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Postnatal Remodeling of Gonadotropin-Releasing Hormone I Neurons: Toward Understanding the Mechanism of the Onset of Puberty

The concept that an increase in pulsatile release of GnRH-I triggers the onset of puberty has been firmly established. Knobil and collaborators (1) have shown that pulsatile infusion of GnRH-I results in precocious menarche followed by first ovulation in sexually immature female rhesus monkeys. GnRH-I analogs have been successfully used for treatments of precocious and delayed puberty in humans (2). We have shown that pulsatile GnRH-I release increases at the onset of puberty in female rhesus monkeys (3). However, the mechanism triggering the pubertal increase in GnRH-I release is still unclear (4).

In primates, GnRH-I neurons appear to be fairly mature before the onset of puberty. The distribution pattern is established well before birth (5), and there are no differences in the number (6) or the shape of GnRH-I neurons (7) or in GnRH-I mRNA levels (8) in the hypothalamus of juvenile and adult monkeys. Functionally, GnRH-I neurons in juvenile monkeys are also mature, because the GnRH-I neuronal system can respond to electrical or neurochemical stimulation, such as NMDA and kisspeptin (9–11).

In contrast, mRNA expression and morphology of GnRH-I neurons in small laboratory rodents appear to be less mature until an age close to puberty. In rats and mice, GnRH-I mRNA levels increase gradually with postnatal age, such that significant increases occur at postnatal d 15–30 (P15–30) depending on sex and experimental conditions (12). Moreover, in rats, the number of GnRH-I neurons with an irregular contour increases, whereas the number of GnRH-I neurons with a smooth contour decreases at the age of puberty (13). The article in this issue by Alan Herbison's group (14) clearly shows that postnatal GnRH-I neurons undergo major structural remodeling and provides new insight into the mechanism of puberty.

As previously used by Campbell *et al.* (15), Cottrell and colleagues (14) filled GnRH-green fluorescent protein (GFP)-labeled neurons with biocytin *in vitro* in juvenile (P10–15) and adult (older than P60) transgenic male mice created by the same laboratory and analyzed cell size, dendritic branching, and the number of somal and dendritic spines using confocal microscopy. Although the soma size (assessed by measurement of circumference) did not differ, GnRH-I neurons in adult mice have a smaller number of dendritic branching points and a larger spine density of the soma and proximal dendrite (0–50 μm from the soma), but not distal dendrite (>50 μm), than those in juvenile mice. Three-dimensional reconstruction images comparing GnRH-I neurons between juvenile and adult are truly striking. Immature GnRH-I neuronal cell soma with a relatively smooth surface extends several dendritic trees with some dendritic spines and filopodia, whereas mature GnRH-I neuronal cell soma with massive spines extends a single dendrite, which is also covered by massive dendritic spines and filopodia. However, these striking results across puberty with the biocytin cell-filling experiment may not reflect *in vivo* events. Accordingly, the authors analyzed GnRH-GFP neurons without dye filling in other transgenic male mice, in which GnRH-I neurons were more intensely GFP labeled (16). The number of GnRH-I neurons in the medial septum and rostral preoptic area forming the inverted Y distribution (17) did not differ among mice at P3, P10, P35, and adults, but GnRH-I neurons in immature mice at P10 had fewer unipolar dendritic processes and more complex processes than those in mature mice at P35 and adult mice, confirming the results of the cell-filling experiment. Finally, because the dendritic spines form predominantly excitatory glutamatergic synapses, the authors further examined whether the number of inhibitory GABAergic inputs to GnRH-I neurons changes across puberty by immunostaining of vesicular GABA transporters. The results indicate that there is no developmental change in GABAergic input to GnRH-I neurons.

Although an increase in GnRH-I release triggers puberty, there is a significant species difference in the neuroendocrine mechanism of the onset of puberty in primates and laboratory rodents (4). In primates, active GnRH-I neurosecretory neurons during the neonatal period are subsequently suppressed by steroid-independent central inhibition until shortly before puberty (18). We have shown that GnRH-I neurons in juvenile monkeys are inhibited by GABAergic neurons, and reduction in GABA tone results in precocious puberty (19, 20). Moreover, the pubertal reduction in GABA inhibition allows an increase in glutamatergic signal that stimulates GnRH-I release (4, 10). In contrast, in rodents, tonic central inhibition, equivalent to that in primates, does not appear to exist, and establishment of excitatory neuronal systems for GnRH-I release, such as glutamatergic (21) stimulation, results in puberty. The study by Cottrell *et al.* (14) provides evidence that postnatal excitatory synaptic remodeling of GnRH-I neurons occurs across puberty, and their finding is consistent with the notion that postnatal excitatory innervation of GnRH-I neurons triggers the onset of puberty in mice. The questions of which excitatory synaptic input (*e.g.* glutamatergic, kisspeptinergic, or other neurochemical signals yet to be discovered) plays a role in the pubertal increase in GnRH-I release and whether the synaptic remodeling observed during pubertal maturation is solely a result of ste-

Abbreviations: GFP, Green fluorescent protein; P, postnatal d.

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roid-independent developmental phenomena or in part a result of the pubertal increase in steroid hormones remain to be investigated. Both estrogens and androgens in adults are involved in synaptic plasticity, modifying spine density, and synaptic formation (22, 23).

In primates, the mechanism of puberty is far from being understood. Although primate GnRH-I neurons may undergo subtle morphological changes during development (24), there is little reported on postnatal ontogeny. Do primate GnRH-I neurons undergo postnatal synaptic excitatory remodeling similar to those observed in mice? Do GABAergic inhibitory synapses on primate GnRH-I neurons undergo a steroid-independent developmental change? If they do, how does inhibitory synaptic remodeling precede excitatory synaptic remodeling? Glial involvement in puberty has been shown (25), but how do nonsynaptic mechanisms contribute to the pubertal change in synaptic plasticity? Answers to these questions should provide the mechanism of the pubertal increase in GnRH-I release.

Postnatal synaptic remodeling through adolescence also appears to occur in the neocortex that controls cognitive functions in humans (26). Although systematic and precise neuroanatomical studies are yet to be conducted, overproduction of synapses of cortical neurons during the early postnatal life are gradually pruned until a specific neural pathway is established, and this process may continue throughout the juvenile period until after puberty (27, 28). Including recent exciting findings on the possible role of kisspeptin in puberty (29, 30), we are now facing another new avenue to discover how synaptic remodeling of GnRH-I neurons occurs at the time of puberty in the primate hypothalamus.

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