c).

**Histology of the kidney.** Kidney specimens were fixed in 10% neutral buffered formalin solution, cryoprotected in 20% sucrose solution, embedded in optimum cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan), snap frozen in liquid nitrogen, and sliced to make 5-µm thick sections. The sections were stained with hematoxylin and eosin.

Severity scores of hydrounephrosis were assigned 0 for no signs of hydronephrosis and +4 for the most severe hydronephrotic kidney, utilizing a scoring system described previously (Bryant et al., 2001). The incidence of hydrounephrosis in the pups was determined by a severity score greater than or equal to 2 on either left or right kidney in accord with previous reports (Bryant et al., 2001; Theobald and Peterson, 1997).

**Quantitative reverse transcription-PCR.** Total RNA was isolated from the kidneys of mice using a column-based purification kit (FastPrep RNA kit; Takara Bio, Otsu, Japan). The complementary DNA (cDNA) synthesis of the messenger RNA (mRNA) was carried out using an oligo-dT\(_20\) primer and Super Script III (Invitrogen, Carlsbad, CA).

The quantitative detection of the gene expression level was performed using a LightCycler System (Roche Molecular Biochemicals, Indianapolis, IN) and SYBR Green-based premix (Thunderbird; Toyobo, Osaka, Japan). The primers for the genes (Supplementary table 2) were designed from the respective cDNA or mRNA sequences using Primer3 software (Rozen and Skaletsky, 2000). The negative controls were analyzed concomitantly to confirm that the samples were not cross-contaminated. To verify the amplification specificity, melting curve analyses of the products were performed in every PCR. The mRNA expression levels were calculated by the ΔC\(_T\) method and normalized using cyclophilin B.

**PGE\(_2\) measurement.** Urine was collected from the neonatal mouse bladder on PND 7. Urinary PGE\(_2\) concentrations were measured using an enzyme immunoassay kit according to the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI).

**Statistical analysis.** To minimize the possible litter effects, the data for individual pups of the same categories, such as sex, age, genotype, and TCDD dose, were averaged within a litter and then averaged among litters. The data are expressed as mean ± SEM for the number of litters. Differences in means between the groups were analyzed by one-way ANOVA followed by pairwise post hoc tests between treatments with Bonferroni’s correction. p values less than 0.05 were considered significant.

**RESULTS**

**Role of mPGES-1 in TCDD-Induced Hydrounephrosis**

Our initial attempt to use the mPGES-1 KO strain with a BALB/c background was not successful because a high dose of TCDD (80 µg/kg) did not induce hydrounephrosis in BALB/c WT mice (data not shown). This observation was unexpected for two reasons. BALB/c has a dioxin-sensitive receptor isofrom, AhR\(^b2\) (Poland and Glover, 1980; Poland et al., 1994). Second, this dose was eight times higher than the one sufficient to induce hydrounephrosis in another dioxin-sensitive strain C57BL/6J (Nishimura et al., 2008) that has the AhR\(^b1\) receptor isofrom (Poland and Glover, 1980; Poland et al., 1994). In extensive trials to determine a condition to induce hydrounephrosis in the mPGES-1 strain, we mated the female mPGES-1 KO mice (F0, mPGES-1(+/−)) with C57BL/6J male mice (F0, mPGES-1(+/+)) to produce hybrid mice (F1,
mPGES-1(+/−)) and subsequently mated the F1 hybrid mice (F1, mPGES-1(+/−)) to each other. After delivery, the dams were administered TCDD (10 \( \mu \)g/kg) on PND 1, and consequently, the mPGES-1(+/+) and mPGES-1(−/−) pups were exposed to TCDD lactationally. In the TCDD-exposed mPGES-1(+/+) pups, a mild degree of hydronephrosis (score 2) and a mild to severe degree of hydronephrosis (score 2–3) were observed on PNDs 7 and 21, respectively (Table 1, Supplementary table 1, and Fig. 1), whereas no hydronephrosis (score 0) or a negligible degree of hydronephrosis (score 1) was observed in vehicle control groups (Table 1). The incidence of hydronephrosis in this study was comparable to previous studies using C57BL/6J (Nishimura et al., 2008) and C57BL/6N (Couture-Haws et al., 1991a). In contrast, lactational TCDD exposure did not induce mild to severe hydronephrosis (score 2–3) in mPGES-1(−/−) mice (Table 1, Supplementary table 1, and Fig. 1).

To analyze the possible relationship between TCDD responsiveness and AhR isoforms, the determinant of dioxin toxicity, the AhR isoform of the pups was analyzed on PND 21. The averaged severity scores for the pups with AhR\textsubscript{b1/b1}, AhR\textsubscript{b1/b2}, and AhR\textsubscript{b2/b2} were 1.6, 1.8, and 2.0, respectively. These results demonstrate no discernible difference in the degree of hydronephrosis between the AhR isoforms, b1 and b2 types. Therefore, the resistance to TCDD-induced hydronephrosis observed in the parental BALB/c strain was not attributable to the AhR\textsubscript{b2} isoform. The reason why BALB/c mice were resistant to dioxin-induced neonatal hydronephrosis shall be reported elsewhere.

Table 1

<table>
<thead>
<tr>
<th>PND (^{a})</th>
<th>Dose (( \mu )g/kg)</th>
<th>mPGES-1 genotype</th>
<th>( N^{b} ) (pup (dam))</th>
<th>Severity (^{c})</th>
<th>Incidence (^{d}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0</td>
<td>+/+</td>
<td>3 (3)</td>
<td>0 1 0 0 0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+/+</td>
<td>5 (4)</td>
<td>0 1 4 0 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>+/+</td>
<td>16 (7)</td>
<td>11 5 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>−/−</td>
<td>15 (7)</td>
<td>11 4 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+/+</td>
<td>19 (15)</td>
<td>0 5 12 2 67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Dams were treated once by gavage on PND 1, and pups were analyzed for hydronephrosis on PNDs 7 or 21.

\(^{b}\)The number of pups and dams in each group.

\(^{c}\)The number of pups of each severity score.

\(^{d}\)Incidence of hydronephrosis with the normalization regarding litters to minimize possible litter effects.
with the substantial increase in renal mPGES-1 mRNA induced by TCDD (Fig. 2B and Supplementary fig. 1B). The detection, albeit very slight, of mPGES-1 mRNA in the mPGES-1(+/−) mice reflects a truncated mPGES-1 mRNA, which produces an enzymatically inactive mutant protein (Uematsu et al., 2002). No alteration in the mRNA levels of the other PGES genes, mPGES-2 and cPGES, was observed in response to TCDD (Figs. 2C and D and Supplementary figs. 1C and D).

**Alterations in Kidney Gene Expression Associated With TCDD-Induced Hydronephrosis**

We quantified the mRNA levels of several genes in the hydronephrotic kidneys. Although TCDD increased mRNA levels of CYP1A1 and AhRR, which are marker genes for AhR transactivation activity, such increases were independent of the mPGES-1 gene (Figs. 3A and B, and Supplementary figs. 2A and B). This result demonstrates that AhR transactivation in response to TCDD in the mice used in the present study is as sensitive as that in the C57BL/6 strain reported previously (Couture-Haws et al., 1991a,b; Nishimura et al., 2008). Another TCDD-responsive gene, cPLA2α (Kinehara et al., 2009; Sciullo et al., 2008), encodes a member of the phospholipase A2 family, which functions by providing arachidonic acid to COX-2. The mRNA of this gene was upregulated similarly in kidneys of TCDD-exposed mPGES-1(+/+) and mPGES-1(−/−) mice (Fig. 3C). In contrast, a discernible increase in the COX-2 mRNA level by TCDD exposure was mPGES-1 gene dependent, though a small but significant level of TCDD-induced upregulation in mPGES-1(−/−) mice was observed (Fig. 3D and Supplementary fig. 2D). The elevated COX-2 gene expression by lipopolysaccharide (LPS) stimulation was reported to be mPGES-1 dependent (Kamei et al., 2004), suggesting that its activity mediates COX-2 induction as a mechanism common to TCDD and LPS stimulation.

Next, we determined whether mPGES-1 gene expression was involved in the gene expression of a tyrosine kinase c-Src, inflammatory cytokines, and electrolyte transporters in the kidney of the TCDD-exposed neonates. Our earlier study (Nishimura et al., 2008) revealed that expression of these genes was altered in TCDD-exposed C57BL/6 mice. In the mPGES-1(+/+) male mice, mRNA levels of c-Src, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α were significantly increased by TCDD (Figs. 4A, B, and C), whereas in TCDD-exposed mPGES-1(−/−) mice, c-Src and TNF-α mRNA levels were almost equivalent to the control group (Figs. 4A and C), and TCDD induced a small but significant increase in IL-1β (Fig. 4B). We observed increasing tendency in IL-6 mRNA level in TCDD-exposed mPGES-1(+/+) male mice. In the female pups, similar results with those in males were obtained, with a significant increase in IL-6 mRNA level in TCDD-exposed mPGES-1(+/+) mice (Supplementary figs. 3A, B, C, and D). These results indicate that TCDD-induced upregulation of c-Src, IL-1β, TNF-α, and possibly IL-6 depends on the mPGES-1 gene. mRNA levels of the electrolyte transporters NKCC2 (Fig. 4E) and ROMK (Fig. 4F) exhibited a decreasing tendency in the TCDD-exposed mPGES-1(+/+) mice compared with control. This tendency was also observed in TCDD-exposed female mouse neonates (Supplementary figs. 3E and F), and a significant reduction in the mRNA levels of these
transporters by TCDD exposure was observed in our previous study (Nishimura et al., 2008) and in the MMDD1, cell line derived from mouse kidney (Dong et al., 2010). In the mPGES-1(C0/C0) mice, TCDD did not induce any change in the mRNA levels of these transporters.

DISCUSSION

The most significant finding in the present study is that mPGES-1 is responsible for a TCDD-induced increase in PGE2 in urine and that suppression of the PGE2 increase in urine by genetic ablation of mPGES-1 fully protects mouse neonates from the onset of TCDD-induced neonatal hydronephrosis (Table 1). Together with our previous finding that TCDD induction of COX-2 is required for the development of hydronephrosis (Nishimura et al., 2008), it is concluded that abnormal activation of the PGE2 production pathway consisting of COX-2/mPGES-1/PGE2 causes TCDD-induced hydronephrosis neonatally.

AhR, mPGES-1, and Incidence of Hydronephrosis in Mice

To elucidate the mechanisms of toxicity in transgenic animal experiments, the identity of the background genetic trait of the transgenic and its corresponding WT strains assures that a given endpoint is induced only by the transgene but not by the background genetic trait. For this purpose, genetically homogeneous animals are usually employed.

In our study, however, a different approach was taken. We used the F2 hybrid of the C57BL/6J and BALB/c strains and carefully examined the validity of the study results. The mice used in the present study possessed either AhRb1/b1, AhRb1/b2, or AhRb2/b2. The observation of nearly identical inductions of mRNAs of CYP1A1 and AhRR genes (Fig. 3), the indicator for AhR activation in these mouse pups, is consistent with the earlier finding that mouse strains with AhRb1/b1 and those with AhRb2/b2 are equally sensitive to TCDD (Poland and Glover, 1980; Poland et al., 1994). In addition, the incidence of hydronephrosis in the F2 hybrid of the C57BL/6J and BALB/c strains in the present study was similar to the incidence of TCDD-induced hydronephrosis in the C57BL/6J strain in our previous study (Nishimura et al., 2008). Therefore, all of the molecules, including AhR isoxforms and COX-2, required for the onset of hydronephrosis were considered functional in the pups that we used. All of the pups used in this study shared the mPGES-1 gene derived from an identical origin because the male mPGES-1(+/+) mice of the C57BL/6J strain were mated to the female mPGES-1(+/C0) mice of the BALB/c strain to produce the F1 hybrid of heterozygous mPGES-1 KO mice. Therefore, the genetic factors responsible for the onset of TCDD-induced neonatal hydronephrosis are thought to have similar functions among the pups. Consequently, the mice used in this study are considered appropriate for the study of the role(s) of mPGES-1 in TCDD-induced hydronephrosis.

TCDD-Induced Increase in PGE2 Production by mPGES-1 and COX-2

There are three important findings regarding mPGES-1 and PGE2. First, TCDD exposure upregulates mPGES-1 expression (Fig. 2B). Second, mPGES-1 has a prominent role in the TCDD-induced increase in urinary PGE2 production, which was demonstrated by lack of the mPGES-1 gene suppressing the TCDD-induced increase in urinary PGE2 concentration (Fig. 2A). Third, an increase in PGE2 production is the cause of the TCDD-induced hydronephrosis. This conclusion was drawn from the observation that suppression of the TCDD-induced increase in PGE2 production by mPGES-1 genetic ablation prevents the onset of TCDD-induced hydronephrosis (Table 1 and Fig. 2). The importance of mPGES-1 as the PGE2 synthase is supported by the fact that the other PGE2 synthases, mPGES-2 (Fig. 2C) and cPGES (Fig. 2D), did not respond to TCDD, although the slight and nonsignificant increase in urinary PGE2 concentration by TCDD in mPGES-1(+/C0) mice (Fig. 2A) could be attributable to cPGES and/or mPGES-2.

The action of mPGES-1 is considered functionally associated with COX-2 for three reasons. First, COX-2 converts arachidonic acid to PGG2/PGH2, providing a substrate for PGE2 synthases, including mPGES-1. Second, COX-2 was
colocalized with mPGES-1 in the macula densa and medullary interstitial cells in the kidney (Schneider et al., 2004). Third, coexpression experiments demonstrate that mPGES-1 is coupled with COX-2, in preference to COX-1, for PGE2 production (Murakami et al., 2000). Therefore, activation of the COX-2/mPGES-1 pathway is considered responsible for the TCDD-induced increase in PGE2 production.

Potential Roles of Other Prostanoids and Positive Feedback Loop of PGE2 Production

The previous finding that COX-2 is indispensable for TCDD-induced neonatal hydronephrosis (Nishimura et al., 2008) has raised a question whether any of the prostanoids, PGD2, PGE2, PGF2α, PGI2, or TXA2, can be responsible for TCDD-induced neonatal hydronephrosis. The present study has revealed that PGE2 is responsible for the pathogenesis of TCDD-induced hydronephrosis. However, the potential roles of other prostanoids are largely unknown. Regulation mechanism of COX-2 gene expression could be involved in the potential roles of other prostanoids in the pathogenesis of hydronephrosis. That is, COX-2 expression was significantly suppressed in the absence of the mPGES-1 gene (Fig. 3C), suggesting that COX-2 expression is regulated by PGE2, the product of mPGES-1. Therefore, mPGES-1 and PGE2 could have responsible roles in upregulating the COX-2, which in turn upregulates the production of other prostanoids potentially involved in eliciting hydronephrosis. The precise mechanism of action of PGE2 in the pathogenesis of hydronephrosis will be clarified by identifying downstream molecules, which connect PGE2 either to pathophysiological changes leading to hydronephrosis or to COX-2 upregulation.

Alterations in Gene Expression in the Hydronephrotic Kidney

We have observed altered expression of a number of genes accompanied by TCDD-induced neonatal hydronephrosis in the C57BL/6J and BALB/c hybrid mouse strain (Figs. 3 and 4). These observations are consistent with our previous study using the C57BL/6J mouse strain (Nishimura et al., 2008). CYP1A1 was upregulated by TCDD regardless of the presence of the mPGES-1 gene and the pathogenesis (Fig. 3A and Table 1). This observation is consistent with CYP1A1 being unnecessary in TCDD-induced teratogenesis, including hydronephrosis (Dragin et al., 2006). The expression pattern of AhRR was equivalent to that of CYP1A1, indicating that CYP1A1 and AhRR belong to a group of genes, which are upregulated by TCDD, but not involved in the TCDD-induced hydronephrosis.

NKCC2 and ROMK are electrolyte transporters expressed at the apical side of distal tubules and involved in NaCl reabsorption in the kidney. These transporters play an important nephrotic role in concentrating urine and are associated with Bartter’s syndrome, which is characterized by a severe salt-wasting state with low blood pressure, metabolic alkalosis, hyperreninemia, and polyuria. Interestingly, mice with a loss of either of these transporters present with several disorders of water and electrolyte handling and hydronephrosis (Lorenz et al., 2002; Takahashi et al., 2000). The decreasing tendency in the NKCC2 and ROMK mRNA levels by TCDD exposure (Fig. 4) is consistent with the significant decrease observed in our previous results. That is, the TCDD-induced decrease in NKCC2 mRNA level depends on COX-2 activity as shown by a COX-2 specific inhibitor experiment (Nishimura et al., 2008). The loss of either the NKCC2 or ROMK gene results in the onset of hydronephrosis (Lorenz et al., 2002; Takahashi et al., 2000), and mice with a mutation in the NKCC2 gene that express a substantially lower level of NKCC2 also develop hydronephrosis (Kemter et al., 2010). Whether the reduced levels of these transporters caused by TCDD exposure correlates with a greater incidence and/or severity of hydronephrosis remains to be examined.

Tyrosine kinase c-Src was revealed to be required for TCDD-induced COX-2 upregulation both in vitro and in vivo (Vogel et al., 2000). Recently, the mechanism involved in the “nongenomic action” of AhR to induce COX-2 via c-Src has been elucidated (Matsumura, 2009). This mechanism resides in various cell types, including the MMD1 cell line (Dong et al., 2010), which is derived from mouse macula densa, in which a TCDD-induced increase in the COX-2 protein (Nishimura et al., 2008) and the presence of mPGES-1 (Schneider et al., 2004) are observed. These findings suggest a potential role of c-Src as the upstream mediator for TCDD-induced COX-2 upregulation in the hydronephrotic kidneys. Our present study indicates that the COX-2/mPGES-1/PGE2 pathway contributes to the TCDD-dependent induction of c-Src (Fig. 4). The mutual dependence of c-Src and COX-2/mPGES-1/PGE2 suggests that a positive feedback loop consisting of c-Src and COX-2/mPGES-1/PGE2 may exist. This putative loop is considered to amplify the response to TCDD. Consequently, the more sensitive response of hydronephrosis than cleft palate, the other well-known teratogenic effect of TCDD in the mouse (Couture et al., 1990; Moore et al., 1973; Thomae et al., 2004), may be explained. Similarly, IL-1β and TNF-α have been shown to act as an upstream mediators of PGE2 production in cells derived from kidney (Benador et al., 1995; Sawano et al., 2002; Sola-Villa et al., 2006), and their upregulation by TCDD is dependent on the COX-2/mPGES-1/PGE2 pathway (Fig. 4). Therefore, c-Src, inflammatory cytokines, COX-2, and mPGES-1 seem to construct a complicated network in the mouse kidney and may regulate the response to TCDD. The mechanism of dioxin toxicity in the developing kidney of mouse neonates will be unraveled by elucidating how this network is regulated.

Collectively, we conclude that PGE2 overproduction by the COX-2/mPGES-1 pathway is responsible for the onset of the TCDD-induced neonatal hydronephrosis in mice. Which types of PGE2-receptor and corresponding downstream effectors are responsible for the onset of TCDD-induced neonatal hydronephrosis remain to be examined. The downstream effectors...
could be unknown molecules or TCDD-responsive transporters, such as NKCC2 and ROMK.

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