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## Review

# Kisspeptin signalling in the brain: Steroid regulation in the rodent and ewe

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### ABSTRACT

The Kiss1 gene encodes a family of peptides called kisspeptins, which are the natural ligands for the receptor GPR54. In humans and mice, inactivating mutations of GPR54 results in hypogonadotropic hypogonadism, indicating that kisspeptins play a vital role in the regulation of GnRH secretion. In many species, centrally administered kisspeptins stimulate gonadotrophin secretion in a GnRH-dependant manner. Moreover, virtually all GnRH neurons coexpress GPR54. In the hypothalamus, the vast majority of kisspeptin producing cells also express sex steroid receptors, particularly estrogen receptor alpha. Thus, sex steroids are able to directly regulate the expression of Kiss1 mRNA, implicating kisspeptins as the 'missing link' between sex steroid feedback and GnRH secretion. Kiss1-expressing cells are localised to various regions of the forebrain in rodents, primates and sheep. In the arcuate nucleus (ARC) of the rodent and the ewe, sex steroids inhibit the expression of Kiss1 mRNA, suggesting that the kisspeptin secreting neurons here are the conduit for the negative feedback regulation of GnRH secretion. However, in the rodent anteroventral periventricular nucleus (AVPV), sex steroids induce the expression of Kiss1, implying that these kisspeptin neurons play a role in the positive feedback regulation of GnRH secretion. In sheep, there are no Kiss1 neurons in the AVPV and Kiss1 mRNA expression in the ARC is stimulated immediately prior to the preovulatory GnRH/luteinising hormone surge. Thus, kisspeptin neurons in the ARC of the ewe appear well placed to play a role in the negative and positive feedback regulation of GnRH exerted by sex steroids.

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Abbreviations: ADP, anterodorsal preoptic nucleus; AR, androgen receptor; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; E, estradiol; ER, estrogen receptor; ER $\alpha$ KO, ER $\alpha$  null mouse; ER $\beta$ KO, ER $\beta$  null mouse; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinising hormone; mRNA, messenger ribonucleic acid; Ob-Rb, signalling form of the leptin receptor; OVX, ovariectomised; P, progesterone; PEN, periventricular nucleus; POA, preoptic area; PR, progesterone receptor; T, testosterone

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## 1. Introduction

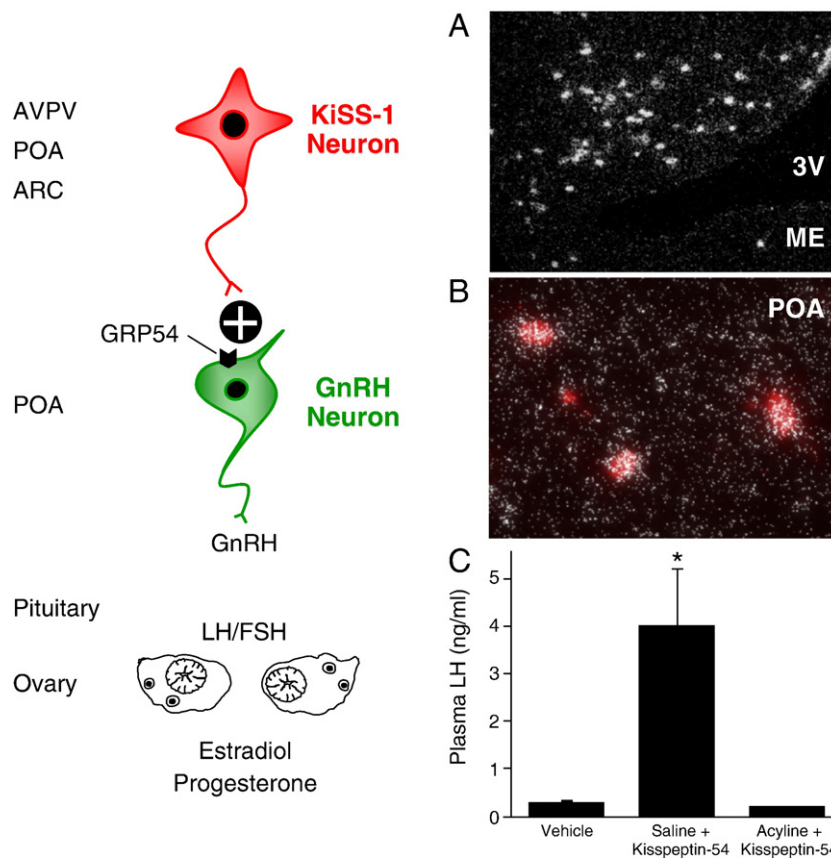
Successful reproduction is dependent on the highly controlled interactions among regulatory signals from the brain, pituitary, and gonads. The release of the pituitary gonadotropins, luteinising hormone (LH) and follicle stimulating hormone (FSH), is pivotal for ovulation and spermatogenesis, and is controlled, in turn, by the feedback effects of gonadal steroids acting on the brain and pituitary (Moore and Price, 1932; Caligaris et al., 1971). Specifically, negative feedback of gonadal steroids from both testes and ovaries controls tonic secretion of LH and FSH necessary for steroidogenesis and gametogenesis, while the positive feedback of ovarian steroids induces the preovulatory LH surge in females (Karsch et al., 1997; Herbison, 1998). The final common factor in the control of the brain–pituitary–gonadal axis was discovered more than 30 years ago as the decapeptide gonadotropin-releasing hormone (GnRH), yet the precise cellular and molecular mechanisms whereby gonadal steroids and other peripheral signals converge in the brain to achieve negative and positive feedback effects on GnRH secretion remain unknown.

In 2003, a major breakthrough occurred toward our understanding of the neural control of signals governing reproduction. Genetic studies from two independent laboratories discovered that disabling mutations and targeted deletions of the G-protein-coupled receptor, GPR54, in humans and mice result in sexual infantilism and hypogonadotropic hypogonadism (de Roux et al., 2003; Seminara et al., 2003). GPR54 was originally identified as an ‘orphan’ receptor in the rat (Lee et al., 1999). This newly discovered link between GPR54 and reproduction brought instant attention to the natural ligands of the GPR54 receptor, known collectively as kisspeptins. Kisspeptins are derived from the translational product of the Kiss1 gene (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001) and belong to a large RF-amide family of peptides, characterised by paired arginine and phenylalanine residues at the amidated C-terminus. In humans, post-translational processing of an initial 145-amino-acid Kiss1 peptide results in the formation of smaller C-terminal peptides (including the 54-amino-acid peptide originally termed metastin), which activate GPR54 with equal efficacy (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001). Advances in kisspeptin research have been rapid and in this short period these peptides are now cast as major players in the control of the hypothalamic–pituitary–gonadal axis.

## 2. Kisspeptins stimulate the reproductive axis

The stimulatory effect of kisspeptins on gonadotropins is unequivocal. Kisspeptins potently elicit the release of gonadotropins in many species (Gottsch et al., 2004; Matsui et al., 2004; Dhillon et al., 2005; Messenger et al., 2005; Shahab et al., 2005) and do so when administered centrally (via intracerebroventricular injection) (Fig. 1) or peripherally. Indeed, intranuclear cannulation and injection of kisspeptin into discrete regions of the hypothalamus stimulate LH secretion in the rat (Patterson et al., 2006). Potent stimulation of LH was noted after injection of kisspeptin into the medial preoptic area (POA), where most GnRH cells reside, bolstering the notion that kisspeptin effects on gonadotropins are GnRH-dependent. This proposal was first put forward by studies showing that the stimulatory effect of kisspeptins is blocked in the presence of a GnRH antagonist (Gottsch et al., 2004; Irwig et al., 2004; Matsui et al., 2004; Shahab et al., 2005) (Fig. 1) and is supported by four lines of evidence: (1) kisspeptin induces expression of the immediate early gene product Fos in GnRH neurons (Irwig et al., 2004; Matsui et al., 2004); (2) the vast majority of GnRH neurons express GPR54 in the rat (Irwig et al., 2004) and mouse (Han et al., 2005) (Fig. 1); (3) kisspeptin-immunoreactive fibres make close appositions to GnRH neuron cell bodies (Kinoshita et al., 2005; Clarkson and Herbison, 2006); and (4) electrophysiological studies show that kisspeptin directly increases GnRH neuron excitability (Han et al., 2005).

Despite these data, it remains possible that kisspeptins also act on pituitary gonadotropes directly, but this remains a matter of controversy. One group has shown that kisspeptins stimulate LH release and augment GnRH-stimulated FSH release from rat pituitary explants *in vitro* (Navarro et al., 2005a,b), whereas others have shown no effect (Matsui et al., 2004; Thompson et al., 2004). However, GPR54 is expressed in the human pituitary (Kotani et al., 2001; Muir et al., 2001) so further experiments are warranted. Kisspeptin-immunoreactive fibres are located in the external zone of the median eminence in sheep (Franceschini et al., 2006; Pompolo et al., 2006), and may release kisspeptin into the hypophyseal portal system to access the pituitary. Also in sheep, primary pituitary cell cultures show elevated LH secretion in response to kisspeptin (J. T. Smith and I. J. Clarke, unpublished observation). However, this response only occurs in cell culture taken from ewes in the follicular phase of the estrous cycle, no response was seen from cells from luteal phase ewes or from



**Fig. 1** – Proposed regulatory action of kisspeptin on GnRH and the hypothalamic–pituitary–gonadal axis. Terminals from kisspeptin producing neurons (Kiss1 neurons) located in the anteroventral periventricular nucleus (AVPV), preoptic area (POA), and the arcuate nucleus (ARC), contact GnRH neurons located in the POA. GnRH neurons are stimulated to release GnRH to the portal circulation via activation of the kisspeptin receptor (GPR54). GnRH then stimulates the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) and in turn ovarian steroids, estradiol (E) and progesterone (P). Panel A is a low power photomicrograph showing Kiss1 mRNA (silver grains) in the ARC of the ewe. Panel B is a high power photomicrograph showing coexpression of GnRH (red) and GPR54 (silver grains) in the POA of the rat. Panel C illustrates the effect of kisspeptin on circulating LH in the rat. Panels B and C are taken from Irwig et al. (2004) with permission from Karger Press.

ovariectomised (OVX) ewes. Kisspeptin also failed to alter GnRH-stimulated LH secretion in hypothalamo-pituitary-disconnected ewes under a range of paradigms (J. T. Smith and I. J. Clarke, unpublished observation) so the physiological significance of any anterior pituitary actions is unclear.

Kisspeptins are able to maintain LH secretory responses in rats, despite differences in pre-existing LH levels, under a range of physiological and experimental conditions (Roa et al., 2006). What is striking is that there appears to be a variation in the kisspeptin responsiveness across the ovarian cycle with maximal responses to kisspeptin (given centrally at 1 nmol) occurring at estrus. However, this patterned sensitivity to kisspeptin was not consistent at lower doses. Hence, a low dose of kisspeptin (0.1 pmol) elicited LH secretion at diestrus but not estrus (Roa et al., 2006). Interestingly, GPR54 mRNA expression appears to be elevated in the hypothalamus of diestrus female rats (Navarro et al., 2004a). It is likely that different mechanisms, such as GnRH response to kisspeptin and possibly pituitary sensitivity to GnRH, contribute to the variable responsiveness to kisspeptin. The precise nature of these mechanisms is yet to be determined.

### 3. Kisspeptin neurons are located in discrete regions of the forebrain

Transcripts for Kiss1 mRNA were first identified in a rudimentary manner in the human brain by RT-PCR (Muir et al., 2001). Later the discrete localisation of Kiss1 mRNA expression was detailed by in situ hybridisation (Gottsch et al., 2004). In the mouse, Kiss1 mRNA was found in cells residing in the anteroventral periventricular nucleus (AVPV), the periventricular nucleus (PEN), the anterodorsal preoptic nucleus (ADP), the medial amygdala, and the arcuate nucleus (ARC) (Gottsch et al., 2004). In the ewe, Kiss1 mRNA-expressing cells are also located in the ARC (Fig. 1) and there is also a much smaller population of positive cells in the POA (Estrada et al., 2006; Smith et al., 2007). Initial detection of kisspeptin-immunoreactive cells by immunohistochemistry yielded mixed results with positive cells found in the dorsomedial hypothalamic nucleus, nucleus of the solitary tract, and the caudal ventrolateral medulla (Brailoiu et al., 2005). Recent studies using a polyclonal rabbit anti-kisspeptin-10 antiserum raised

against the amino acid residues 443–52 of mouse kisspeptin (which shares complete homology to that of the sheep), show immunoreactive cells in the mouse AVPV and ARC (Clarkson and Herbison, 2006) and immunopositive cells in the ovine POA and ARC (Franceschini et al., 2006) — consistent with mRNA data. However, in the mouse and sheep immunoreactive cells were also detected in the dorsomedial hypothalamic nucleus (Clarkson and Herbison, 2006; Franceschini et al., 2006), an area void of any Kiss1 mRNA (Gottsch et al., 2004; Estrada et al., 2006; Smith et al., 2007). Such a discrepancy may indicate a degree of non-specificity of kisspeptin antisera, particularly to related C-terminal RF amide peptides. Cross-reactivity studies of known RF amides to kisspeptin antisera are warranted.

#### 4. Are kisspeptins the ‘missing link’ in steroidal control of GnRH secretion?

GnRH neurons do not express estrogen receptor alpha (ER $\alpha$ ) (Shivers et al., 1983; Herbison and Theodosis, 1992), or progesterone receptor (PR) (Skinner et al., 2001). They do express estrogen receptor beta (ER $\beta$ ) (Skynner et al., 1999; Hrabovszky et al., 2001), but the functional importance of this receptor to the central control of the reproductive axis is doubtful (Dorling et al., 2003). Hence, other steroid-sensitive neurons must mediate the feedback effects of sex steroids on GnRH secretion (Wintermantel et al., 2006). Kisspeptin cells are ideally placed to fill this void. Based simply on the distribution of Kiss1 mRNA-expressing cells, the ability of kisspeptin to stimulate GnRH secretion and the observation that a high percentage of kisspeptin-expressing cells express steroid receptors, it is possible to speculate that kisspeptin may relay information of the sex steroid milieu to the GnRH neuron.

##### 4.1. Sex steroid regulation of Kiss1 in the male

Testosterone (T) regulates the expression of Kiss1 mRNA in the forebrain of the male. In the rat, whole hypothalamic expression of Kiss1 mRNA is upregulated by castration and inhibited by T (Navarro et al., 2004a). A more complete histological examination (by *in situ* hybridisation) revealed that this was indeed the case in the ARC of the mouse (Smith et al., 2005b) and rat (Irwig et al., 2004). However, in the AVPV (and PEN) castration reduced expression of Kiss1 mRNA and treatment with T stimulated expression (Smith et al., 2005b). Thus, it can be suggested that the kisspeptin cells residing in the ARC relay the negative feedback effects of T on GnRH secretion, an assertion that is bolstered by the fact that cells in the ARC project to the POA (Canteras et al., 1994; Simonian et al., 1999) and that kisspeptin-immunoreactive boutons are found in close association with GnRH neuronal cell bodies (Clarkson and Herbison, 2006) (although the exact anatomical origin of these kisspeptin terminals is yet to be determined). The kisspeptin cells in the AVPV are clearly different to those of the ARC and may play a role in other T-mediated processes, such as sex behaviour, a proposition that remains to be tested.

Cell signalling mechanisms mediating the actions of steroids in the male also appear to differ between these two subpopulations of kisspeptin cells. The effects of T on Kiss1

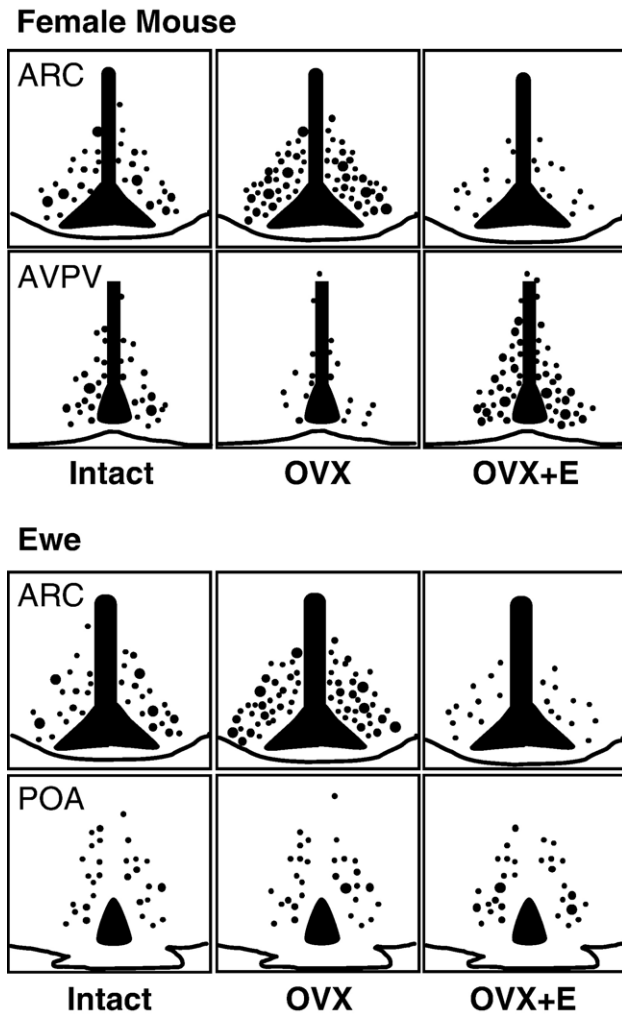
mRNA in the AVPV appear to be mediated exclusively by ER $\alpha$  (or ER $\beta$ ), as estradiol (E) treatment fully mimicked the effect of T (Smith et al., 2005b). Treatment with dihydrotestosterone (DHT, a non-aromatisable androgen) had no effect on Kiss1 in the AVPV, indicating that here regulation was not mediated by androgen receptor (AR) (Smith et al., 2005b). Using mice with targeted deletions of ER $\alpha$  (ER $\alpha$ KO), the effect of T regulation of Kiss1 in the AVPV was determined. Here ER $\alpha$ KO mice responded similarly to their wild-type counterparts indicating that the effect in the AVPV was not mediated by ER $\alpha$  and therefore was either possibly due to ER $\beta$  (Smith et al., 2005b), or some other compensatory system that is activated in these knockout mice. In contrast, both E and DHT appeared to mimic the effect of T on Kiss1 expression in the ARC, indicating that here regulation of Kiss1 is mediated by both the ER and the AR (Smith et al., 2005b). In both ER $\alpha$ KO mice and mice lacking complete functional AR (Ar<sup>invflox(ex1)-neo</sup>) there was no disturbance in the T-mediated regulation of Kiss1 in the ARC (Smith et al., 2005b). Furthermore, the majority of Kiss1 mRNA-positive cells in the ARC of the male mouse also coexpress ER $\alpha$  and AR mRNA (Smith et al., 2005b).

##### 4.2. Sex steroid regulation of Kiss1 in the female

Circulating concentrations of E define the levels of Kiss1 mRNA expression in the female brain in a site-specific manner that appears to relate to the negative and positive feedback actions of this steroid. Ovariectomy stimulates Kiss1 expression in the ARC of the female mouse (Smith et al., 2005a), rat (Smith et al., 2006b) and ewe (Smith et al., 2007), while E replacement diminishes this effect (Fig. 2). As discussed above in the male, kisspeptin producing cells in the ARC are poised to play a role in the negative feedback control of GnRH secretion, and this also appears to be the case in the female. In the ewe, the mediobasal hypothalamus is the major site of steroid-negative feedback action (Blache et al., 1991; Caraty et al., 1998). Other steroid receptive cellular populations that reside in the ARC (such as dynorphin and tyrosine hydroxylase) project from here to GnRH-rich regions of the POA (Dufourny et al., 2005a,b).

In contrast to the effects on Kiss1 neurons in the ARC, E robustly stimulates the expression of Kiss1 in the AVPV of the female rat and mouse (Smith et al., 2005a,b) (Fig. 2). These and other observations led to the hypothesis that these AVPV kisspeptin neurons mediate the positive feedback effect of E (see below). In the ewe, Kiss1 cells are not found in the AVPV (Estrada et al., 2006), but a small population is located in the POA; these Kiss1 cells do not appear to be regulated by circulating E (Smith et al., 2007).

In the ewe, progesterone (P) treatment only partially counters the effect of ovariectomy in the ARC (Smith et al., 2007). This result was unexpected, as P exerts strong negative feedback effects on LH secretion (Goodman and Karsch, 1980; Skinner et al., 1998). It is likely that the lesser effect of P on Kiss1 may be a result of reduced progesterone receptor (PR) expression in OVX ewes. PR expression is regulated by variations in circulating E (Shughrue et al., 1997). Moreover, the negative feedback effect of P is lost over time in OVX ewes (Skinner et al., 1998). P treatment in OVX female rats had no effect on whole hypothalamic expression of Kiss1 mRNA, and



**Fig. 2** – Kiss1 is differentially regulated by estrogen in the female mouse and ewe. In female mice, Kiss1 mRNA (black dots) is upregulated in the arcuate nucleus (ARC) in ovariectomised (OVX) mice. Estradiol replacement (+E) diminishes this effect. In the anteroventral periventricular nucleus (AVPV), OVX mice have decreased Kiss1 mRNA, while E treatment stimulates Kiss1 mRNA. In the ewe, Kiss1 mRNA is upregulated in the ARC in OVX ewes. E replacement diminishes this effect. There was no effect of OVX or OVX+E on Kiss1 in the preoptic area (POA).

no additional effect when paired with E treatment (Roa et al., 2006). P effects on Kiss1 expression in discrete hypothalamic areas of the rodent are yet to be fully examined.

Given the profound effect of E on Kiss1 expression in the rodent ARC and AVPV, it is not surprising that virtually all Kiss1 cells here coexpress  $ER\alpha$  in the female mouse (Smith et al., 2005a), and rat (Smith et al., 2006b). Additionally, the majority of Kiss1 cells in the ewe also express  $ER\alpha$  (Franceschini et al., 2006) and also PR (Smith et al., 2007). Whether Kiss1 cells in the rodent express PR is unknown. The importance of  $ER\alpha$  expression on Kiss1 cells in the female is unquestionable, because female  $ER\alpha$ KO mice are unable to regulate Kiss1 mRNA expression in the face of OVX and E

replacement (Smith et al., 2005a). It is interesting to note that in the mouse, approximately 25–30% of Kiss1 cells in the ARC and AVPV contain  $ER\beta$  (Smith et al., 2005a). However, the significance of  $ER\beta$  is questionable because female mice with targeted deletions of this receptor are still able to regulate Kiss1 mRNA expression in response to E manipulation similarly to their wild-type littermates (Smith et al., 2005a). Thus Kiss1-expressing cells are geared to directly receive information on the circulating sex steroid milieu and relay this information directly to GnRH neurons.

#### 4.3. Sex differences in Kiss1 gene expression

In all mammalian species, the brain is structurally and functionally differentiated between sexes. In terms of reproductive neuroendocrinology, the most dramatic functional difference is the ability of adult females, but not males, to display an E-induced preovulatory GnRH and LH surge. This sexual dimorphism is due to the ‘organisational’ effects of T during a critical period of development. The anatomical substrates underlying these differences in control of the GnRH/LH surge remained largely unknown until recent work on sex differences in Kiss1 gene expression. In the mouse and rat, both kisspeptin protein and Kiss1 mRNA expression are higher in the AVPV of the female than the male (Clarkson and Herbison, 2006; Gottsch et al., 2006; Kauffman et al., 2007). This difference, at least in the rat, appears to be due to the organisational effect of circulating sex steroids during a neonatal critical period. Thus, in female neonatal rats androgenised with exogenous administration of T, the adult level of Kiss1 mRNA expressed in the AVPV was similar to that of males (Kauffman et al., 2007). Interestingly, there was neither sex difference nor an effect of neonatal T treatment on Kiss1 expression in the ARC (Kauffman et al., 2007). Whether this effect of neonatal T treatment in the AVPV is due to the aromatisation of T to E is unknown, but it is worthy to note that in male  $ER\alpha$ KO mice, where any effect of E exposure during the neonatal period is presumably blocked, there appears to be an increased number of Kiss1 mRNA-positive cells in the AVPV (Smith et al., 2005b).

There is also an apparent sex difference in the receptors employed by the steroid-mediated regulation of Kiss1. As described above (Sex steroid regulation of Kiss1 in the male and Sex steroid regulation of Kiss1 in the female sections), female  $ER\alpha$ KO mice are unable to regulate Kiss1 expression in response to E treatment in the either the AVPV or ARC (Smith et al., 2005a). On the other hand, Kiss1 expression in male  $ER\alpha$ KO mice is able to respond to E treatment (Smith et al., 2005b). Thus,  $ER\alpha$  is critical for Kiss1 regulation in the female, but not the male.

## 5. Kisspeptins are critical to the preovulatory GnRH/LH surge

There is compelling evidence to suggest that the sexually differentiated population of Kiss1 cells in the AVPV of the female mouse and rat are critical to the positive feedback induced preovulatory LH surge. GnRH neurons are not direct targets for the actions of E (Shivers et al., 1983; Herbison and Theodosios, 1992), so another population of afferents must act

indirectly to relay these effects. Kiss1 cells appear well placed to fill such a void for many reasons. Kisspeptin is a potent secretagogue for GnRH (Gottsch et al., 2004; Matsui et al., 2004; Dhillon et al., 2005; Messager et al., 2005; Shahab et al., 2005). There is evidence to suggest that in the rodent the GnRH/LH surge is generated by E-sensitive neurons residing in the AVPV (Gu and Simerly, 1997; Wintermantel et al., 2006). Lesions of the AVPV block the GnRH/LH surge in rodents (Wiegand et al., 1978). Kiss1 neurons are direct targets for E and expression of Kiss1 is stimulated by E in the AVPV. Most important is that expression of Kiss1 is upregulated in the AVPV concomitantly with the preovulatory GnRH/LH surge (Smith et al., 2006b; Adachi et al., *in press*) (Fig. 3). Moreover, these cells become transcriptionally activated at the time of the surge (Smith et al., 2006b). Thus, kisspeptin producing cells in the AVPV are well placed to provide critical inputs toward the preovulatory GnRH/LH surge (Fig. 4). However, increased Kiss1 expression in the AVPV does not simply preclude a rise in LH. In OVX mice treated with E, Kiss1 mRNA is elevated in the AVPV, yet these animals have suppressed levels of LH (Smith et al., 2005a). Interestingly, the increase in Kiss1 expression in the rat over the transition between the morning of proestrus to the evening is associated with relatively stable concentrations of E (Smith et al., 2006b). This induction in Kiss1 may suggest additional afferent input to Kiss1 cells from other circuitry that is activated prior to the GnRH/LH surge. Projections from the suprachiasmatic nucleus to the AVPV are known to constrain the GnRH/LH surge to the evening of proestrus (Gu and Simerly, 1997; Barbacka-Surowiak et al., 2003; de la Iglesia and Schwartz, 2006), and hence these circadian signals may form additional regulatory afferents to Kiss1 cells.

In the ewe, there is no population of Kiss1 neurons in the AVPV (Estrada et al., 2006). Interestingly, the mediobasal hypothalamic region of the ewe brain (and not the AVPV) is recognised as the site that E acts to evoke positive feedback effects on GnRH/LH secretion (Blache et al., 1991; Caraty et al., 1998). It is, therefore, significant that expression of Kiss1 in the ARC is also upregulated immediately prior to, and during the preovulatory GnRH/LH surge (Estrada et al., 2006) (Fig. 3). This suggests that, in the ewe, Kiss1 cells in the ARC participate in both the negative and positive feedback regulation of GnRH secretion (Fig. 4). Moreover, it appears that only the Kiss1 neurons located at the caudal portion of the ARC are activated just prior to the surge (Estrada et al., 2006), yet Kiss1 cells across the whole ARC appear to be responsive to ovariectomy and chronic E replacement (Smith et al., 2007). This leads to the enticing proposition that populations of Kiss1 cells in discrete regions of the ARC are involved in negative and positive feedback regulation of GnRH (Fig. 4).

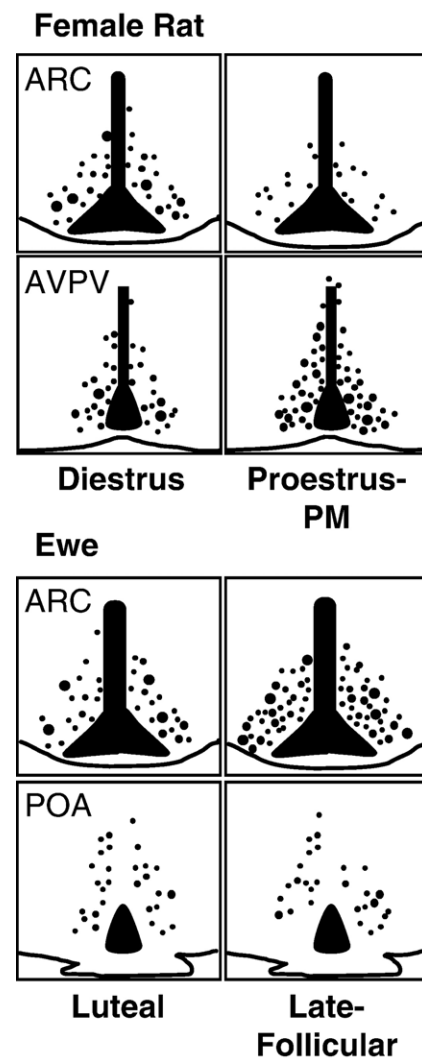
## 6. Kisspeptins as central mediators for control of fertility

Because it is not essential for the survival of the individual, reproductive function may not operate during periods of essential growth or cease under physiologically adverse conditions. Thus, reproductive function is quiescent prior to puberty, which occurs in all mammals and also during the annual non-breeding season in seasonally breeding mammals.

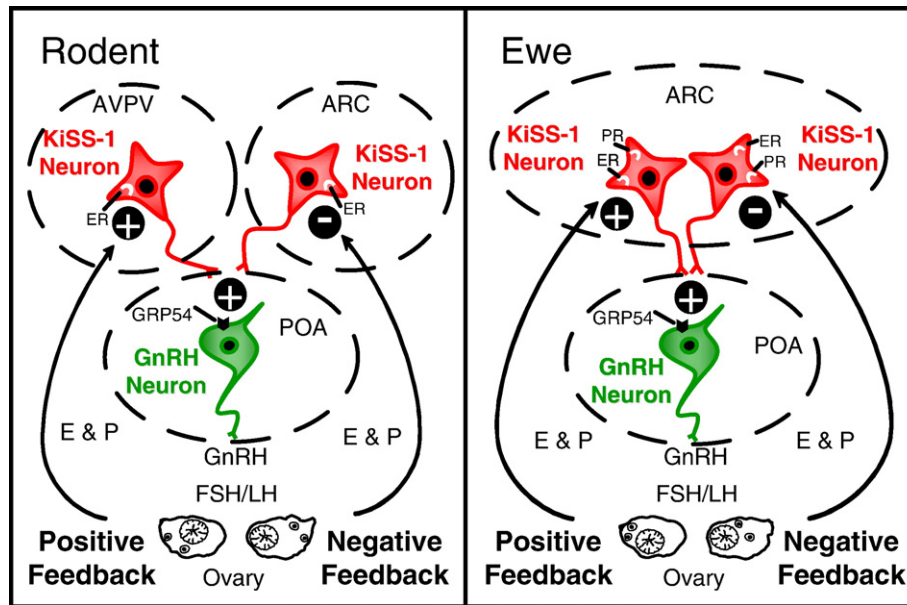
Both these phenomena involve a physiological transition between sexual inactivity and sexual activity. Interestingly, increases in kisspeptin have been proposed to play a key role in both the onset of puberty and the reinitiation of reproductive function at the beginning of each breeding season.

### 6.1. Kisspeptins and the onset of puberty

The onset of puberty is triggered by the resurgence of GnRH secretion at an appropriate stage of development. Kisspeptin-GPR54 signalling appears critical to this process, because mice and men with disabling mutations of GPR54 fail to reach puberty and are forever fated to hypogonadotropic-hypogonadism (de Roux et al., 2003; Seminara et al., 2003). Kisspeptin treatment,



**Fig. 3** – Kiss1 is upregulated during the GnRH/LH surge in the female rat and ewe. In female rats, Kiss1 mRNA (black dots) is stimulated in the anteroventral periventricular nucleus (AVPV) at the time of the preovulatory GnRH/LH surge (Proestrus PM). Whereas in the arcuate nucleus (ARC), Kiss1 mRNA is reduced at this time. In the ewe, Kiss1 mRNA is stimulated in the ARC immediately prior to the GnRH/LH surge (Late-Follicular). There was no change in Kiss1 seen in the preoptic area (POA).



**Fig. 4** – Proposed mechanism by which ovarian sex steroids mediate positive and negative feedback regulation of Kiss1, and in turn GnRH secretion, in the female rodent and ewe. In rodents, estradiol (E) stimulates expression of Kiss1 in the anteroventral periventricular nucleus (AVPV) potentially stimulating GnRH secretion and mediating positive feedback regulation. In the arcuate nucleus (ARC), E inhibits Kiss1, potentially leading to reduced GnRH secretion forming the circuitry for negative feedback regulation. In the ewe, E inhibits Kiss1 in the ARC, consistent with negative feedback regulation. We hypothesise that E is additionally able to stimulate Kiss1 in a discrete population of cells in the ARC, mediating positive feedback regulation.

both central and peripheral, advances the onset of puberty (Navarro et al., 2004b), and stimulates ovulation in juvenile rats (Matsui et al., 2004). Central administration of submaximal doses of kisspeptin stimulate LH in adult mice and rats but not juveniles (Han et al., 2005; Castellano et al., 2006), indicating that the sensitivity of GnRH neurons is increased across the pubertal transition. This is further illustrated by electrophysiological examination of GnRH neurons and their response to kisspeptin across puberty. Hence, the proportion of GnRH neurons in mice that respond to kisspeptin increases with pubertal development (Han et al., 2005). One might infer from this developmental recruitment of GnRH neurons that the expression of GPR54 on GnRH neurons also increases concurrently with puberty, however, this was not the case; both the percentage of GnRH neurons expressing GPR54 and the content of GPR54 mRNA per GnRH cell were identical before and after puberty (Han et al., 2005). Contrary to this, within whole brain regions containing GnRH neurons, GPR54 mRNA was increased with puberty in the rat (Navarro et al., 2004a) and Rhesus monkey (Shahab et al., 2005). Specifically, in the Rhesus monkey, GPR54 only increased in the intact female and not in the agonadal male (Shahab et al., 2005).

If kisspeptins were a trigger for puberty onset, one would expect to see an increase in Kiss1 expression during this time. This was shown to be true in both the male and female rat where whole hypothalamic expression of Kiss1 mRNA increased from pre- to post-puberty (Navarro et al., 2004a). Likewise, expression of Kiss1 mRNA increased in the ARC of the agonadal male and intact female Rhesus monkey (Shahab et al., 2005) during the puberty-related increase in LH secretion that occurs in both models. In the mouse, Kiss1 mRNA in the

AVPV increased significantly across puberty (Han et al., 2005). Notably, Kiss1 mRNA expression was unchanged in the ARC (Han et al., 2005). Additionally, recent data have revealed that the number of kisspeptin-immunoreactive fibres making close appositions to GnRH neurons increases from pre- to post-puberty (Clarkson and Herbison, 2006). Thus it appears that the net result of these changes may equate to an ‘attack on two fronts’ involving an increase in kisspeptin output and supply to the GnRH neuron, and an increase in the response of the GnRH neuron, both of which potentially lead to puberty onset.

## 6.2. Kisspeptins and seasonal breeding

Sheep are seasonally breeding mammals, with an annual reproductive cycle under the control of photoperiod. This is reflected by the increased activity of GnRH neurons and LH secretion during short-day photoperiod and inhibition by long days (Legan et al., 1977; Robinson et al., 1985; Barrell et al., 1992; Karsch et al., 1993; Barker-Gibb and Clarke, 2000). Because there is no morphological difference in GnRH neurons across the seasons (Lehman et al., 1986), it has been proposed that the function of afferents regulating GnRH release is of critical importance. Indeed, synaptic inputs to GnRH neurons in the sheep undergo pronounced seasonal rearrangements (Lehman et al., 2002; Pompolo et al., 2003). The identity of these inputs remains virtually unknown. Expression of Kiss1 mRNA in the ARC of OVX ewes increases significantly during the transition from the anestrus season to the breeding season (Smith et al., 2007). This change in Kiss1 may implicate kisspeptin as a ‘neuroendocrine switch’ stimulating the renaissance of ovulatory cycles in ewes at the

onset of the breeding season. Such an effect would imply the role of kisspeptin in the steroid-independent mechanisms in seasonal activation of GnRH in the ewe (Robinson et al., 1985; Barrell et al., 1992; Barker-Gibb and Clarke, 2000). Season breeding in the ewe is known to also involve a steroid-dependent mechanism (Legan et al., 1977; Karsch et al., 1993). Whether kisspeptin also participates in the seasonal shift in E feedback sensitivity of the reproductive neuroendocrine axis is under active investigation in our laboratory.

Similar observations to that of the ewe have been made in the male Syrian hamster brain (Revel et al., 2006). In contrast to the sheep, the breeding season of the hamster is stimulated by long-day photoperiod and accordingly, Kiss1 mRNA expression and kisspeptin protein (assessed by immunohistochemistry) are increased in the ARC under this photoperiod (Revel et al., 2006). In contradiction, however, a later study, in male Siberian hamsters reported that there was a reduction in kisspeptin-immunoreactive cells in the ARC during long-day photoperiod (i.e., the breeding season), but an increase in cell number was seen in the AVPV during the breeding season (Greives et al., 2007). Whether this contradiction reflects the fact that one study was conducted in Syrian hamsters and the other in Siberian hamsters or that different kisspeptin antisera were used was not addressed. Furthermore, neither study controlled for endogenous steroid concentrations (by using castrated and T replacement groups) that profoundly regulate Kiss1, so it is difficult to determine whether the changes in Kiss1/kisspeptin expression in these studies are an organisational effect of season or simply an activational response to seasonal changes in T concentrations. Clearly, additional studies in the hamster are required to clarify this matter.

### 6.3. What awakens the kisspeptin circuitry at the reinitiation of fertility?

The question remains as to what stimulus awakens kisspeptin-GPR54 signalling to potentially initiate the onset of puberty? There are clear links between nutrition and reproduction (Clarke and Henry, 1999; Cunningham et al., 1999). Moreover, there is a mandatory requirement for the satiety hormone leptin to allow puberty to proceed (Cheung et al., 1997). How leptin exerts its effects on reproduction – in particular the onset of puberty – is unclear, but it most certainly involves intermediary neurons, because compelling evidence suggests that leptin does not act directly on GnRH neurons (Finn et al., 1998; Hakansson et al., 1998). There are firm links between leptin and kisspeptin. Kiss1 mRNA expression is reduced in the hypothalamus of fasted rats (Castellano et al., 2005). Indeed, mice genetically lacking leptin (*ob/ob* mice), experiencing a state of perceived starvation, have significantly less Kiss1 mRNA in the ARC (Smith et al., 2006a). Leptin treatment restores Kiss1 expression in these mice and the majority of Kiss1 cells in the ARC express the signalling form of the leptin receptor (*Ob-Rb*) (Smith et al., 2006a). Thus, kisspeptin producing cells may form part of this regulatory system by which leptin is thought to permissively gate the onset of puberty.

Regarding seasonal breeding, it is thought that yearly melatonin rhythms govern this seasonal transition (Malpoux et al., 1993, 1998). It is possible, but yet to be determined, that

melatonin regulates kisspeptin signalling. Melatonin receptors are located in the basal hypothalamus (Malpoux et al., 1998) and melatonin acts in this region to control reproduction in the sheep (Lincoln and Maeda, 1992; Lincoln, 1994; Malpoux et al., 1998).

## 7. Conclusion

Remarkable advances in our understanding of reproductive neuroendocrinology have been made through research on kisspeptin and GPR54. In the short period since the discovery of the link between kisspeptins and reproduction, this peptide has been demonstrated to be one of the most pivotal components of the neuroendocrine system. Kisspeptins are clear and vital GnRH secretagogues and Kiss1-expressing cells in the hypothalamus are likely to be the ‘missing link’ in the steroid feedback control of GnRH secretion. There is compelling evidence that kisspeptins act as the ‘trigger’ to the onset of puberty and to initiate each breeding season, although more evidence is required to substantiate this notion. Nevertheless, it is clear that the newly discovered kisspeptin system represents a major control mechanism for the reproductive system and future work should expand our knowledge of its involvement in a range of processes.

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