Kiss and tell in the brain – kisspeptins and the neural control of fertility

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The gonadotropin-releasing hormone (GnRH) neurons represent the final output cells of a complex neuronal network regulating fertility. As such, activation of the GnRH neurons in late postnatal development is critical for initiating the process of puberty. The mechanism through which GnRH neuron activation is achieved is presently under intense investigation.

In 2003, two clinical studies reported that mutations in a G-protein-coupled receptor, GPR54, were associated with the absence of puberty and consequent infertility in humans (de Roux *et al.*, 2003; Seminara *et al.*, 2003). Earlier studies had identified the product of the *Kiss-1* gene, the kisspeptin family of peptides, to be the ligands for GPR54. Interestingly, the *Kiss-1* gene was named by cancer biology investigators located in Hershey, Pennsylvania after the Hershey's Kisses chocolate product. The *Kiss-1* gene encodes a 54-amino-acid peptide (kisspeptin-54, also known as metastin) that is cleaved to shorter C-terminal peptides kisspeptin-14, -13 and -10 that all activate the kisspeptin receptor GPR54 with equal potency (Kotani *et al.*, 2001; Muir *et al.*, 2001; Ohtaki *et al.*, 2001). Kisspeptin and GPR54 are expressed in a variety of tissues but most notably in the placenta, pancreas and brain (Kotani *et al.*, 2001; Muir *et al.*, 2001; Ohtaki *et al.*, 2001).

Also in 2003, GPR54 knockout mice were reported to have a phenotype identical to that of humans with GPR54 mutations, with mice showing an absence of puberty and failure to reproduce (Funes *et al.*, 2003; Seminara *et al.*, 2003). Together, these studies strongly suggested that kisspeptin-GPR54 signalling was critical for puberty and opened a completely new line of research in reproductive physiology.

Several reproductive neurobiology laboratories quickly moved to investigate the possibility that kisspeptin-GPR54 signalling in the brain was involved in the regulation of puberty. Using mouse, rat, sheep and monkey experimental models for their different attributes, investigators quickly gained *in vivo* evidence that kisspeptin is an extremely potent activator of GnRH neurons and consequently gonadotrophin secretion (Gottsch *et al.*, 2004; Han *et al.*, 2005; Messager *et al.*, 2005; Navarro *et al.*, 2005a; Navarro *et al.*, 2005b; Shahab *et al.*, 2005). Kisspeptin's site of action to stimulate GnRH/gonadotropin secretion is very likely to be the GnRH neuron itself as these cells express GPR54 mRNA in several species (Irwig *et al.*, 2004; Parhar *et al.*, 2004; Messager *et al.*, 2005; Shibata *et al.*, 2005) and respond directly to kisspeptin with a marked increase in electrical excitability (Han *et al.*, 2005).

In situ hybridization and immunocytochemical studies in mice, rats, sheep and primates have shown that kisspeptin neurons are clustered in three regions of the hypothalamus; the periventricular preoptic area, dorsomedial hypothalamus and arcuate nucleus (ARN) (Gottsch *et al.*, 2004; Kinoshita *et al.*, 2005; Shahab *et al.*, 2005; Smith *et al.*, 2005; Franceschini *et al.*, 2006; Pompolo *et al.*, 2006). Recent immunocytochemical investigations have also now shown kisspeptin protein expression in these same three areas in the mouse (Clarkson & Herbison, 2006). Precisely which of these populations provide the afferent kisspeptin input to the GnRH neurons is unresolved, although indirect evidence (Clarkson & Herbison, 2006) and our unpublished observations indicate that it is the periventricular kisspeptin neuronal population.

How do the kisspeptin neurons activate GnRH neurons at the correct developmental time point? This important question is currently being addressed by several laboratories. Recent immunocytochemical data show that kisspeptin protein expression begins immediately prior to the onset of puberty in the periventricular neurons of the mouse and that kisspeptin fibers become evident around GnRH neuron cell bodies at the same time (Clarkson & Herbison, 2006). The GnRH neurons themselves are known to express GPR54 mRNA from early in development (Han *et al.*, 2005) so it seems likely that the critical switch for puberty is the rather abrupt onset of kisspeptin secretion onto GnRH neurons. How this is achieved remains unknown. Consistent with this observation, the administration of kisspeptin into the brains of prepubertal rodents and primates activates gonadotropin secretion (Navarro *et al.*, 2004; Shahab *et al.*, 2005; Plant *et al.*, 2006).

The absolute necessity of kisspeptin-GPR54 signalling for puberty to occur in mice and humans (Seminara *et al.*, 2003), coupled with the abrupt onset of kisspeptin synthesis in periventricular neurons innervating GnRH neurons (Clarkson & Herbison, 2006), indicate a critical role for kisspeptin in the activation of GnRH neurons at puberty. Although work continues to explore the mechanisms involved in this activation, studies have also branched out to evaluate the potential role of kisspeptin in regulating GnRH neuron activity following puberty. At the mid-point of each cycle in adult females, the GnRH neurons are activated intensely for several hours to initiate the GnRH surge that, in turn, generates the luteinizing hormone surge that triggers ovulation. Exciting work has now suggested that kisspeptin-GPR54 signaling is also employed to activate the GnRH neurons to initiate the GnRH surge (Han *et al.*, 2005; Kinoshita *et al.*, 2005; Smith *et al.*, 2006).

The kisspeptin-GPR54 saga so far has been remarkable for several reasons. First, because it was

discovered in cancer biology but has turned out, thus far, to have its biggest impact in neuroscience. Second, because it was brought to the attention of unsuspecting reproductive neurobiologists by clinical studies. Third, because kisspeptin turns out to be the most potent activator of GnRH neurons yet discovered and is almost certainly an important physiologically-relevant regulator of these cells. Fourth, because it shows what can be achieved by a concerted world-wide effort in less than three years. Kisspeptin is now back in the clinic and being trialled in humans.

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