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Brain-derived neurotrophic factor (BDNF) ameliorates the suppression of thyroid hormone-induced granule cell neurite extension by hexabromocyclododecane (HBCD)

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ABSTRACT

Thyroid hormone (TH) plays an essential role in growth and differentiation of the central nervous system. Deficiency of TH during perinatal period results in abnormal brain development known as cretinism in human. We recently reported that an environmental chemical 1,2,5,6,9,10-α-hexabromocyclododecane (HBCD) suppressed TH receptor (TR)-mediated transcription. To examine the effect of HBCD on cerebellar granule cells, we used purified rat cerebellar granule cells in reaggregate culture. Low dose HBCD (10^{-10} M) significantly suppressed TH-induced neurite extension of granule cell aggregate. To clarify further the mechanisms of such suppression, we added brain-derived neurotrophic factor (BDNF) into culture medium, since BDNF plays a critical role in promoting granule cell development and is regulated by TH. BDNF completely rescued HBCD-induced suppression of granule cell neurite extension in the presence of T3. These results indicate that HBCD may disrupt TH-mediated brain development at least in part due to a disruption of the T3 stimulated increase in BDNF and BDNF may possess ability to ameliorate the effect of HBCD in granule cells.

Thyroid hormones (L-triiodothyronine, T3; thyroxine, T4; THs) are essential for normal brain development and function in animals and humans. During the perinatal period, TH influences neuronal and glial proliferation in definitive brain regions, mediates neuronal migration and differentiation [34,35]. From the animal studies, hypothyroidism especially during the period of brain growth spurt [6] leads to neurological defects, growth retardation and impaired learning and memory [21,36,40] as well as aberrant behavioral patterns [11]. Specifically, perinatal hypothyroidism causes decreased number of synapses between Purkinje and granule cell axons [29,30], delayed disappearance of the external granule cell layer (EGL), delayed migration of granule cells into the internal granule cell layer (IGL) [30,31], and delayed synaptic connection among cerebellar neurons and afferent neuronal fibers from other brain regions [12].

We have recently tried to identify environmental chemicals affecting TH-mediated brain development, and identified polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and 1,2,5,6,9,10-α-hexabromocyclododecane (HBCD) as potent candidates [16,17,26,27]. HBCD is an additive flame retardant that has been extensively used since the early 80s [4] and is the third most widely used brominated flame retardant (BFR) [22]. HBCD is lipophilic and can bioaccumulate. It is persistent in the environment and detectable levels have been found in abiotic and biotic samples including human blood and breast milk [4,22,23].

It has been suggested that HBCD could have endocrine disruptive and neurotoxic effects. Impaired oligodendroglial development in the brain [38], impairment in learning and memory, and aberrant spontaneous behavior [7,8] have also been reported following HBCD exposure in rodent. We have recently reported that HBCD suppressed TH receptor (TR)-mediated gene expression in CV-1 cells and impaired TH-mediated dendrite arborization of Purkinje cells [17]. These studies indicate that HBCD may disrupt TH homeostasis, and affect normal developmental activities in the brain. However, the mechanisms of HBCD action on TH-mediated brain development are still largely un-clarified. Cerebellum is essential for motor learning, memory and vestibular functions [18]. TRs are widely expressed in granule cells [19]. The cerebellar granule cell provides an opportune model system for studies

Abbreviations: HBCD, 1,2,5,6,9,10-α-hexabromocyclododecane; TH, thyroid hormone; TR, thyroid hormone receptor; T3, 3,5,3′-triiodo-L-thyronine, triiodothyronine; T4, 3,5,3′,5′-tetraiodo-L-thyronine, thyroxine; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; BFR, brominated flame retardant; BSA, bovine serum albumin; PBS, phosphate buffered saline; DMSO, dimethylsulphoxide; FITC, fluorescein isothiocyanate; GCP, granule cell precursor.

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on mammalian central nervous system neurogenesis [10]. Their characteristic morphology, cell size and large numbers during early postnatal development in rodents allow for their purification for *in vitro* analysis [9,10,13].

TH regulates transcription of target gene by binding to TR, which is a ligand-regulated transcription factor. Although factors that play a key role in TH-mediated cerebellar development have not yet been identified, neurotrophins such as brain-derived neurotrophic factor (BDNF) may play such role. Neurotrophins (NTs) are a family of related homodimeric protein factors. They comprise of nerve growth factor (NGF) [24], BDNF [2], NT-3 [15], and NT-4/5 [1]. NTs profoundly affect the development of the central nervous system. In the developing cerebellum, BDNF is widely implicated in regulation of neuronal proliferation and differentiation during neurogenesis [14,33,37]. Particularly, BDNF promotes migration and neurite elongation of external granule cells [39], affects Purkinje cell survival in culture, and acts as a neuro-protective factor [3]. Furthermore, mice harboring a deletion of the BDNF gene show abnormal cerebellar development similar to those seen in hypothyroid animals [2]. We and others have previously reported that the expression of BDNF is depressed in hypothyroid rodent cerebellum [20,28]. Replacing BDNF, in part, prevents hypothyroidism-induced abnormal cerebellar development [28], indicating that BDNF may play a crucial role in TH-mediated cerebellar development. Thus, it is important to investigate the involvement of BDNF on disruption of TH-mediated cerebellar development by environmental chemicals.

In the present study, we therefore examined the effect of HBCD on TH-mediated granule cell neurite extension. We further inves-

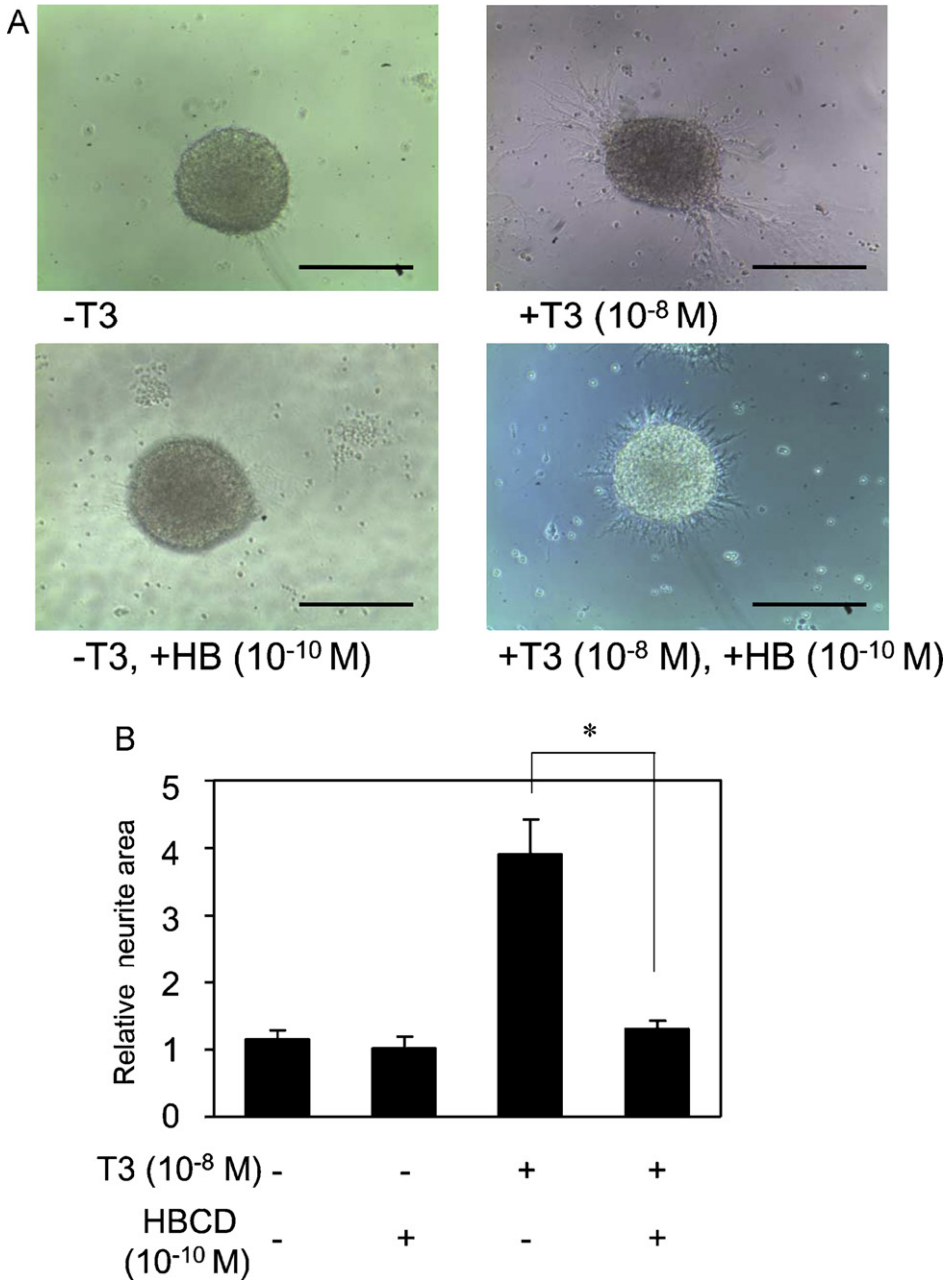

Fig. 1. Suppression of TH-induced neurite extension of granule cells by low dose HBCD. (A) Photomicrographs showing the effect of 10^{-10} M HBCD on granule cell neurite extension (2 Days *in vitro*) in the absence or presence of T3 (10^{-8} M). Bars indicate 50 μm. (B) Effect of HBCD on granule cell aggregate neurite extension. Neurite areas were quantified using ImageJ software and were normalized by circumference of aggregate. Data are expressed as mean ± S.E.M. ($n = 6$ determinations). *statistically significant, $p < 0.01$ by ANOVA for T3 (+), HBCD (−) vs. T3 (+), HBCD (+). Data shown are representative of at least three independent experiments.