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Low dose hydroxylated PCB induces c-Jun expression in PC12 cells

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Abstract

Polychlorinated biphenyls (PCBs) are known as environmental pollutants that may cause adverse health problems. Recently, accumulating evidence shows that PCBs express neurotoxicity through alteration of gene expression and signal transduction. On the other hand, c-Jun, a component of AP-1, is likely to coordinate transcription programs in response to various extracellular signals. However, little is known about the effects of PCBs on c-Jun expression. Here we investigated the expression of c-Jun in response to PCB. PC12 cells were incubated with hydroxylated PCB (4(OH)-2′,3,3′,4′,5′-penta chlorobiphenyl, OH-PCB) at a final concentration from 10^{-8} to 10^{-5} M. The level of c-Jun expression was increased by OH-PCB at relatively low-dose; concentration of OH-PCB at 10^{-8} M and 10^{-7} M produced a 2.4- and 3.5-fold increase of c-Jun expression in respectively, compared with the values without OH-PCB treatment. Thyroid hormone (T3) did not induce such c-Jun expression, indicating that the effect of OH-PCB is not mediated through thyroid hormone signaling pathway. OH-PCB also enhanced phosphorylation of c-Jun NH_2-terminal kinases. To determine whether the activation of Ca^{2+} channel is involved in the OH-PCB-induced c-Jun expression, we examined it using a L-type voltage-gated Ca^{2+} channel blocker nimodipine. Nimodipine partially inhibited OH-PCB-induced c-Jun expression by 50%. Moreover, Na^+ channel antagonist tetrodotoxin inhibited OH-PCB-induced c-Jun expression completely. Taken together, our results indicate that exposure to OH-PCB induces c-Jun expression, and the response may be triggered by depolarization of a plasma membrane via Na^+ influx, followed by Ca^{2+} influx partially through voltage-gated Ca^{2+} channels.

Keywords: Hydroxylated PCB; Neurotoxicity; c-Jun expression; Phosphorylation of c-Jun NH_2-terminal kinases; Voltage-gated Ca^{2+} channels; PC 12 cells

1. Introduction

Polychlorinated biphenyls (PCBs) belong to a large group of polycyclic chlorinated hydrocarbons, and are ubiquitous and serious environmental contaminants. These compounds contain 209 congeners, each of which is chlorinated to various degrees (Safe, 1994; Tilson et al., 1990; Tilson and Kodavanti, 1997). They are known bioaccumulators because of their extreme stability and lipophilicity, and have been detected in almost every natural medium including water, air and soil as well as in human and animal tissues (Kimbrough and Jensen, 1989; Kimbrough, 1995). Despite widespread environmental contamination by PCBs, their adverse health effects are only partially understood.

As toxicity of PCB become clear, it is pointed out that PCB may be involved in adverse effects on neural development and function. From analysis of PCB concentration in umbilical-cord serum, prenatal exposure to PCB may affect the intellectual functions such as the acquisition of reading and arithmetic skills in children (Jacobson and Jacobson, 1996). The mechanisms that underlie the neurotoxicity of PCBs are not clear. Perinatal exposure of laboratory animals to PCBs results in neurobehavioral disorders including motor dysfunction (Pantaleoni et al., 1988; Tilson et al., 1979) and cognitive deficits such as spatial learning, memory and intellectual function (Levin et al., 1988; Lilienthal and Winneke, 1991; Schantz et al., 1992, 1995). Exposure to PCBs gives rise to regionally specific decrease in the concentration of dopamine in the rat brain (Seegal et al., 1991). Developmental exposure to PCB produces persistent decrements in hippocampal synaptic plasticity in rats (Gilbert and Crofton, 1999). As shown in some in vitro experiments, the ortho-substituted PCB congeners decrease the cellular dopamine content in PC12 cells (Angus and Contreras, 1996; Seegal et al., 1989).

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The molecular mechanism(s) and structure–activity relationship that underlie the neurotoxicity of PCBs are not clear. However, there is accumulating evidence that shows PCBs induce neurotoxicity through alteration in gene expression and signal transduction. Recently, we reported that PCBs suppress thyroid hormone receptor (TR)-mediated transcription through partial dissociation of TR from the thyroid hormone-response element (TRE) (Iwasaki et al., 2002; Miyazaki et al., 2004). On the other hand, Aroclor 1254 (A1254), a mixture of PCB congeners used commercially in various products, causes intracellular dopamine depletion and cell death via the depletion of intracellular Ca^{2+} stores and the alteration of nitric oxide production in the immortalized catecholaminergic cell-line (Kang et al., 2002, 2004). A1254 also alters intracellular calcium buffering and protein kinase C translocation (Tilson and Kodavanti, 1998). A1254 perturbs Ca^{2+} homeostasis and increases phosphorylation of cyclic AMP-responsive element-binding protein (CREB) in cultured cortical neurons derived from the neonatal rat brain (Inglefield et al., 2001). Moreover, non-coplanar PCB95 (2,2′,3,5′,6-pentachlorobiphenyl) disrupts intracellular Ca^{2+} signaling in PC12 cells by interaction with the FK506 binding protein/ryanodine receptors complex (Wong et al., 2001).

Jun is a component of the nuclear transcription factor activator protein 1 (AP-1), and constitutes AP-1 activity either as a homodimers or as a heterodimer with other transcription factors such as Fos and ATF-2. c-Jun is a major stress-activated protein and is likely to coordinate transcription programs in response to various extracellular stimuli. c-Jun must be phosphorylated at Ser63 and Ser73 on its NH_2-terminal transactivation domain for augmentation of the transcriptional activity (Binetruy et al., 1991; Pulverer et al., 1991; Smeal et al., 1991). This phosphorylation is mediated by c-Jun NH_2-terminal kinase (JNK), which itself need to be phosphorylated at Thr183 and Tyr185 by the upstream dual specificity kinases for the activation of its kinase domain (Lin et al., 1995; Lu et al., 1997; Sanchez et al., 1994). This phosphorylation event can be induced by a variety of extra- and intra-cellular stress signals including UV radiation (Derijard et al., 1994; Hibi et al., 1993), osmotic (Galcheva-Gargova et al., 1994) and oxidative stress (Inoshita et al., 2002), extracellular acidification (Shimokawa et al., 2004), alkalinization (Shrode et al., 1997) and cytokines (tumor necrosis factor-α) (Ichijo, 1999). Once activated, JNK translocates to the nucleus where it regulates the activity by phosphorylation of several transcription factors such as the Jun family proteins, ATF-2 (activating transcription factor-2) and p53 (Ip and Davis, 1998). Therefore, JNK plays key roles to regulate stress-induced c-Jun activity.

In the present study, we examined whether PCB induces c-Jun expression in PC12 cells. Since our previous study has shown that hydroxylated form of PCB (OH-PCB), a major metabolite of PCB in mammals is more potent to suppress TR-mediated transcription, we used 4(OH)-2′,3,3′,4′,5′-penta chlorobiphenyl (Miyazaki et al., 2004). Here we show that exposure to OH-PCB induces JNK phosphorylation and c-Jun expression. The effects may be mediated by membrane depolarization via activation of Na^+ channels and by extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels. Thus, OH-PCB may be involved in transcriptional regulation of genes having AP-1 site through expression of c-Jun.

2. Materials and methods

2.1. Reagents and antibodies

OH-PCB (4(OH)-2′,3,3′,4′,5′-penta chlorobiphenyl) (purity $\geq$ 99%) was purchased from AccuStandard Chemicals (New Haven, CT). Nimodipine, tetrodotoxin (TTX) and triiodothyronine (T3) were obtained from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, horse serum (HS), fetal calf serum (FCS) and antibiotics (penicillin and streptomycin) were purchased from Invitrogen (Carlsbad, CA). c-Jun polyclonal antibody (H-79) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibody to phospho-JNK (Thr-183/Tyr-185; G9) and rabbit polyclonal antibody to JNK were purchased from Cell Signaling Technology Inc. (Beverly, MA). Horseradish peroxidase (HRP)-linked secondary antibodies (anti-mouse IgG and anti-rabbit IgG) and a chemiluminescent detection system (ECL Western blotting detection kit) were all obtained from Amersham Biosciences (Buckinghamshire, UK).

2.2. Cell culture and exposure to OH-PCB

PC12 cells were provided by the RIKEN Cell Bank (Tsukuba, Japan). All cells used in the present study were with passage number lower than 16. PC12 cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated HS and 5% FCS. Since the cells are weakly adherent, they were plated on poly-D-lysine coated dishes (Biocoat, Becton Dickinson, Bedford, MA). Cells were plated at an intensity of 0.2×10^6 cells per 35-mm dish and were incubated at 37 °C in an atmosphere of 95% air/5% CO_2.

To extinguish c-Jun expression and JNK phosphorylation that might have been present before exposure to OH-PCB, cells at 70–80% confluence were made quiescent by incubation in a suitable culture medium (pH 7.40, 37 °C) for 2 h. OH-PCB was dissolved in dimethyl sulfoxide and added to culture medium at final concentration 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M. In the experiment on effect of the OH-PCB-induced c-Jun expression by the Ca^{2+} channel blocker nimodipine or Na^+ channel blocker TTX, cells were treated with or without nimodipine (from 10^{-6} to 10^{-4} M) or TTX (from 0.05 to 5 μM) for 15 min. After treatment with nimodipine or TTX, OH-PCB was added to the culture medium, and cells were incubated for additional 60 min.

2.3. Western blot analysis

Following exposure, cells were rinsed with ice-cold phosphate-buffered saline (PBS) and lysed in ice-cold 1% Triton X-100 lysis buffer (pH7.4, 50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol) with protease inhibitors (10 μg/ml aprotinin, 2.5 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride), 25 mM NaF, 10 μM $ZnCl_2$ and