MicroRNAs involvement in expression of NMDAR1 in Hypothalamus of Central Precocious Puberty Female Rat

Minda Ju, Mizhen Zhang, Zhanzhuang Tian

Abstract: Background: Central Precocious Puberty (CPP), one of an abnormal development disease, was due to the early activation of Hypothalamus-pituitary-gonad axis (HPGA). Gonadotropin-releasing hormone (GnRH) is the initial factor of HPGA and play an important role in puberty onset and reproduction. The regulation of GnRH synthesis and pulsatile secretion become a central issue in the study of puberty onset and its related diseases. Abnormal changing of gamma-aminobutyric acid (GABA), Kisspeptin/GPR54, dynorphin/κ opioid receptor (KOR-1), neurokinin B (NKB), somatostatin (SS), glutamate (Glu) and etc, could alter GnRH secretion and lead to CPP. N-methyl-D-aspartate receptor (NMDARs) is a kind of ionotropic glutamate receptor and wide spread in the central nervous system (CNS). NMDAR1 is considered to be the necessary subunit of NMDARs. MiRNAs play an important role in CNS development and some diseases. Studies found that miR-200 and miR-155 could rise the hypothalamic GnRH expression before puberty. However, the mechanism of NMDARs especially NMDAR1 and its relative miRNAs in pathogenesis of CPP remains obscure. To investigate the role of NMDAR1 and its miRNAs in mechanisms of CPP, hyporhalamic NMDAR1 and its miRNAs expression were probed in female CPP model rats induced by danazol.

Methods: Firstly, hypothalamic NMDAR1 expression, as well as the serum GnRH, E₂, LH and FSH levels were detected in normal female Sprague-Dawley rats at 7, 14, 21, 28, 35, 42 days of age by means of western blot, RT-PCR and RIA. Secondly, female Sprague-Dawley rats were randomly divided into normal (N), vehicle (V) and model (M) groups. The model litters at postnatal day 5 were given a single subcutaneous injection of 300ug of danazol. The characteristic of hypothalamic NMDAR1 expression, as well as the serum GnRH, E₂, LH and FSH levels on the day of prepuberty, onset-puberty and post-puberty were investigated. The relevant miRNAs of NMDAR1 were screened out by bioinformatics, their inhibitors and mimics were transfected to the cultured primary neurons of hypothalamus. The miRNAs related to NMDAR1 and GnRH were detected by RT-PCR, WB and Dual-Luciferase reporter assay system.
**Results:** The serum GnRH, \(E_2\), LH and FSH levels increased with age and reached the top level on the day of vaginal opening (at approximately 35 days of age) in normal female rats. Hypothalamic NMDAR1 expression was in accord with the trend of serum hormones change. The serum GnRH, LH, FSH and \(E_2\) levels of the CPP model increased more obviously than those of vehicle ones, with the days of vaginal opening advancing. The NMDAR1 expression of the CPP model increased more significantly than normal ones. By means of bioinformatics and transfecting miRNA inhibitors and mimics, miR-664-2, miR-326, miR-92a were screened out related to NMDAR1 in primary neurons of hypothalamus.

**Conclusions:** The up regulation of hypothalamic NMDAR1 might involve in the onset-puberty in normal female rats. NMDAR1 might participate in pathogenesis of CPP. MiR-664-2, miR-326 and miR-92a were related to NMDAR1 in hypothalamus.

**Key words:** NMDAR1; miRNAs; hypothalamus; danazol; rat