

Is precocious puberty linked to hypothalamic expression of arginine-phenylalanine-amide-related peptide?

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ABSTRACT

The up-regulation and down-regulation of gonadotropin-releasing hormone (GnRH) in central precocious puberty is not yet known. However, recent advances in neuroendocrinology have shown the controlling role of arginine-phenylalanine RF-amide-related peptides (RFRPs) on GnRH secretion in different phenomenon of reproduction such as estrus cycle and pregnancy, but the exact role of RFRPs in puberty and its related pathologic condition, precocious puberty, is not clear yet. This paper hypothesizes that RFRP is a regulatory peptide of puberty and might prevent the precocious puberty. On the basis of previous studies on hormonal fluctuations at the time of puberty, RFRP might have a role on controlling of premature secretion of GnRH and avoiding central precocious puberty.

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Introduction

Puberty is a complex developmental physiologic process in the development that can be affected by genetic and environmental factors (1) and internal changes and other factors such as body weight and fat content of the body. On the other hand, development of secondary sexual characteristics before the age of 9 years in boys and 8 years in girls have been defined as precocious puberty (2). Because the onset of pulsatile secretion of gonadotropin-releasing hormone (GnRH) in hypothalamus is the major event for start of the complex process of puberty in primates (3), disturbance in GnRH release causes the gonadotropin-dependent type of precocious puberty. Premature activation of the hypothalamic-pituitary-gonadal (HPG) axis is one of the possible predisposing factors of the gonadotropin-dependent (central) precocious puberty (2).

Different hypothalamic neuropeptides have been investigated in animal models regarding to the onset of puberty. It has been shown that the number of kisspeptin-immunoreactive cells and level of hypothalamic Kiss-1 mRNA in the arcuate nucleus (Arc), preoptic area (POA) and periventricular nucleus (PeN) and NKB/NK3R mRNA and protein in the Arc and medial preoptic area (MPO) were increased by induction of precocious puberty (4, 5). Furthermore, induction of precocious puberty increased the hypothalamic contents of ghrelin, the

number and activity of growth hormone secretion peptide receptor 1-alpha (GHSR1- α), mTOR mRNA and p-mTOR protein in rat model (6, 7). Therefore, hypothalamus neuropeptides controls puberty with different pathways that have role in HPG axis and controlling gonadotropin secretion. In this review on the basis of previous investigations on sex steroid hormonal fluctuations at the time of puberty, we discussed that an inhibitory neuropeptide might have a role on inhibition of premature secretion of GnRH and avoiding central precocious puberty.

Inhibition of GnRH secretion in hypothalamus

Existence of an inhibitor of HPG axis by controlling gonadotropin secretion had been suspected earlier (8). Then, discovery of a 12 amino acid peptide (SIKPSAYLPLRFamide), gonadotropin inhibitory hormone (GnIH), which could directly inhibit releasing of GnRH in the quail brain (9), opened a new window for better understanding of the HPG axis function. After discovery of GnIH role in the control of GnRH during the current decade, the same function was reported for the avian homologues of GnIH in mammals, arginine-phenylalanine-amide (RFamide)-related peptides (RFRP). Presence and expression of RFRPs have been reported in rodents (10, 11), ruminants (12-15), primates (16) and human (17). The RFRPs are a peptide family with an arginine-phenylalanine (RF-NH₂) sequence at their carboxyl

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terminals. The first member of this family was discovered in shell ganglions (FMRFamide) (18) and then in vertebrates was known in the avian brain (LPLRFamide) (19). Between the members of these family, RFRP-3 has several reproductive functions (20) and has affinity to NPFFR1 (21). In all vertebrate species from fish (22) to human (17), the GnIH/RFRP peptides inhibit the gonadotropins secretion.

RFRP expression in hypothalamus

To determine the exact function of RFRP in puberty or precocious puberty, detection of the cell bodies and fibers of RFRP neurons in hypothalamus and its nuclei is necessary. For instance, for detection of neuronal cell bodies of RFRP in the dorsomedial hypothalamus (DMH) several types of antibodies have been used, including an antibody against the sparrow GnIH produced in rabbits (23), an antibody against the quail GnIH produced in rabbits (24), and an antibody produced against the sequence 119-132 of prepeptide RFRP (25). In addition to DMH, Arc, paraventricular nucleus (PVN), POA, and PeN in rodents, ruminants and primates have the RFRP neuronal bodies (11, 14, 16, 17, 23, 26-29). Furthermore, RFRP neuronal fibers have been detected in most parts of the brain, including the hemispheres or telencephalon, middle areas of the brain, limbic areas (POA, septal and amygdala), rostral hypothalamus, septal nuclei and accumbens, habenular nuclei, thalamus, upper colliculi, Raphe nuclei and the pons in rodents and primates (11, 16, 23).

A direct connection between GnRH neurons and RFRP neuronal terminals may show a direct effect of RFRP neurotransmitter on GnRH neurons. For instance, in the POA of male rats, RFRP fibers have close associations with approximately 75% of GnRH cell bodies (30). Similar connections have been reported in female rodents (23). Furthermore, GnRH neurons of POA in the rodents expressed NPFFR1 (11, 31). In another study, there was communication of RFRP fibers with GnRH neurons observed in the anterior hypothalamic area (AH), medial basal hypothalamus (MBH) and POA of sheep (32, 33). In addition, in the POA of primates, GnRH neurons have connections with RFRP fibers (16, 17). Exposing of GnRH neurons to RFRP *in vitro* demonstrated the inhibitory effect of RFRP on the GnRH neurons (34, 35).

RFRP expression and inhibition of gonadotropins

On the other hand, inhibitory effects of RFRP administration on gonadotropins have been shown. Decrease of plasma LH concentration has been observed after intraventricular administration of RFRP in male and female rodents (23, 30) and after intravenous injection of RFRP (molecular weight: 25 kDa) in rodents (23, 36) and ruminants (14, 37). In

addition, intraventricular administration of an antagonist of the RFRP receptor, RF9, resulted in a rapid, dose-dependent increase in gonadotropin secretion in male and female rats (38). However, some inconsistent findings on the direct effect of RFRP administration on gonadotropin secretions, including positive or no effects, are also available in hamster or ovariectomized ewes, respectively (39, 40). Therefore, these findings suggested that RFRP can inhibit GnRH secretion via a direct action on the GnRH neuronal system (41), that represent the probable role of RFRP in puberty.

In addition to the presence of RFRP neurons in hypothalamus, RFRP also has inhibitory effect on gonadotropin secretion in the pituitary. Presence of close association of hypothalamic RFRP neuronal terminals with GnRH neurons in the median eminence (ME) and/or presence of RFRP receptors in this area might lead to change the onset of puberty. The RFRP neuronal terminals are found in the external layer of the ME in rodents (11, 23, 42), ruminants (14), and primates (16, 17). Presence of RFRP fibers in ME of male (43) and female (24) rats has been shown. In addition, NPFFR1 expression has reported in the pituitary of rodents (42, 44, 45) and primates (17). Taken together and considering evidences such as abundant RFRP-ir fibers in the ME of mammals (14, 16, 17) and also peripheral administration of RFRP-3 in pituitary that inhibited gonadotropin release (14, 36, 37) show that RFRP can also directly inhibit pituitary function.

On the other hand, gonads are also affected directly by RFRP via neuropeptide FF receptors (NPFFR1) previously was known as G protein-coupled receptor, GPR147, on male or female gonadal cells and through the gametogenesis (46-49). Spermatogenesis in male also can be affected by RFRP alterations that shows the possible role of this peptide in male puberty (50).

RFRP, nutrition and puberty

Regarding to the role of nutrition in precocious puberty (51), the mediator effect of RFRP in the relationship of reproductive phenomena and nutrition (52, 53) might demonstrate the possible role of RFRP on the onset of puberty. Extension of the RFRP neuronal terminals to neurons of orexin, melanin, proopiomelanocortin and neuropeptide Y has been shown (53). It also has been shown that two weeks malnutrition increased RFRP-3 mRNA expression in DMH of the hypothalamus in female rats (52). Furthermore, it has been recently shown that intracerebroventricular injection of RFRP delayed the puberty onset in female rats and increased the growth hormone secretion (54). Although, increase in growth hormone secretion was observed in male rat after RFRP injection but no effect on puberty onset is reported in male rats. Therefore, RFRP neurons may

have a role in the regulation of energy balance, and possibly function as a link between nutrition and puberty.

RFRP, prolactin and puberty

On the other hand, it is shown that serum prolactin concentrations increase in girls between 7.5 and 8.5 years old (55). In addition, the relationship of prolactin secretion and RFRP alterations in adult rats has been reported (56). During pregnancy, after parturition and then with increase of milk secretion by suckling the RFRP increased in DMH of rats (57, 58). Therefore, it can be supposed that during female puberty RFRP might play its role via hypothalamic stimulation of prolactin secretion.

Conclusion

It can be concluded that expression of RFRP in the hypothalamic nuclei including DMH, Arc, PVN, POA, and Pe might have effect on the occurrence of puberty in a rodent model. Furthermore, using a Danazol-induced central precocious puberty in a female rat model (59), the role of RFRP in the premature function of HPG axis can be evaluated in the same hypothalamic nuclei. Performing that investigations, it will be clarified, first, RFRP secretion might play role on the onset of puberty, and second, the precocious puberty might have relationship with lower levels of RFRP secretion before puberty, which might not inhibit premature functions of the HPG axis. If this hypothesis stands, it might explain one of the possible mechanisms of occurrence of precocious puberty.

Therefore, it can be suggested clinical trials on RFRP agonists, for prevention of precocious puberty in girls and boys. Furthermore, a RFRP agonist might affect the HPG axis at the brain level and probably would result in the lowest side effects. However, there is a hypotheses about the possible role of RFRP during mammalian development (60), but there is no definite and objective explanation for RFRP secretion malfunction on the precocious puberty. RFRP expression has been investigated in hypothalamus of different species using different methods and techniques, for example, using immunohistochemistry in rodents (61), ruminants (26), and primates (16, 17), using *in situ* hybridization in rodents and primates (62-64), and using RT-PCR in rodents (44, 65). Furthermore, as an *in vivo* method, RFRP concentration in portal blood vessel can be measured in hypothalamus (66). To test the current hypothesis, expression of RFRP peptide or gene and its portal system concentration can be evaluated before and after puberty in normal and precocious puberty rat model (59).

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Conflict of interest statement

The authors have no conflict of interest to declare.

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