HHS Public Access

Author manuscript

J Biochem Mol Toxicol. Author manuscript; available in PMC 2018 December 28.

Published in final edited form as:

J Biochem Mol Toxicol. 2017 October ; 31(10): . doi:10.1002/jbt.21947.

TCDD induces UbcH7 expression and synphilin-1 protein degradation in the mouse ventral midbrain

Emmanuel González-Barbosa[1], **Alejandro Mejía-García**[1], **Elizabeth Bautista**[2], **Frank J. Gonzalez**[3], **José Segovia**[2], and **Guillermo Elizondo**[1]

[1]Departamento de Biología Celular, CINVESTAV-IPN, México D.F, México

[2]Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN, México D.F, México

[3]Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, MD, USA

Abstract

UbcH7 is an ubiquitin-conjugating enzyme that interacts with parkin, an E3 ligase. The UbcH7–parkin complex promotes the ubiquitination and degradation of several proteins via the 26S proteasome. Cellular accumulation of the UbcH7–parkin targets alpha-synuclein and synphilin-1 has been associated with Parkinson disease. In mouse liver, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, an aryl hydrocarbon receptor ligand, induces UbcH7 expression. Therefore, the aim of the present study was to determine whether 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induces *Ubch7* mRNA and UbcH7 protein expression in the mouse brain, to characterize the molecular mechanism, and the effect on synphilin-1 half-life. We found that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin promotes the aryl hydrocarbon receptor binding to *Ubch7* gene promoter as well as its transactivation, resulting in an induction of UbcH7 levels in the olfactory bulb, ventral midbrain, hippocampus, striatum, cerebral cortex, brain stem, and medulla oblongata. In parallel, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin promoted synphilin-1 degradation in an aryl hydrocarbon receptor-dependent way.

Keywords

aryl hydrocarbon receptor; midbrain; Synphilin-1; UbcH7

1 | INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a halogenated aromatic hydrocarbon and an environmental contaminant. Exposure to TCDD, the most toxic dioxin, results in several deleterious effects, including wasting syndrome, immunotoxicity, hepatotoxicity, teratogenicity, and cancer.[1] These effects are mediated by the aryl hydrocarbon receptor (AHR) a ligand-dependent transcription factor that is a member of the bHLH-PAS (basic-helix-loop-helix-Per-ARNT-Sim) superfamily. Upon binding to TCDD, AHR translocates from the cytoplasm to the nucleus and binds to xenobiotic response elements (XREs) located in the promoter regions of its target genes. The result is an upregulation of the expression of

Correspondence Guillermo Elizondo. Departamento de Biología, Celular, Cinvestav, Apto. 2508, México D.F., México. gazuela@cinvestav.mx.

a battery of genes encoding xenobiotic-metabolizing enzymes[2] as well as genes involved in liver development,[3] homeostasis of the immune system,[4–6] neurogenesis,[7] cholesterol and glucose metabolism,[8,9] and cell proliferation and apoptosis.[10]

Recently, we reported that induction of *Ubch7* gene expression through the activation of the AHR results in ubiquitin proteasome system-dependent p53 and c-Fos degradation in mouse liver and Hepa-1c1c7 cells.[11,12] UBCH7[1] is a ubiquitin-conjugating enzyme with the conserved catalytic domain UBC and is classified as an E2 class I enzyme.[13] UbcH7 is necessary for normal mouse development, and its inactivation results in abnormal placental development, causing a lethal phenotype.[14] UbcH7 interacts with parkin a ubiquitin E3 ligase that catalyzes the ubiquitination of several proteins including α-synuclein, Pael-R, Crel-1, and synphilin-1.[15] The ubiquitinated target protein is usually recognized and degraded by the 26S proteasome.[16] It was shown that the interaction between parkin and the UBCH7 is required for substrate ubiquitination.[17] UBCH7 transfers ubiquitin (Ub) to substrates identified by parkin, and therefore plays an important role in the degradation of several parkin targets, including those whose accumulation leads to dopaminergic cell death.[18,19] Accumulation of UBCH7–parkin substrates such as synphilin-1 in dopamine neurons has also been associated with Parkinson disease (PD).[20,21].

Because UbcH7 is essential for parkin function, the aim of the present study was to determine whether TCDD induces UbcH7 expression in the mouse brain, particularly in the ventral midbrain where dopaminergic neurons are housed, and, if so, to determine whether this induction results in degradation of synphilin-1.

2 | MATERIALS AND METHODS

2.1 | Materials

TCDD was purchased from AccuStandard (New Haven, CT). Phenylmethanesulfonyl fluoride (PMSF) and dimethyl sulfoxide (DMSO) were acquired from Sigma (St. Louis, MO).

2.2 | Animals and treatments

The development of *Ahr*-null mice was described previously.[[4] Wild-type (WT) littermates on a C57BL/6J background were used as controls. The animals were housed in a pathogen-free facility and fed with autoclaved Purina rodent chow (St. Louis, MO) with water available ad libitum. All animal procedures were performed according to the *Guide for the Care and Use of Laboratory Animals*, as adopted and enforced by the U.S. National Institutes of Health and the Mexican Regulation of Animal Care and Maintenance (NOM-062-ZOO-1999, 2001). Mice were distributed randomly into TCDD and vehicle treatment groups (N=3/group), and treatments were carried out as described previously.[22] TCDD was dissolved in corn oil, and a single dose of TCDD (250 μg/kg) or corn oil alone (vehicle) was administered by gavage to C57BL/6J WT and *Ahr*-null male mice (8–9 weeks

[1]UbcH7, also known as UbcM4, is the mouse ortholog of the human protein UBCH7, which is also known as UBE2L3. For practical purposes, we will refer to the E2 human protein and gene as UBCH7 and *UBCH7*, respectively, and the mouse protein as UbcH7 and its gene and mRNA as *Ubch7*.

old). Seven days later, the mice were anesthetized with pentobarbital and killed by decapitation. The brains were rapidly removed, and the olfactory bulb, ventral midbrain, hippocampus, striatum, cortex, stem, and medulla oblongata dissected out. Liver sections were also extracted. Brain and liver samples were snap frozen and stored at –70°C.

2.3 | Real-time quantitative PCR

Total RNA was prepared from mouse liver and dissected brain region sections using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Camarillo, CA). RNA was quantified spectrophotometrically at an optical density of 260. RNA integrity was evaluated by electrophoresis on 1% agarose gels. cDNA was prepared for the quantitative PCR from 2 μg of total RNA using the SuperScript First-Strand Synthesis kit (Invitrogen, Camarillo, CA) and oligo dT. PCRs were performed in a StepOne Real-Time PCR System (Applied Biosystems, Branchburg, NJ) and analyzed using the comparative threshold cycle (C_T) method. The mRNAs encoding *Ahr* and *Ubch7,* with 18S ribosomal RNA (rRNA, endogenous) were amplified in a single PCR reaction to allow for normalization of the mRNA data. The PCR reaction mixture contained 2 μL of cDNA, 1xTaqMan universal PCR master mix (Applied Biosystems, Branchburg, NJ) and 0.9 and 0.25 μM primers and probes, respectively. The primer and probe sequences used for *Ubch7* were as follows: 5′-TGCCAGTCATTAGTGCTGAAAACT-3′ (forward), 5′-GGGTCATTCACCAGTGCTATGAG-3′ (reverse), and probe (FAM): AAGACTGACCAAGTAATCC. The probes used for *Ahr* mRNA and the 18S rRNA were obtained from Applied Biosystems (Branchburg, NJ) with identification numbers Mm01291777_m1 and Mm00507222_s1, respectively.

2.4 | Western blotting

Brain sections were homogenized in buffer containing 20 mM HEPES, 350 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1% Non-idet P40, 0.5 mM DTT, 0.1% PMSF, and Mini cOmplete protease inhibitor cocktail (1 tablet/10 mL; Roche, Mannhein, Germany). The protein suspension was homogenized completely by sonication for 30 s. Protein concentrations were determined using the Bradford assay (Bio-Rad, Hercules, CA). Aliquots (40 μg) were solubilized in sample buffer (60 mM Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 20% glycerol, 2% mercaptoethanol, and 0.001% bromophenol blue) and subjected to 12% SDS polyacrylamide gel electrophoresis. Protein extracts were transferred to a nitrocellulose membrane using a mini trans-blot (Bio-Rad, Hercules, CA). The transfer was performed at a constant voltage of 80 V for 2 h in transfer buffer (48 mM Tris– HCl, 39 mM glycine, pH 8.3, and 20% methanol). Following the transfer, membranes were blocked overnight at 4°C in the presence of 2% nonfat dry milk and 0.5% bovine serum albumin (BSA) in blocking buffer (25 mM Tris–HCl, pH 7.5, 150 mM NaCl) and subsequently incubated at 4°C for 3 h with goat polyclonal anti-UbcH7 (1:2000; Abcam, Cambridge, MA), rabbit anti-synphilin-1 (1:500; Santa Cruz Biotechnology, Visalia, CA) or β-actin (1:1000; Zymed, San Francisco, CA) diluted in buffer (25 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20, 0.05% nonfat dry milk, and 0.05% BSA). After washing, the membranes were incubated with the relevant horseradish peroxidase (HRP)-conjugated secondary antibodies HRP-goat anti-rabbit IgG (Zymed, San Francisco, CA), or HRP-rabbit anti-goat IgG (Pierce, Rockford, IL), for 2 h at 4°C. The membranes were washed and the

immunoreactive protein detected using an ECL Western blotting detection kit (Amersham, Arlington Heights, IL). The integrated optical density of the bands was quantified using scanning densitometry (GS-800 calibrated densitometer; Bio-Rad, Hercules, CA).

2.5 | Immunofluorescence

Localization of UbcH7 protein was determined by inmmunofluorescence in *substantia nigra pars compacta* of adult mouse brain and to identify dopaminergic neurons tyrosine hydroxylase (TH) was used. Mice were injected with an overdose of pentobarbital (80 mg/kg) and perfused intracardially with saline, subsequently the brains were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and postfixed in the same solution for 48 h, then incubated in 20% sucrose PBS. Coronal sections of 30-μm thick were cut in a cryostat and used for immunohistochemical assays. Briefly, free-floating sections were incubated at room temperature for 1 h in 0.25% Triton X-100/PBS buffer and subsequently in 5% BSA, 0.25% Triton X100/PBS buffer for 3 h. Then, sections were incubated in 3% BSA-0.25% Triton X-100/PBS solution containing the UbcH7 (1:100) and TH (1:1000) antibodies (Cell Signaling Technologies, MA). After a 48-h incubation, sections were incubated with secondary antibodies (1:400) (Alexa 488 anti-rabbit and Alexa 594 anti-mouse IgG; Thermo Fisher Scientific, Waltham, MA) diluted in PBS with 0.25% Triton X-100. Sections were counterstained with (4′,6-Diamidino-2-Phenylindole, Dihydrochloride) DAPI to reveal the nuclei and observed under a confocal microscope. Triple-labeled images were obtained using a confocal laser-scanning microscope (Leica TCS-SPE, Wetzlar, Germany) in the XYZ (Z-stacks) mode using a 63× objective. The Z-stacks (three to four optical slices) were then converted into three-dimensional projection images using the Leica LAS AF lite software. Quantification of UbcH7 fluorescence levels in dopaminergic cells was determined as described in Burgess et al. Briefly, using Image J software, mean fluorescence measurements were obtained, along with several adjacent background readings. Fluorescence readings of eight ROIs (defined as *Substantia nigra*) per treatment were calculated.[23]

2.6 | *In silico* analysis

The mouse *Ubch7* promoter was analyzed using the web-based bioinformatics tool TRANSFAC (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Score >75.

2.7 | Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed according to the kit protocol (Santa Cruz Biotechnology, Visalia, CA) with an anti-AHR antibody (Thermo Scientific, Rockland, IL). The PCR product corresponding to the *Ubch7* proximal gene promoter was generated from an aliquot of immunoprecipitated material. Brain homogenates from WT mice treated with a single oral dose of TCDD or vehicle (corn oil) were washed with PBS buffer and cross-linked with 1% formaldehyde. After chromatin isolation, the DNA was fragmented, and immunoprecipitation was performed. The cross-linking was reversed, the DNA was purified, and PCR amplification was performed as follows: initial denaturation at 94°C for 3 min, 25 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 3 s. A final extension cycle at 72°C for 10 min was added to the end of

the program. The oligonucleotides used for PCR amplification were 5′-GGCTAGAACCCCCTCACTTC-3′ (forward) and 5′-GGCTAGAACCCCCTCACTTC-3 (reverse).

2.8 | Cell culture and transfections

SH-SY5Y cells were obtained from American Type Culture Collection (CRL-2266; Manassas, VA). The cells were grown in 100-mm dishes with DMEM high glucose medium (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco, Logan, UT) and 1% antibiotic/antimycotic (Invitrogen, Carlsbad, CA) at 37°C in a humidified incubator with a 5% CO_2 atmosphere. The reporter plasmid pGL4/-832UbcH7 containing the *Ubch7* gene promoter was described previously.[12] SH-SY5Y cells were cultured in DMEM high glucose medium as described above. Transfection was performed using Escort IV (Sigma, San Louis, MO). Each culture (1×10^6 cells/ml) was transfected with 5 μg of pGL4/-832UbcH7 and 1 μg of pRL-CMV as an internal control. The media was replaced with fresh media containing TCDD for 48 h posttransfection. After 48 h, the cells were homogenized by incubation with Passive Lysis Buffer (Promega, Madison, WI) for 15 min at room temperature. Luciferase activity was performed using the Dual-Glo Luciferase Reporter Assay System (Promega, Madison,WI) according to the manufacturer's instructions in a Modulus luminometer (Turner Biosystems, Sunnyvale, CA). Blanks were obtained by performing the luciferase activity assays in mock-transfected cells. Firefly luciferase activity levels were normalized by comparison to the activity levels of *Renilla* luciferase.

2.9 | Statistical analysis

Results are presented as the mean values ± standard deviation (SD). The statistical significance of the data was evaluated using the Student's *t*-test. In all cases, the differences between groups were considered to be statistically significant when the p value was less than 0.05.

3 | RESULTS

A previous study showed that activation of hepatic AHR results in *UbcH7* gene induction.[11] UBCH7 is the reported E2 binding partner of parkin[17] and the combined activities of these enzymes conjugate Ub onto several substrates such as synphilin-1 for subsequent degradation by the 26S proteasome.[15] Accumulation of UBCH7–parkin substrates was also associated with PD.[18] Therefore, we investigated whether activation of AHR by TCDD regulates UbcH7 expression in the mouse ventral midbrain, thereby promoting synphilin-1 protein degradation.

Initially, the *Ahr* mRNA was measured in olfactory bulb, ventral mid-brain, hippocampus, striatum, cortex, brain stem, and medulla oblongata tissue samples, with liver serving as a positive control reference tissue (Figure 1). *Ahr* mRNA was observed in all brain regions analyzed, with the highest levels found in the cortex followed by the ventral midbrain, striatum, and hippocampus. Olfactory bulb, brain stem, and medulla oblongata samples had the lowest levels of *Ahr* mRNA expression among the brain regions analyzed. Notably, the

Ahr mRNA levels in the cortex and ventral midbrain were quite high at 70% and 40% that found in liver levels, respectively.

Liver and cortex samples exhibited a fivefold *Ubch7* mRNA induction upon TCDD treatment compared to their respective controls (Figure 2). *Ubch7* mRNA was increased approximately twofold after TCDD treatment in samples isolated from the other brain regions including the ventral midbrain. In contrast, TCDD treatment failed to induce *Ubch7* mRNA expression in brain samples from *Ahr*-null mice, indicating that this effect is AHR dependent.

Furthermore, Western blot analysis showed that the TCDD treatment increased UbcH7 protein levels in the ventral midbrain (where dopaminergic neurons are present) to more than twofold of control levels. Confocal microscopy showed that UbcH7 immunopositivity colocalized with TH (dopaminergic cell marker) immunopositivity, indicating that this ubiquitin-conjugating enzyme is indeed expressed in dopaminergic neurons. Moreover, quantification of the fluorescence intensity of the UbcH7 signal showed that TCDD treatment increased UbcH7 levels relative to control levels in WT mice (data not shown). In contrast, TCDD treatment fails to induce UbcH7 in *Ahr*-null dopaminergic neurons (Figure 3).

In silico analysis of the mouse *Ubch7* gene promoter for identification of potential transcription factor binding sites revealed common regulatory sequences, such as TATA box, Oct-1, GATA, c-ETS, as well as Pbx-1 and Cdxa binding sites between position –595 and the putative transcription start site (+1). Four putative (XREs) were also identified (data not shown). ChIP assays of brain extracts from WT mice treated with TCDD or vehicle revealed that AHR bound the *Ubch7* gene promoter in the control sample and that this interaction increases notably after TCDD treatment (Figure 4). After confirming, by con-focal microscopy that SH-SY5Y cell expresses AHR (data not shown), transactivation studies were performed. Following TCDD treatment, the luciferase activity of SH-SY5Y cells that had been transfected with a pGL4 reporter vector containing the *Ubch7* gene promoter upstream from the luciferase open reading frame (pGL4/-832UbcH7) was elevated in a dose response manner, reaching threefold increase, compared to untreated controls, with the 50 nM dose, indicating that the TCDD treatment trans-activated the *Ubch7* gene promoter (Figure 4).

We then examined whether the induction of UbcH7 correlates with a decrease on synphilin-1 protein levels. Western blots analysis revealed that TCDD treatment resulted in a 40% decrease in levels of the UbcH7–parkin substrate synphilin-1 in ventral midbrain of WT mice (Figure 5). In contrast, TCDD treatment failed to decrease synphilin-1 protein levels in *Ahr*-null mice, indicating that this effect is AHR-dependent.

4 | DISCUSSION

In agreement with previous studies reporting the expression of *Ahr* mRNA in the rodent brain,[24] in this study *Ahr* mRNA was detected in all brain regions analyzed. The highest *Ahr* mRNA expression levels were observed in the cortex and might be linked to NMDA

receptor expression and activity as suggested previously by Lin et al.[25] High levels of *Ahr* mRNA were also detected in the ventral midbrain. This result agrees with earlier findings, where AHR was detected in the substantia nigra in rats.[26] The above results suggest that AHR may play an important role in brain physiology by regulating the expression of genes in several brain regions, in particular in the ventral midbrain.

Activation of AHR by TCDD resulted in an increase in *Ubch7* mRNA levels in all brain regions analyzed, including the ventral midbrain, that were approximately double the control levels. This induction is likely AHR dependent because no increases were observed in the *Ahr*-null mice after TCDD treatment. According to the in silico study, the *Ubch7* promoter contains four putative XREs. The ChIP assay suggested that, most likely, activation by TCDD causes AHRs to bind one or more of these XREs. Interestingly, AHR-*Ubch7* promoter interaction was detected in controls, indicating that this transcription factor might control not only *Ubch7* induction but also its basal expression. Regarding this point, several endogenous AHR ligands, derived from tryptophan metabolism, have been identified[27] and may regulate *Ubch7* basal expression through their binding to the AHR. Moreover, transfection studies established that AHR activation and binding to the *Ubch7* promoter, after TCDD treatment, resulted in promoter transactivation. Taken together, these results demonstrated that the *Ubch7* gene is under control of the AHR not only in the liver but also in the mouse brain.

Because UbcH7 is the partner required for proper parkin function, we focused on the ventral midbrain region to evaluate UbcH7 protein levels. TCDD treatment resulted in a twofold increase in ventral midbrain UbcH7 protein levels. Additionally, confocal microscopy indicated that AHR activation induces UbcH7 expression only in dopamine neurons from WT mice. Although previous reports have shown expression of UBCH7 and the rat ortholog UbcR7 in the substantia nigra,[28,29] the present study demonstrated expression of UbcH7 specifically in dopaminergic neurons.

The increase in AHR activation observed in response to TCDD treatment might augment ubiquitination and degradation of UbcH7– parkin substrates. In agreement with this possibility, TCDD treatment decreased synphilin-1 protein levels in the ventral midbrain in an AHR-dependent way.

To our knowledge, there is no information regarding *UBCH7* gene variations (such as polymorphisms) that have been associated with any neuropathology, such as PD. However, some *UBCH7* gene variant haplotypes that result in increased levels of this E2 enzyme were associated with autoimmune diseases, such as lupus erythematosus and Crohn's disease.[28,30] Therefore, UBCH7 gene polymorphisms or exposure to chemical agents such as TCDD that modify UBCH7 levels might promote the development of certain pathologies.

In conclusion, these results identify the AHR as an indirect modulator of the degradation of UbcH7–parkin substrates in the mouse brain. Future studies are needed to characterize the consequences of AHR-mediated induction of UbcH7 and to explore both the toxicological implications and the therapeutic potential of this pathway.

ACKNOWLEDGMENTS

This work was supported by CONACYT grant 153377.

REFERENCES

[1]. Huff J, Lucier G, Tritscher A, Ann. Rev. Pharmacol. Toxicol 1994, 34, 343–372. [PubMed: 8042855]

[2]. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ, J. Biol. Chem 2004, 279, 23847–23850. [PubMed: 15028720]

[3]. Lahvis GP, Lindell SL, Thomas RS, McCuskey RS, Murphy C, Glover E, Bentz M, Southard J, Bradfield CA, Proc. Natl. Acad. Sci. U. S. A 2000, 97, 10442–10447. [PubMed: 10973493]

[4]. Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ, Science 1995, 268, 722–726. [PubMed: 7732381]

[5]. Rodriguez-Sosa M, Elizondo G, Lopez-Duran RM, Rivera I, Gonzalez FJ, Vega L, FEBS Lett. 2005, 579, 6403–6410. [PubMed: 16289099]

[6]. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL, Nature 2008, 453, 65–71. [PubMed: 18362915]

[7]. Latchney SE, Hein AM, O'Banion MK, DiCicco-Bloom E, Opanashuk LA, J. Neurochem 2013, 125, 430–445. [PubMed: 23240617]

[8]. Sato S, Shirakawa H, Tomita S, Ohsaki Y, Haketa K, Tooi O, Santo N, Tohkin M, Furukawa Y, Gonzalez FJ, Komai M, Toxicol. Appl. Pharmacol 2008, 229, 10–19. [PubMed: 18295293]

[9]. Reyes-Hernandez OD, Mejia-Garcia A, Sanchez-Ocampo EM, Castro-Munozledo F, Hernandez-Munoz R, Elizondo G, Toxicology 2009, 266, 30–37. [PubMed: 19850099]

[10]. Elizondo G, Fernandez-Salguero P, Sheikh MS, Kim GY, Fornace AJ, Lee KS, Gonzalez FJ, Mol. Pharmacol 2000, 57, 1056–1063. [PubMed: 10779392]

[11]. Reyes-Hernandez OD, Mejia-Garcia A, Sanchez-Ocampo EM, Cabanas-Cortes MA, Ramirez P, Chavez-Gonzalez L, Gonzalez FJ, Elizondo G, Biochem. Pharmacol 2010, 80, 932–940. [PubMed: 20478272]

[12]. Mejia-Garcia A, Gonzalez-Barbosa E, Martinez-Guzman C, Torres-Ramos MA, Rodriguez MS, Guzman-Leon S, Elizondo G, Toxicology 2015, 337, 47–57. [PubMed: 26318284]

[13]. Moynihan TP, Ardley HC, Nuber U, Rose SA, Jones PF, Markham AF, Scheffner M, Robinson PA, J. Biol. Chem 1999, 274, 30963–30968. [PubMed: 10521492]

[14]. Harbers K, Muller U, Grams A, Li E, Jaenisch R, Franz T, Proc. Natl. Acad. Sci. U. S. A 1996, 93, 12412–12417. [PubMed: 8901595]

[15]. Rankin CA, Roy A, Zhang Y, Richter M, Open Biochem. J 2011, 5, 9–26. [PubMed: 21633666]

[16]. Salomons FA, Acs K, Dantuma NP, Exp. Cell Res 2010, 316, 1289–1295. [PubMed: 20149791]

[17]. Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ, Science 2001, 293, 263–269. [PubMed: 11431533]

[18]. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, Suzuki T, Nat. Genet 2000, 25, 302–305. [PubMed: 10888878]

[19]. Dawson TM, Dawson VL, Mov. Disord 2010, 25 (Suppl 1), S32–39. [PubMed: 20187240]

[20]. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N, Nature 1998, 392, 605–608. [PubMed: 9560156]

[21]. LaVoie MJ, Ostaszewski BL, Weihofen A, Schlossmacher MG, Selkoe DJ, Nat. Med 2005, 11, 1214–1221. [PubMed: 16227987]

[22]. Huang P, Ceccatelli S, Hoegberg P, Sten Shi TJ, Hakansson H, Rannug A, Toxicol. Appl. Pharmacol 2003, 192, 262–274. [PubMed: 14575644]

[23]. Burgess A, Vigneron S, Brioudes E, Labbe JC, Lorca T, Castro A, Proc. Natl. Acad. Sci. U. S. A 2010, 107, 12564–12569. [PubMed: 20538976]

[24]. Huang P, Rannug A, Ahlbom E, Hakansson H, Ceccatelli S, Toxicol. Appl. Pharmacol 2000, 169, 159–167. [PubMed: 11097868]

[25]. Lin CH, Chen CC, Chou CM, Wang CY, Hung CC, Chen JY, Chang HW, Chen YC, Yeh GC, Lee YH, Neurochem J 2009, 111, 777–789.

[26]. Akahoshi E, Yoshimura S, Uruno S, Ishihara-Sugano M, Environ. Health 2009, 8, 24. [PubMed: 19500377]

[27]. Stejskalova L, Dvorak Z, Pavek P, Curr. Drug Metab 2011, 12, 198–212. [PubMed: 21395538]

[28]. Wang S, Adrianto I, Wiley GB, Lessard CJ, Kelly JA, Adler AJ, Glenn SB, Williams AH, Ziegler JT, Comeau ME, Marion MC, Wakeland BE, Liang C, Kaufman KM, Guthridge JM, Alarcon-Riquelme ME, Biolupus, Networks G, Alarcon GS, Anaya JM, Bae SC, Kim JH, Joo YB, Boackle A, Brown EE, Petri MA, Ramsey-Goldman R, Reveille JD, Vila LM, Criswell LA, Edberg JC, Freedman BI, Gilkeson GS, Jacob CO, James JA, Kamen DL, Kimberly RP, Martin J, Merrill JT, Niewold TB, Pons-Estel BA, Scofield RH, Stevens AM,Tsao BP,Vyse TJ,Langefeld CD,Harley JB,Wakeland EK, Moser KL, Montgomery G, Gaffney PM, Genes Immun. 2012, 13, 380–387. [PubMed: 22476155]

[29]. Schlossmacher MG, Frosch MP, Gai WP, Medina M, Sharma N, Forno L, Ochiishi T, Shimura H, Sharon R, Hattori N, Langston JW, Mizuno Y, Hyman BT, Selkoe DJ, Kosik KS, Am. J. Pathol 2002, 160, 1655–1667. [PubMed: 12000718]

[30]. Fransen K, Visschedijk MC, van Sommeren S, Fu JY, Franke L, Festen EA, Stokkers PC, van Bodegraven AA, Crusius JB, Hommes DW, Zanen P, de Jong DJ, Wijmenga C, van Diemen CC, Weersma RK, Hum. Mol. Genet 2010, 19, 3482–3488. [PubMed: 20601676]

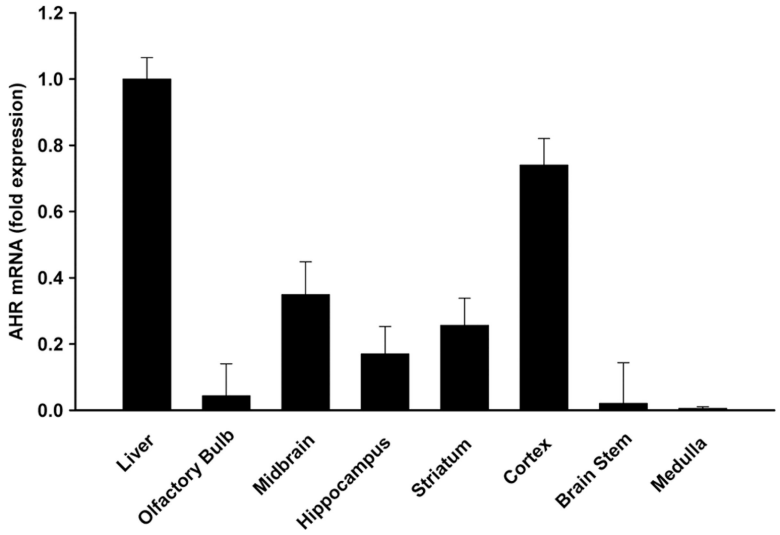

FIGURE 1.

AHR mRNA expression in brain. Total mRNA was extracted from the liver, olfactory bulb, ventral midbrain, hippocampus, striatum, cortex, brain stem, and medulla oblongata from sacrificed mice. *Ahr* mRNA levels were determined by qPCR and normalized to 18S ribosomal RNA. *Ahr* mRNA expression levels for each brain section are reported relative to expression in the liver. Results are expressed as the mean ± SD of samples from three different mice

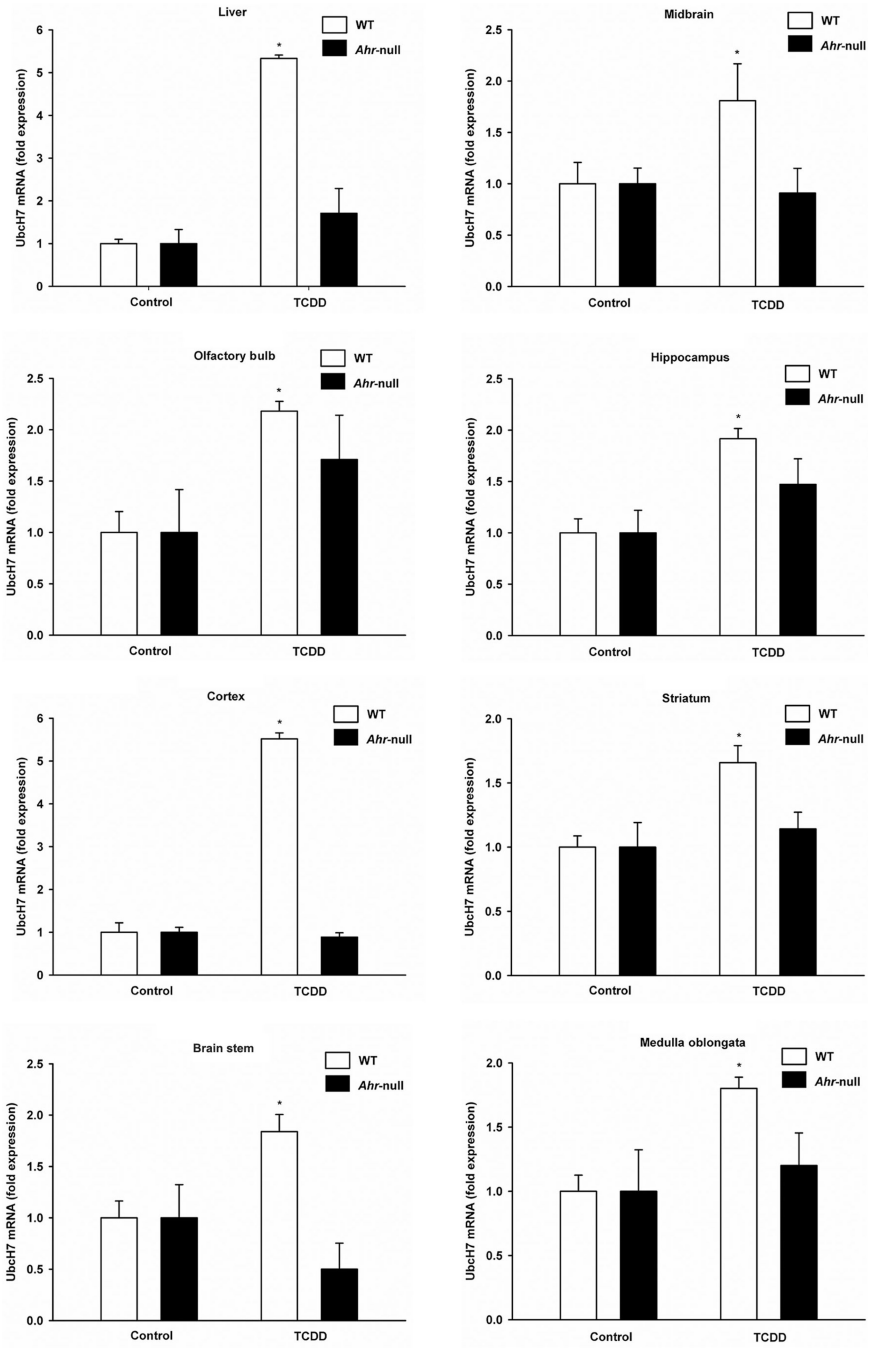

FIGURE 2.

AHR-dependent induction of brain *Ubch7* mRNA levels. WT and *Ahr*-null mice were treated with a single oral dose of TCDD or corn oil (vehicle). Total mRNA was extracted from the liver, ventral midbrain, olfactory bulb, hippocampus, cortex, striatum, brain stem, and medulla oblongata. *Ubch7* mRNA levels were determined by qPCR and normalized to 18S ribosomal RNA. Results are expressed as the mean ± SD of samples from three different mice. $*p \leq 0.05$, treatments vs. control

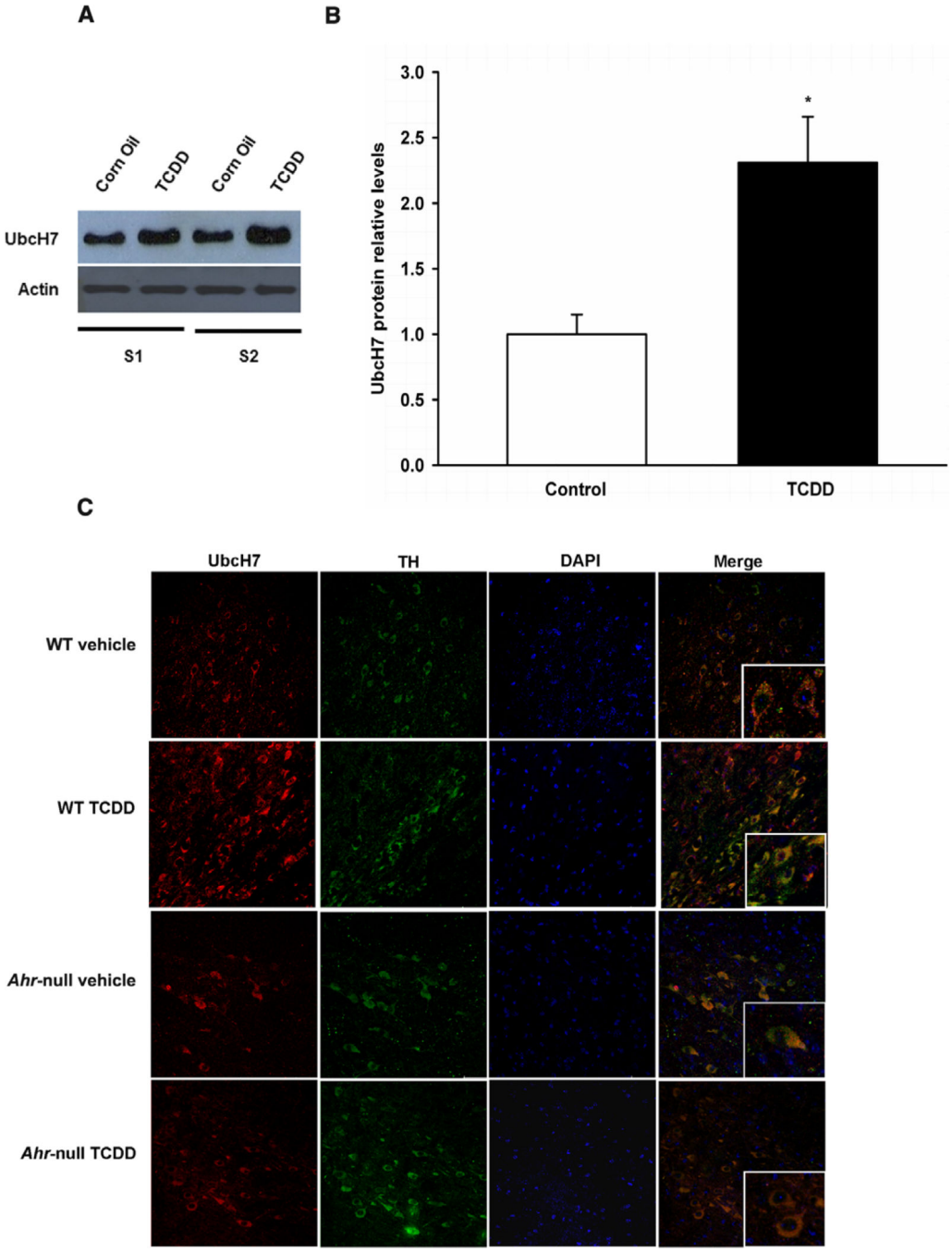

FIGURE 3.

TCDD treatment induced UbcH7 protein expression in the ventral midbrain. WT and *Ahr*-null mice were treated with TCDD or corn oil (vehicle). (A) Western blot of UbcH7 from ventral midbrain samples of WT mice. β-actin was used as a loading control. S1 = sample 1 and S2 = sample 2. (B) Relative expression of UbcH7 protein levels. Results are expressed as the mean±SD of samples from three different mice. *$p \leq 0.05$, treatments vs. control. (C) Representative confocal microscopy images of UbcH7 expression in the ventral midbrain

region. TH, UbcH7, and DAPI were visualized as green, red, and blue, respectively. Insets show in more detail the colocalization of the TH and UbcH7

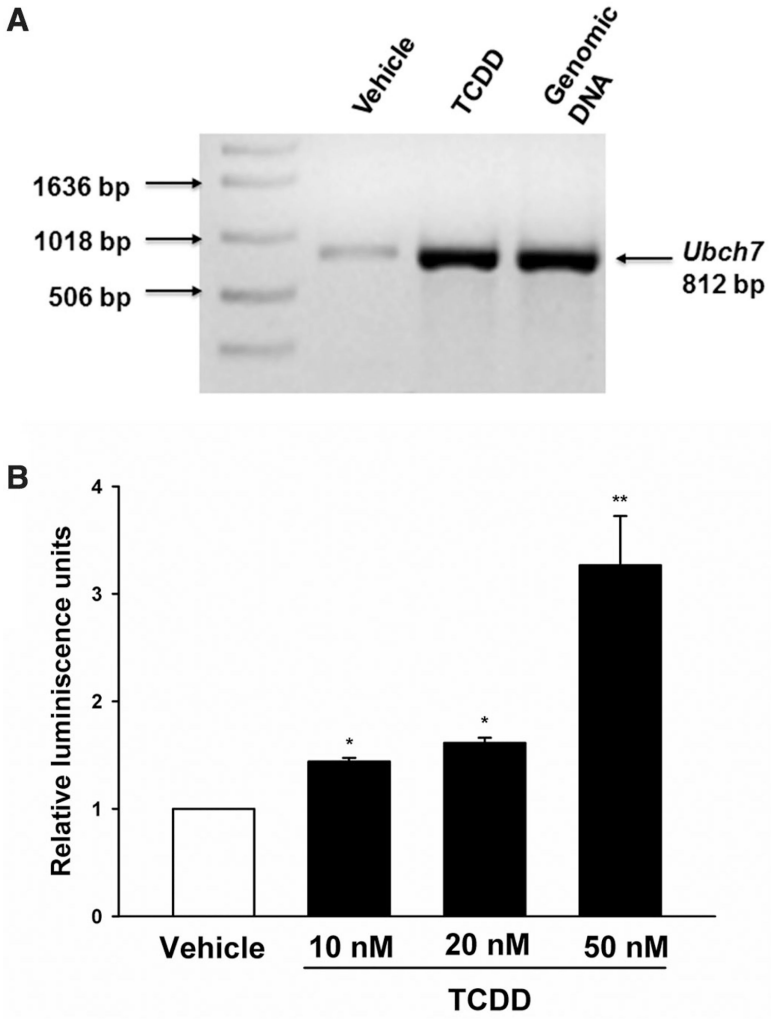

FIGURE 4.

AHR binds to and transactivates the *Ubch7* promoter. (A) ChIP analysis of AHR binding to the *Ubch7* gene promoter region. Immunoprecipitated material was obtained from brain samples from mice treated with vehicle (line 2) or TCDD (line 3). PCR products corresponding to the –832/–20 bp of the *Ubch7* promoter region were generated. Genomic DNA was used as positive control (line 4). (B) Luciferase activity of SH-SY5Y cells (1×10^6) transfected with 5 μg of the pGL4/-832UbcH7 vector containing the firefly luciferase open reading under the control of the *Ubch7* promoter and then treated with TCDD or DMSO (vehicle) for 48 h. Luciferase activity was normalized to that of *Renilla* luciferase and expressed as the mean ± SD of three independent experiments. * $p \leq 0.05$; ** $p \leq 0.01$, control vs. treatments

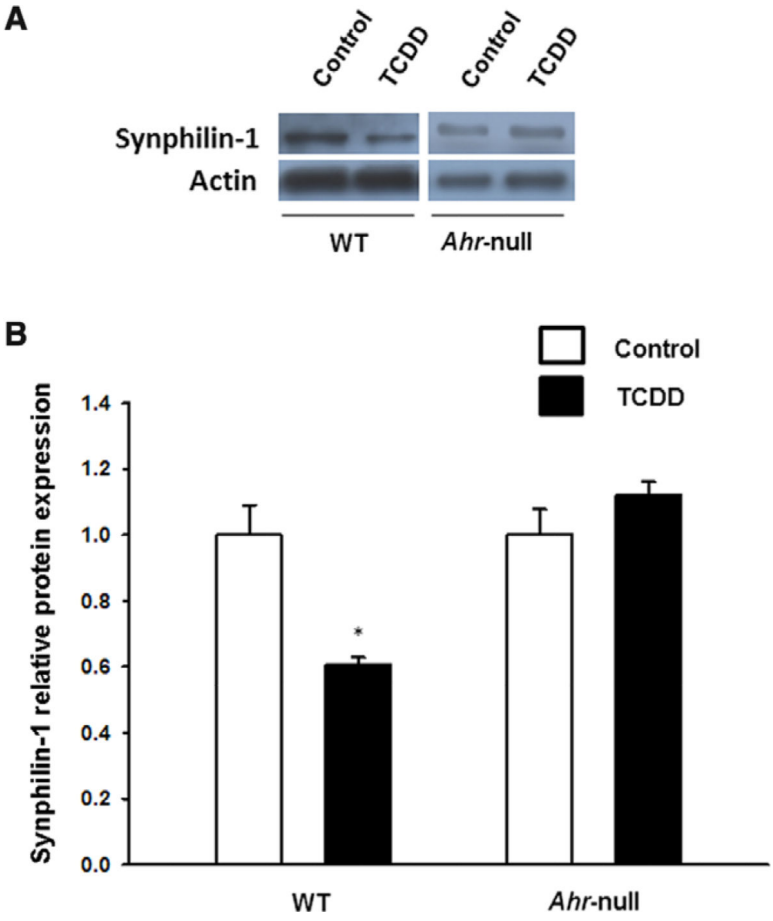

FIGURE 5.

TCDD treatment decreased synphilin-1 protein levels in an AHR-dependent way. A) Western blot of synphilin-1 from ventral midbrain samples of WT and *Ahr*-null mice treated with TCDD or corn oil (control). β-actin was used as a loading control. B) Relative expression of synphilin-1 protein levels. Results are expressed as the mean ± S.D. of samples from three different mice. * $p \leq 0.05$, treatments *vs.* control