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Sex-specific effects of early neonatal progesterone treatment on dopamine and serotonin metabolism in rat striatum and frontal cortex

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ABSTRACT

Aims: The early neonatal period is critical for the development of the rodent brain. Neurosteroid levels in the brain decline from the late gestation to the neonatal period. Previous studies indicate effects of neurosteroid treatment during the neonatal period on the development of the dopaminergic system. In this study, we investigated the sex-specific effects of neonatal treatment with the neurosteroid progesterone on monoamine metabolism. Separately, we examined the contribution of pre-pubertal castration on the effect of neonatal treatment of pregnenolone (a neurosteroid precursor).
Main methods: Progesterone (Experiment 1) or pregnenolone (Experiment 2) treatments in Sprague-Dawley rats were performed from postnatal days 3 through 7. Castration in experiment 2 was performed in male rats at postnatal day 21. We measured the brain tissue contents of dopamine, serotonin (5-HT), and their metabolites in rats at age 10 weeks.
Key findings: Results showed that neonatal progesterone treatment altered striatal 5-hydroxy-3-indolacetic acid/5-HT ratios in males and females in opposite directions, in addition to dopaminergic effects. The treatment also influenced dopamine and 5-HT metabolism without sex-specificity in the frontal cortex. In addition, there was no significant difference in striatal monoamine metabolism between sham-operated, castrated and castrated pregnenolone-treated group.
Significance: The present result indicates a sex-specific influence of progesterone during the early neonatal period on the development of the serotonergic system, depending on brain region in addition to of the dopaminergic system.

Introduction

The rodent early neonatal period is critical to determining normal behavior or endocrine responses after maturation (Ogawa et al., 1994). Pregnenolone-derived neurosteroids, including progesterone (PROG) and allopregnanolone (ALLO), occur at high levels in dams during pregnancy and fetus (Concas et al., 1999; Grobin and Morrow, 2001; Ward and Weisz, 1984), which attenuates excessive stress responses such as activation of the hypothalamo–pituitary–adrenal axis or oxytocin secretion (Russell and Brunton, 2006). Neurosteroids levels in dams and neonates then decrease drastically around delivery (Concas et al., 1999; Grobin and Morrow, 2001; Ward and Weisz, 1984). The early neonatal period can thus be designated as a withdrawal period from the prenatal high neurosteroid condition.

Data have indicated that neonatal treatment with neurosteroids can modulate dopaminergic functions. Neonatal administration of ALLO (Muneoka et al., 2009b) or pregnenolone (Muneoka and Takigawa, 2002) alters dopamine (DA) metabolism in adult striatum. Neonatal treatment with another neurosteroid, dehydroepiandrosterone (DHEA), increases DA transporter density in the striatum (Muneoka et al., 2009a). Furthermore, neonatal administration of ALLO affects amphetamine-induced locomotor activity and prepulse inhibition in adulthood (Gizerian et al., 2006). While, previous data have not clearly demonstrated a consistent influence of neonatal neurosteroids on serotonergic functions (Muneoka et al., 2009b; Muneoka et al., 2002; Shirayama et al., 2001) although neonatal pregnenolone transiently alters striatal serotonin (5-HT) (Muneoka and Takigawa, 2002).

A role for PROG in the development of the brain is beginning to be investigated despite that cases of PROG exposure on the developing human brain is increasing (Wagner, 2008); PROG is used for the prevention of premature birth (Ness et al., 2006) and the treatment of premature infants (Trotter et al., 2007). In addition, breast-fed infants may be exposed to progestins from the use by lactating women as contraceptives (Wagner, 2008). Animal studies indicate that the brain is sensitive to PROG during development and PROG receptors transiently express in the cerebral cortex during perinatal period

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(Jahagirdar and Wagner, 2010). Furthermore, there are sex differences in PROG receptor expression (Wagner, 2008). These evidence suggests that PROG may play an important role in the sexual differentiation of brain. Effects of neurosteroids on monoamine neuronal system have been confirmed by treatment with ALLO or DHEA from postnatal day (P) 3 to 7 (Muneoka et al., 2009a,b; Muneoka et al., 2002; Muneoka and Takigawa, 2002). In this study, we addressed sex specificity in the effects of PROG on striatal and cortical monoamine metabolism on the same treatment schedule.

Furthermore, gonadal hormones during puberty are critically involved in the maturation of monoaminergic function (Knoll et al., 2000). Indeed, recent studies have demonstrated that neonatal administration of pregnenolone (Muneoka et al., 2002), pregnenolone sulfate (Jorge et al., 2005), and ALLO (Zimmerberg and Kajunski, 2004) influence behavior sex specifically. Therefore, we examined pre-pubertally castrated animals that were treated with pregnenolone neonatally to investigate a relationship between gonadal hormones and neurosteroids. Pregnenolone is considered proper for a primary study of sexual dimorphism in the effects of neurosteroid because it is the precursor for all neurosteroids.

Experimental methods

Animals

Sprague-Dawley rats were purchased from Charles River Laboratories (Tsukuba, Japan). They were housed in metal cages in a room with temperature and relative humidity controlled at 24 ± 1 °C and 50 ± 5%, respectively. Lights were turned on from 0700 to 1900 h daily, and food and tap water were supplied ad libitum. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the Food and Drug Safety Center, Hatano Research Institute. At 11 weeks of age, female rats were cohabited overnight with males. Females with sperm in their vaginal smears were regarded as pregnant. Within 24 h after birth (P 0), the litters were culled to 10 pups with an equal as possible number of male and female pups. Pups were weaned at P 21. For the neurochemical assays, at 10 weeks of age, animals obtained from independent litters were sacrificed by decapitation and their brains removed between 1600 and 1800 h. The frontal cortex and striatum were subsequently dissected on ice. All tissues were stored at −80 °C until the assay. Seven litters each were used for Experiments 1 and 2.

Experiment 1. Neonatal treatment with PROG in male and female rats.

Male and female pups were injected with PROG (Sigma, 5 mg/kg, subcutaneously) suspended in sesame oil, at 1300 h, from P 3 through 7 (PROG-group). Controls were injected with an equivalent volume of sesame oil (5 μl/g) on the same time schedule as the PROG-group.

Experiment 2. Pre-pubertal castration and neonatal pregnenolone treatment.

Male rats in the Castration + Pregnenolone group were injected with pregnenolone (3β-hydroxy-5-pregnen-20-one, Sigma; 10 mg/kg, subcutaneously) suspended in sesame oil, at 1300 h, from P 3 through 7 while the Sham and Castration groups were injected with an equivalent volume of sesame oil (5 μl/g) on the same time schedule as the Castration + Pregnenolone group. Castration and Castration + Pregnenolone groups received the castration operation at P 21 while the Sham group underwent a sham castration operation at P 21.

Measurement of brain monoamines

All tissues were stored at −80 °C until the assay. Tissue levels of DA, 5-HT, and their metabolites, dihydroxyphenylacetic acid

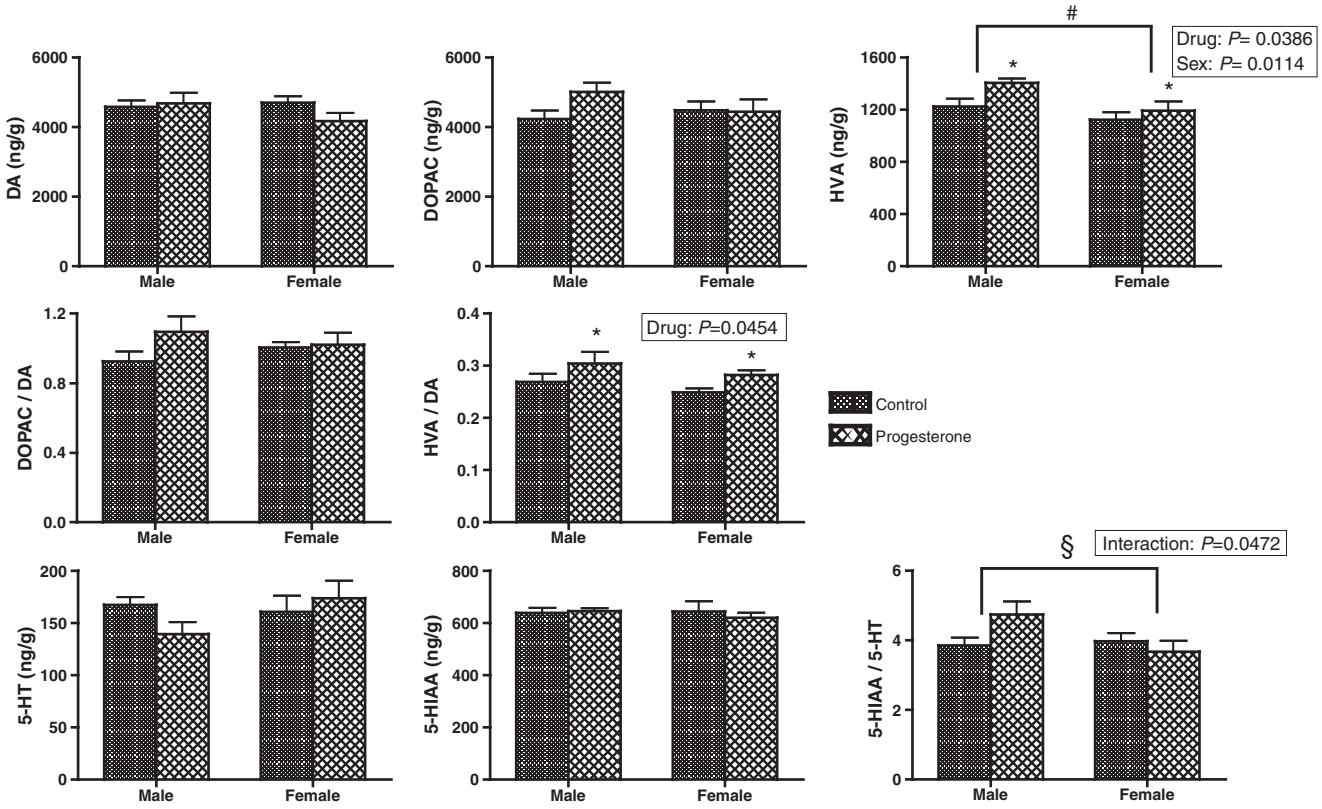

Fig. 1. Effects of neonatal PROG treatment on monoamine metabolism in the striatum of 10 weeks-aged male and female rats. Monoamine contents (ng/g) and turnovers are indicated as mean ± SEM. *P* values are indicated when significant effects of Drug, Sex or Interaction (Drug × Sex) are detected in two-way ANOVAs. Significant effects of Drug, Sex and Interaction (Drug × Sex) are indicated in the figure as *, # and §, respectively. Sample numbers are 4 to 6.