The role of kisspeptin in reproductive function in the ewe

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Summary

Kisspeptin is a hypothalamic neuropeptide that is critical for fertility. In virtually all species, kisspeptin neurons stimulate gonadotrophin-releasing hormone (GnRH) secretion and act as transmitters for sex-steroid feedback to GnRH neurons. In sheep, kisspeptin neurons are located in the preoptic area and the arcuate nucleus (ARC), with the latter involved in both oestradiol positive and negative feedback regulation of GnRH. In addition, sheep are seasonal breeders, with an annual cycle controlled by changes in the pulsatile secretion of GnRH. Kisspeptin neurons are also important in this phenomenon showing increased expression and terminal apposition to GnRH neurons during the breeding season. Reduced kisspeptin expression during the non-breeding season can be overcome by administration of kisspeptin, which causes ovulation in seasonally acyclic females. On the other hand, kisspeptin neurons do not appear to express the melatonin receptor, so the transduction of photoperiod to these neurons must be indirect, perhaps involving dopaminergic suppression during the non-breeding season. Importantly, kisspeptin neurons of the ARC do not operate in isolation. Autoregulation of kisspeptin expression by the neuropeptides neurokinin B and dynorphin is a key contributor to the "KNDy neuron" concept and the hypothesis that these neurons comprise the GnRH pulse generator. Indeed, the pheromone-induced interruption of seasonal anestrus, known as the male effect, appears to be mediated by KNDy signalling. However, the 'KNDy hypothesis' for GnRH pulse generation is still unproven and, indeed, the precise role of KNDy cells in seasonal breeding has yet to be determined.

Introduction

In mammals, the reproductive process is governed through intricate neural and hormonal communication between the brain, pituitary gland and gonads. At the top of this hierarchical regulatory system is the release of gonadotrophin-releasing hormone (GnRH) pulses from neurons in the preoptic-hypothalamic continuum that are obligatory for the secretion of luteinizing hormone (LH) pulses from the anterior pituitary gland (Clarke & Pompolo 2005). Changes in the frequency or amplitude of GnRH pulses have a profound effect on the reproductive system, so it is not surprising that the origin and control of the pulsatile pattern have been the focus of research for nearly four decades (Martin 1984, Clarke 2011). One major puzzle has been the fact that, in both sexes, sex steroids are major drivers of change in the pulsatile release of GnRH, yet GnRH neurons lack the receptors responsible for steroid feedback control. This

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conundrum led to a prolonged search for cells that could fill this gap in communication between the gonads and the brain's GnRH neurons. Many candidates, focused within the mediobasal hypothalamus (Blache *et al*. 1991, Caraty *et al*. 1998), with both inhibitory and stimulatory effects on GnRH secretion, have been assessed (Clarke & Pompolo 2005), and kisspeptin is currently leading the field.

In sheep, there is also an annual pattern in reproduction that is controlled primarily by the annual photoperiodic cycle, with modulation by other inputs such as nutrition and socio-sexual signals (Scaramuzzi & Martin 2008). The photoperiodic cues are perceived and translated into a key physiological signal that is mediated through the secretion of melatonin from the pineal gland during periods of darkness. Melatonin cannot act on GnRH neurons directly (Goodman *et al*. 2010) so, as for the sex steroids, melatonin appears to need other cells within the mediobasal hypothalamus (Malpaux *et al*. 1998, Migaud *et al*. 2005) to exert its influence on GnRH secretion. Nevertheless, GnRH and LH pulses appear to be the over-riding determinant of seasonal reproductive function. Photoperiod-driven changes in pulsatile GnRH secretion are evident in both oestradiol negative feedback independent and dependent mechanisms (Karsch *et al*. 1980, Martin *et al*. 1983). The former is evidenced by a change in LH pulse frequency in ovariectomised (OVX) ewes, the latter by a profound increase in responsiveness to oestradiol negative feedback on GnRH/LH during the non-breeding season (Fig. 1). Thus, neuropeptides governing the pulsatile release of GnRH/LH, and/or those involved in transmitting oestradiol negative feedback signals to GnRH neurons, are likely to be key systems in the control of the seasonal reproductive pathway. Again, kisspeptin is currently leading the field.

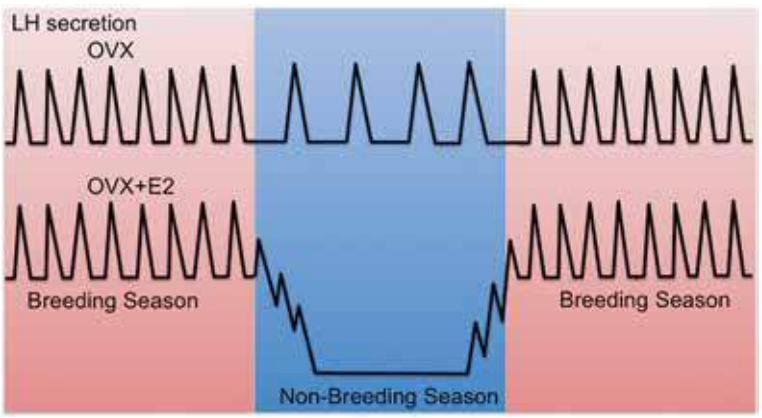

Fig. 1. Schematic representation of the seasonal change in LH secretion in ewes. LH profiles are shown for ovariectomised (OVX) ewes and OVX plus oestradiol (E2) treated ewes – demonstrating the oestradiol independent and dependent mechanisms for seasonal regulation of GnRH/LH secretion. The figure is derived from Karsch et al. (1980).

Kisspeptin

There is absolutely no doubt that kisspeptin, the product of the *Kiss1* gene, and its receptor, Kiss1r (previously Gpr54) play a major role in the control of GnRH secretion (Gottsch *et al*. 2004, Oakley *et al*. 2009). In sheep, the evidence for this includes: i) kisspeptin neurons project to GnRH neurons (Smith *et al*. 2008); ii) virtually all GnRH neurons express *KISS1R* mRNA (Smith *et al*. 2009a); iii) kisspeptin rapidly and robustly stimulates GnRH and gonadotrophin secretion (Smith *et al*. 2011); iv) kisspeptin neurons are located in regions of the hypothalamus

involved in sex steroid feedback (including the arcuate nucleus, ARC) in both males and females, and express oestrogen, progesterone and androgen receptors (Lehman *et al.* 2013); and v) in turn, sex steroids, particularly oestradiol, regulate kisspeptin neurons in a manner consistent with negative and positive feedback effects on GnRH neurons (Smith 2013), vital for tonic gonadotrophin secretion and the preovulatory LH surge.

In sheep, kisspeptin neurons are found in the preoptic area (POA) and the ARC, as shown with *in situ* hybridization (Estrada *et al.* 2006, Smith *et al.* 2007) and immunohistochemistry (Franceschini *et al.* 2006, Smith *et al.* 2008) (Fig. 2). Expression of *KISS1* mRNA in the ARC increases after ovariectomy in ewes, and is normalized in ovariectomized ewes after chronic oestradiol replacement (Smith *et al.* 2007, Smith *et al.* 2008). Chronic progesterone replacement also inhibits *KISS1* expression in ovariectomized sheep (Smith *et al.* 2007). Virtually all kisspeptin neurons in the ovine ARC co-express oestrogen receptor alpha (ESR1) and progesterone receptor (Franceschini *et al.* 2006, Smith *et al.* 2007) and steroid-sensitive neurons projecting from the ARC have been implicated for a role in the negative feedback control of GnRH secretion by oestradiol (Blache *et al.* 1991, Caraty *et al.* 1998, Simerly 2002). In sheep, the ARC is also thought to relay oestradiol positive feedback signals to GnRH neurons (Blache *et al.* 1991, Caraty *et al.* 1998). Again this appears to be mediated by kisspeptin neurons because kisspeptin gene expression and peptide production are increased in the ARC, particularly the middle-to-caudal areas, during the late-follicular phase of the oestrous cycle (Estrada *et al.* 2006, Smith *et al.*

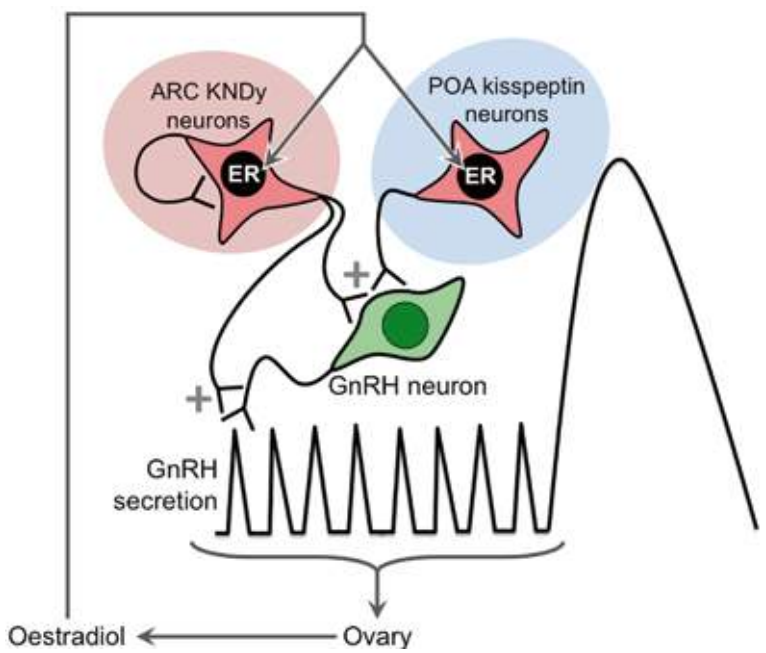

Fig. 2. The proposed anatomical relationship between kisspeptin and GnRH neurons in the ewe. Caudal kisspeptin neurons in the arcuate nucleus (ARC) co-express neurokinin B and dynorphin and are thus termed "KNDy" neurons. KNDy neurons appear to project to both the GnRH neuron cell body (Lehman et al., 2013) and GnRH terminals in the median eminence (Smith et al., 2011) to participate in both GnRH pulsatile secretion and the preovulatory LH surge. Rostral kisspeptin neurons located in the preoptic area (POA) project directly to GnRH neurons (Backholer et al., 2009) and appear to solely participate in the preovulatory LH surge. Both populations express oestrogen receptor alpha (ER) and modulate oestradiol feedback.

2009a). Moreover, kisspeptin neurons in the middle and caudal ARC show robust induction of FOS (a marker for neuronal activation) following a surge-inducing oestradiol injection (Smith *et al.* 2009a, Merkley *et al.* 2012). How this single population of neurons responds to oestradiol, but with two opposing outcomes, has puzzled researchers in the kisspeptin field. Data from studies with mice suggest alternative intracellular pathways (genomic vs non-genomic) post-ESR1 binding (Gottsch *et al.* 2009) but such observations only provide few clues for the ovine model. It is however possible that an indirect, multi-synaptic activation pathway rather than direct effect of oestradiol is central to the preovulatory LH surge generation in ewes. A temporal aspect to the oestradiol response has also been proposed (Clarke & Caraty 2013). Central to this hypothesis is that the positive feedback event is transient, but negative feedback is continuous. Thus, an acute rise in oestradiol temporarily activates the ARC kisspeptin cells culminating in the LH surge, while constant levels of oestradiol inhibit the activity of ARC kisspeptin neurons (Smith et al., 2009a). Given the current data in the sheep, this appears to be the most feasible explanation.

Kisspeptin neurons in the POA are now also known to play an important role in mediating positive feedback in sheep (Smith *et al.* 2009a, Hoffman *et al.* 2011, Merkley *et al.* 2012), suggesting a parallel with the rostral population of kisspeptin neurons in rodent species, located in the anteroventral periventricular (AVPV) region. In both neuronal populations, oestradiol can stimulate the expression of *Kiss1* mRNA (Smith *et al.* 2005, Smith *et al.* 2006, Smith *et al.* 2008). Importantly, the positive feedback mechanism in ewes does not change between breeding seasons. Thus, during seasonal anoestrus, increased oestradiol negative feedback prevents the sequence of events leading to positive feedback, but the neuroendocrine ability to produce a surge is intact (Clarke 1988).

It is clear that sex steroids are major regulators of kisspeptin neurons in the ARC. Focusing on negative feedback, which is pivotal for the pulsatile release of GnRH, a strong case can be made for these steroid-responsive kisspeptin neurons playing a central role. The secretion of LH pulses in female monkeys and ovariectomized sheep is inhibited by central administration of a selective kisspeptin antagonist (Roseweir *et al.* 2009), suggesting that GnRH pulse frequency is dependent on kisspeptin signaling. In goats, indirect evidence suggests the GnRH pulse generator is located in the caudal ARC, where kisspeptin cell bodies are located (Ohkura *et al.* 2009). Thus, in primates and ruminants, oestradiol negative feedback signals are sensed by kisspeptin neurons in the ARC that, in turn, stimulate GnRH neurons appropriately to control the pulsatile secretion of GnRH. However, the exact mechanism through which kisspeptin can so profoundly affect GnRH secretion is only now beginning to emerge, primarily with the help of the 'KNDy hypothesis'.

The KNDy hypothesis

Despite the KNDy hypothesis being the latest chapter in the kisspeptin field, clues of its existence date back to the 1990s when neurokinin B (NKB, product of the *TAC3* gene) expression in the ARC was correlated with LH concentrations in humans and rats (Rance & Young 1991, Rance & Bruce 1994). In sheep, these NKB neurons shared a close anatomical distribution with dynorphin neurons (Foradori *et al.* 2006) and all appear to contain ESR1 (Goubillon *et al.* 2000) and progesterone receptor (Foradori *et al.* 2002). With the discovery of kisspeptin the final piece of the puzzle fell into place and it was subsequently shown that a single population of neurons contained all three peptides – first demonstrated in sheep (Goodman *et al.* 2007) and then expanded to rodents (Navarro *et al.* 2009). This specific population of cells, now commonly referred to as 'KNDy neurons', has moved to the frontline in discussions of the control

of GnRH secretion (Lehman *et al.* 2010, Goodman *et al.* 2013). KNDy neurons were showed to have remarkable reciprocal projections, forming a KNDy-KNDy network (Burke *et al.* 2006), with terminals also projecting predominantly to the median eminence (ME), but also to a lesser degree to the POA (Smith *et al.* 2011) (Fig. 2). These observations formed the foundation of the KNDy hypothesis and were further supported by the knowledge that KNDy neurons contained the NKB receptor (TACR3) and GnRH neurons do not (Amstalden *et al.* 2010). Studies in mice showed there is also potential for communication within and between KNDy neurons because they express NKB and dynorphin receptors (Navarro *et al.* 2009). Interestingly, they do not express *Kiss1r* mRNA (Smith *et al.* 2011) leading to the proposition that kisspeptin was the final output signal of the KNDy neuron. The essence of the KNDy hypothesis is that NKB acts as a 'pace-setter' for kisspeptin release, dynorphin acts as a 'brake', and kisspeptin acts as the final step in communication between the KNDy neurons and GnRH neurons, thus affecting changes in the pulsatile pattern of secretion of GnRH (Okamura *et al.* 2013).

The KNDy neurons and their fibres are well placed to fulfil the role of a 'pulse generator' that drives GnRH secretion. In the ewe: i) KNDy neurons receive feedback signals from sex steroids and express the required receptors (Smith 2013); ii) KNDy neurons express FOS during both surge and pulsatile secretion of LH (Smith *et al.* 2009a, Merkley *et al.* 2012); iii) LH pulse frequency is reduced by administration of NKB receptor antagonists into the ARC, but increased by administration of NKB and dynorphin receptor antagonists (Goodman *et al.* 2013); and iv) Kiss1r antagonists blocked the secretion of LH pulses (Roseweir *et al.* 2009). Furthermore, in the monkey, kisspeptin content in the ME correlates with GnRH pulses (Keen *et al.* 2008). Finally, periodic bursts of multiple-unit activity (referred to as 'MUA volleys') in the vicinity of KNDy neurons in goats are temporally associated with the secretion of LH pulses (Wakabayashi *et al.* 2010, Wakabayashi *et al.* 2013). Moreover, an icv administration of NKB induces and dynorphin inhibits MUA volleys. All of these observations are consistent with the basic tenets of the hypothesis that KNDy neurons act as a putative GnRH pulse generator.

Despite this evidence, there is still serious controversy in the literature. For example, there is debate about the role of KNDy neurons in inhibition of GnRH secretion by sex steroids, in both males and females, and in different species and about the mechanism through which three neuropeptides interact and are released with temporal specificity to change the pattern of secretion of a single GnRH pulse. There is also controversy surrounding the very existence of a 'GnRH pulse generator' in the brain – many researchers are of the view that KNDy neurons fulfil this role, while others reject the very notion of the existence of a "single" pulse generator arguing that is a property of a distributed network that includes GnRH neurons (Goodman *et al.* 2014). Regarding the latter, it should also be noted that the response to a constant infusion of kisspeptin – increased LH pulse frequency (George *et al.* 2011) – suggests that KNDy neuronal output (kisspeptin 'pulses') may not function as the 'GnRH pulse generator', but as a modulator of the pulses intrinsic to GnRH neurons (Martinez de la Escalera *et al.* 1992, Richter *et al.* 2002). On the other hand, data from rats (Roa *et al.* 2008), sheep (Caraty *et al.* 2007) and monkeys (Seminara *et al.* 2006) indicate that constant elevation of kisspeptin is unable to maintain sustained elevated LH secretion. Whether kisspeptin neurons truly drive GnRH pulses, or whether kisspeptin is simply a permissive signal to allow expression of GnRH pulses, needs to be rigorously tested.

The role of kisspeptin in seasonal breeding

In ovary-intact ewes, *KISS1* mRNA expression in the ARC is higher during the breeding season than the non-breeding season (Wagner *et al.* 2008). As previously stated, the seasonal change in GnRH pulse frequency is the outcome of interactions between steroid-dependent and

steroid-independent mechanisms, both of which appear to be mediated by kisspeptin neurons. With regard to the steroid-independent mechanism, the breeding season elevation in *KISS1* is apparent in the absence of sex steroid feedback in ovariectomized ewes (Smith *et al.* 2007). With regard to the steroid dependent mechanism, *KISS1* is also elevated during the breeding season in ovariectomized ewes provided with oestradiol replacement (Smith *et al.* 2008). Most importantly, the inhibitory effect of oestradiol on *KISS1* expression in the ARC is greater during the non-breeding season than the breeding season (Smith *et al.* 2008), consistent with the view that the seasonal change in responsiveness to oestradiol is affected by changes in the response of kisspeptin neurons to oestradiol.

Kisspeptin neurons in the POA also appear to be regulated by season. A modest but significant increase in the number of POA kisspeptin neurons is apparent in ewes after they have been shifted into a short-day photoperiod, although this change was only significant when the data were corrected for the total number of neurons in the POA (Chalivoix *et al.* 2010). Importantly, this observation was made in ovariectomized-oestradiol treated ewes, indicating a photoperiod-driven change in oestradiol responsiveness in POA kisspeptin neurons, as observed in the ARC. However, other studies have led to the conclusion that kisspeptin in the POA is not regulated by seasonal status (Smith *et al.* 2007, Smith *et al.* 2008) – importantly, this work was done by two separate but collaborating laboratory groups, using both *in situ* hybridization and immunohistochemistry, and in ovariectomized ewes in the presence and absence of oestradiol (Smith *et al.* 2008). Our view is, therefore, that the two populations of kisspeptin neurons play different roles in the regulation of GnRH secretion in the ewe. In the ARC, there are more kisspeptin neurons and these mediate both negative and positive feedback regulation of GnRH secretion by oestradiol (Smith 2008, Smith 2009, Merkley *et al.* 2012), with the negative feedback action being key to the regulation of seasonal reproduction. In the POA, on the other hand, kisspeptin neurons appear to be involved only in positive feedback and therefore the induction of the preovulatory surge of GnRH and LH (Smith *et al.* 2009a, Hoffman *et al.* 2011, Merkley *et al.* 2012).

The combination of increases in *KISS1* expression, and in the number of kisspeptin neurons during the transition from non-breeding to breeding season, suggests an increase in neuroanatomical communication between kisspeptin neurons and GnRH neurons. The number of GnRH neurons that receive 'input' (defined by the close apposition of kisspeptin terminals) and the number of kisspeptin appositions on each GnRH neuron, are higher during the breeding season than during the anoestrus season (Smith *et al.* 2008). The origin of these appositions is subject to considerable debate. Presumably, this extra kisspeptin input arises from the ARC, consistent with the increased *KISS1* expression in this region. However, previous data utilizing anterograde and retrograde tracers suggests that ARC kisspeptin neurons do not project directly to GnRH neurons, in contrast with POA kisspeptin neurons (Backholer *et al.* 2009). Indeed, few projections from the ARC terminate at GnRH neurons (Pompolo *et al.* 2001), and there are similar observations for mice (Wintermantel *et al.* 2006). It is, however, possible that neuronal tracing is not sufficiently sensitive to reveal appositions to specific neurons. In line with this, a large percentage of GnRH neurons in the ovine POA and MBH are shown to have appositions from kisspeptin terminals, which co-express dynorphin, thus confirming their origin from ARC KNDy neuronal population (Lehman *et al.* 2013).

In ewes, the GnRH/LH response to kisspeptin is greater during the non-breeding season than during the breeding season (Smith *et al.* 2009b, Li *et al.* 2012). This difference might be a consequence of changes in the releasable pool of GnRH accumulating between pulses, which would be greater in the anoestrus season. A reduction in GnRH/LH pulse frequency leads to an increase in LH pulse amplitude due to effects at the level of the gonadotroph (Clarke & Cummins

1985). Despite this, recent evidence shows that the expression of KISS1R on GnRH neurons is greater during the non-breeding season than during the breeding season (Li *et al.* 2012). The role of this increase in responsiveness of GnRH neurons to kisspeptin during anoestrus is not clear, but one possibility is that it allows for a greater 'perception' of the increasing levels of ARC kisspeptin expression at the onset of the breeding season (Smith *et al.* 2007, Smith *et al.* 2008), enabling the return of pulsatile GnRH secretion.

Can kisspeptin prevent seasonal anoestrous?

Given the discussion above, a strong case can be made for an increase in kisspeptin signalling driving the return of GnRH pulses at the onset of the breeding season. If this were to be true, then we would expect exogenous administration of kisspeptin to reactivate the gonadotrophic axis and allow ovulation in ewes. This outcome was indeed demonstrated by two collaborating research groups using an intravenous infusion of kisspeptin over 48 h (Caraty *et al.* 2007). In anoestrus ewes, kisspeptin transiently restored LH concentrations and induced ovulation in the majority of animals (Caraty *et al.* 2007). It was proposed that the kisspeptin infusion induced a follicular phase-like state, culminating in oestradiol-positive feedback and ovulation. This was further detailed in a follow-up study, showing elevated oestradiol levels were a prerequisite for kisspeptin to induce ovulation (Sebert *et al.* 2010).

An interesting aspect to both these studies was that a peripheral infusion of kisspeptin was adequate for initiating ovulation despite the fact that kisspeptin acts centrally to stimulate GnRH neurons. In sheep, kisspeptin neurons extend down through the internal zone of the ME to the external zone and are thought to mingle with GnRH terminals outside the blood-brain barrier stimulating the release of GnRH (Smith *et al.* 2011). It is probable that kisspeptin in the periphery may cross the fenestrated capillaries to also stimulate the GnRH terminals. Equally, recent data (albeit in mice) has shown GnRH neurons extend complex highly branched dendritic trees to the organum vasculosum of the lamina terminalis (OVLT) outside the blood-brain barrier (Herde *et al.* 2011). Although a peripheral infusion offers a technological advantage over a cerebroventricular infusion, it still offers little for industry where extensive grazing systems are not conducive to reproductive technologies (Martin 2014).

In sheep, the introduction of a novel male stimulates the secretion of GnRH pulses in females during the non-breeding season, causing the resumption of follicle maturation and ovulation (Hawken *et al.* 2009). This 'male effect' has been well characterized in sheep and is known to be effected predominantly by the action of pheromones (Delgadillo *et al.* 2009) initiated through activation of the main and accessory olfactory systems (Hawken & Martin 2012). Moreover, this naturally occurring phenomenon affect draws some similarities to the above-mentioned response to exogenous kisspeptin treatment. It is therefore not surprising that, in anoestrous ewes exposed to rams, there was an increase in the activation of kisspeptin neurons in the ARC, as detected by FOS protein expression (De Bond *et al.* 2013). Moreover, the administration of a kisspeptin antagonist completely blocked the response to male exposure, demonstrating the requirement of kisspeptin signaling. Given that the 'male effect' appears to rely on ARC kisspeptin neurons, the question remains as to whether activation of the KNDy network is driving the response. While this has not yet been addressed in sheep, a recent study in female goats showed that MUA-volleys in the vicinity of KNDy neurons and LH pulses are also stimulated acutely by pheromone exposure. Most importantly, this action was blocked by treatment with an NKB receptor antagonist (Sakamoto *et al.* 2013). Although at this stage circumstantial, and not immune to the uncertainty continuing to surround the KNDy hypothesis, as noted above,

these results implicate the KNDy system in mediating the pheromone effect on GnRH secretion seen in anoestrous ewes (Fig. 3).

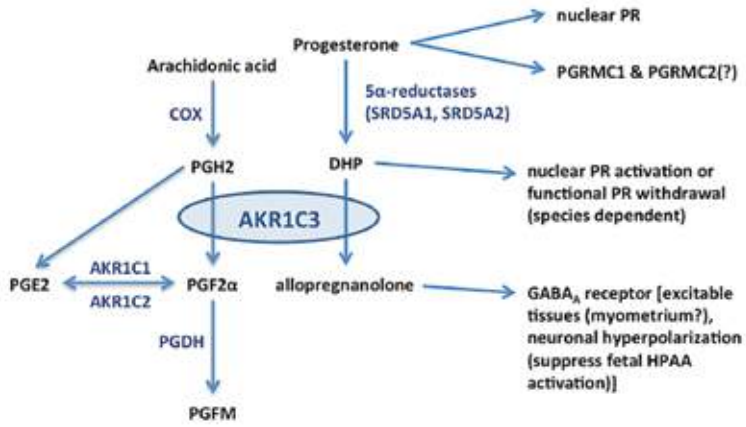

Fig. 3. Schematic representation of the possible signalling pathway leading to increased gonadotrophin-releasing hormone (GnRH) pulses in ewes exposed to males. Male odor (indicated by the male symbol) results in activation of the main and accessory olfactory systems (MOB and AOB)(Hawken and Martin, 2012). This signal is transmitted, via the amygdala (AMG), to kisspeptin, neurokinin B (NKB), dynorphin (Dyn) neurons (KNDy neurons) in the arcuate nucleus (ARC) of the hypothalamus. KNDy neurons receive autoregulatory input from NKB and Dyn, forming a local circuit. To increase GnRH secretion, kisspeptin release is enhanced through NKB and a reduction in Dyn. The resultant increases in oestradiol release initiate the resumption of oestrous cycles.

The pathway from photoperiod to kisspeptin neurons

Photoperiod appears to be the major factor governing seasonal change in kisspeptin expression, as evidenced by studies using controlled light/dark cycles in seasonal rodents (Revel *et al.* 2007, Simonneaux *et al.* 2009) and sheep (Wagner *et al.* 2008, Chalivoix *et al.* 2010). When ewes were shifted from a short day photoperiod (8:16 h light/dark) to a long day photoperiod (16:8 h light/dark), *KISS1* mRNA expression in the ARC declined (Wagner *et al.* 2008). Conversely, when ewes were shifted from long days (16:8 h light/dark) to short days (8:16 h light/dark), the number of identifiable kisspeptin neurons in the ARC increased (Chalivoix *et al.* 2010). Photoperiodic information is transduced into a neuroendocrine signal by the secretion of melatonin by the pineal gland at night. In ewes, removal of the pineal gland prevents the seasonal breeding response to photoperiod (Malpaux *et al.* 2002, Biebermann *et al.* 2006) and a similar response in seasonal rodents is linked to kisspeptin neurons (Greives *et al.* 2007, Ansel *et al.* 2010). In sheep, melatonin acts at the level of the premammillary and mediobasal hypothalamic areas (Malpaux *et al.* 1993, Malpaux *et al.* 1998), two regions that are near the ARC kisspeptin neurons. If such regulation does take place, it must be indirect because ARC kisspeptin neurons do not express the signaling form of the melatonin receptor (Li *et al.* 2011).

The pathway from photoperiod to kisspeptin neurons remains to be elucidated. One possibility is the dopaminergic neurons within the A14/A15 region (Goodman *et al.* 2010)

that are recognised as inhibitors of LH pulse frequency during the non-breeding season (Meyer & Goodman 1985, Havern *et al.* 1994). Moreover, they appear to only exert their influence during the non-breeding season and they are oestradiol-dependent (Meyer & Goodman 1986). Oestradiol induces FOS protein expression in A14/A15 dopaminergic neurons during the non-breeding season but not during the breeding season (Lehman *et al.* 1996). Most importantly, these cells send projections to the ARC (Havern *et al.* 1991) and thus potentially to kisspeptin neurons. Recently, ovine kisspeptin neurons were shown to possess the dopamine receptor (DRD2) that is responsible for the inhibition of LH secretion in anoestrous ewes (Goodman *et al.* 2012). Moreover, DRD2 antagonist treatment increased LH pulse frequency in ewes during the non-breeding season, but the effect was completely blocked by a central infusion of a kisspeptin antagonist (Goodman *et al.* 2012). These observations support a role for A14/A15 dopaminergic neurons in relaying seasonal information to kisspeptin neurons. Where melatonin intervenes is yet to be determined.

Conclusions

Since 2003, kisspeptin has been in the spotlight of neuroendocrine research and remarkable advances in our understanding of fertility have been made, many utilising the ovine model. The weight of evidence indicates, but is yet to prove conclusively, that kisspeptin neurons, operating through the KNDy unit, play the role of a GnRH pulse generator. Equally, kisspeptin neurons are well placed to govern the seasonal switch in reproduction that occurs in sheep. Manipulation of the breeding season is possible with kisspeptin treatment and also the introduction of novel males, a phenomenon that may operate through KNDy neurons. The precise mechanisms and neuroanatomical pathway by which this is achieved remain to be determined.

Acknowledgments

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