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Original article

Prenatal and lactational bisphenol A exposure does not alter serotonergic neurons morphologically in the murine dorsal raphe nucleus

Shoko Goto [a,b], Hiroshi Ogi [a], Shinji Fushiki [a], Kyoko Itoh [a,*]

[a] Department of Pathology and Applied Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan
[b] Department of Pathology, Meiji University of Integrative Medicine, Japan

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Abstract

Objective: There is concern that bisphenol A (BPA), an endocrine-disrupting chemical, affects brain development when exposed to a fetus and/or infant. We previously reported that increased serotonin (5-HT) and its metabolite (5-HIAA) in the dorsal raphe nucleus (DRN) in murine adult brains when they were prenatally exposed to low doses of BPA. This study investigates the morphological alteration of the dorsal raphe nucleus (DRN) in order to explain the disrupted serotonergic system after prenatal and lactational exposure to bisphenol A (BPA).

Methods: The murine dams were orally administrated with 500 μg/kg/day of BPA from embryonic day 0 to postnatal 3 weeks. The DRN, the main region of serotonin production, was morphometrically analyzed at 14 weeks, using immunohistochemistry and image analysis combined with 3-dimensional reconstruction.

Results: No significant differences were revealed in the number of tryptophan hydroxylase 2-immunoreactive neurons in any of the DRN sub-regions or the morphometric parameters, including the whole volume, ventrodorsal, longitudinal, and wing lengths of the DRN among the BPA treatment and sex groups.

Conclusions: The murine DRN was not morphologically affected by prenatal and lactational exposure to low doses of BPA. Further studies are necessary regarding the function of serotonergic neurons and the activity of different kinds of related receptors in the brain.

Keywords: Serotonin; Tryptophan hydroxylase 2; Morphometry; 3D-reconstruction; Development

1. Introduction

Bisphenol A (BPA), an endocrine-disrupting chemical, has been extensively used in polycarbonate plastic products and epoxy resins. Concerns have been expressed regarding the effects on brain development when BPA was exposed especially to human fetus and young children [1–5]. Braun et al. reported a positive association between a high maternal urinary concentration of BPA and psychiatric behavioral alteration in young girls, including more anxious and depressed behavior, poorer emotional control and inhibition [6]. Furthermore, some epidemiological studies demonstrated the significant association of maternal urine

* Corresponding author at: Department of Pathology and Applied Neurobiology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. Fax: +81 75 251 5849.
E-mail address: kxi14@koto.kpu-m.ac.jp (K. Itoh).

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BPA concentration and neurobehavior of children, in that increased emotional reactivity, aggressive behavior, internalizing or externalizing problems, anxiety, depression and attention problems were noted in boys; decreased anxious/depressed and increased internalizing or externalizing problems, hyperactivity and conduct problems in girls [2].

Previously, we reported that prenatal exposure to low doses of BPA affected murine fetal brain development, including accelerated radial migration of cortical neurons associated with abnormal genes expression responsible for cell differentiation, which resulted in abnormal cortical cytoarchitecture and thalamocortical projections [7,8], decreased dopaminergic neurons in the substantia nigra [9], reversed sexual differentiation in tyrosine hydroxylase-immunoreactive neurons of the locus coeruleus [10]. In addition, we demonstrated the epigenetic modifications of some genes as one of the underlying mechanisms of the BPA effects on developing brain [11]. Embryonic exposure to BPA caused both hyper- and hypomethylation at the promotor-associated CpG islands of multiple unique loci, which resulted in aberrant gene expression in the developing mouse forebrain.

Matsuda et al. demonstrated that perinatal exposure to bisphenol A enhanced contextual fear memory and affected the serotonergic system by inducing increased levels of serotonin metabolite (5-HIAA) and 5-HIAA/5-HT(serotonin) ratio associated with an increase of the expression levels of Tryptophan hydroxylase 2(*Tph2*), Solute Carrier Family 6, Member 4 (*Slc6a4*), and Monoamine oxidase A (*Maoa*) mRNA in the hippocampus of juvenile female mice [12]. We reported increased 5-HT and its metabolite, 5-HIAA in the caudate/putamen complex, the dorsal raphe nucleus and the substantia nigra at both postnatal 3 weeks and 14–15 weeks when mice were prenatally exposed to low doses of BPA [13]. In addition, they showed suppressed motor activity in behavioral tests, such as the plus maze test and the open field test [14]. These results suggested that prenatal and lactational BPA-exposure might perturb the serotonergic system in adult mice.

In the central nervous system, serotonin originates in the dorsal raphe nucleus (DRN) and median raphe nucleus in the midbrain and widely innervates the forebrain, midbrain and spinal cord through different kinds of serotonin receptors [15–18]. The DRN is divided into sub-regions such as the dorsal raphe (DR) rostral part, the DR ventral part, the DR dorsal part, the DR caudal part, the DR lateral part and the posterodorsal raphe nucleus, and each sub-region projects to selective brain areas so that distinct functions are executed [19,20]. The function of serotonin is complicated but mainly involved in the inhibitory system both in sensory input, behavioral output such as social function, repetitive behavior, and sensory development [21]. The serotonin system is reported to be involved in autism spectrum disorder (ASD) due to its pleiotropic role through multiple brain systems both dynamically and during development, although the contribution of the serotonin system to ASD pathophysiology remains to be understood. Taken together, the mechanisms how embryonic exposure to BPA affects the developing raphe nucleus and disrupts the serotonergic system are required to be scrutinized.

In the present study, we hypothesized that prenatal and lactational exposure to BPA might affect the morphological development of dorsal raphe nucleus (DRN), one of the main origins of serotonin. We evaluated the morphological changes in the dorsal raphe nucleus (DRN), with special reference to the number of tryptophan hydroxylase 2-immunoreactive neurons and GABAergic neurons in the different subnuclei of DRN, the volume and three dimensional shape of DRN in BPA-exposed mice compared with the controls.

2. Materials & methods

2.1. Animals and treatment

C57BL/6J mice (CLEA Japan, Tokyo, Japan) were housed in an animal facility and maintained under a 12:12-h light-dark cycle in a conditioned environment of 24 °C and 50% humidity. The animals were fed standard rodent diet CE-2 (CLEA Japan, Tokyo, Japan) upon arrival and for the duration of the experiment. All of the animal experiments were approved by the Institutional Review Board for Biomedical Research using Laboratory Animals at Kyoto Prefectural University of Medicine, and the animals were handled in accordance with the institutional guidelines and regulations.

The morning when a vaginal plug was observed after mating was designated embryonic day 0 (E0). In the BPA-exposed group, the dams were treated by feeding tube with 500 μg/kg body weight/day of BPA (Wako, Osaka, Japan) dissolved in 0.01% ethanol daily from E0 to postnatal 3 weeks (P3W). In the vehicle control group, the dams were treated by the same amount of 0.01% ethanol for the same period. The dose of 500 μg/kg body weight/day of BPA is less than one-hundredth of no-observed-adverse-effect level (NOAEL; 5 mg/kg body weight/day) in the Food and Drug Administration (FDA) safety assessment of BPA (2008). The offspring were weaned at P3 W and housed separately for each sex (4–5 mice in each cage) until P14W.

In the control cohorts, 8 female were randomly chosen from 5 dams and 8 male pups were chosen from 3 dams. BPA cohorts had 6 dams. 8 female pups were randomly chosen from 3 dams and 9 male pups were chosen from 5 dams.