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Towards new strategies to manage livestock reproduction using kisspeptin analogs

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ABSTRACT

The discovery of the hypothalamic neuropeptide kisspeptin and its receptor (KISS1R) have dramatically improved our knowledge about the central mechanisms controlling reproduction. Kisspeptin neurons could be considered the hub where internal and external information controlling reproduction converge. The information is here elaborated and the command dispatched to GnRH neurons, the final output of the brain system controlling reproduction. Several studies have shown that in mammals administration of kisspeptin could finely modulate many aspects of reproduction from puberty to ovulation. For example in ewes kisspeptin infusion triggered ovulation during the non-breeding season and in prepubertal rat repeated injections advanced puberty onset. However, especially in livestock, the suboptimal pharmacological properties of endogenous kisspeptin, notably it short half-life and consequently its poor pharmacodynamics, fetters its use to experimental setting. To overcome this issue synthetic KISS1R agonists, mainly based on kisspeptin backbone, were created. Their more favorable pharmacological profile, longer half-life and duration of action, allowed to perform promising initial experiments for controlling ovulation and puberty. Additional experiments and further refinement of analogs would still be necessary to exploit fully the potential of targeting the kisspeptin system. Nevertheless, it is already clear that this new strategy may represent a breakthrough in the field of reproduction control.

1. Kisspeptin system in mammals

In the ventral hypothalamus of mammals distinct, but interconnected, neuronal populations gather and elaborate internal and external inputs controlling reproduction. The main output of this integrated system is the secretion of gonadotropins releasing hormone (GnRH). GnRH release in the portal blood triggers gonadotropins (luteinizing hormone, LH; and folliculo-stimulating hormone, FSH) secretion from the pituitary. Gonadotropins will in turn stimulate the gonads to secrete sex steroids. An impressive amount of data collected in the last decade point to kisspeptin (Kp), a neuropeptide expressed in the ventral hypothalamus, as the major positive modulator of GnRH release (Fig. 1). For an exhaustive review on the subject see Pinilla et al., 2012 [1]. Hence, Kp is fundamental to induce hormonal conditions favorable to reproduction. In human the *KiSS1* gene is translated into a 145 amino acids long precursor, which is cleaved into a C-terminally amidated 54 amino acids peptide, called Kp54. Additional cleavage of Kp54 leads to the production of shorter peptides that, based on their amino acid length, were named Kp16, Kp14, Kp13, and Kp10. All these peptides, collectively called Kps, have been detected in the brain, are considered endogenous ligands and share the last 10 C-terminal amino acids sequence. In primate the C-terminal amino acid is a Phe^{10}, whereas in all other mammals is a Tyr^{10}. In the horse and in the dog there is an additional amino acid difference (Arg replacing Asn^{2} and Val replacing Ser^{5} respectively). Otherwise, the rest of sequence is highly conserved in mammals corroborating the hypothesis of a conserved physiological function. For convenience we will refer to the sequence terminating with Phe as human Kp10 (hKp10) and that terminating with Tyr as ovine Kp10 (oKp10).

Kps receptor, KISS1R, was identified in 2001 [2–4] and is a member of the G-protein coupled receptor (GPCR) superfamily. Activation of KISS1R triggers intracellular calcium mobilization and

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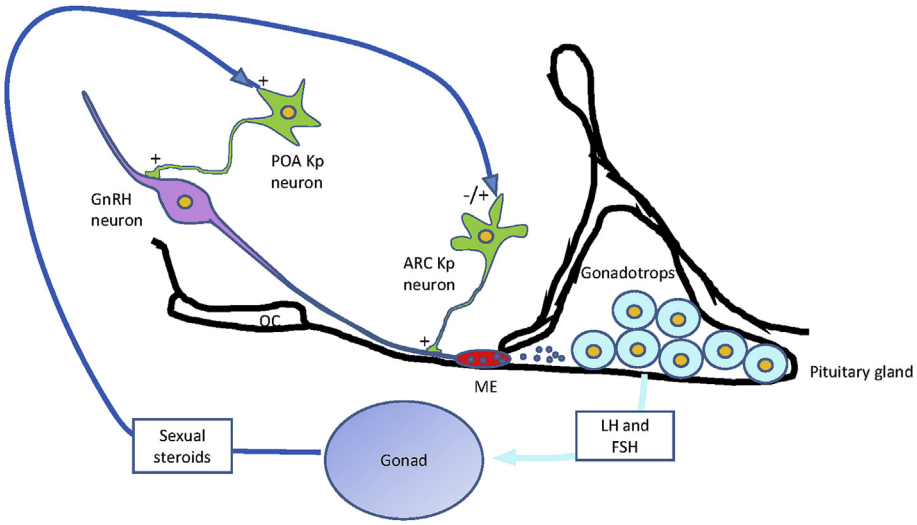

Fig. 1. Schematic representation of the mechanism controlling reproduction. The kisspeptin (Kp) neurons located in the preoptic area (POA) and arcuate nucleus (ARC) project stimulatory afferents (+) to the body and to the axon of the Gonadotropin Releasing Hormone (GnRH) neurons. GnRH is released in the median eminence (ME) and reaches the gonadotrops in the pituitary gland triggering the release of the luteinizing and folliculo-stimulating hormones (LH and FSH). Once released in the blood stream LH and FSH activate the gonads that secrete sexual steroids having a positive feedback (+) on POA Kp neurons and, pending on species, a negative (−) or positive (+) one on ARC Kp neurons.

cell activation.

Kisspeptin neurons are mainly located in two distinct hypothalamic regions: the arcuate nucleus (ARC) and the preoptic area (POA). Neurons from these regions send projections contacting either the cell bodies of GnRH neurons or their terminals in the median eminence [1,5]. There is consensus about Kp acting directly on GnRH neurons expressing KISS1R to stimulate GnRH release (Fig. 1).

Several lines of evidence show the paramount importance of an intact Kp system for normal fertility and reproduction. In human loss of function mutation in KISS1R results in hypogonadotropic hypogonadism and infertility [6,7]. Consistently, KISS1R ablation in mice results in a dramatic size reduction and impaired functioning of the genitalia, which underlie lack of puberty and infertility [7]. On the other hand, evidence exists that mice lacking Kp neurons or neurons expressing KISS1R are fertile. However, acute ablation of Kp neurons in adult mice inhibits fertility, suggesting the existence of a developmental compensatory mechanism [8].

The presence of Kp neurons in the ARC is a common feature in mammals. This neuronal population expresses the estrogen receptor (ERα) and is influenced by estradiol [9]. The second Kp population is located in the POA region, but its precise neuroanatomical distribution is species-dependent. In rodents, it is localized in the hypothalamic rostral periventricular area of the third ventricle (RP3V) and it is sexually dimorphic, with females having far more neurons than males. In sheep and primates, this population is mainly localized in the medial POA [9,10]. This POA population express ERα and progesterone receptor [11]. In general, ARC Kp neurons mainly innervate the median eminence, whereas POA neurons afferents chiefly project to GnRH cell bodies, even though difference exists between species (Fig. 1). The innervation and estradiol feedback patterns led to the hypothesis that in rodents ARC neurons would be mainly responsible for driving or regulating GnRH pulsatile secretion. Conversely, POA/RP3V neurons would have a dominant role in the induction of preovulatory GnRH surge that is also under the control of circadian input from the suprachiasmatic nucleus. What it is known regarding the mechanisms and neural sites responsible for the control of the pre-ovulatory GnRH surge in rodents may not be transposable to other species. In sheep, there are data supporting a rodent-like mechanism [12] with a preponderant role for the POA, but most data are consistent with either a combined contribution from the ventromedial hypothalamus/ARC region and the POA [13,14] or from the ventromedial hypothalamus/ARC region alone [15,16].

2. Kisspeptin and reproduction in livestock

The Kp system has been mainly studied in rodents, nevertheless significant amount of data have also been obtained using livestock (sheep, goat, cow, horse and pig). Below we will focus on results obtained in livestock and relevant to proof that Kp system is a valuable target from a drug discovery perspective. For a broader overview on the subject the reader is referred to general [1,17–21] or to specific reviews dedicated to domestic animals [22–25].

The vast majority of livestock studies focused on females and most particularly on ewes. Indeed the first evidence of a direct action of Kp on GnRH neurons was obtained by intracerebroventricular (icv) administration of hKp10 into ovariectomized and estradiol replaced ewes. In this experimental setting was possible to demonstrate a parallel increase of GnRH in the cerebrospinal fluid and of LH in the blood [26]. Additional experiment showed that intravenous (iv) injection of oKp10 induced a rapid, albeit transient, increase of GnRH and LH [27] at doses as low as 6.2 nmol/ewe (around 0.1 nmol kg^{-1} of body weight). In hypothalamo-pituitary-disconnected ewes, a model in which neuronal input to the median eminence is eliminated, oKp10 injection was unable to modify LH plasma concentration [28]. Taken together these data strongly suggest that Kp acts upstream to LH and GnRH cells.

Additional studies confirmed that bolus iv injection of Kp10 triggered a similar pattern of LH secretion in female of various livestock species [29–32]. Administration of hKp10 (0.77, 3.85 or 7.69 nmol kg^{-1} of body weight) to female goats during the luteal phase resulted in a rapid increase of LH, with the maximal effect observed about 20 min after injection, and subsequent decrease to basal level in a couple of hours [29]. On the other hand, in adult Jersey cows (ovariectomized and replaced or not with estrogen, progesterone or both) much lower doses of hKp10 (0.1–0.2 nmol kg^{-1} of body weight) were sufficient to increase LH even though to a lesser extent [30]. Also in the horse, either oKp10 or equine Kp10 (eKp10) administration, encompassing doses

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