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Recently we demonstrated that exonic splicing enhancers (ESEs) located in the gonadotropin-releasing hormone (GnRH) exons 3 and 4, were involved in enhanced excision of the first intron (intron A) of the GnRH gene. To elucidate the functional importance of the ESEs in excision of the intron A from the GnRH primary transcripts, we employed hypogonadal (hpg) mutant mice. Hpg mice lack the ability to synthesize the GnRH peptide due to a deletion of two exons (exons 3 and 4) in the GnRH gene. Intron A excision rate of GnRH pre-mRNA was examined by ribonuclease protection assay and competitive reverse transcription-polymerase chain reaction (RT-PCR) with three RNA samples from the preoptic area (POA) where the majority of GnRH-synthesizing cell bodies and GnRH mRNA are concentrated, olfactory bulb (OB), and cerebral cortex (CTX) derived from the adult hpg (hpg/hpg), heterozygous (hpg/+), and normal (+/+) mice. Intron A excision rate in the POA of hpg mice was severely lower than that of normal and heterozygous mice but similar to that in other tissues such as CTX and olfactory bulb. In contrast, the level of GnRH pre-mRNA containing intron A in hpg mice was not significantly different from that of heterozygous and normal mice. It appears that ESEs are important for excision of intron A of the GnRH primary transcripts.

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First Intron Excision of Gonadotropin-Releasing Hormone Pre-mRNA during Postnatal Development of Normal Mice

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Gonadotropin-Releasing Hormone (GnRH) is a hypothalamic decapeptide that plays a pivotal role in the neuroendocrine regulation of pituitary functions, and GnRH gene is consisted of four exons and three introns. We recently found that intron A excision appears a rate limiting step for GnRH pre-mRNA splicing. In the present study we explored whether the splicing rate of GnRH pre-mRNA is developmentally regulated. The preoptic area (POA) where the majority of GnRH synthesizing cell bodies and GnRH mRNA are concentrated and cerebral cortex (CTX) as a control were removed from mice at the age of 1 to 7 weeks. GnRH pre-mRNA splicing rate was examined by competitive reverse transcription-polymerase chain reaction (RT-PCR) using a variety of primer sets. An intron A excision rate of GnRH pre-mRNA in the POA was altered during mouse postnatal development: The intron A excision rate in the POA was significantly increased in 3 week-old mice and further increased until adulthood. In contrast, in the CTX, intron A excision rate was extremely low, drastically decreased in 3 week-old mice, and remained at low levels until adulthood. Intron B excision rate in the POA was not significantly changed during development. GnRH mRNA levels in the POA were gradually increased during development as similarly as the intron A excision rate, but pre-mRNA containing intron A (1A23) levels was not significantly changed. It suggests that the alteration in GnRH mRNA levels during development roughly parallel with changes in intron A excision rate. Collectively, the present study indicates that intron A excision rate is developmentally regulated and involved in the function of GnRH neurons.