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Role of Kisspeptin and NKB in Puberty in Nonhuman Primates: Sex Differences

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Abstract

To understand the roles of kisspeptin and neurokinin B (NKB) in puberty and sex differences in their involvement, we conducted a series of experiments measuring the release of GnRH and kisspeptin in the median eminence of the hypothalamus in male and female monkeys throughout sexual development. Results indicate that kisspeptin-10 and the NKB agonist, senktide, stimulated GnRH release in males and females at the prepubertal and pubertal stages, but females are much more sensitive to kisspeptin signaling than males. Moreover, throughout the progress of puberty, major remodeling of kisspeptin and NKB signaling pathways for the regulation of GnRH release takes place. In females during puberty reciprocal pathways, i.e., kisspeptin signaling mediated through NKB neurons and NKB signaling mediated through kisspeptin neurons, are established, to provide powerful and flexible mechanisms for GnRH neurosecretory activity necessary for complex female reproductive function in adulthood. By contrast, during puberty in males reciprocal pathways are consolidated to a simpler kisspeptin dominant signaling pathway. Nevertheless, in primates both kisspeptin and NKB signaling are contributing factors for the pubertal increase in GnRH release, rather than initiating puberty.

Keywords

kisspeptin; neurokinin B; puberty; nonhuman primate; sex difference

1. Introduction

An increase in pulsatile gonadotropin-releasing hormone (GnRH) release from the median eminence (ME) of the hypothalamus heralds a series of events associated with puberty¹. However, the signal triggering the pubertal increase in GnRH release remains unclear. In non-human primates, the prepubertal low levels of GnRH release have been attributed to the

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gonadal steroid independent “central inhibition”^{1, 2} and this concept has been confirmed in human puberty³.

Studies in human genetics showing that mutations in the genes encoding *kisspeptin* (*KISS1*) or *neurokinin B* (*NK3*, NKB) and their receptors (*KISS1R* or *NK3R*, respectively) result in delayed puberty or hypogonadotropic hypogonadism^{4–6} suggesting that kisspeptin and NKB signaling each play roles in puberty. In fact, it has been shown that in female mice the developmental increase in kisspeptin neurons in the anterior ventral periventricular nucleus is critical to puberty⁷. However, a series of studies in non-human primates that directly measured release of GnRH and kisspeptin in the ME across puberty indicate that kisspeptin and NKB are merely contributors, and not initiators of puberty in female⁸, as kisspeptin and NKB are released before the onset of puberty, and reduction in central inhibition has to precede the pubertal increase in release of kisspeptin and NKB. Moreover, application of the direct measurement approach of neuropeptides in the ME to both sexes has revealed similarities and differences in the underlying mechanisms of neurocircuitry formation between the kisspeptin and NKB networks during the pubertal period that ensures adult regulation of the GnRH neurosecretory system. This review summarizes our recent findings.

2. Staging of puberty in male and female rhesus monkeys

To discuss sex differences in the hypothalamic mechanism of puberty in rhesus monkeys, we need to define the stages of puberty in both sexes.

Females:

Based on developmental changes in LH and FSH levels and external signs of puberty, the Terasawa lab established the stages of puberty in female rhesus monkeys over 35 years ago (Fig. 1)⁹. The “prepubertal stage” is defined as no signs of puberty -- low gonadotropin and gonadal steroid levels lasting through ~20 months of age. The “early pubertal stage” is defined as the time when the first external signs of puberty become apparent, but menarche has not occurred yet. The first external sign of female puberty is seen as a slight increase in nipple size and subsequent swelling of the perineal sex-skin, which occurs at ~22 months of age. These external signs of puberty are a consequence of increased levels of circulating ovarian estrogens due to an increase in gonadotropins. The LH pulse frequency and pulse amplitude increase, and a nocturnal elevation of gonadotropin levels becomes prominent. Subsequently, menarche occurs at 26–30 months of age, entering the “midpubertal stage”. During the midpubertal stage, pulse amplitude of LH further increases. Monkeys start to ovulate at 38–45 months of age, thereby entering the post pubertal period/adulthood.

Males:

External signs of puberty in male rhesus monkeys are not as clear as those in females. Nevertheless, based on physiological and hormonal changes we are able to establish the developmental stage of puberty in male rhesus monkeys (Fig. 1)¹⁰. The “prepubertal stage” is defined as no signs of puberty, which is characterized by low morning and evening LH and testosterone (T) levels (< 1 ng/ml for both), and a testicular volume of < 1 cm³, lasting through ~25 months of age. The “early pubertal stage” is defined by physiological signs of

puberty -- a slight increase in evening LH levels that starts at ~26 months of age¹⁰. The “midpubertal stage” is characterized by elevated evening LH levels as well as an increase in evening T levels and a significant increase in testicular volume, which occurs at ~34 months of age. In the later part of the midpubertal stage, between 40–48 months of age, morning T levels are also elevated and a large increase in testicular volume is achieved. By 48 months of age, male monkeys typically attain the capacity of full spermatogenesis¹¹.

Previously, Plant and colleagues have defined puberty onset in male rhesus monkeys to occur at ~42 months of age based on the testicular development and spermatogenesis^{11, 12}. However, as described above, a pubertal increase in LH starts to occur between 25 and 28 months of age^{10, 13}, indicating that pubertal events in the hypothalamo-pituitary axis in males start much sooner than previously thought. Moreover, spermatogenesis in males occurs shortly after first ovulation in females (Fig. 1). Therefore, it appears that although in rhesus monkeys, male puberty starts several months later than in females, there is little sex difference in the period between initiation of puberty and full maturation of reproductive function.

3. Release of GnRH and kisspeptin increases during the progress of puberty

The description of developmental changes in release of GnRH and kisspeptin is the first step to understand the mechanism underlying puberty onset. For measurements of GnRH and kisspeptin we insert a microdialysis probe into the ME of the hypothalamus, where neuroterminals of GnRH and kisspeptin are concentrated, and collect dialysate samples, in which concentrations of GnRH and kisspeptin are assessed by RIA¹⁴.

GnRH Release

An increase in pulsatile GnRH release in the hypothalamus is essential for initiating puberty and subsequent maturation of reproductive function. This concept is based on experiments showing that pulsatile infusion of GnRH in prepubertal female monkeys results in precocious puberty¹⁵ and that an increase in GnRH release occurs at puberty onset in female monkeys¹⁶. In both females and males, gradual increases in baseline GnRH release occur from the prepubertal to the early pubertal stage, and then the early pubertal to the mid pubertal stage^{10, 16, 17}. Importantly, there was no sex difference in the developmental changes in baseline GnRH release in gonadally intact monkeys (Fig. 2).

The pulse frequency and pulse amplitude of GnRH release are low in prepubertal females and both increase when females reach the early pubertal stage. While the pulse frequency stays the same throughout the remaining pubertal period, the pulse amplitude keeps increasing from the early pubertal stage to the midpubertal stage until the age of first ovulation¹⁸. These pubertal changes in GnRH pulsatility are independent of the pubertal increase in circulating gonadal steroid hormones. In fact, we previously documented in ovariectomized (OVX) female monkeys that the pubertal increase in GnRH release, as a consequence of baseline and pulse amplitude increase¹⁹, occur similarly to those in gonadally intact females¹⁸. Developmental changes in the GnRH pulse pattern in males are

speculated to be similar to those in females, but 10-min dialysate sampling needs to be conducted. Nevertheless, orchidectomy is unlikely to alter the pubertal increase in GnRH release²⁰, as the Plant lab has shown that the pubertal increase in LH release is independent of gonadal status in males²¹.

Kisspeptin Release

Mutations in the gene encoding *KISS1* and its receptor *KISS1R* result in delayed puberty and hypogonadotropic hypogonadism in humans^{4,5}. In rodents kisspeptin appears to play a role in puberty onset²². Therefore, it is important to examine the release pattern of kisspeptin in the ME.

In females, the mean release, pulse frequency, and pulse amplitude of kisspeptin release in ovarian intact pubertal female monkeys are all higher than those in prepubertal females²³. That is, kisspeptin release in prepubertal females is characterized by low mean release, low pulse frequency, and low pulse amplitude, similar to those seen with GnRH release in prepubertal females. Importantly, the developmental increases in pulse frequency of kisspeptin and GnRH release are almost identical^{17, 23}, as kisspeptin pulses coincide with GnRH pulses²⁴. In pubertal females mean kisspeptin release and pulse frequency of kisspeptin release further increase. In pubertal females mean kisspeptin release and pulse frequency of kisspeptin further increase. The pubertal changes in kisspeptin release in primates is ovarian steroid independent, as the developmental pattern of kisspeptin release in OVX females is very similar to those in intact counter parts, except for much higher pulse amplitude and mean release in OVX females²³

Although changes in pulsatile kisspeptin release in males have not been systematically examined, a gradual increase in baseline kisspeptin release from the prepubertal to early pubertal and then mid pubertal stage is seen in both female and male monkeys (Fig. 2)^{10, 23}. Again, the pattern of gradual increase in baseline kisspeptin release is parallel to increases in baseline GnRH release in both sexes (Fig. 2). Preliminary data in orchidectomized males further indicate that the pubertal increase in kisspeptin release is independent of the pubertal increase in T²⁰.

4. GnRH neurons respond to kisspeptin-10 and the responsiveness increases through the progress of puberty

Kisspeptin is one of the most powerful secretagogues of GnRH release. As described above, there is a parallel increase in kisspeptin release and GnRH release during puberty. Moreover, we have previously shown that kisspeptin release is pulsatile and kisspeptin pulses occur in high concordance with GnRH pulses²⁴. If kisspeptin signaling plays any role in the pubertal increase in GnRH release, it is possible that the GnRH response to kisspeptin undergoes pubertal change. Thus, the effect of human kisspeptin-10 (hKP10) was examined by local infusion into the ME through the microdialysis probe. Note that concentrations of neurochemical substances including kisspeptin at the ME are approximately one-tenth of the infused concentrations, because small neurochemicals are reduced by 90% when they pass through a semi-permeable membrane¹⁴. In this article, the concentration stated is the infused

concentration, not the actual concentration at the ME, i.e., the cells in the ME would be exposed to approximately one digit less concentration than the statement.

The results indicate that in both prepubertal and pubertal females hKP10 at 0.01 and 0.1 μM doses stimulate GnRH release in a dose-responsive manner¹⁷. Similarly, in both prepubertal and pubertal males hKP10 at 0.1 and 1 μM doses also stimulate GnRH release in a dose-responsive manner¹⁰. Note that in males, GnRH neurons did not respond to hKP10 at 0.01 μM . Importantly, in both sexes pubertal monkeys had a larger GnRH response than their prepubertal counter parts, when the effect of the same dose within the same sex (1 μM in males prepubertal vs. pubertal and 0.01 μM in females prepubertal vs. pubertal) on GnRH release is compared (Fig. 3A). A similar developmental increase in GnRH sensitivity to KP10 has been described in mice²⁵. Nevertheless, there are clear sex differences in the sensitivity of GnRH neurons to hKP10. First, the minimum dose required in males (0.1 μM) is 10-fold larger than in females (0.01 μM). Second, at the both developmental stages the GnRH responses to 0.1 μM in males are significantly smaller than those in females. Third, GnRH responses to the highest dose examined in males are much smaller than those in females at both stages (Fig. 3A). Therefore, 1) female GnRH neurons are more sensitive to kisspeptin signaling than males and 2) during the progress of puberty GnRH neurons become more sensitive to kisspeptin in both sexes.

Because it is possible that developmental differences in neuropeptide release and GnRH response to kisspeptin are due to the pubertal increase in circulating gonadal steroids, we have examined the effects of gonadectomy. Results indicate that hKP10 does not stimulate GnRH release in OVX pubertal females¹⁷. Similarly, in orchidectomized pubertal males the hKP10-induced GnRH release is greatly attenuated¹⁹. It appears, therefore, that the enhanced response of GnRH neurons to kisspeptin during the pubertal period is, in part, due to circulating gonadal steroids. Collectively, we can conclude that in both sexes the pubertal increase in GnRH release is due to 1) an increase in kisspeptin release in the hypothalamus as well as to 2) increases in sensitivity of GnRH neurons to kisspeptin signaling.

5. GnRH neurons respond to senktide, but the responsiveness does not increase during the progress of puberty

Because mutations of the gene encoding NKB (*TAC3*) and its receptor (*TAC3R*) are associated with hypogonadotropic hypogonadism⁶, NKB signaling has been implicated in the mechanism of puberty onset. Accordingly, we examined the possible role of NKB signaling in the pubertal increase in GnRH release by assessing the developmental changes in GnRH release in response to the NKB agonist, senktide. In females senktide at 0.1 and 10 μM doses induces GnRH release in a dose-responsive manner at both the prepubertal and pubertal stages²⁶. Note that initially we examined the effects of senktide at 0.1 and 1 μM , but there were only marginal GnRH dose-responses in females. As such, we chose to examine the 0.1 and 10 μM doses in both sexes. In males, similar to that seen in females, senktide also induces dose-dependent responses in GnRH release at both developmental stages¹⁰. Interestingly, the effect with 0.1 μM senktide in males is larger than in females, whereas 10 μM senktide effects in males are smaller than in females (Fig. 3B). However, in

contrast to the developmental amplification of GnRH response to hKP10, no developmental amplification is seen in GnRH responses to senktide in both sexes.

6. Kisspeptin neurons respond to senktide in both sexes, but the responsiveness increases through the progress of puberty only in females.

As discussed in the next section in detail, NKB signaling to GnRH neurons can be mediated through kisspeptin neurons. We next examined the effects of senktide at 0.1 and 10 μ M, on kisspeptin release during pubertal progress. As seen in Fig. 3C, senktide stimulates kisspeptin release in both sexes^{10, 26}. However, the dose-dependent increase is only seen in females at both developmental stages and the senktide-induced kisspeptin release at the pubertal stage is much larger than those at the prepubertal stage with both doses²⁶. By contrast, in males while senktide at both doses also induces kisspeptin release, there are no dose-responses -- even though the senktide concentration is 100-fold different¹⁰. Moreover, there is no developmental difference in the senktide-induced kisspeptin release, i.e., the kisspeptin response to senktide in pubertal males is similar to that in prepubertal males (Fig. 3C). Therefore, there is a clear female biased sex difference in kisspeptin response to NKB signaling. The question of whether and how circulating steroid hormones, i.e., estrogens in females and androgens in males, play a role in these sex differences remain unclear at this time, as we have not conducted a similar experiment in gonadectomized monkeys with or without steroid replacement. Nevertheless, kisspeptin neurons in female monkeys appear to be very sensitive to estradiol, as 1) GnRH response to both kisspeptin and senktide stimuli in females are larger than in males and 2) the pubertal amplification of the kisspeptin response to senktide is only seen in females, and not in males.

7. Sex difference in the neurocircuitry governing the pubertal increase in GnRH release

The concept that a group of neurons in the arcuate nucleus (ARC) expressing kisspeptin, NKB, and dynorphin (also called as **KNDy** neurons) are the pulse-generator for GnRH release has been proposed by Goodman and his colleagues. In fact, the co-localization rate of these three neuropeptides is 100% in the hypothalamus in species including sheep and mice²⁷. According to this concept, within a single kisspeptin neuron, excitatory kisspeptin and NKB and inhibitory dynorphin signal generate a kisspeptin pulse, which results in a GnRH pulse. Because in primates, the co-localization rate of the three peptides is considerably lower^{28, 29}, we have hypothesized that kisspeptin, NKB, and dynorphin neurons in the hypothalamus form a network for GnRH pulse-generation. We thought that with this hypothesis, we might be able to clarify the hierarchy between kisspeptin and NKB signaling over the GnRH neurosecretory system. A series of experiments are conducted with infusion of kisspeptin agonists and antagonists or NKB agonists and antagonists into the ME, while microdialysates are continuously collected for measurement of GnRH.

Females:

As described earlier, both hKP10 and senktide induce GnRH release in prepubertal as well as pubertal female monkeys. The results of the kisspeptin and NKB agonists with

antagonists experiments²⁶ show that the 0.1 μM hKP10-induced GnRH release is blocked by the NKB antagonist, SB222200 (1 μM), in pubertal, but not prepubertal females (Fig. 4A). Similarly, the 0.1 μM senktide-induced GnRH release is blocked in the presence of the kisspeptin antagonist, peptide 234 (0.1 μM), in pubertal, but not in prepubertal female monkeys (Fig. 4B). Together, these results indicate that while in prepubertal female monkeys, kisspeptin and NKB signaling influences GnRH release as independent units, there are no reciprocal interactions (Fig. 5). By contrast, in pubertal females, reciprocal pathways between kisspeptin and NKB neurons are established, i.e., NKB signaling is mediated through kisspeptin neurons and kisspeptin signaling is mediated through NKB neurons. Emergence of this cooperative mechanism formed by the kisspeptin and NKB networks seems to underlie the developmental increase in release of GnRH and kisspeptin in females.

Males:

As we have described earlier, both hKP10 and senktide induce GnRH release in male monkeys at the prepubertal and pubertal stages. The results of the kisspeptin and NKB agonists with antagonists experiments⁹ show that in prepubertal males the 0.1 μM hKP10-induced GnRH release is blocked by the NKB antagonist, SB222200 (1 μM). This indicates that kisspeptin induced GnRH release in prepubertal males is, in part, mediated through NKB signaling (i.e., NKB neurons and/or NK3R mediated mechanism). Similarly, in prepubertal males the 0.1 μM senktide-induced GnRH release is greatly attenuated in the presence of the kisspeptin antagonist, peptide 234 (0.1 μM), suggesting that the senktide-induced GnRH release in prepubertal males is also, in part, mediated through kisspeptin signaling. In contrast, in pubertal males the 0.1 μM hKP10-induced GnRH release is not altered by the SB222200, suggesting that kisspeptin induced GnRH release in pubertal males is independent of NKB signaling (Fig. 4A). Additionally, the senktide-induced GnRH release is completely blocked by the kisspeptin antagonist, peptide 234, indicating that NKB signaling to GnRH neurons is dependent on kisspeptin neurons (Fig. 4B).

These results are interpreted to mean that in prepubertal males both kisspeptin signaling and NKB signaling can alter GnRH release directly, or indirectly, i.e., kisspeptin signaling through NKB neurons or NKB signaling through kisspeptin neurons, whereas in pubertal males NKB signaling to GnRH neurons is mediated solely through kisspeptin neurons and kisspeptin signaling plays a major role in the pubertal increase in GnRH release (Fig. 5). Similar upstream NKB signaling over kisspeptin signaling in pubertal male monkeys has also been reported³⁰. Therefore, in males NKB signaling to GnRH neurons mediated through kisspeptin neurons is present throughout sexual maturation, but kisspeptin signaling mediated through NKB neurons is limited to the prepubertal period. It is speculated that the reciprocal pathways are important during the neonatal “mini-puberty” period in males, when activity of GnRH neurons (and circulating LH release) is as high as that in adults²¹. Perhaps subsequent central inhibition during the prepubertal stage masks the activity of the reciprocal pathways until the time of puberty onset. After the onset of puberty, the reciprocal pathways in the prepubertal stage are remodeled to a simpler kisspeptin-dominant pathway. Nonetheless, neurocircuitry in the hypothalamus governing the GnRH neurosecretory system in males is quite different from that in females before and after puberty onset and

remodeling of this neurocircuitry occurs during the pubertal period to sculpt the fully functional adult neurocircuitry in both sexes.

8. Conclusions and Future Directions

A series of parallel studies in male and female monkeys revealed similarities and differences in the process of initiating puberty onset. In both sexes, release of GnRH and kisspeptin increases at puberty onset and these pubertal increases in release of GnRH and kisspeptin are independent of the pubertal increase in gonadal steroids, estrogens and androgens. Moreover, despite the facts that there are sufficient amounts of kisspeptin and NKB in the hypothalamus, and prepubertal GnRH neurons are able to respond to both hKP10 and senktide, GnRH levels during the prepubertal period remain low. As we have shown in previous and ongoing studies, the low levels of GnRH measured before puberty onset is due to the tonic inhibition by elevated levels of gamma-aminobutyric acid (GABA) in the ME¹. Infusion of the GABA_A receptor blocker bicuculline to the ME of prepubertal females stimulates GnRH release³¹ and kisspeptin release³². Moreover, pulsatile infusion of bicuculline resulted in precocious puberty in females³³. A recent preliminary observation further suggests that bicuculline infusion into the ME induces increased release of GnRH, kisspeptin and NKB in the prepubertal male hypothalamus³⁴. Importantly, the bicuculline-induced release of GnRH and kisspeptin is blocked by peptide 234 in females³² and the bicuculline-induced release of GnRH, kisspeptin, and NKB is blocked by SB222200 in males³⁴. Although symmetric experiments in both sexes have not been conducted, it appears that GABA inhibition over the GnRH, kisspeptin and NKB circuit is present during the prepubertal period in both sexes of non-human primates. Therefore, tonic GABA inhibition has to be removed or diminished prior to the pubertal increase in GnRH release, and consequently, the pubertal increase in kisspeptin or NKB is not responsible for the initiating puberty in both sexes. This concept is consistent with a report showing that in orchietomized male monkeys the number of kisspeptin neurons in the hypothalamus at the neonatal period is as high as that in adults parallel to elevated LH, yet LH decreases during the transition to the prepubertal low LH period³⁵. Nevertheless, throughout the progress of puberty, both kisspeptin and NKB signaling contribute to the pubertal increase in GnRH release independently or in concert. Currently, the question of how central GABA inhibition is regulated by the *makorin RING finger protein 3 (MKRN3)* gene³⁶ or the zinc finger gene *GADAD1*³⁷, which have been reported as genes involved in prepubertal inhibition, is unknown and thus, answering this question remains.

In this review, we have highlighted sex differences in the underlying mechanism and neurocircuitry regulating GnRH release. While in females the sensitivity of GnRH neurons to both kisspeptin and NKB signaling increases during the progress of puberty, in males only GnRH sensitivity to kisspeptin signaling increases. Moreover, female kisspeptin signaling is much more sensitive to developmental factors, such as the pubertal increase in circulating estradiol, than those in males. Notably, throughout the progress of male puberty the regulatory mechanism for GnRH neurosecretion becomes a kisspeptin dominant simpler system. In females, by contrast, during the progress of puberty a collaborative network between kisspeptin and NKB signaling for regulation of the GnRH neurosecretory system is established and this network provides flexibility to govern the complex female reproductive

function in adult life. The questions of how kisspeptin and NKB signaling plays a role in the fine tuning of the menstrual cycle, especially at the time of the preovulatory surge, and whether a greater sensitivity of kisspeptin and NKB signaling to estradiol plays a role in the negative energy balance need to be answered. Collectively, it is remarkable to learn that after the onset of puberty the neurocircuitry regulating GnRH release undergoes pubertal changes, tailoring to the reproductive function in adulthood. Although so far we studied the role of kisspeptin and NKB signaling in the pubertal GnRH increase, it will be imperative to understand the role of dynorphin, another component of KNDy networks, in this context in the future.

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Figure 1:

Developmental stages of puberty in male and female rhesus monkeys. Prepubertal, early pubertal, and midpubertal stages are defined based on physical and physiological changes in circulating hormone levels. Note that the age of puberty onset and subsequent progress varies among animals. Abbreviations: LH=luteinizing hormone; PM=evening; T=testosterone. Modified from^{8, 10}.

Figure 2:

Baseline release of GnRH and kisspeptin increases during pubertal progression in male and female monkeys. Mean $\pm$ SEM peptide levels at the prepubertal, early pubertal and midpubertal stages in males (**A** and **C**) and females (**B** and **D**) are calculated from dialysate samples collected from the ME of the hypothalamus during the first 2–4 h period before agonists/antagonists challenges. In both sexes, GnRH and kisspeptin levels in early pubertal and midpubertal monkeys are significantly greater than those in prepubertal monkeys. Note that there are no sex differences in baseline GnRH and kisspeptin levels at each stage. aa, $P < 0.01$; aaa, $P < 0.001$ vs. prepubertal stage. Modified from^{10, 23, 26}.

Figure 3:

GnRH (A and B) and kisspeptin (C) responses to direct infusion of kisspeptin and NKB agonists into the ME of prepubertal and pubertal male and female monkeys. Mean $\pm$ SEM values of GnRH and kisspeptin are derived from the area under the curve of GnRH and kisspeptin responses to human kisspeptin-10 (hKP10) at 0.01 and 0.1 μ M in females and 0.1 and 1 μ M in males (A) or the NKB agonist, senktide, at 0.1 and 10 μ M in both sexes (B and C). A. Developmental changes in GnRH response to hKP10. Note that hKP10 stimulates GnRH release in a dose-responsive manner in males and females at both stages. Importantly, the female GnRH neurons are much more sensitive to hKP10 than that in the males, as 1) the minimum effective dose in females is 10-fold less than that in males and 2) GnRH responses at 0.1 μ M hKP10 in females are greater ($p < 0.001$) than those in males at both developmental stages. Moreover, a significant developmental increase in hKP10-induced GnRH response is observed at both doses in females, whereas a developmental increase is

observed only at the 1 μ M in males. **B.** Developmental changes in GnRH response to senktide. Note that senktide stimulates GnRH release in both sexes, but a dose response is only seen in females at the prepubertal and pubertal stages. Interestingly, the GnRH response to 10 μ M dose senktide in pubertal females is greater ($p < 0.001$) than that in pubertal males, whereas the GnRH response to 0.1 μ M senktide in prepubertal males is greater ($p < 0.01$) than that in prepubertal females. **C.** Developmental changes in the kisspeptin response to senktide. Note that senktide stimulated kisspeptin release both sexes. Again, a dose-dependent response is only seen in females at both developmental stages and a significant developmental amplification in kisspeptin responses to senktide occur only in females. ***, $p < 0.001$; ****, $p < 0.0001$ vs. vehicle infusion; #, $p < 0.05$; ###, $p < 0.001$; ####, $p < 0.0001$ vs. low dose at the same stage; aa, $p < 0.01$; aaa, $p < 0.001$ vs. the corresponding dose in the prepubertal stage; ††, $P < 0.01$; †††, $P < 0.001$; ††††, $p < 0.0001$ vs. the corresponding dose and stage in the opposite sex. Modified from^{10, 26} with original data from¹⁶.

Figure 4:

GnRH responses to the kisspeptin agonists 0.1 μ M hKP10 in the presence and absence of the NKB antagonists SB222200 (1 μ M, **A**) and to 0.1 μ M sentkide in the presence or absence of the kisspeptin antagonist, peptide 234 (P234, 0.1 μ M, **B**) in male and female monkeys. Mean $\pm$ SEM was calculated from the area under the curve of GnRH responses after the agonist and antagonist challenges. **A.** There are significant sex differences in kisspeptin signaling through NKB neurons to the GnRH neurosecretory system. The hKP10-induced GnRH release is blocked in presence of SB222200 in prepubertal males, but not in pubertal males. hKP10-induced GnRH release is blocked in presence of SB222200 in pubertal females, but not in prepubertal females. **B.** There are also significant sex differences in NKB signaling through kisspeptin neurons to the GnRH neurosecretory system. The sentkide-induced GnRH release is attenuated or blocked in the presence of P234 in males at both developmental stages, whereas the sentkide-induced GnRH release is greatly attenuated by

P234 only in pubertal females, but not in prepubertal females. *, $p < 0.05$; ***, $p < 0.001$ vs. vehicle infusion; a, $P < 0.05$; aa, $P < 0.01$; aaa, $P < 0.001$ vs. same sex prepubertal stage; †, $P < 0.05$; ††, $P < 0.01$; †††, $P < 0.001$ vs. the corresponding dose and stage in the opposite sex; bb, $P < 0.01$; bbb, $P < 0.001$ vs. in the presence of antagonist in the same sex. Modified from^{10, 26}.

Figure 5:

Schematic illustration summarizing developmental changes in the neuroendocrine mechanism of puberty. Possible interactions between kisspeptin (**red**), NKB (**blue**), and GnRH (**white**) neurons in the ME in prepubertal and pubertal male and female monkeys are shown. A black X indicates signaling pathways are not present. The number of colored dots reflects estimated amount of neuropeptide release at the neuroterminals, based on the results from a series of experiments. Note that, major remodeling of kisspeptin and NKB signaling pathways takes place during puberty, such that their regulation of the GnRH neurosecretory system is most effective in adult reproductive function. Modified from^{10, 26}.