Applicability of tyrosine hydroxylase promoter activation assay (TH assay) for screening of dioxin-like compounds

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Abstract

Several environmental such as polycyclic aromatic hydrocarbons (PAHs), affect human health. These chemicals also influence brain function. Thus, the development of methods for screening toxic chemicals and detecting their toxic potency is important.

PAH toxicities are mainly due to the disruption of gene expression. A major participant in the process is the aryl hydrocarbon receptor (AhR) and AhR-mediated gene expression.

Previously, we reported that the *tyrosine hydroxylase (TH)* gene was a target of AhR activated by dioxins and that a novel AhR binding element, AhRE-TH, upstream of the *TH* gene was identified. Since TH is a rate-limiting enzyme of dopamine synthesis, abnormal *TH* gene expression is thought to be correlated to the disturbance of brain function. Using the transcriptional mechanism of the *TH* gene, we developed a TH promoter activation assay (TH assay), which measures the activity of compounds against transcriptional regulation of the *TH* gene.

In this study, to verify the applicability of the TH assay, we investigated the role of PAHs using this assay and calculated the 2,3,7,8-tetrachlorodibenzo-*p*-dioxintoxic equivalency factors (TH-TEFs). The obtained TH-TEF values corresponded to the reported WHO-TEF values. Therefore, the results demonstrate the suitability of the TH assay in assessing the toxic potency of PAHs.

Introduction

Several environmental and industrial chemicals affect human health. These include polycyclic aromatic hydrocarbons (PAHs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated biphenyls (PCBs), and brominated flame retardants (BFRs). These chemicals cause significant adverse health effects such as oncogenesis, reproductive toxicity, immunosuppression, and neurological dysfunction. Thus, effective methods are required for screening toxic chemicals and detecting their toxic potency.

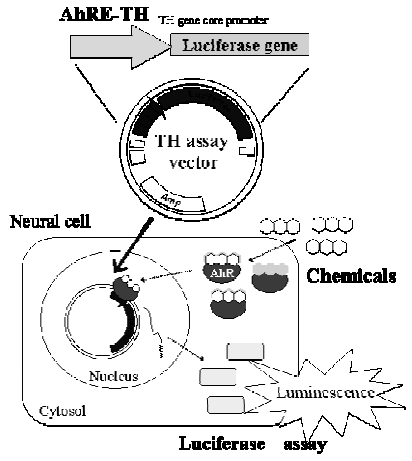

Fig. 1 Principle of TH assay

Toxic chemicals have severe neurotoxic potency. One of the PCDDs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and PCBs lead to neurobehavioral abnormalities associated with both cognitive and locomotor systems [1-2]. Experimental animal and epidemiological studies have indicated that TCDD and related

compounds potentially cause neurodevelopmental disabilities such as learning disabilities and attention-deficit hyperactivity disorder (ADHD), [3-5] and clinical studies have shown that disabilities such as ADHD are closely related to the dopamine system [6-7].

Although the precise mechanisms of their action have not been fully elucidated, a major participant in the process by which dioxins exert toxicity is the aryl hydrocarbon receptor (AhR) [8, 9]. AhR is a receptor for PAHs and a ligand-activated transcription factor. The disruption of transcription of several genes by ligand-activated AhR is thought to be a key process in the toxic action of PAHs.

In previous studies, we demonstrated that tyrosine hydroxylase (TH), a functional marker of catecholaminergic neurons, was a target of AhR activated by dioxins [10, 11]. *TH* gene expression is thought to be upregulated by TCDD through AhR in undifferentiated neuroblastomas. Since TH is a rate-limiting enzyme of dopamine synthesis and a functional marker of the dopamine system, abnormal transcription of the TH gene will reflect disturbance of the dopamine system.

At a previous conference, DIOXIN 2008, we presented a paper showing that AhRE-TH, the responsive element of TCDD, is located upstream of the TH gene. Using AhRE-TH fused to the luciferase gene as a reporter gene, we developed a novel reporter gene assay (Fig. 1), named the TH promoter activation assay or "TH assay" [12].

This TH assay can measure the activity of compounds against transcriptional regulation of the TH gene, making it useful for detecting neurotoxic effects. In this study, we show the applicability of the TH assay to screen PAHs by calculating TCDD equivalency factors (TEFs).

Materials and Methods

Chemicals and cell cultures

PCDDs, PCBs, and polybrominated dibenzo-*p*-dioxin (PBDD) (Kanto Chemical Co. Inc., Tokyo, Japan) were maintained as stock solutions in dimethyl sulfoxide.

Neuro2a, a murine neuroblastoma cell line, was purchased from the American Type Culture Collection. The cells were routinely grown in a Dulbecco's modified Eagle's medium and a F12 medium in a 1:1 ratio, supplemented with 10% fetal calf serum at 37°C in humidified CO_2-air (5:95).

Reporter vector

A reporter vector, which contained the 25-bp fragment of the *TH* gene 5′upstream region (AhRE-TH) and the TH gene core promoter region upstream of the luciferase gene in a PGV vector (TOYO-B-Net Co., Tokyo, Japan), was developed.

Luciferase assay

Neuro2a cells were exposed to various concentrations of compound. After exposure for 20–24 h, the cells were lysed with PicaGene (R) Cell Culture Lysis Reagent Luc (TOYO-B-Net Co., Tokyo, Japan). To measure luciferase activity, the cell extract was pipetted into a 96-well microtiter plate, and a PicaGene LT2.0 Luminescence Kit (TOYO-B-Net CO., Tokyo, Japan) was used. The light produced was measured in a Mithras LB940 Luminometer (Berthold Technologies, Bad Wildbad, Germany). The induction factor was calculated by measuring the ratio between luciferase activity of the compounds) and that of the vehicle. Values represent the mean ± S.D. for triplicates.