The role of *KiSS-1* in the regulation of puberty in higher primates

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Abstract

Puberty in higher primates is triggered by resurgence in the pulsatile secretion of hypothalamic GnRH after a hiatus in the robust release of this hypophysiotropic signal during childhood and juvenile development. Interestingly, the prepubertal decline in GnRH release is not associated with a marked reduction in the expression of either the gene that codes for GnRH (*GnRH-1*) or the decapeptide itself, and the network of GnRH neurons in the hypothalamus of the juvenile may be activated prematurely and with surprising ease by intermittent neurochemical stimulation with *N*-methyl-D-aspartate (NMDA), a glutamate receptor agonist. *KiSS-1*, a gene that encodes for kisspeptin-121, which is proteolytically cleaved to a 54 amino acid peptide, metastin, was initially studied in the context of tumor suppression. In 2003, however, inactivating mutations in the metastin receptor, GPR54, were reported to be associated with hypogonadotropic hypogonadism and absent puberty in man. Subsequent studies in the rhesus monkey have shown that GPR54 and *KiSS-1* are expressed in the mediobasal hypothalamus (MBH), *KiSS-1* expression in the MBH increases at the time of the pubertal resurgence in GnRH release and pulsatile, but not continuous, i.v. administration of metastin 45–54 in the juvenile male monkey elicits sustained GnRH release precociously. The significance of these findings in the context of the initiation of the onset of puberty is discussed.

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Introduction

The gene, *KiSS-1*, was cloned in 1996 at the Pennsylvania State College of Medicine by a group interested in the suppression of metastasis (1). Apparently, the gene was named so because this Institution is located in Hershey, Pennsylvania, in the United States, a town famous for its confectionary products, in particular Hershey Chocolate Kisses (D R Welch, personal communication, 2005). *KiSS-1* codes for a 121 amino acid protein, kisspeptin-121, which is proteolytically cleaved in man to a 54 amino acid peptide, metastin (2–4). The name metastin derives from the ability of the peptide to suppress proliferation in cell lines derived from human melanomas and breast carcinomas (1, 5). The receptor mediating the action of metastin was recognized in 2001 to be GPR54, a G-protein-coupled receptor (2–4), which had been identified 2 years earlier as an ‘orphan receptor’ in rat (6). GPR54 is coupled to the Gq subclass of G-proteins and activation of the receptor promotes phosphatidylinositol turnover, calcium mobilization,

and signaling in pathways involving MAP kinases. Signal transduction in the metastin–GPR54 pathway has recently been reviewed (7).

Interestingly, a neuroendocrine role of GPR54 signaling only emerged in 2003, when it was reported that several members of two large consanguineous families presenting with hypogonadotropic hypogonadism and absent puberty were found to carry homozygous mutations for GPR54 (8, 9). In one other subject bearing a compound heterozygote mutation of GPR54, administration of a pulsatile regimen of GnRH reversed the hypogonadotropism (9), indicating a hypothalamic locus for the deficit associated with this genetic disorder. One of these signal papers (8) concluded with the statement ‘a new chapter may thus be opened in the physiology of the gonadotropic axis’, and the other (9) with the notion ‘GPR54 is a key regulator of the biology of puberty’. The present review examines the latter, more circumspect, and perhaps the more intriguing suggestion.

Puberty and its neuroendocrine determinants

Puberty in man is a period of development that results from the expression of two physiological processes,

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namely adrenarche and gonadarche (10). Adrenarche, the increase in adrenal androgen secretion typically observed at approximately 8–9.5 years of age in girls and boys, is only observed in man and the great apes (11). Of the two processes, only gonadarche is critical to the onset of fertility and, from a biological perspective, may therefore be considered fundamental to the process of puberty (12). Adrenarche, on the other hand, may be viewed as a temporally related corollary of puberty. Throughout the present review, gonadarche and puberty will be used interchangeably although this is not strictly correct in the case of human development.

Gonadarche is triggered by resurgence in the pulsatile pattern of hypothalamic GnRH secretion after a hiatus or dampening of this neuroendocrine activity during childhood and the juvenile years that intervene between infancy and puberty (11, 12). The diminished activity in the hypothalamic–pituitary axis during the greater part of prepubertal development guarantees the relative quiescence of both the male and female gonad of the child and the juvenile. Interestingly, the network of hypothalamic GnRH neurons that discharges pulses of the decapeptide during infancy and puberty appears to be held in a state of functional readiness during the intervening period of childhood and juvenile development. In the monkey, hypothalamic levels of mRNA coding for GnRH and the content of the peptide in this region of the brain during juvenile development when GnRH pulsatile release is restrained, is similar to that during infancy and puberty when pulsatile GnRH release is robust (13–16). Moreover, a pattern of GnRH secretion similar to that of the pubertal state may be precociously elicited with remarkable ease from the hypothalamus of the juvenile by applying a repetitive intermittent chemical stimulation with the glutamate-receptor agonist, NMDA (17, 18). Thus, it may be concluded that, the molecular and cellular machinery for generating an adult hypophysiotropic drive to the gonadotrope is extant throughout childhood and juvenile development and the signal responsible for the initiation of gonadarche must originate upstream from the hypothalamic network of GnRH neurons. The upregulated state of the GnRH neuron in the hypothalamus of the juvenile is an interesting contrast to the pituitary gonadotrope in which, at the same stage of development, expression of the gonadotropin subunits is minimal and the cell is hyporesponsive to stimulation by GnRH ((19), Winters and Plant, unpublished observations).

As implied, the ability of the hypothalamus to generate a pulsatile GnRH discharge originates well before the onset of puberty, actually during fetal development at the time when these hypothalamic neurons complete their migration from the olfactory bulb (20). Although fetal GnRH release is suppressed during the latter stages of gestation, probably by the rising levels of fetoplacental steroids, following birth and the loss of the inhibitory action of these hormones, GnRH pulsatility is expressed in a robust fashion (11). Thus, the ontogeny of GnRH release

during postnatal development in higher primates is characterized by an ‘on–off–on’ pattern. Since the pituitary–gonadal axis in both the male and the female is capable of responding to pulsatile GnRH stimulation well before the onset of puberty (11), the neural mechanism that times the initiation of gonadarche in primates may be viewed as one that regulates a delay (rather than onset) in this developmental process (21).

The role of *KiSS-1* in dictating the on–off–on pattern of GnRH drive to the pituitary–gonadal axis during postnatal development

To date, studies on the role of *KiSS-1* in dictating the postnatal ontogeny of the hypophysiotropic drive to the pituitary–gonadal axis of the primate have focused primarily on the ‘off–on’ transition that occurs at the initiation of puberty. Following the initial observations in man that inactivating mutations of GPR54 were associated with hypogonadotropic hypogonadism and absent puberty, it was reasoned that if an increase in metastin–GPR54 signaling was the proximate trigger for increased pulsatile GnRH release at this stage of development, then the following conditions should apply: (i) *KiSS-1* and GPR54 should be expressed in the medial basal hypothalamus (MBH), the area of the primate brain containing the majority of neuroendocrine GnRH neurons (22); (ii) an increase in GPR54 signaling should occur in association with the onset of puberty; and (iii) a premature increase in metastin tone in the MBH during juvenile development should elicit a precocious pubertal pattern of GnRH release. Using the rhesus monkey (*Macaca mulatta*), a species of higher primate in which puberty occurs at 3–4 years of age (11), and in collaboration with the laboratories of W R Crowley and S R Ojeda, all three of these conditions have been confirmed. Hybridization histochemistry demonstrated that *KiSS-1* is discretely expressed in neurons in the region of the arcuate (infundibular) nucleus of the MBH (Fig. 1). Expression of the receptor was also observed in this hypothalamic region, although the pattern of expression was more generalized than that of the ligand (23). Real-time PCR revealed that hypothalamic *KiSS-1* expression increased at the time of pubertal resurgence in GnRH release in both male and female, while an increase in GPR54 expression during this developmental transition was observed only in the female (23). At present, it is not known whether this male/female difference represents a consequence of testicular-dependent programming of hypothalamic function during fetal development. This is because our male monkeys were studied in the agonadal condition (castration between 16 and 21 months of age), while the females were ovarian intact. Thus, the peripubertal sex differences that have been observed in the developmental expression of GPR54 in the monkey may simply be related to the difference in hormonal status of the animals

Figure 1 Selective expression of *KiSS-1* mRNA in neurons of the arcuate nucleus of the female monkey. (A) Low magnification view. Bar = 400 μ m, (B) merged bright- and dark-field higher magnification image showing the presence of *KiSS-1* mRNA transcripts in neurons of the arcuate nucleus (arrows). Bar = 20 μ m. Reprinted from Ref. (23).

studied. In this regard, *KiSS-1* expression is modulated by both testicular and ovarian steroids (24). Finally, administration of brief i.v. infusions of human metastin 45–54 (2 μ g/monkey over 1 min) every hour, for 48 h, to agonadal juvenile males, 20–24 months of age, but in which pituitary responsiveness to GnRH had been increased by a ‘priming’ infusion of exogenous GnRH (0.15 μ g/min for 2 min every hour), elicited a sustained train of endogenous hypothalamic GnRH discharges, as reflected by the pulsatile pattern of circulating luteinizing hormone (LH) concentrations ((25), Fig. 2). That the site of this action of metastin is hypothalamic was indicated by the finding that metastin-induced LH release was abolished by treatment with acyline, a GnRH-receptor antagonist (25). Since GnRH neurons in the hypothalamus of both the rodent and the primate express GPR54 mRNA (26, 27), it is reasonable to propose that

the stimulatory action of metastin on GnRH release represents a direct action on GnRH neurons.

The ability of repetitive hourly activation of hypothalamic GPR54 in the juvenile monkey to elicit robust trains of GnRH discharges without evidence of decrement is reminiscent of the action of NMDA (17), which when administered in a pulsatile manner for several months drives the hypothalamic–pituitary–testicular axis of the juvenile monkey into an adult mode of operation with the onset of episodic testicular testosterone secretion and initiation of spermatogenesis (18). The authors predict that similar chronic, repetitive stimulation of hypothalamic GPR54 in the juvenile monkey would also result in precocious gonadarche, and have previously argued that the role of kisspeptinergic neurons in hypothalamic function may be restricted to that of regulating GnRH release, while that of glutamatergic interneurons may be more promiscuous (28).

In contrast to the ability of pulsatile stimulation with metastin to elicit a sustained hypophysiotropic drive to the gonadotrope in the juvenile monkey, in which endogenous GnRH release is greatly dampened (the ‘off’ phase of postnatal development), continuous i.v. infusions of metastin 45–54 ranging in dose from 1 to 100 μ g/h, during this phase of development, failed to sustain GnRH release in the same experimental model (29). Further study is required to determine the significance of the difference in the effectiveness of the hypothalamic actions of these two modes of i.v. metastin 45–54 administration.

Parenthetically, it deserves to be mentioned that the high dose, continuous metastin 45–54 infusion (100 μ g/h) to the juvenile monkey, although producing an initial stimulation of GnRH release lasting, approximately, 1–3 h, resulted, after 4 days of uninterrupted exposure, in desensitization of GPR54 ((30), Fig. 3). Metastin 45–54-induced desensitization, however, neither compromised the ability of the GnRH neuronal network to respond to stimulation with the glutamate agonist, NMDA, nor impaired the pituitary’s ability to respond to physiologic stimulation with a bolus of synthetic GnRH (30). GPR54 was re-sensitized within 24 h following termination of the continuous metastin 45–54 infusion. The ability of metastin 45–54 to downregulate GPR54 offers potential new approaches to probe the control of the hypothalamic–pituitary axis and perhaps to the development of therapeutic approaches for the treatment of reproductive disorders (30).

As described earlier, changes in metastin–GPR54 signaling at the GnRH neuron in the primate hypothalamus during the infantile–juvenile ‘on–off’ transition are yet to be described. However, based on the report of hypogonadotropism and low-circulating testosterone concentrations at 2 months of age in one infantile boy with a homozygous mutation for GPR54 (31) it would seem reasonable to propose that GPR54

Figure 2 Discharges of luteinizing hormone (LH), as reflected by circulating concentrations of the gonadotropin, during the last two priming pulses of synthetic GnRH (*open arrows*) on day 1 in agonadal male rhesus monkeys were sustained without decrement by an intermittent i.v. infusion of 2 μ g metastatin 45–54/monkey administered as a 1 min pulse every hour for 48 h from 1100 h on day 1, to 1000 h on day 3 (*solid data points*) after withdrawing GnRH treatment on day 1, but not during vehicle administration (*open data points*). Although metastatin 45–54 or vehicle was administered every hour, the LH response was tracked for only two or three pulses each day. The LH response to re-initiation of pulsatile GnRH priming at 1100 h on day 3 was monitored after both metastatin 45–54 and vehicle treatment, but only shown for the former treatment because of the exaggerated response in the vehicle group. *Solid arrows* indicate times of pulse infusions of metastatin 45–54 that were selected for tracking LH responses. *Open arrows* indicate time of GnRH pulse infusions. Values are mean $\pm$ S.E.M. Reprinted with permission from Ref. (25).

signaling in the GnRH neuron is critical for a functional GnRH drive to the pituitary–gonadal axis during infancy, as it is during puberty. This being the case, it is to be anticipated that during the transition from infancy to the juvenile state, expression of *KISS-1* would decline and, therefore, mirror the upregulation in expression of this gene at puberty.

Is GPR54 therefore a key regulator of the biology of puberty?

Compelling evidence from studies of several mammalian species is now at hand demonstrating that metastatin signaling at GPR54 represents a critical, and probably obligatory, component of the neuroendocrine control

Figure 3 Effect of continuous administration of metastatin 45–54 on LH release in agonadal juvenile male rhesus monkeys, in which pituitary responsiveness to GnRH had been previously increased by pulsatile GnRH treatment. Dark circles represent LH levels (mean $\pm$ S.E.M.) from three monkeys receiving 100 μ g/h metastatin 45–54 over a 98 h infusion period (shaded horizontal bar). Open circles represent mean LH levels during vehicle infusion. Administration of metastatin 45–54 or vehicle was initiated at 1000 h on day 1. White arrow indicates i.v. administration of last GnRH-priming pulse at 0800 h on day 1. Black arrow indicates i.v. administration of single bolus of metastatin 45–54 at 0900 h on day 1. Reprinted with permission from Ref. (30).

system regulating GnRH release (8, 9, 24, 32–35), and hence it is reasonable to conclude, as done by Seminara *et al.* (9) that this membrane receptor is a key regulator of the biology of puberty. However, after three further exciting years of probing the physiology of kisspeptin in the context of the neuroendocrine axis governing reproduction, it is time to rephrase the question with more focus, namely, 'Will GPR54 lead us to a resolution of the fascinating mystery of the timing of puberty?'

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