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Does it really all start with a Kiss?

Activating the neuroendocrine reproductive axis

Kisspeptin is a relatively recent discovery in the hormonal control of reproduction, and arguably the most important finding in the last decade in the field of reproductive biology. Before kisspeptin came onto the scene, the kick-start needed for the key event of puberty - pulsatile secretion of gonadotrophin-releasing hormone (GnRH) - remained a mystery. In a mere five to six years there has been a massive explosion of research and major advances in our understanding of this process. Besides the understandable excitement about furthering our understanding of the hormonal control of reproductive physiology, there are also clinical implications. Elucidation of the mechanisms of kisspeptin signaling and the functional significance of this signaling could lead to new therapies for precocious puberty and delayed or absent puberty.

<u>What is kisspeptin?</u>

A team of researchers in Hershey, Pennsylvania originally identified *Kiss1*, the gene encoding kisspeptin. They decided to name this gene in recognition of the famous Hershey chocolate kisses. In 2001, three groups independently showed that the orphan G protein-coupled receptor 54 (GPR54) had high affinity for kisspeptin. The major breakthrough in the field of reproduction came in 2003, when two groups showed that GPR54 was crucial for normal puberty(de Roux *et al*. 2003, Seminara *et al*. 2003). They found mutations in the gene encoding the receptor in members of two consanguineous families suffering from idiopathic hypogonadotropic hypogonadism (IHH). Mice lacking GPR54 had a similar phenotype to humans. Further evidence came to light in 2007 as investigators found that kisspeptin knockout mice had an almost identical phenotype to mice lacking the receptor(d'Anglemont de Tassigny *et al*. 2007). Though kisspeptin is expressed by a number of tissues, including the placenta, the human mutations and animal models suggest that it is critical only in the neuroendocrine reproductive axis.

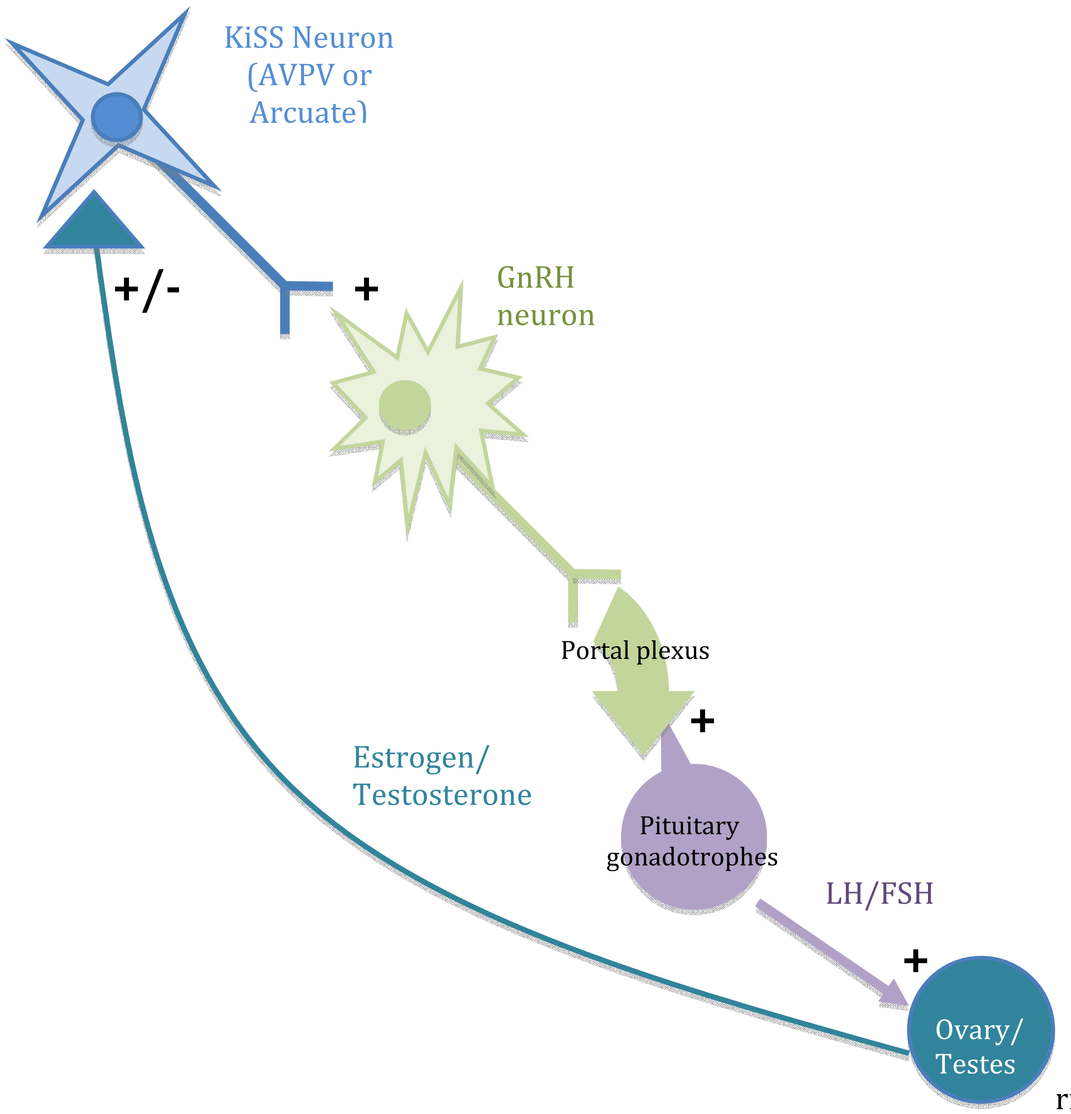
Research concerning kisspeptin's role in cancer metastasis lead scientists in the field of reproduction to ask the question whether kisspeptin had a similar role to anosmin-1. Anosmin-1, the gene product of *KAL1*, directs the migration of GnRH neurons during development. Mutations in *KAL1* cause Kallman syndrome, a type of hypogonadotropic hypogonadism associated with a lack of sense of smell (anosmia), with defects in migration of GnRH neurons and olfactory tract progenitors. However, those suffering from IHH with GPR54 mutations are normosmic. Additionally, it has been shown using immunohistochemistry that GnRH neurons are found in their correct location in preoptic nuclei of the hypothalamus with projections to the median eminence in mice lacking functional GPR54(Messager *et al*. 2005). Naturally, the next question was what then did kisspeptin do?

The function of kisspeptin/GPR54 signaling

Subsequent studies have shown that administering kisspeptin either centrally, via the ventricles of the brain, or peripherally via blood vessels, leads to an increase in the gonadotrophins LH (luteinizing hormone) and FSH (follicle-stimulating hormone), and that this is dose-dependent. These studies have been conducted in many different species and in both sexes e.g. the male rat(Thompson *et al*. 2004), female sheep(Messager *et al*. 2005), and human male(Dhillo *et al*. 2005). The release of gonadotrophins is dependent on the release of GnRH, and LH and FSH act on steroidogenic cells in the gonad to stimulate sex steroid synthesis.

The effect of kisspeptin on gonadotrophin secretion is via GnRH secretion as GnRH antagonists such as acyline inhibit the ability of kisspeptin to induce LH and FSH release in the mouse and rat(Gottsch *et al*. 2004, Irwig *et al*. 2004). Messager et al(Messager *et al*. 2005) demonstrated that GPR54 transcripts are colocalized with GnRH neurons in the mouse hypothalamus, providing evidence of a direct effect of kisspeptin on GnRH neurons. This group also showed that kisspeptin administration lead to an increase in GnRH release. Eight ewes were ovariectomized with estradiol implants introduced immediately in order to depress endogenously regulated GnRH and gonadotrophin levels. Tubes were inserted into the third and lateral ventricles of the brain in order to centrally administer kisspeptin. Human kisspeptin or vehicle (PBS) were administered for four hours, with CSF (cerebrospinal fluid) and jugular blood samples collected simultaneously both before and during the infusion. They detected a significant rise in CSF GnRH concentration when kisspeptin was infused, and a corresponding increase in plasma LH concentration. Irwig and colleagues (Irwig *et al*. 2004) have also found that a high percentage (77%) of GnRH neurons coexpress GPR54 mRNA, using a radiolabelled

The neuroendocrine reproductive axis.



riboprobe for GPR54 and a digoxigenin-labelled riboprobe for GnRH in the male rat. Additionally, they used immunohistochemistry to show that administration of kisspeptin increased Fos expression, a marker of neuronal activation, in 86% of GnRH neurons after 2 hours compared to less than 1% of GnRH neurons in animals treated with vehicle alone.

<u>Do kisspeptin neurons act as a 'switch' for puberty?</u>

There has been a great deal of excitement regarding the possibility that all the signals for puberty converge on kisspeptin neurons to activate the system at puberty. The initial finding that inactivating GPR54 mutations were responsible for IHH in two consanguineous families indicated that kisspeptin was crucial in the onset of puberty and the activation of pulsatile GnRH secretion. An activating mutation of the receptor has subsequently been associated with precocious puberty(Teles *et al*. 2008), as has an activating mutation of the Kiss1 gene(Silveira *et al*. 2010). This has allowed us to form a schema where kisspeptin neurons are upstream of GnRH neurons and act on them directly, with a role in switching on GnRH secretion at puberty. Chronic central administration of kisspeptin in prepubertal female rats advances the age of puberty (defined as the age of vaginal opening)(Navarro *et al*. 2004). Recent work by Pineda and colleagues using a newly developed kisspeptin antagonist, p234, shows that blocking kisspeptin action delays puberty in female rats(Pineda *et al*. 2010). The role of kisspeptin in puberty in the primate is supported by an increase in Kiss1 mRNA in the hypothalamus of pubertal monkeys compared to juvenile monkeys of both sexes(Shahab *et al*. 2005). Recent work has found that Neurokinin B and Neurokinin 3 receptor mutations are also implicated in cases of IHH(Topaloglu *et al*. 2009). Interestingly, Neurokinin B expression is colocalized with kisspeptin in ARC kisspeptin neurons(Goodman *et al*. 2007).

The role of body mass and leptin, the cytokine produced by white adipose tissue, in puberty and the control of the reproductive axis has been investigated for many years. We know that low body mass, for example associated with eating disorders, is responsible for delayed puberty and oligo- or amenorrhoea. Furthermore, leptin and leptin receptor mutations have been found in human patients with disorders of puberty. A high proportion of kisspeptin neurons express leptin receptors(Smith *et al*. 2006), suggesting that leptin acts through kisspeptin neurons to influence the reproductive axis. Smith and colleagues also demonstrated that Kiss1 mRNA is reduced in the arcuate nucleus in the hypothalamus of mice deficient in leptin (ob/ob mice). The Navarro et al study (Navarro *et al*. 2004) found that administration of kisspeptin could advance the age of puberty despite food deprivation, immunizing the rat against leptin, or in obese Zucher rats (a model of leptin resistance). These studies may indicate one therapeutic use of kisspeptin in the future.

Kisspeptins are crucial in the onset of puberty. The fact that not all normosmic IHH cases are explained by defects in kisspeptin or GPR54 strongly suggests that this is not the entire story, and that it may not all start with a ‘Kiss’. However, we may find that other discoveries will also be associated with kisspeptin signaling, as was the case for mutations of Neurokinin B and its receptor. The rapid advances in this field so far allows us to be hopeful that the next five years will see similar gains in our knowledge.

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