Aryl Hydrocarbon Receptor Expression and Activity in Cerebellar Granule Neuroblasts: Implications for Development and Dioxin Neurotoxicity

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INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent teratogen that produces neurobehavioral abnormalities associated with both cognitive and locomotor systems, yet the precise regional and cellular targets of developmental neurotoxicity remain largely unknown. Most, if not all, TCDD-induced pathology is mediated via binding to the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) superfamily. Upon ligand binding, AhR translocates to the nucleus, dimerizes with the AhR nuclear translocator protein (Arnt), and regulates transcription by interaction with dioxin-response elements (DREs) in target genes, most notably specific cytochrome P450 (CYP) family members.

To assess whether developing cerebellar granule neuroblasts are potential direct targets for TCDD toxicity, AhR expression and transcriptional activity were examined. AhR and Arnt proteins were present in mouse cerebellum from birth throughout postnatal development. AhR protein levels peaked between postnatal day (PND) 3–10, a critical period for granule neuroblast growth and maturation. Transcriptionally active AhR was detected in immature cerebellar granule cells in a transgenic dioxin-responsive lacZ mouse model after acute TCDD exposure. AhR and Arnt were also expressed in cerebellar granule neuroblast cultures. AhR localized to the nucleus in granule cells 15 min after TCDD treatment. TCDD elicited time-dependent and concentration-dependent increases in CYP1A1 and IBI mRNA and protein levels. Moreover, TCDD treatment reduced both thymidine incorporation and granule neuroblast survival in a concentration-dependent manner. These data suggest that (1) granule neuroblasts are direct targets for developmental AhR-mediated TCDD neurotoxicity and (2) TCDD exposure may disrupt granule cell neurogenesis.

Key Words: neurogenesis; bHLH/PAS; Arnt; CYP 1A1/1BI; dioxin.

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interaction with the AhR, is also known to regulate the expression of a wide array of additional drug-metabolizing enzymes, genes that participate in cell cycle regulation, and inflammatory mediators (Lai et al., 1996; Nebert et al., 2000; Puga and Elferink, 2002). Despite the understanding of the molecular mechanisms by which TCDD modulates gene regulation, specific roles for AhR during brain development and in neurotoxicity are poorly understood.

Evidence at the cellular and molecular levels supports the contention that inappropriate AhR activation by xenobiotics during development could lead to neurotoxicity in the cerebellum. For example, prenatal exposure to 7,12-dimethylbenz[a]anthracene, an Ah-R ligand and a known carcinogen, was shown to profoundly disrupt cerebellar cytarchitecture (Kellen et al., 1976). At the molecular level, a recent study revealed that the cerebellum contained the highest levels of DRE binding in the adult rat brain and that indigo, a putative endogenous AhR ligand, stimulated DRE binding in cerebellar granule neurons (Kuramoto et al., 2003). Furthermore, gestational TCDD exposure was shown to modulate the developmental expression profile of Sp1, a transcription factor involved in growth and differentiation, in rat cerebellum and cerebral cortices (Nayyar et al., 2002), but the biological significance requires clarification. Although the developing cerebellum is potentially vulnerable to AhR-mediated neurotoxicity, the precise cellular localization and transcriptional activity of AhR in this brain region requires additional study.

Considering that AhR is present during critical phases of histogenesis in several organs (Abbott et al., 1995; Abbott and Probst, 1995) and is widely expressed throughout the adult central nervous system (CNS) (Petersen et al., 2000), it is conceivable that AhR might normally regulate neurogenesis and differentiation during brain development. It is hypothesized that perinatal exposure to TCDD disrupts endogenous AhR signaling events during brain development. This study determined that AhR is expressed and transcriptionally active in cerebellar neuronoblasts throughout a critical period of postnatal neurogenesis. Several processes, which include proliferation, migration, differentiation, synaptogenesis, and programmed cell death (apoptosis), occur during this time (Altman and Bayer, 1997; Goldowitz and Hamre, 1998; White and Barone, 2001). Furthermore, TCDD was shown to reduce DNA synthesis and cell survival in a granule precursor cell model system that maintains the ability to proliferate in vitro. These observations suggest potential roles for AhR in cerebellar granule neuron maturation and raise the possibility that TCDD might interfere with these actions by displacing an endogenous ligand from its normal developmental functions.

**MATERIALS AND METHODS**

**Reagents.** 2,3,7,8-tetrachlorodibenz-p-dioxin (TCDD) was obtained from Cambridge Isotopes (Cambridge, MA) and solubilized in dimethyl sulfoxide (DMSO). Triton X-100, Bovine Serum Albumin (BSA), and DMSO were purchased from Sigma (St. Louis, MO). Dulbecco’s phosphate buffered saline (DPBS), minimal essential medium (MEM), B27, and N2 supplements were purchased from Gibco (Grand Island, NY). Supplies for RNA isolation and DNA synthesis were obtained from Invitrogen (Carlsbad, CA). Trypsin and DNase were purchased from Worthington (Lakeewood, NJ).

**Experimental animals.** All animals were maintained on a 12-h light/dark cycle with food and water provided ad libitum and kept in accordance with the guidelines set by the University of Rochester University Committee on Animal Resources and the American Association for Laboratory Animal Science. Five- to six-day-old C57Bl/6 J mice were purchased from Jackson Laboratories (Bar Harbor, ME). AhR+/– mice modified at exon 2 (Schmidt et al., 1996) were also obtained from Jackson Laboratories and backcrossed onto the wild-type C57Bl/6 J background for at least 10 generations. C57Bl/6 J mice were purchased from Jackson Laboratory, and DRE-lacZ animals were generated as previously described (Willey et al., 1998). Animals were generated using the p21lac plasmid construct, which consists of 2 DRE-Ds, a TATA box, lacZ reporter, and the SV40 intron and polyadenylation signal. Mice were maintained as a heterozygous colony backcrossed onto wild-type C57Bl/6 J background for greater than 10 generations. Polymerase chain reaction (PCR) analysis of tail DNA was performed to determine transgene status of animals.

**In vivo TCDD treatment.** DRE-lacZ mice were injected with 30µg TCDD/kg dissolved in olive oil or vehicle alone when AhR protein is at peak levels on postnatal day 10 in the developing cerebellum. This dose has been previously shown to produce maximal induction of aryl hydrocarbon hydroxylase activity in C57Bl/6 J mice (Poland and Glover, 1975). Animals were perfused 18–24 h later with saline, followed by 4% paraformaldehyde. This time period after TCDD exposure was previously reported to be sufficient for detection of β-galactosidase activity in tissue sections obtained from transgenic animals (Nazarenko et al., 2001).

**β-galactosidase staining.** In situ localization of β-galactosidase (β-gal) activity was determined on a histochemical substrate, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). After perfusion, whole brains were fixed in 4% paraformaldehyde overnight. Brains were transferred to 30% sucrose for 24 h. Cerebella were cut into 30-µm sagittal sections with a freezing sliding microtome. Sections were incubated with X-gal for 16 h at 37°C in a humidified chamber and then rinsed with DPBS before mounting. Wild-type animals were injected with olive oil to evaluate endogenous β-galactosidase activity.

**Cell culture.** Primary granule cell cultures were prepared as previously described (Gao et al., 1991; Opanashuk and Hauser, 1998; Opanashuk et al., 2001). Briefly, cerebella were quickly dissected from postnatal day 5–6 C57Bl/6 mice and the meninges were removed prior to the addition of trypsin and DNase. Dissociated cells were then passed through a 30-µm nylon mesh (Spectrum Laboratories) and centrifuged through a 35%/60% Percoll gradient (Amersham Biosciences, Piscataway, NJ). Cells were then rinsed and preplated for 90 min in poly-t-lysine (0.1 mg/ml; Sigma, St. Louis, MO) coated flasks. Cells were then resuspended in MEM containing 10% horse serum, 5% fetal bovine serum, glucose (9 mg/ml), glutamine (292 mg/ml), and penicillin (0.1%). Cells were plated in 96-well plates at a density of 3 × 10^4 cells/well and maintained in a humidified atmosphere of 5% CO2/95% air at 37°C to facilitate reaggregate formation. After 24 h, reaggregates were then transferred to serum-free MEM (SFM) containing B27 and N2 supplements, glucose (9 mg/ml), glutamine (292 mg/ml), and penicillin (0.1%), and plated on high molecular weight poly-t-lysine–coated plates (0.1 mg/ml). This time point is designated day in vitro 1 (DIV 1). The characterization of these cultures, as previously described (Gao et al., 1991) by morphological and immunocytochemical criteria, indicated that >98% of these cells were of the granule neuron lineage.

**Western blot analysis.** Cerebellar tissue or cells were homogenized in phosphate buffered saline (PBS) containing 0.1% Triton X-100 and antiprotease cocktail (Roche Molecular Biochemical, Manheim, Germany). Total protein concentrations were determined by the microBCA assay (Pierce, Rockford, IL). Proteins (20–75 µg) were fractionated on 7% acrylamide gels and transferred to Immobilon-PVDF membranes (BioRad, Hercules, CA). Membranes were
standards and samples were run in duplicate.

36 h and cells were labeled with 2

et al, 1991; Opanashuk and Hauser, 1998; Opanashuk,

measured by procedures similar to those previously described (Gao et al., 1991; Opanashuk and Hauser, 1998; Opanashuk et al., 2001). After the filters dried, thymidine labeling was measured by liquid scintillation counting. At least four independent determinations from separate cell preparations were evaluated for each treatment.

Cell survival assay. Neuron viability was assessed with a commercially available kit (Live/Dead Cytotoxicity Kit, Molecular Probes, Eugene, OR), as previously described (Opanashuk and Hauser, 1998; Opanashuk et al., 2001). EGL reaggregates were transferred to SFM and plated onto poly-l-lysine–coated glass coverslips at the same densities used for the thymidine incorporation. After 24 h in SFM, EGL cells were treated with TCDD for 24 h. Cultures were then rinsed three times with DPBS (Gibco BRL) and incubated for 40 min at 34–35°C in DPBS containing ethidium homodimer and calcein-AM. Ethidium binds to DNA in dead neurons and emits red fluorescence, while calcein-AM is converted by esterases within living cells and emits a green fluorescence. The proportion of surviving neurons was determined as a percentage of the number of live + dead neurons. About 800 neurons were counted in each culture using a Nikon Eclipse T100 fluorescence microscope (40×) with the experimenter blinded to the conditions. At least four cultures were evaluated for each experimental treatment and each culture consisted of cells pooled from separate litterers.

Statistical analyses. Data were expressed as mean ± standard error of the mean (SEM). All experiments were completed at least four times. Data were analyzed by analysis of variance (ANOVA) with Statview (version 5.0). The Fisher’s post hoc test was used for individual comparisons.

RESULTS

AhR and ARNT Proteins Are Expressed in the Cerebellum During a Critical Period of Neuron Development

To determine whether the developing cerebellum is a potential target for AhR-mediated gene expression or TCDD toxicity, cerebellar homogenates were analyzed for AhR and Arnt protein content between day of birth (PND 0) and adulthood by Western blot analysis. Both AhR and Arnt proteins were expressed throughout postnatal development and in adult cerebellum (Fig. 1). AhR protein levels peaked during PND 3–10, a critical period of granule neuron development (Altman and Bayer, 1997; Goldowitz and Hamre, 1998). Arnt protein was detected on PND 3 and expression was maintained at similar levels from

Thymidine incorporation. Thymidine incorporation into DNA was measured by procedures similar to those previously described (Gao et al., 1991; Opanashuk and Hauser, 1998; Opanashuk et al., 2001). After reaggregation for 24 h, EGL cells were transferred to SFM. TCDD was added to cultures for 36 h and cells were labeled with 2 μCi/ml 3H-thymidine for the last 12 h of the

FIG. 1. AhR and ARNT proteins are expressed during mouse postnatal cerebellar development. Mouse cerebellar homogenates (50 μg) were examined for AhR and ARNT protein expression between day of birth (PND 0) and adulthood by immunoblot analyses. Hepa1c17 (Hepa) protein lysates (5 μg) served as a positive control. Similar results were obtained in cerebellar lysates prepared from 4–5 separate litters.
PND 3 throughout adulthood. Arnt, but not AhR, was detected in the cerebellum of AhR −/− animals (data not shown). These findings suggest that the developing cerebellum is a potential target for AhR-mediated TCDD neurotoxicity.

**Transcriptionally Active AhR is Localized to Developing Granule Neurons in TCDD-Responsive lacZ Reporter Mice**

The transgenic lacZ-DRE mouse model provides a unique system to determine the temporal and spatial expression of transcriptionally active AhR following agonist exposure in vivo (Willey et al., 1998). To identify cell types in which AhR is activated to a transcriptionally functional form in the cerebellum during a critical period of granule neuron development, transgenic LacZ-DRE mice were injected with oil or 30 μg/kg TCDD on PD 10 and analyzed for activity 18 h later. This TCDD dosing regimen was chosen to ensure maximal responsiveness of AhR transcriptional activity (Poland and Glover, 1975). Following TCDD exposure, β-gal activity was detected within pre-migratory granule neuron precursors in the external granule cell layer (EGL) and in molecular layer (ML) of the cerebellum (Fig. 2B, D). Less intense staining was also detected within the internal granule cell layer (IGL). Staining was not observed in the Purkinje cell layer. In addition, β-gal staining was present in deep cerebellar nuclei and in perivascular cells located throughout the cerebellum. Endogenous β-gal activity was not detected within the vehicle control treated lacZ-DRE (Fig. 2A, C) or wild-type (data not shown) mouse cerebellum. As previously reported (Willey et al., 1998), endogenous β-gal activity was detected in the choroid plexus of vehicle control lacZ-DRE (Fig. 2A) and wild-type animals (data not shown). Together, these data indicate that AhR is present and can become transcriptionally active in cerebellar granule neurons after agonist binding.

**AhR and ARNT Proteins Are Expressed by Cerebellar Granule Neuroblasts In Vitro**

Although developing granule neurons contained transcriptionally active AhR and Arnt in vivo, it is not known whether expression of these molecules is retained in cultured granule neuroblasts. Primary granule neuroblast reaggregate cultures were analyzed to confirm that both AhR and Arnt are present
in vitro. AhR and Arnt mRNA were detected in granule neuroblasts by RT-PCR (data not shown). Western blot analyses indicated that both AhR and Arnt proteins were expressed in cerebellar granule cells that were cultured for 3 days (Fig. 3A). Additionally, AhR was not detected in cultures prepared from AhR null mice (Fig. 3B), but was present in wild-type (WT) neuroblasts (Fig. 3B). These findings indicate that cerebellar granule neuroblasts are potentially direct targets for AhR-mediated TCDD neurotoxicity.

AhR Undergoes Nuclear Localization After TCDD Treatment

Previous investigations in a variety of cell types indicate that AhR resides primarily within the cytosol and upon ligand binding, it translocates to the nucleus and dimerizes with Arnt (Gu et al., 2000). To determine whether AhR is activated in cultured cerebellar granule neurons, the cellular localization of AhR cultures was resolved by immunocytochemistry under both basal conditions and after treatment with TCDD for time intervals ranging from 15 to 60 min. AhR was typically present within the cytosol of both untreated and vehicle control-treated granule cells (Fig. 4A, E). After treatment with 10 nM TCDD for 15 min on DIV 3, AhR expression co-localized with the nuclear DAPI stain (Fig. 4B, F), indicating that the receptor translocated to the nucleus within 15 min and remained there for at least 60 min (data not shown). Occasional less intense nuclear staining was detected in untreated and vehicle control granule neurons.
TCDD Treatment Induces CYP1A1 and CYP1B1 mRNA and Protein Expression

After translocating to the nucleus and dimerizing with ARNT, the AhR heteromeric complex binds to DREs of target genes, such as certain members of the cytochrome p450 (CYP) family of xenobiotic metabolizing enzymes (Gu et al., 2000; Whitlock, 1999). To confirm that TCDD is transcriptionally active for endogenous genes present in cultured cerebellar granule neurons, CYP1A1 and CYP1B1 expression was evaluated after 10 nM TCDD treatment for various time intervals. CYP1A1 mRNA was induced 3–5-fold and peaked between 4 and 8 h after 10 nM TCDD treatment (Fig. 5A). CYP1B1 gene expression was significantly elevated 1.5–2-fold in response to 10 nM TCDD treatment (Fig. 5B). To determine whether elevated protein levels accompanied alterations in CYP gene expression, granule neurons were treated with 0.1–10 nM TCDD for 24 h, and CYP expression was analyzed by Western blot. Both CYP1A1 and CYP1B1 proteins were upregulated in a concentration-dependent manner after TCDD treatment (Fig. 6). CYP1A1 protein expression was slightly induced in vehicle (DMSO)-treated preparations (Fig. 6, upper panel); however, to a lesser extent than observed in TCDD-treated cells. Neither CYP isoform was induced by TCDD treatment in cultures prepared from AhR null mice (data not shown).

TCDD Decreases ³H-Thymidine Incorporation in Granule Neuroblasts

Prior to differentiating, dividing neuroblasts receive a signal to exit the cell cycle. To evaluate whether TCDD exposure affects DNA synthesis, ³H-thymidine incorporation into DNA was determined as previously described (Opanashuk and Hauser, 1998; Opanashuk et al., 2001). After a 24-h reaggregation period, granule neuroblasts were exposed to DMSO or TCDD 0.1–10 nM for 24 h then labeled with 2 µCi/ml ³H-thymidine for 12 h, resulting in a 36-h treatment period. Heparin-binding epidermal growth factor (HB-EGF; 10 ng/ml) served as a positive control for growth (Opanashuk and Hauser, 1998) and was added at the same time as TCDD. Whereas, 10 ng/ml HB-EGF stimulated a 50% elevation in thymidine incorporation, TCDD treatment reduced DNA synthesis by 10–30% in a concentration-dependent manner (Fig. 7).
TCDD Attenuates Granule Neuroblast Survival in a Concentration-Dependent Manner

During the normal course of development, granule neurons undergo a tightly regulated program of apoptosis (White and Barone, 2001). To ascertain whether TCDD affected granule precursor cell survival, EGL neuroblast viability was assessed in response to 0.1–10 nM TCDD after 24 h on DIV 2 using the live-dead assay (Molecular Probes, Eugene OR) as previously described (Opanashuk and Hauser, 1998; Opanashuk et al., 2001). As controls for the viability assay, cultures were treated with staurosporine for 24 h or lysed with 0.1% Triton-X-100, resulting in 100% neuronal death (data not shown). The live-dead markers did not overlap in the same cell, and all cells were labeled as living or dead. In general, there was a low percentage of cell death, ranging between 5% and 10%, in untreated or vehicle control cultures after the 24-h treatment period. However, TCDD treatment decreased granule neuroblast survival by 10–20% after a 24-h exposure (Fig. 8).

DISCUSSION

AhR and Arnt genes were recently identified in the internal granule cell layer of adult rat cerebellum (Petersen et al., 2000), yet the developmental expression of these proteins has not been examined. The observations in this study are the first to suggest potential roles for the AhR/Arnt complex during the course of cerebellar granule neuron development, which could be disrupted in response to TCDD exposure. Immunoblot analyses revealed that AhR and Arnt proteins were present in cerebellar tissue from birth throughout adulthood. Whereas Arnt levels remained constant throughout postnatal ontogeny, AhR expression was detected on the day of birth and peaked between PND 3 and PND 10 in cerebellar homogenates. AhR protein was upregulated during a critical window of granule neuron maturation, a time period in which several processes are coordinately regulated, including proliferation, migration, differentiation, and programmed cell death (apoptosis) (Altman and Bayer, 1997; Goldowitz and Hamre, 1998; White and Barone, 2001). The presence of both AhR and Arnt in cultured cerebellar granule cells indicates that these developing neuroblasts have the potential to be direct targets for AhR-mediated neurotoxicity in response to TCDD or related PAH xenobiotics.

For AhR to disrupt the molecular events that regulate brain development after TCDD exposure, the receptor must be transcriptionally functional. The data from this study strongly indicate that cerebellar granule neuroblasts are responsive to dioxin and that AhR is transcriptionally active. The transgenic DRE-LacZ reporter mouse (Willey et al., 1998) served as an ideal system with which to explore the spatial distribution of transcriptionally active AhR in the developing cerebellum. A maximally inducing dose of TCDD (Poland and Glover, 1975) administered on PND 10 revealed activation of the DRE-LacZ reporter gene in cerebellar granule neuroblasts residing in the EGL, molecular layer, and IGL, suggesting that an AhR-mediated signaling pathway was stimulated. The absence of detectable DRE-LacZ reporter gene activation in tissues from mice not treated with TCDD does not exclude the possibility that AhR normally participates in granule neuron development.
Only a single time point was examined in the present investigation, and the sensitivity of the exogenous LacZ gene may be lower than particular endogenous DRE-containing genes. Nevertheless, the DRE-LacZ reporter mouse will be useful to elucidate endogenous roles for AhR by examining the spatiotemporal expression of transcriptional activity throughout brain development. Furthermore, this transgenic model will facilitate identifying additional cellular and anatomical targets of AhR-mediated developmental TCDD neurotoxicity.

At the cellular level, ligand interaction stimulates AhR to translocate from the cytosol into the nucleus, where the receptor dimerizes with Arnt prior to activating gene transcription by binding to DREs (Gu et al., 2000). TCDD stimulates nuclear translocation of AhR in cultured granule neuroblasts rapidly following treatment. Interestingly, AhR was intermittently expressed in the nuclei of vehicle-treated and untreated control granule cells. This phenomenon has been previously been observed in other tissue types (Chang and Puga, 1998; Singh et al., 1996). It is not clear whether nuclear expression can be attributed to the presence of an endogenous ligand or a ligand-independent process. After entering the nucleus, AhR regulates the expression of several genes, termed the “Ah target gene battery” (Nebert et al., 2000), of which the best characterized are certain CYP metabolic enzymes. The CYP family members are heme-containing enzymes that are responsible for the metabolic oxidation of a wide variety of exogenous and endogenous substrates (Whitlock, 1999). Although TCDD was recently shown to induce CYP1A1 mRNA in adult rat cerebellar granule neurons (Huang et al., 2000), the developmental regulation of AhR target gene expression has not been examined at the cellular level. In the current study, TCDD stimulated concentration-dependent and time-dependent increases in CYP1A1 and IB1 mRNA in cerebellar granule neuroblast cultures. Elevated CYP1A1 and IB1 protein levels accompanied alterations in gene expression. These findings indicate that TCDD modulates CYP expression, most likely via interaction with the AhR, in developing cerebellar granule neuroblasts.

Although TCDD served as a means to examine the transcriptional activity of AhR by monitoring CYP 1A1 and IB1 expression, the altered profiles of these metabolic enzymes produced after environmental exposures could impair normal brain function. The CYP family members are heterogeneously distributed at modest levels in several brain regions (Hedlund et al., 2001), which suggests that the CNS participates in bioactivation or detoxification of environmental toxicants and the regulation of endogenous substances. Previous studies have indicated that the CYP isozymes normally participate in the metabolism of neurotransmitters, endogenous steroids, and neurosteroids in the brain (Miksys and Tyndale, 2002). Often inert exogenous or endogenous compounds are bioactivated to reactive metabolites by CYP1A1, which can lead to oxidative stress production and cell death (Nebert et al., 2000), potentially mechanisms by which TCDD could mediate neurotoxicity. The relationships between AhR-mediated CYP induction, neuronal development, and TCDD neurotoxicity obviously require further study.

The most compelling issues that emerge from this study are related to the impact of reduced granule neuroblast DNA synthesis and survival on cerebellar development and ultimately function. Cerebellar granule neuron precursors (GNP) originate in the rostral aspect of the rhombic lip, and then migrate laterally to form the external granule cell layer (EGL) on the dorsal surface of the cerebellum during the embryonic period in rodents (Altman and Bayer, 1997; Goldowitz and Hamre, 1998). During the postnatal period in rodents, GPNs proliferate in the outer EGL, leave the cell cycle, and move into the inner EGL before migrating through the molecular and Purkinje cell layers and settling in the IGL, where terminal differentiation and synaptogenesis occur. A tightly regulated spatiotemporal program of gene expression orchestrates these molecular events during granule neuron maturation. Considering that TCDD treatment decreased DNA synthesis and cell survival in granule neuroblast cultures, environmental exposure could influence final granule neuron numbers in the cerebellum by interfering with normal proliferative and apoptotic programs. Such disruption of granule neuron production could adversely affect cell interactions and neural circuitry formation, thereby leading to functional abnormalities.

Although it is premature to speculate about functional consequences of TCDD exposure, our observations are the first to demonstrate that AhR and ARNT proteins are expressed and transcriptionally active in cerebellar granule cells during a critical period of neuronal maturation. Most importantly, the reduction in DNA synthesis and cell survival in cultured granule neuroblasts after TCDD treatment leads to the speculation that TCDD may impede granule neuron production by diverting AhR from its endogenous function. Findings from ongoing and future studies will provide information regarding the mechanisms by which perinatal TCDD exposure affects neurogenesis, perhaps by dysregulating the expression of candidate genes involved in the normal proliferation, differentiation, and apoptosis programs during granule neuron development. Inappropriate activation of AhR after exposure to an environmental toxicant such as TCDD could interfere with the requisite gene profiles for neuronal maturation and disrupt the proper formation of brain circuitry, which might ultimately engender adverse neurobehavioral or neurological outcomes.

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