論 文 内 容 の 要 旨

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論文題目　Studies on the Effect of Perinatal Exposure to Phthalate/Adipate Esters on Sexual Differentiation of the Brain
（フタル酸/アジピン酸エステル周産期暴露の脳の性分化に対する影響）

In mammals, gonadal steroids play an essential role in the process of sexual differentiation of the brain during embryonic development and early postnatal life, known as the critical period. During this period, testosterone secreted from the testes in males acts to masculinize and defeminize the developing brain after conversion to estradiol by the enzyme aromatase in the brain. Our previous research has identified granulin (grn) and p130 genes as sex steroid-regulated genes in neonatal rat hypothalamus, which are probably involved in sexual differentiation of the brain. According to our observations regarding the androgen- and estrogen-dependent regulation of grn and p130 genes and the sexually dimorphic patterns of their expression in the hypothalamus during the critical period, these genes could be good parameters for assessing the androgenic and/or estrogenic properties of potential environmental endocrine disrupting chemicals (EDCs) in the neonatal brain.

EDCs include a number of environmental chemicals that interact with an endocrine system, often due to their activity as a hormonal mimic. EDCs are supposed to act genomically with agonistic or antagonistic effects on sex steroid receptors, and alter reproductive function. For example, phthalate esters that are used as plasticizers and also found at low levels in foods such as dairy products are often mentioned as suspected endocrine disrupters having (anti)-estrogenic or (anti)-androgenic properties.

Moreover, since phthalates have been shown to cross the placenta and pass into breast milk in animal studies, prenatal exposure and exposure from breastfeeding may occur in humans. Therefore, there is a risk that brain sexual differentiation is affected by exposure to EDCs during the critical period. The purpose of the present study is to elucidate whether perinatal exposure to di-n-butyl phthalate (DBP), diisononyl phthalate (DINP) and di-2-ethylhexyl adipate (DEHA) affects several aspects of reproductive function in rats, especially sexual differentiation of the brain. To this end, the dams were provided with pulverized soy-free diet (low phytoestrogen diet) containing 20, 200, 2,000 and 10,000 ppm of DBP, 40, 400, 4,000 and 20,000 ppm of DINP, or 480, 2,400 and 12,000 ppm of DEHA from gestational day (GD) 15 to postnatal day (PDN) 21, the day of weaning, and anogenital distance (AGD), serum sex steroid levels, hypothalamic gene expression of grn and p130 during neonatal period and serum gonadotropin levels and sexual behaviors after maturation were assessed.

Exposure to the high doses of DBP, DINP and DEHA during gestational period significantly decreased food consumption and body weight gain of dams, while a significant reduction of maternal food consumption during lactation period (PND 9-17) was observed in only 10,000 ppm DBP-exposed group. At PND 1, body weights of DBP-, DINP- or DEHA-exposed pups were also significantly decreased compared with those of control pups of corresponding sexes. When AGD was normalized by the cube root of body weight, exposure to DINP of all the doses used and the higher doses (2,400 and 12,000 ppm) of DEHA decreased AGD in male neonates, though that to DBP did not affect AGD in males. In female neonates, an increase in AGD was observed in DBP- and DINP-exposed animals at the highest doses. At PND 3 and 7, perinatal exposure to DBP, DINP and DEHA did not substantially affect serum concentrations of testosterone and estradiol as compared with the control group in both sexes. On the other hand, at PND 3, the expression of grn mRNA levels in males was decreased by DEHA, and that of p130 was decreased by DBP, DINP and DEHA, though the effects were not dose-dependent. At PND 7, the expression of grn gene in female pups was increased by higher doses (2,000 and 10,000 ppm) of DBP and all the doses, except for 4,000 ppm, of DINP, while that in male pups decreased by 480 and 12,000 ppm of DEHA. Hypothalamic expression of p130 mRNA in males was increased by lower doses (20 and 200 ppm) of DBP and all the doses of DINP, whereas that of females was decreased by 480 and 2,400 ppm of DEHA.

After maturation, serum LH, FSH and testosterone levels in male rats at postnatal week (PNW) 20 were not affected by perinatal exposure to any of the chemicals used. Although the numbers of both mount and intromission in 40 ppm DINP- and 480 ppm DEHA-exposed rats were significantly decreased, perinatal exposure to DBP and DINP at other doses and DEHA at any doses used did not affect the number of mount and intromission. On the other hand, a decrease in the number of ejaculation was observed in 200 and 2,000 ppm DBP-, 40 ppm DINP-, and 48 and 12,000 ppm DEHA-exposed rats, while in 10,000 ppm DBP-exposed rats, the number of ejaculation was increased. A decrease in post ejaculation interval was observed only in 10,000 ppm DBP-exposed rats. At both PNW 8-9 and 19-20, all the control and chemical-exposed females showed regular estrous cycles. Perinatal exposure to the chemicals also did not affect serum levels of LH, FSH and estradiol at both 1100 and 1600 h on the proestrous day at PNW 20. On the other hand, lordosis quotient examined on the proestrous day was significantly decreased in all the groups of animals perinatally exposed to DBP, DINP and DEHA at any doses.

The present results indicate that gestational exposure to these chemicals affects fetal development and AGD. Since AGD is commonly regarded as an androgen-sensitive developmental measure in rodents, the antiandrogenic effect of DINP and DEHA appears to be more potent than that of DBP. On the other hand, DBP and DINP at the highest dose used increased AGD in female neonates, suggesting a large amount of these phthalates may also have a weak androgenic property. Gene expression of grn and p130 in the hypothalamus was also affected by perinatal exposure to the chemicals. Although the effects of chemicals varied depending on the sexes of neonates, but not on the doses of chemicals, the increase in grn gene expression in female hypothalamus may be due to the estrogenic properties of the pthalates, and antiandorogenic properties of these compounds may account for the changes in p130 gene expression in males. Contrary to the pthalates, the adipate might exert antiestrogenic effects on males and androgenic effects on females, but further studies are needed to clarify the precise mechanisms underlying the actions of pthalate/adipate esters on sex steroid-dependent gene expression in the hypothalamus. After maturation, serum levels of sex steroids and gonadotropins in both male and female rats, as well as estrous cyclicity in females, were not changed by perinatal exposure to DBP, DINP and DEHA, indicating that these chemicals do not affect sexual differentiation of

the brain controlling the endocrine system of hypothalamo-pituitary-gonadal (HPG) axis. On the other hand, inhibitory influences on sexual behaviors, especially on ejaculation in males and lordosis in females, were observed by perinatal exposure to these chemicals. Since it has been demonstrated that ejaculation-specific circuit exists within the larger circuitry for male sexual behavior, the chemicals may directly affect the ejaculation-specific circuit within the brain by altering the expression of grn and/or p130 genes. In females, the brain region responsible for inducing preovulatory GnRH surge is supposed to be the medial preoptic area (MPOA), and that responsible for inducing lorsosis is the ventromedial nucleus of the hypothalamus (VMH). The chemicals used in the present study may directly affect the organization of neuronal circuits in the VMH, but not MPOA, during the processes of the sexual differentiation of the brain.

In conclusion, the present study showed that inappropriate expression of grn and/or p130 genes in the hypothalamus of neonatal rats by perinatal exposure to DBP, DINP and DEHA may results in a decrease in sexual behaviors after maturation in both sexes without affecting the endocrine system of the HPG axis. The results also suggest that grn and p130 may be involved in the processes of not only masculinization (increase in male-type sexual behavior), but also defeminization (decrease in female-type sexual behavior) of the rat. The phthalate/adipate esters may act directly on discrete regions of the hypothalamus regulating sexual behaviors, but not regulating gonadotropin secretion, thereby affect sexual differentiation of the brain with a resultant decrease in sex-specific behaviors in adulthood.