Mechanism of gonadotropin releasing hormone (GnRH) pulse generation: A review

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Abstract— Gonadotropin releasing hormone (GnRH) surge is important for the puberty onset in animals. This pulse is under the control of the neurons present in the suprachiasmatic neuclei (SCN) and Pre optic area (POA). These neuclei are expressing kisspeptin neurons in which the pulse generation is initiated. However data regarding pulse generation in the kisspeptin neurons are not yet well understood. In this review it is hypothesized that the presence of certain factors like NKB/dynorphin and different micro RNA are involved in the pulse generation in the kisspeptin neurons and are important for the regulation of puberty. In this review, role of these factors in the involvement of GnRH pulse generation at puberty are summarized.

Index terms- Suprachiasmatic neuclei, Pre optic area, Pulse generation.
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1 INTRODUCTION

The reproductive function in mammals is determined by hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator. The generation of GnRH pulse is responsible for the release of GnRH into portal vessels. This GnRH then regulates the secretion of pulsatile luteinizing hormone (LH) and follicle stimulating hormone (FSH) in peripheral circulation [1]. In medio basal hypothalamus (MBH), multi-unit activity monitored the activity of GnRH pulse generations [2]. But within MBH, specific neuronal population responsible for GnRH pulse generation has not been identified. In GnRH pulse generation, kisspeptin plays a very crucial role. It has been reported in goats that, very close to the KNDy neurons, GnRH pulse generating activity can be recorded [3]. This study confirmed that, for GnRH pulse generation, KNDy neuronal population is a likely intrinsic candidate [4]. All KNDy neuronal populations express androgen receptors in male and progesterone receptor and estrogen receptor alpha in females, and is therefore negatively regulated by gonadal steroid.

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Same is the case with GnRH. In rats, total failure of negative feedback action of E2 on LH secretion was reported when; all KNDy neurons were pharmacological ablated [5]. Therefore, it is seems that the activity of GnRH pulsatility originated in KNDy neurons and is transmitted to median eminence. Moreover, it is also evident that the timing of puberty does not exclusively depend upon the transcriptional control of multiple pathways but may also depend on other regulatory elements, such as epigenetics and microRNAs (miRNAs). But physiological roles of these alternative mechanisms are still under study.

Physiology of GnRH pulse generator

GnRH pulse generator is the key determinant of puberty. In the basal forebrain, during puberty onset the neurosecretory activity of GnRH neuronal population increases. This rise in GnRH secretion then activates gonadotropins, leading to adult functions and gonadal maturation [6]. For the proper stimulation and secretion of gonadotropins and adult functions episodic secretion of GnRH is mandatory. All this is achieved by a very complex interplay between extensive array of excitatory and inhibitory afferents and the intrinsic oscillatory nature of GnRH neurons, which is called GnRH pulse generator [7]. Kisspeptin, Neurokinin B (NKB) and Dynorphin A (Dyn) are coexpressed along with their receptors [G-protein-coupled receptor-54 (GPR54), neurokinin 3 receptor (NK3R) and opioid receptor (KOR), within kisspeptin/NKB/Dyn (KNDy) neurons in the hypothalamic arcuate nucleus (ARC), which is designated as the site of the GnRH pulse generator. The information on regulatory signals that projects on GnRH and their mechanism of action is still incomplete. However identification of kisspeptin has provided certain cues for further studies in this field.

Role of kisspeptin in GnRH pulse generator

Kisspeptin is considered to be the gate keeper of puberty. It is a very strong stimulator of GnRH secretion that has been associated in the feedback actions of gonadal steroids. In ewes ARC nucleus, majority of hypothalamic kisspeptin neuronal populations are found, but in preoptic area a very smaller neuronal population is present. Majority of ARC kisspeptin neuronal population express receptors for estrogen. The Kiss 1 gene encodes large precursor molecules

(132–145 amino acids) that add to a family of smaller peptides, ranging from 10–54 amino acids, which stimulate GnRH and LH release in rodents [8], sheep [9], monkeys [10], and humans [11] by acting via a G-coupled protein receptor (GPR54). Mutations of GPR54 prevent the onset of puberty in humans [12] and mice [13].

Two populations of kisspeptin neuronal populations have been identified in rats and mice: a caudal group present in the ARC nucleus and a rostral group located in the anteroventral periventricular nucleus (AVPV) [14]. AVPV kisspeptin neurons appear to have a role in mediating the positive feedback action of estradiol (E2). It has been found that preovulatory LH surge is blocked by kisspeptin antisera [15]. In the AVPV, Kiss 1 expression is stimulated by E2 [14],[16] and these kisspeptin neuronal populations are sexually differentiated, with more kisspeptin-containing neurons in females than males [17]. In contrast in rodents, the ARC kisspeptin neuronal population mediates the negative feedback actions of steroids largely because, in the ARC kiss1 expression is inhibited by E2 and testosterone. In ewes before LH surge, an increase in Kiss 1 mRNA levels is reported in ARC neuronal population [18], indicating a likely role in the positive feedback actions of E2 in this species. The ARC kiss 1 gene expression increases in rodents, and in menopause women [19], suggesting that these neuronal populations may have a role in steroid negative feedback. Kisspeptin induced LH release is mediated via GnRH, is supported by the finding that GnRH release into the median eminence of the monkey is interrupted following local administration of a kisspeptin receptor (KISS1R) antagonist to this region of the hypothalamus [20]. Kisspeptin induced LH release has also been reported in men and women [21], [22]. Furthermore, kisspeptin has the striking property of resetting the hypothalamic clock that causes pulses of GnRH secretion. It has been shown that estrogen, progesterone, testosterone, opioids, and their agonists and antagonists can modulate GnRH pulse frequency and amplitude [23], kisspeptin is the first known agent that can acutely reset the hypothalamic GnRH clock.

Role of neurokinin B in GnRH pulse generator

Human genetics have shown that kisspeptin signaling and neurokinin B (NKB) signaling are both required for robust pulsatile gonadotropin-releasing hormone (GnRH) release, and therefore for puberty and maintenance of adult gonadal function in adults. How these two peptides interact to affect GnRH pulse generation remains a mystery. The expression of neurokinin B (NKB) has been demonstrated in a subset of Kiss1 neurons in numerous species, including the sheep [24], the goat, the mouse [25],[26], the rat [27], and the monkey (Ramaswamy et al., 2010) [28]. Both neuropeptides are co-synthesized in a population of neurons located in the arcuate nucleus as these cells also express Dynorphin [24], [29], they have been termed KNDy neurons (Lehman et al., 2013) [30]. Radio frequency lesions of the arcuate nucleus in the monkey, which would likely destroy the majority of KNDy neurons, abolish gonadotropin secretion [31], while i.v. administration of either kisspeptin or NKB in this species stimulates LH release in a gonadotropin releasing hormone (GnRH) dependent manner. On the other hand, actions of NKB on gonadotropin secretion in non-primate species are less clear. Genetic disruption of the NKB pathway in mice does not lead to infertility [32], and initial studies of rodents indicated that NKB had an inhibitory or no action on LH release [33], [34]. However, more recent studies of the rodent [25] and of sheep and goat [35], [36] indicate a stimulatory action of this peptide on GnRH/LH release. The immuno histochemical study has revealed the presence of the NKB receptor (neurokinin 3 receptor, NK3R) on GnRH terminals in the median eminence of rat [37], although such co-expression has not been observed in sheep hypothalamus [38].

In the agonadal juvenile monkey, intermittent i.v. injections of kisspeptin-10 show a sustained train of GnRH discharges [39], while similar treatment with the NK3R agonist senktide [40] fails to do so and is associated with a progressive blunting of the GnRH releases (Ramaswamy et al., 2010) [28]. While the site of action of NKB to induce GnRH release in the monkey remains to be established, the arcuate nucleus is considered the most likely. It is hypothesized that GnRH pulse generation originates within the network of KNDy neurons because of coordinated and alternating stimulatory (NK3R) and inhibitory (kappa opioid receptor) signaling, and that the output of the pulse generator is relayed from the KNDy neurons to the GnRH network by kisspeptin. DYN is an important regulator of the pulse generator system. In sheep, KOR antagonists stimulate the episodic secretion of LH during the luteal phase [24]. In ovariectomized goats, central administration of DYN decreases and KOR antagonist increases the frequencies of the multiunit activity volleys and of the LH secretory pulses [26].

Role of micro RNAs in GnRH pulse generator

The complex nature of puberty is also dependent on additional regulatory elements, such as micro RNAs and epigenetic mechanisms. But physiological role of this complementary element is still under study as their involvement in mammalian puberty and concepts about their mechanism are challenging. By means of genome-wide association study, recently new studies are in progress to find new candidate genes and pathways that control pubertal onset. Initial investigations found a link between the age at menarche and variability at 6q21, in or near the Lin28B gene [41], [42].

Adult height and breast development had also been linked to Lin28B. Both Lin28B and Lin28A are RNA binding proteins. The major function of these two RNA binding protein is to inhibit the processing of miRNAs belonging to let 7 family, through inhibition of let 7 precursor maturation. Similarly, Lin28A over-expression in mice has shown delay puberty onset [43]. It leads to the possibility that either miRNAs of the let7 family or other miRNAs may have a role in controlling puberty in humans and other mammals. Let7 miRNAs were initially listed as putative tumor suppressors [44]. Interestingly, tumor-suppressor genes have been involved in the transcriptional control of puberty and, at the time of puberty detectable increase in its expression

is observed [45]. Whether let7 miRNAs fit into this category remains to be elucidated.

Role of epigenetic factors in GnRH pulse generator

As epigenetic is defined as, the inheritable information not encoded by a given gene nucleotide sequence only [46].The epigenetic changes might participate in long-term developmental modifications produced by gene–environment interactions, as well as in rapid changes of specific pathways, as those seen at the time of puberty [47]. But its potential role in mammalian puberty, very little attention has been given. There are different mechanisms for epigenetic control of gene expression but histone modifications and DNA methylation are well characterized [46], [48], [49]. Its role in pubertal onset is obvious because, in juvenile female rats delay pubertal timing is reported when pharmacologically DNA methylation or histone deacetylation is blocked [47]. One possibility is that kiss 1is under epigenetic control. Latest investigations strongly recommend that the differential methylation of the Kiss1 gene might add to the sexual dimorphism in the expression levels of Kiss1 in the RP3V [50]. But estrogen-induced alterations in histone acetylation at the Kiss1 promoter in this nucleus may have a role in the positive feedback that is liable for the generation of the preovulatory surge [51]. During pubertal transitions, these discoveries pave the way for specific analyses on the alterations of epigenetic marks on the Kiss1 gene in the ARC and RP3V.

Conclusion

The pulse mode of GnRH secretion stimulates tonic luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion which drives folliculogenesis, spermatogenesis and steroidogenesis and is negatively fine-tuned by estrogen or androgen. Pulsatility originates in the ARC kisspeptin neurons containing neurokinin B and dynorphin by reciprocal interaction, and intermittent output to the GnRH neuronal network is mediated by KNDY nurons. KNDY neurons are functionally and anatomically interconnected to generate discrete neural signals. Abundance of these peptides and their overlap are species, sex, and age dependent that govern pulsatile GnRH secretions. Neurokinin plays an important role in pulsatile GnRH/LH release through multiple receptors present in both males and females. Using continuous infusion of respective agonists to desensitize KISS1R or NK3R signaling in the male monkey, evidence is provided to support the view that NK3R is upstream of KISS1R in the signaling cascade within the hypothalamus that leads to pulsatile GnRH release. Inhibitory GABA neurotransmission is an important component in the upstream suppression of the GnRH pulse generation mechanism during the juvenile development in primates. Similarly the role of micro RNA in the GnRH pulse generation cannot be neglected, because, these micro RNA are involved in kisspeptin gene silencing. It needs to be further elucidated by experimental methods to determine, whether these micro RNA are involved in puberty onset or operating via other type of mechanism regulating GnRH pulse generation.

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