Identification of Granulin Gene as a Sex-Steroid Inducible Gene Involved in Sexual Differentiation of the Rat Brain

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Mechanisms of sexual differentiation of the brain by sex steroids seem conserved throughout the mammalian species, although there may be some species difference. In rat, sex-dependent differentiation of the brain occurs in a sex steroid-dependent manner during the perinatal period known as the critical period. For the differentiation of the brain, it is reasonably premised that sex steroids would induce some specific gene expression in the hypothalamus during the critical period. We attempted to characterize sex steroid-inducible genes that are involved in the sexually dimorphic function of the brain. For a trial to identify the sex steroid-inducible genes that are involved in the sexual differentiation of the hypothalamus, we employed cDNA subtraction. Following the cDNA subtraction between hypothalami of 5-day-old intact and neonatally androgenized female rats, a granulin (grn) precursor gene was identified. Grn encodes a 6 kDa polypeptide known as a growth modulating factor of epithelial cells in vitro.

Next, we determined the localization of grn mRNA in the brain of neonatal rats. In situ hybridization with the grn probe was performed on the brain tissue obtained from a 5-day-old male rat. The strong grn mRNA signals were obtained in the ventromedial hypothalamic nucleus (VMH) and the arcuate nucleus (ARC) of the hypothalamus, although weak signals were widely expressed throughout the brain.

To determine changes in grn gene expression in the hypothalamus of intact male and female rats during a perinatal period, northern hybridization was carried out on hypothalamic samples from intact males and females at -1, 1, 3, 5, 7 and 10 days after birth. Between days -1 to 1, there was no difference in grn gene expression in the hypothalamus between males and females. While the expression level of the grn gene in the male hypothalami remained almost constant until day 10, that in the female hypothalami gradually decreased and reached about 1/4 of the initial value on day 10.

From above results, we hypothesized that higher grn gene expression in the neonatal hypothalamus during the critical period is important for the process of masculinization of the brain in the rat. To ascertain this hypothesis, we adapted the antisense oligodeoxynucleotide (ODN) method. Antisense ODN complementary to grn mRNA was synthesized and infused into the third ventricle of male rats at 2-day old. Two different control treatments were used; the first consisted of a control sequence ODN that had little homology to known mRNAs, and the second of vehicle alone. After maturation, subject animals treated with antisense ODN of grn displayed significantly lower scores than control males on various parameters assessing sexual behavior; i.e. mounting, intromission and ejaculation.

The present results suggest that the grn gene, expression of which is induced by testosterone in the neonatal hypothalamus, plays a crucial role in the process of functional masculinization of the rat brain. Supported by JSPS-RFTF 97L00904.

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