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Research Focus

A KiSS to remember

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The *Kiss1* gene encodes a family of neuropeptides named kisspeptins, which bind to a (former orphan) G-protein-coupled receptor called GPR54. Recent investigations suggest that kisspeptins play a vital role in regulating the secretion of gonadotropin-releasing hormone (GnRH). New evidence confirms that kisspeptins act through GPR54 to stimulate GnRH secretion. Kisspeptins and GPR54 are crucial for pubertal maturation in the primate. *Kiss1* mRNA is differentially regulated by sex steroids in distinct hypothalamic nuclei.

New candidates for the regulation of gonadotropin-releasing hormone secretion

Reproductive endocrinologists stay up late wondering how sex steroids and environmental cues converge in the forebrain to govern the activity of the few thousand gonadotropin-releasing hormone (GnRH) neurons. Recently, a family of neuropeptides and their receptor – already familiar to cancer researchers – jumped on to the center stage of reproductive science. Kisspeptins are encoded by a gene with the irresistible name of *Kiss1*. The receptor (or at least one of the receptors) for kisspeptins is a G-protein-coupled receptor that is commonly known by its former orphan name, GPR54. In 2004, *Trends in Endocrinology and Metabolism* reviewed the role of GPR54 in the onset of puberty [1]. We will focus on recent publications that have helped to elucidate how kisspeptins and GPR54 regulate GnRH secretion and helped to establish that both the ligand and its receptor are themselves targets for regulation by sex steroids [2–7].

Kisspeptins stimulate the reproductive axis

In 2003, three teams of investigators discovered that mutations in GPR54 lead to a complete impairment of reproductive function in humans and in mice [8–10]. Subsequently, several groups demonstrated that kisspeptins stimulate GnRH-mediated gonadotropin secretion – and do so at astonishingly low doses and for prolonged periods of time [7,11,12]. Neuroendocrinologists then wondered whether the effect of kisspeptin is mediated by GPR54 or if kisspeptins signal through other, unidentified receptors. Furthermore, the potent effects of kisspeptins raised the question of whether kisspeptins could directly stimulate GnRH neurons or if they acted through a population of interneurons. A recent publication by Messager *et al.* [2] has addressed these questions.

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When kisspeptin was administered to mice lacking a functional GPR54, it had no effect on gonadotropin secretion, confirming that GPR54 is necessary and sufficient for the stimulatory effect of kisspeptins on gonadotropin secretion. Furthermore, Messager *et al.* showed that GPR54 colocalizes with GnRH neurons in mice, as had been shown earlier in cichlid fish and rats [11,13], implying that kisspeptins directly stimulate GnRH neurons. Perhaps the most important discovery about GPR54 by Messager *et al.* is that there are no other obvious abnormalities in the GnRH neurons of GPR54 knockout mice: the location and morphology of GnRH neurons are the same as those in wild-type animals. Unlike other mutations leading to reproductive dysfunctions, such as the *kal1* mutation, *GPR54* mutations do not prevent the migration of GnRH neurons from the olfactory placode to the forebrain [14]. This finding suggests that GPR54 is required for the normal physiological function of GnRH neurons after they have migrated and innervated their targets.

Puberty: is a KiSS enough?

Is it conceivable that the onset of puberty is gated by the awakening of a functional connection between KiSS-1 neurons and GPR54 expressed on GnRH neurons? If this were the case, an increase in KiSS-1 neuronal activity or increased sensitivity of GnRH neurons to kisspeptins could be responsible for increased GnRH release at pubertal onset. Some evidence consistent with this model derives from a recent study by Shahab *et al.* [3] and from an earlier study by Navarro *et al.* [6]. Shahab *et al.* found increases in *Kiss1* mRNA associated with puberty in agonadal male and intact female rhesus macaques and found increased GPR54 mRNA in females. Likewise, Navarro *et al.* found increases in the expression of both KiSS-1 and GPR54 in male and female rats around the time of puberty. Thus, it seems plausible that these increases in the expression of both kisspeptin and its receptor play some role in initiating the onset of puberty. However, most measurements of KiSS-1 and GPR54 expression have been derived from gross hypothalamic fragments, with no attention paid to individual hypothalamic nuclei. KiSS-1 neurons are located discretely in many hypothalamic nuclei. It will be essential to conduct a more detailed analysis of possible changes in expression patterns of both KiSS-1 and GPR54 among these nuclei before conclusions can be drawn about the role of kisspeptins and GPR54 in timing the onset of puberty. It will also be important to study the effects of kisspeptins on

the electrophysiological properties of GnRH neurons as a function of development before it can be known for certain whether kisspeptin–GPR54 signaling has a role in sexual maturation.

Although neuroendocrinologists have yet to unravel the cellular physiology of the effects of kisspeptins on GnRH neurons, they have already uncovered evidence that kisspeptins can stimulate precocious GnRH secretion in prepubertal macaques. Shahab *et al.* [3] demonstrated that kisspeptin stimulates luteinizing hormone (LH) secretion in the juvenile male monkey, thus establishing that GPR54 is functional in prepubertal animals (and presumably expressed by GnRH neurons). Whether prepubertal animals are as sensitive as adults to the stimulatory effects of kisspeptins or if KiSS-1 neuronal activity differs between prepubertal animals and adults has yet to be discovered.

A related study by Navarro *et al.* [5] showed that kisspeptin administered into the lateral cerebral ventricle advances vaginal opening, increases uterine weight and triggers LH responses in prepubertal female rats. These findings nicely complement an earlier study by Matsui *et al.* [7] who reported that subcutaneous injections of kisspeptin induce ovulation in prepubertal rats. It remains to be determined whether a change in kisspeptin–GPR54 signaling is the principal endogenous instigator of increased GnRH release at puberty.

A link between sex steroids and GnRH secretion?

Recent literature suggests that kisspeptins and GPR54 might also be important for maintaining normal reproductive function in the adult. It has long been known that sex steroids exert negative feedback effects on GnRH secretion in males and that they exert both positive and negative feedback effects in females [15,16]. However, the identity of the cells that relay these signals to GnRH neurons remains a mystery. Recently, Smith *et al.* [4] postulated that these cells might be KiSS-1 neurons. KiSS-1 is expressed in the arcuate nucleus, which has been associated with negative feedback regulation of LH secretion, and in the anteroventral periventricular nucleus (AVPV), which is thought to be involved in the positive feedback effects of sex steroids associated with the preovulatory LH surge. Smith *et al.* [4] showed that, in the male mouse, testosterone suppresses *Kiss1* mRNA expression in the arcuate nucleus but stimulates it in the AVPV. If *Kiss1* mRNA levels correlate with neuronal activity in these regions, it is possible that tonic stimulatory kisspeptinergic input from the arcuate nucleus to GnRH neurons could be suppressed by testosterone, reducing the overall activity of GnRH neurons. However, the responsiveness of GnRH neurons to kisspeptins might also be influenced by sex steroids because they can also regulate GPR54 expression [6]. The recent findings by Smith *et al.* [4] suggest a possible mechanism of negative feedback but they also raise questions about positive feedback in the AVPV. Are KiSS-1 neurons in the AVPV involved in generating the preovulatory LH surge in the female? What is the function, if any, of the inductive effects of testosterone on KiSS-1 expression in the AVPV of the male? What factor or factors are responsible for the differential regulation of KiSS-1 between the arcuate nucleus and AVPV?

Early advances into understanding the world of kisspeptins and GPR54 have been rapid and exhilarating, having inspired fantasies about answers to old questions. Is this truly the stuff that turns children into adults, mediates negative feedback in the male and female, positive feedback in the female and turns frogs into princes? Only time and further research will tell.

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