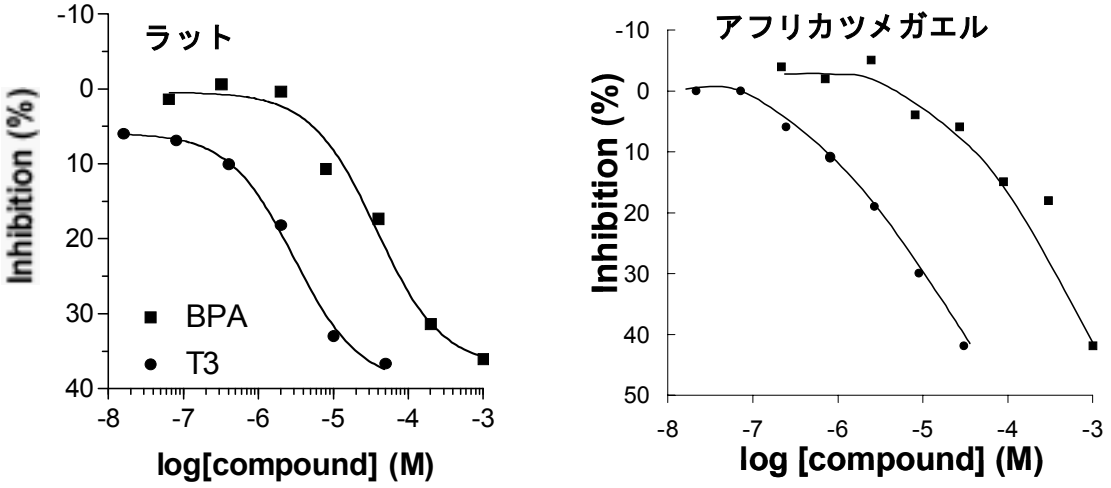

図３　ラットおよびアフリカツメガエルリコンビナントPDIに対するT3結合阻害実験

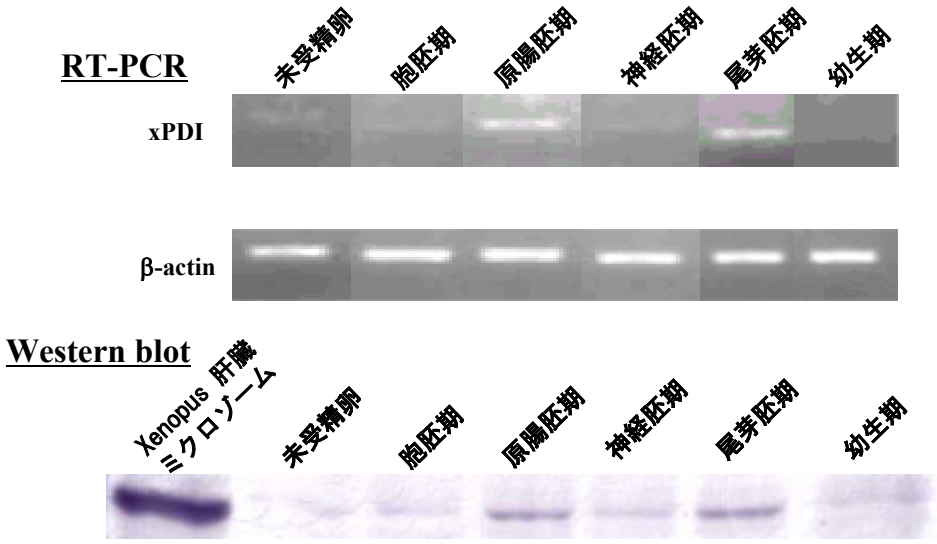

図４　発生過程におけるXenopus PDIの発現量変化

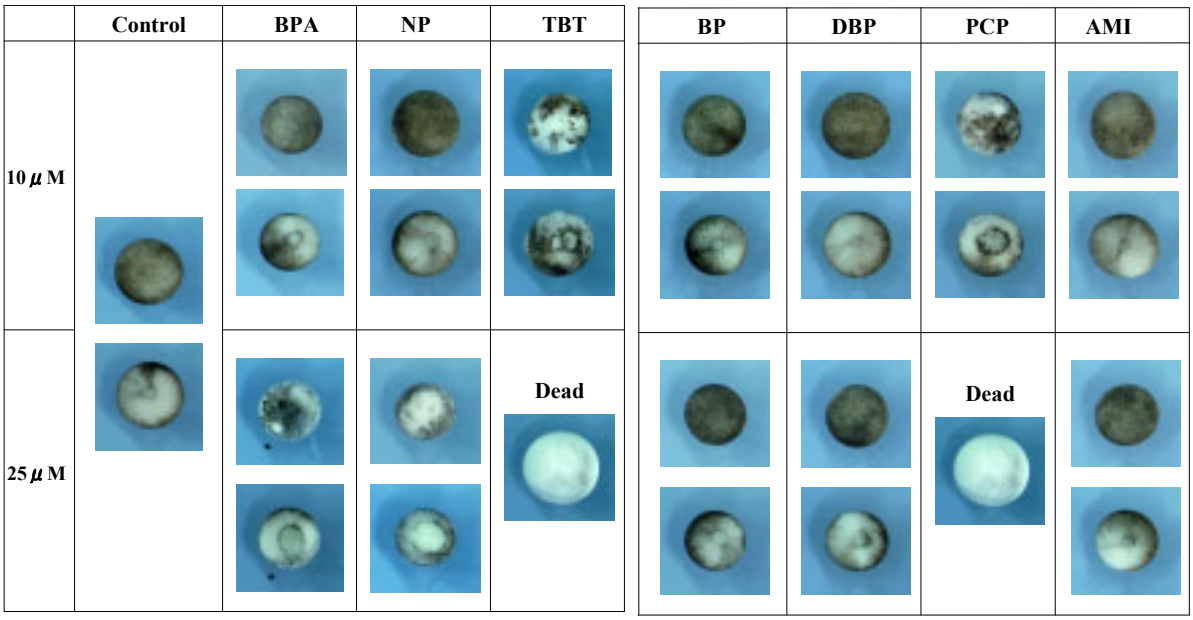

図５　アフリカツメガエル発生過程に及ぼす影響

Molecular biological investigations of bisphenol A receptor.

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Key Word : bisphenol A, central nervous system

Abstract

Environmental chemicals have been known to affect not only reproduct systems but also central nervous systems, which is caused by disruption of thyroid hormone action. In order to clarify their relevance to behavior disorder or learning disorder, it is important to elucidate the mechanism of disrupting activity of environmental chemicals toward thyroid hormone action, essential for normal development of brain function. Previously, we isolated and purified the novel bisphenol A (BPA) binding protein and found that it is the protein disulfide isomerase (PDI). We characterized that the triiodothyronine (T3) binding was inhibited by BPA. BPA may interfere with the function of thyroid hormone via PDI. In this study, to investigate T3 disrupting effects on neuronal development via PDI, we established three assay methods, the competitive binding assay with PDI, the induction of T3-dependent growth of GH3 cells, and the evaluation of thyroid hormone disrupting effect on early development of *Xenopus* embryos. By using these assay methods, we examined and compared the thyroid hormone disrupting activity of seven chemicals listed in "Chemicals to be Addressed in Prioritized Risk Assessments" selected 2000 and 2001 at ministry of environment as chemicals suspected of having endocrine disrupting effects.

In the first assay, the inhibition of T3-binding to PDI was investigated by using BPA, *p*-nonylphenol, tributyltin, benzophenone, dibutyl phthalate, pentachlorophenol, and amitrole. As the results, BPA, *p*-nonylphenol, and pentachlorophenol inhibited the T3 binding. These chemicals may possible to interfere with the function of thyroid hormone. We further investigated the thyroid hormone disrupting activity using GH3 cells, a rat pituitary cell line which grow and produce growth hormone (GH) depending on physiological concentrations of thyroid hormone. The cell growth is induced by the treatment of BPA and *p*-nonylphenol. Amphibians appeared in the course of vertebrate evolution between aquatic branchial respiration and quantic or terrestrial pulmonary respiration. *Xenopus laevis* has been studied extensively in developmental biology. First, *Xenopus* PDI (xPDI) cDNA was isolated and PDI protein was expressed in *E. coli*. xPDI interacted with T3 and BPA inhibited the binding of T3 to xPDI. xPDI revealed the similar properties with that of rat and human. xPDI was expressed at the stages of gastrula and tail

bud during the early development of *Xenopus*. In the third assay, we investigated effects of the chemicals on embryos during developmental stages from egg to gastrula. BPA and *p*-nonylphenol induced apoptosis at the stage of gastrula. These chemicals may possible to interfere with the function of thyroid hormone and then may affect the development of the embryos.