

players in the reproductive brain clearly exceeds the scope of this review, and can be found elsewhere. On the contrary, this work intends to identify *hot areas* of kisspeptin physiology, aiming to provide a last-minute view of recent progress and predictable pathways for future research in these particular lines. For sake of uniformity, we will adopt herein the recent proposal for nomenclature by Gottsch et al. (2009), where *KISS1* and *Kiss1* are used to name the human and non-human genes, whereas GPR54 is termed KISS1R or Kiss1R, depending on the species. For global reference to the system (i.e., not for a given species), the term KISS1 will be used. Finally, as consensus nomenclature, the peptide products of *KISS1* will be globally termed kisspeptins (Kp), with a numeric extension to indicate the amino acid length, when considered relevant.

2. Kisspeptin signaling: molecular biology of KISS1R

Identification of KISS1R as the cognate receptor for kisspeptin dates back to 2001, when three independent groups de-orphanized the former GPR54 and initially addressed the molecular analysis of its major signaling pathways (Ohtaki et al., 2001; Muir et al., 2001; Kotani et al., 2001). Curiously enough, upon disclosure of the 'reproductive' dimension of kisspeptins, largely based on the characterization of the reproductive phenotypes of humans and mice with null mutations of their receptor, efforts devoted to unravel key aspects of brain kisspeptin distribution and biological actions have been disproportionately larger than those focused on KISS1R. For instance, while mapping of kisspeptin neurons has been conducted in several species, from fish to rodents and humans (Roa et al., 2008; Roa and Tena-Sempere, 2007), data on the major sites of expression of KISS1R in hypothalamic and other brain areas are still scarce. Similarly, while the ability of kisspeptins to potently elicit GnRH and gonadotropin secretion has been conclusively documented in mammals and non-mammalian species (Roa et al., 2008; Felip et al., 2008), our knowledge on the molecular mechanisms for KISS1R signaling, from receptor activation to signal transduction and desensitization, remains largely incomplete. Considering the obvious therapeutic potential of kisspeptins for pharmacological

manipulation of the gonadotropic axis, it is predictable that this area will draw considerable attention in the near future.

Given that kisspeptins act primarily on GnRH neurons to activate the gonadotropic axis, analysis of the intracellular signaling pathways responsible for such stimulatory effects has been conducted using hypothalamic explants and protocols of pharmacological blockade of key intracellular signals/factors following *ex vivo* stimulation with kisspeptin (Castellano et al., 2006a). This approach evidenced that the stimulatory effects of Kp-10 on GnRH secretion require the activation of phospholipase-C (PLC), mobilization of intracellular Ca^{2+} stores and recruitment of ERK1/2 and p38 kinases, while kisspeptin-induced GnRH release was preserved in spite of the blockade of adenylate cyclase (Fig. 1); features that are grossly similar to those initially reported for KISS1R signaling using heterologous cell systems (Kotani et al., 2001). Likewise, recent studies involving electrophysiological recordings and/or calcium imaging in GnRH neurons have supported and extended those observations, showing that long-term excitation of GnRH neurons by kisspeptin is conveyed through a PLC/calcium dependent pathway regulating multiple ion channels, including the closing of potassium channels and the activation of non-selective cation (NSC) channels, which likely include canonical transient receptor potential (TRPC) channels (Liu et al., 2008; Zhang et al., 2008; Dumalska et al., 2008).

In contrast to the above progress in terms of receptor signaling, dissection of the functional anatomy of KISS1R is still at its infancy, but it will be certainly assisted by analyses of the phenotypic and biochemical consequences of known mutations of this receptor in humans (see Fig. 2). Although globally rare, these have been enormously instrumental to identify crucial areas for receptor activation and signal transduction (Roseweir and Millar, 2009). For instance, molecular analysis of the disease-causing mutation L148S at the second intracellular loop of KISS1R has allowed to reveal the importance of this domain for ligand-induced catalytic activation of $\text{G}\alpha$ subunits, but not for receptor expression or ligand binding (Wacker et al., 2008). Predictably, analyses on other inactivating mutations of KISS1R will help to identify residues critical for different aspects of receptor function. On the other hand, the first

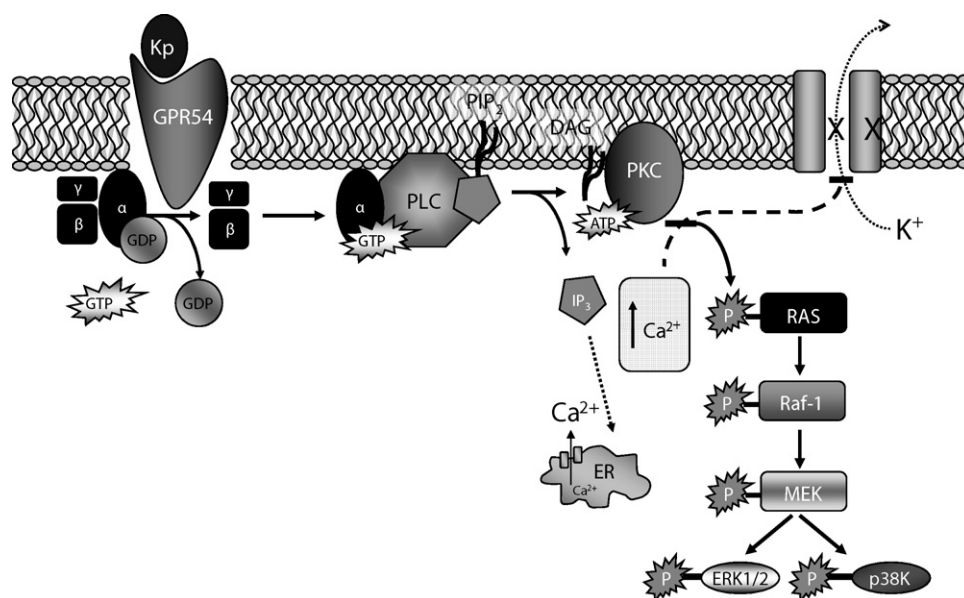


Fig. 1. Signaling pathways tentatively recruited following activation of KISS1R by kisspeptin (Kp) at the hypothalamus. Based on data from experiments involving stimulation of hypothalamic explants with Kp *ex vivo*, it is proposed that the GnRH releasing effect of kisspeptin involves the activation of phospholipase-C (PLC), mobilization of intracellular Ca^{2+} stores and recruitment of ERK1/2 and p38 kinases, but is independent of adenylate cyclase/cAMP pathways. In addition, electrophysiological studies have demonstrated that long-term excitation of GnRH neurons by Kp is conveyed through modulation of multiple ion channels, including closing of potassium channels (dotted line) and the opening of non-selective cation (NSC) channels, likely of the TRPC-like type; for sake of simplicity, only the mechanism involving closing of K channels is depicted in the scheme. For further details, see text. Adapted from reference (Roa et al., 2009), with substantial modifications.

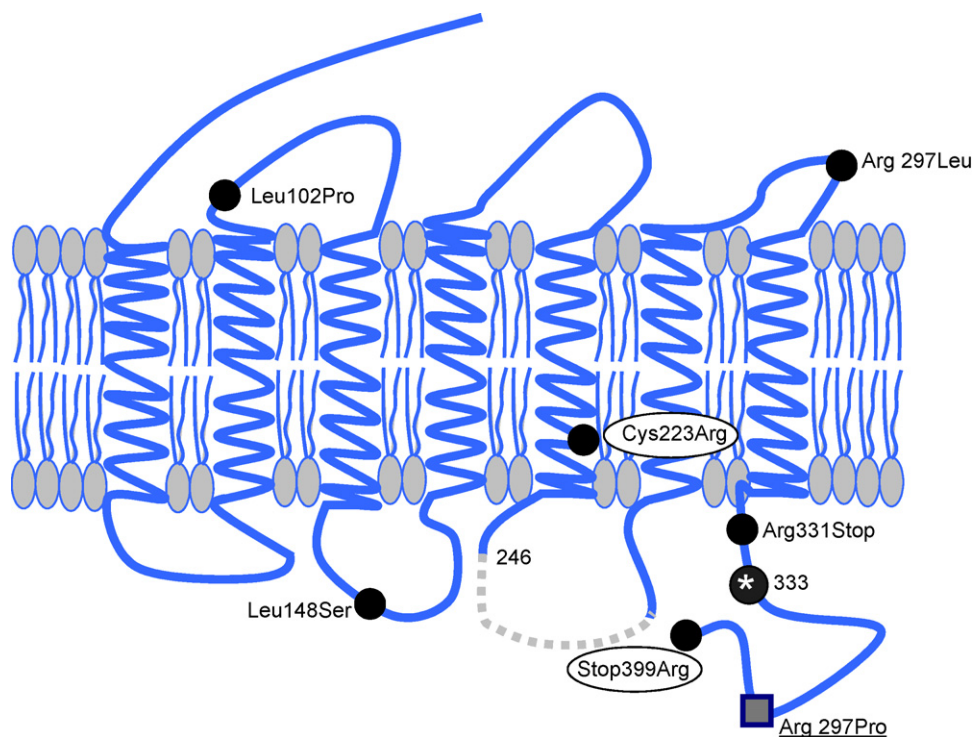


Fig. 2. Schematic representation of the activating and inactivating mutations of *KISS1R* gene so far reported. Inactivating mutations are indicated as black dots, with reference to the corresponding amino acid substitution. In addition, the location of the predicted deletion reported in reference (de Roux et al., 2003) is marked by grey dots. Likewise, the predicted insertion (1001..1002insC), which results in a shift of the open reading frame (Roseweir and Millar, 2009), is denoted by an asterisk. Finally, the first activating mutation of *KISS1R*, recently found in a patient suffering precocious puberty, is depicted as a grey box. Adapted from reference (Roa et al., 2008), with substantial modifications.

activating mutation of *KISS1R* (R386P) has been recently reported as cause for precocious puberty (Teles et al., 2008). Interestingly, this amino acid substitution does not affect receptor signaling machinery itself, but apparently prolongs receptor activation upon ligand binding, probably due to a reduction in the rate of desensitization of the mutant (Teles et al., 2008). This observation underscores a key domain for *KISS1R* desensitization; a phenomenon whose mechanisms (e.g., internal phosphorylation and/or arresting binding) remain unexplored. Finally, as additional source of signaling complexity, molecular analyses in HEK293 cells have recently suggested the potential hetero-oligomerization of *KISS1R* and GnRH receptors (Quaynor et al., 2007); yet, the relevance of this putative interaction in physiological conditions (i.e., in real GnRH neurons) is completely unknown.

3. Control of GnRH neurons by kisspeptin pathways

As essential afferents controlling GnRH neurons, extensive efforts have been recently devoted to identify the location of neurons expressing *KISS1*/kisspeptins in the basal forebrain of a large diversity of species, from non-mammals (e.g., fish) to rodents and humans. Despite some minor discrepancies between RNA and protein data, mapping of kisspeptin neurons in rodents has conclusively documented the existence of two major neuronal populations located at the arcuate (ARC) and anteroventral periventricular (AVPV) nuclei of the hypothalamus (Mikkelsen and Simonneaux, 2009); the latter extending probably as a continuum along the rostral periventricular area of the third ventricle (RV3P) (Herbison, 2008). Besides important functional divergences in sex steroid regulation (see Section 4), these two populations appear to display important anatomical differences in terms of projections to GnRH neurons, as direct appositions have been reported only from the AVPV (Clarkson and Herbison, 2006). Pending of further refinement of tracing analyses, it is highly plausible, although yet to

be proven, that kisspeptin neurons from ARC interact with elements of the pre-synaptic network controlling GnRH neurons (if not GnRH neurons themselves). Indeed, both direct and trans-synaptic (indirect) actions of kisspeptins on GnRH neurons have been documented in female mice (Pielecka-Fortuna et al., 2008). Of important note, while AVPV neurons have been involved in the generation of the pre-ovulatory surge of gonadotropins in the female by mediating positive feedback effects of estradiol, kisspeptin neurons at the ARC have been proposed to convey the negative feedback effects of sex steroids (Roa et al., 2008) (Fig. 3). The molecular basis for such disparate functions is yet to be defined (see Section 4).

In contrast to rodents, in sheep and primates, hypothalamic neurons expressing *KISS1*/kisspeptin appear to be concentrated mostly at the ARC/infundibular region. Interestingly, in the sheep, kisspeptin neurons mediating positive and negative feedback effects of sex steroids seem to be intermingled within the ARC; neurons responding positively to estrogen being mostly located at its caudal portion (Estrada et al., 2006). In primates, including humans, only inhibitory responses to sex steroids have been documented in ARC/infundibular neurons, as major mechanism for their negative feedback on gonadotropin secretion (Shibata et al., 2007; Rometo et al., 2007; Kim et al., 2009) (see Section 4). It remains to be defined whether and how kisspeptin neurons contribute to the generation of the pre-ovulatory surges in primates.

In addition to neuroanatomical evidence for physical contacts between kisspeptin and GnRH neurons, initial expression analyses demonstrated that >85% of GnRH neurons in the rat forebrain express *Kiss1R* (Irwig et al., 2004), thus providing the basis for direct excitatory effects of kisspeptins. This contention was reinforced by the observation of long-lasting depolarizing responses in GnRH neurons following exposure to Kp-10 (Han et al., 2005). Intriguingly, however, despite robust GnRH neuron activation and secretion induced by kisspeptins in different species, electrophysio-

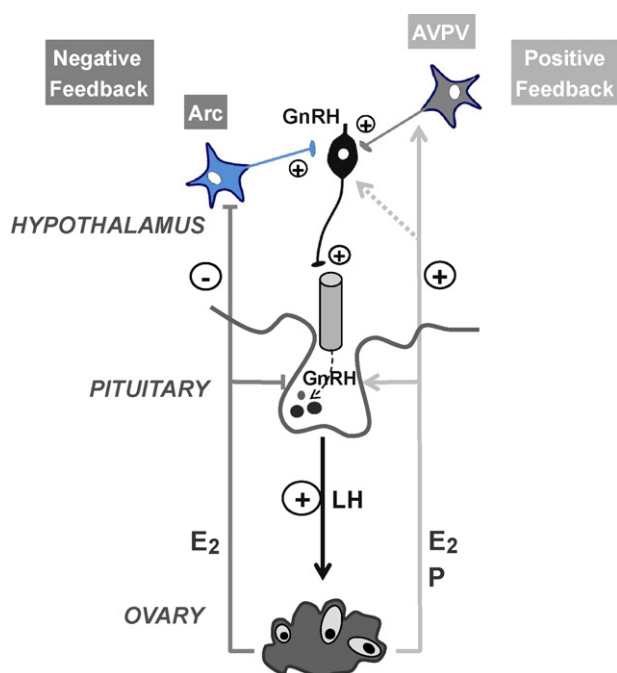


Fig. 3. Proposed roles of kisspeptin neurons at the arcuate (ARC) and anteroventral periventricular (AVPV) nuclei in mediating the negative and positive feedback effects of ovarian sex steroids on GnRH and gonadotropin secretion, as suggested on the basis of rodent data. Estrogen (E2) input exerts a predominant inhibitory action on *Kiss1* expression at the ARC, which contributes to negative feedback control of GnRH/LH. In contrast, the rise of E2 levels at the pre-ovulatory period stimulate *Kiss1* expression at the AVPV that, in the presence of activated receptors for progesterone (P), contributes to the induction of pre-ovulatory surge of LH (positive feedback). In addition to transcriptional effects, E2 seems to elicit a state of enhanced responsiveness to kisspeptin, likely at the level of GnRH neurons, during the peri-ovulatory period (dotted line). For further details, see the text. Adapted from reference (Roa et al., 2008), with modifications.

logical studies have recently revealed the existence of two distinct populations of GnRH neurons at least in rodents; one being (surprisingly) insensitive to kisspeptin but highly responsive to group I metabotropic glutamate receptor agonists (Dumalska et al., 2008). The physiological relevance of such a subset of GnRH neurons insensitive to kisspeptin awaits to be further elucidated.

4. Major regulators of *KISS1* expression: central and peripheral factors

As complement to neuroanatomical analyses, increasing efforts have been devoted to understand the mechanisms and signals whereby hypothalamic expression of *KISS1*/kisspeptins is regulated, as a mean to unravel their major physiological functions within the reproductive brain. As clear example, initial studies on this system were focused in the analysis of the regulatory roles of sex steroids (Roa et al., 2008). As indicated above, compelling evidence gathered mostly in rodents, sheep and primates has demonstrated that *KISS1* expression is under the control of estrogens and androgens, with divergent effects depending on the hypothalamic site. Thus, it is well documented that expression of *Kiss1*/kisspeptins in ARC neurons is tonically repressed by sex steroids in rodents (Smith et al., 2005a,b). Likewise, *Kiss1* mRNA levels were found to be inhibited by androgen, estrogen and progesterone in sheep and/or monkeys (Shibata et al., 2007; Smith et al., 2007). Indirect proof for the presence of a similar mechanism in humans comes from expression analyses in postmenopausal women that showed increased *KISS1* mRNA expression at the infundibular region of the hypothalamus (Romero et al., 2007). Intriguingly, sex steroid regulation of *Kiss1* mRNA expression at the

AVPV region, at least in rodents, seems to be diametrically opposite, with androgens and estrogens carrying stimulatory actions (Smith et al., 2005a,b). The molecular basis for such a disparate regulation of gene expression by similar signals in these discrete neuronal populations remains unclear. Thus, promoter analyses have revealed that through Sp1 sites estradiol up-regulates *KISS1* promoter expression via estrogen receptor (ER) α ; a mechanism that may contribute to its reported effects at the AVPV (Li et al., 2007). However, the inhibitory mode of action of sex steroids at the ARC cannot be explained by this set of data and remains to be elucidated.

While the nature of some of the peripheral regulators of *KISS1*, such as sex steroids and leptin (see Section 5), has been unraveled in recent years, our knowledge on the major central regulators of hypothalamic kisspeptin neurons is much more limited. These may include, among others, melatonin, neuropeptide Y and IGF-1 (Revel et al., 2006; Luque et al., 2007; Hiney et al., 2009), although their roles in the physiologic control of the *KISS1* system in different conditions (from changes in photoperiod to metabolic stress) have not been conclusively defined and merit further investigation. Intriguingly, recent work in sheep has revealed that *Kiss1* neurons at the ARC co-express dynorphin (Dyn) and neurokinin-B (NKB) (Goodman et al., 2007), which might operate as auto-regulatory signals for kisspeptin neurons in the mouse also. The importance of such co-expression has been recently reinforced by the observation that humans with genetic inactivation of *TAC3* or *TACR3* genes (which encode for NKB or its putative receptor) suffer from hypogonadotropic hypogonadism (Topaloglu et al., 2009). The neuroendocrine basis for such phenomenon, however, is still unclear, as NKB had been reported as inhibitory factor in the control of gonadotropin secretion in different species.

Finally, recent studies in ewes suggested a reciprocal interplay between kisspeptins and the mammalian homologue of gonadotropin-inhibitory hormone (GnIH), termed RFRP, in the central control of GnRH neurons; *Kiss1*/kisspeptin levels at the ARC being elevated, but RFRP levels at the dorsomedial hypothalamus decreased, at states of maximal activation of the gonadotropic axis (i.e., during the breeding season) (Smith et al., 2008). Notably, both kisspeptins and RFRP belong to the super-family of RFamide neuropeptides and, based on their opposite roles in terms of control of gonadotropin secretion, the appealing hypothesis that they are responsible for dynamically driving reproductive function, as a result of the balance between stimulatory (kisspeptin) and inhibitory (RFRP) influences, has been proposed (Kriegsfeld, 2006). Yet, the relative potency of these factors, and even their major sites of action within the reproductive axis, seem to be quite different and likely depends on the species; e.g., GnIH appears to play a crucial role in reproductive control in birds, but the *Kiss1* gene has not been so far identified in the avian genome (Felip et al., 2008). In this scenario, it will be crucial to define whether, in mammals, kisspeptins and RFRP neurons reciprocally regulate each other.

5. Molecular mechanisms for the metabolic regulation of hypothalamic *KISS1*

In recent years, the metabolic regulation of the hypothalamic *KISS1* system has been exposed, mainly by experimental studies in rodents, where conditions of negative energy balance were shown to induce variable degrees of inhibition of *Kiss1* mRNA expression (Luque et al., 2007; Castellano et al., 2005, 2006b, 2009a,b). These analyses, together with the rescue of gonadotropic function by exogenous kisspeptin in these conditions, have allowed us to suggest that kisspeptin neurons at the hypothalamus operate as sensor and neuroendocrine conduit for conveying metabolic information onto brain reproductive centers (likely, GnRH neurons),

thereby contributing to the well-known coupling between body energy status, puberty onset and fertility (Castellano et al., 2009a; Tena-Sempere, 2006).

The regulatory network responsible for the metabolic control of the KISS1 system is far from being fully elucidated. Compelling evidence, though, has demonstrated that the adipose hormone, leptin, is a master hormonal regulator of hypothalamic Kiss1, as evidenced by rodent studies, where conditions of hypoleptinemia were linked to decreased hypothalamic expression of *Kiss1* mRNA, which could be rescued by central administration of leptin (Castellano et al., 2006b; Smith et al., 2006). However, some crucial facets of this regulatory pathway remain to be unfolded. For instance, whether leptin operates in physiologic conditions as elicitor or permissive factor for kisspeptin expression and/or release remains to be defined; the latter would fit well with the proposed role of leptin as permissive (but not trigger) for puberty onset, but would necessarily require the concurrent action of additional regulators of kisspeptin neurons, whose nature is yet to be characterized.

Likewise, the molecular mechanisms whereby leptin activates *Kiss1* gene expression remain largely unknown. Admittedly, however, our knowledge on this area has recently enlarged by the demonstration of the putative role of the *Crt1* coactivator, *Crtc1*, as positive transcriptional regulator of *Kiss1* gene expression at discrete hypothalamic areas (Altarejos et al., 2008). Importantly, genetic inactivation of *Crtc1* in mice results in infertility and decreased levels of expression of *Kiss1* mRNA, whereas leptin increased recruitment of *Crtc1* onto the *Kiss1* promoter (Altarejos et al., 2008). These data suggest the existence of a leptin-*Crtc1*-kisspeptin signaling pathway, as indispensable for normal reproductive function. In the same vein, we have recently obtained evidence that brain signaling through sensors of the intracellular energy state, such as the mammalian target of rapamycin (mTOR) and AMP kinase (AMPK), is likely to play a crucial role in the central control of puberty onset, eventually via modulation of *Kiss1* gene expression at the hypothalamus, as we have preliminarily observed at least for mTOR signaling (unpublished data). Of note, both mTOR and AMPK have been proposed as transducer of leptin effects in terms of energy homeostasis (Cota et al., 2006). Altogether, these observations disclose an additional level of complexity in the metabolic regulation of hypothalamic KISS1 system, whereby a set of intracellular sensors and transcriptional factors are likely to cooperate in order to mediate the effects of extracellular signals (e.g., leptin) on the expression of *KISS1* gene.

6. Open questions and future directions

As summarized in previous sections, our understanding of the roles and modes of action of kisspeptins as indispensable regulators of the reproductive brain has dramatically expanded in the last 5 years. Of note, identification of the crucial functions of kisspeptin neurons in discrete hypothalamic areas has not only allowed to propose discernible neural pathways for key regulatory phenomena of the reproductive axis, such as positive and negative feedback as well as metabolic control of fertility, but has also forced the scientific community to reassess the functional roles and mechanisms of action of many classical neurotransmitters and neuropeptides, already known to modulate GnRH function, within this novel conceptual framework.

Anyhow, despite the enormous advancements in the field, several key aspects of kisspeptin physiology remain to be fully elucidated and will certainly concentrate considerable attention in the near future. These will likely include (i) better characterization of the functional anatomy, biochemical function and neuronal distribution of KISS1R in different mammalian species, including humans; (ii) mapping of the afferents to and projec-

tions of kisspeptin neurons in the brain, and particularly within the hypothalamus, in relation to the pre-synaptic networks controlling GnRH secretion; (iii) identification of additional central and peripheral regulators of hypothalamic KISS1 expression, as well as their roles in the physiologic control of the KISS1 system; (iv) unraveling of the molecular mechanisms for sex steroid (i.e., disparate effects of estrogen at ARC and AVPV) and metabolic control of KISS1 expression; and (v) definition of the actual roles of kisspeptins (trigger vs. amplifier) in the control of puberty onset and the GnRH pulse generator. In parallel, (vi) it is anticipated that further progress will be made towards the characterization of kisspeptins as targets for pharmacological intervention of the reproductive system, as well as the identification/generation of analogs of kisspeptins, with either agonist or antagonist activities at the KISS1R level. Overall, it is foreseen that such research efforts will result in a substantial progress of our understanding of the physiological basis, and eventual physiopathological implications, of kisspeptin signaling in the brain, and how it contributes to the dynamic regulation (and potential alteration) of reproductive maturation and function along the life-span.

Disclosure statement

The author has nothing to disclose.

Acknowledgments

The author is indebted with the members of the research team at the Physiology Section of the University of Cordoba, who actively participated in the generation of experimental data discussed herein. The assistance of J. Roa in the preparation of some of the figures of this paper is cordially appreciated. The work from the author's laboratory reviewed in this article was supported by grants BFU 2005-07446 and BFU 2008-00984 (Ministerio de Ciencia e Innovación, Spain), funds from Instituto de Salud Carlos III (Red de Centros RCMN C03/08 and Project PI042082; Ministerio de Sanidad, Spain); Project P08-CVI-03788 (Junta de Andalucía, Spain) and EU research contract DEER FP7-ENV-2007-1. CIBER is an initiative of Instituto de Salud Carlos III (Ministerio de Sanidad, Spain).

References

- Altarejos, J.Y., Goebel, N., Konkright, M.D., Inoue, H., Xie, J., Arias, C.M., Sawchenko, P.E., Montminy, M., 2008. The *Crt1* coactivator *Crtc1* is required for energy balance and fertility. *Nat. Med.* 14, 1112–1117.
- Castellano, J.M., Navarro, V.M., Fernandez-Fernandez, R., Nogueiras, R., Tovar, S., Roa, J., Vazquez, M.J., Vigo, E., Casanueva, F.F., Aguilar, E., Pinilla, L., Dieguez, C., Tena-Sempere, M., 2005. Changes in hypothalamic KISS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology* 146, 3917–3925.
- Castellano, J.M., Navarro, V.M., Fernandez-Fernandez, R., Castano, J.P., Malagon, M.M., Aguilar, E., Dieguez, C., Magni, P., Pinilla, L., Tena-Sempere, M., 2006a. Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat. *Mol. Cell. Endocrinol.* 257–258, 75–83.
- Castellano, J.M., Navarro, V.M., Fernandez-Fernandez, R., Roa, J., Vigo, E., Pineda, R., Dieguez, C., Aguilar, E., Pinilla, L., Tena-Sempere, M., 2006b. Expression of hypothalamic KISS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. *Diabetes* 55, 2602–2610.
- Castellano, J.M., Roa, J., Luque, R.M., Dieguez, C., Aguilar, E., Pinilla, L., Tena-Sempere, M., 2009a. KISS-1/kisspeptins and the metabolic control of reproduction: physiologic roles and putative physiopathological implications. *Peptides* 30, 139–145.
- Castellano, J.M., Navarro, V.M., Roa, J., Pineda, R., Sanchez-Garrido, M.A., Garcia-Galiano, D., Vigo, E., Dieguez, C., Aguilar, E., Pinilla, L., Tena-Sempere, M., 2009b. Alterations in hypothalamic KISS-1 system in experimental diabetes: early changes and functional consequences. *Endocrinology* 150, 784–794.
- Clarkson, J., Herbison, A.E., 2006. Postnatal development of kisspeptin neurons in mouse hypothalamus: sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology* 147, 5817–5825.
- Cota, D., Proulx, K., Smith, K.A., Kozma, S.C., Thomas, G., Woods, S.C., Seeley, R.J., 2006. Hypothalamic mTOR signaling regulates food intake. *Science* 312, 927–930.
- de Roux, N., Genin, E., Carel, J.C., Matsuda, F., Chaussain, J.L., Milgrom, E., 2003. Hypogonadotropic hypogonadism due to loss of function of the KISS1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10972–10976.

- Dumalska, I., Wu, M., Morozova, E., Liu, R., van den Pol, A., Alreja, M., 2008. Excitatory effects of the puberty-initiating peptide kisspeptin and group I metabotropic glutamate receptor agonists differentiate two distinct subpopulations of gonadotropin-releasing hormone neurons. *J. Neurosci.* 28, 8003–8013.
- Estrada, K.M., Clay, C.M., Pompolo, S., Smith, J.T., Clarke, I.J., 2006. Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/luteinising hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. *J. Neuroendocrinol.* 18, 806–809.
- Felip, A., Zanuy, S., Pineda, R., Pinilla, L., Carrillo, M., Tena-Sempere, M., Gomez, A., 2008. Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. *Mol. Cell. Endocrinol.*, doi:10.1016/j.mce.2008.11.017, in press.
- Goodman, R.L., Lehman, M.N., Smith, J.T., Coolen, L.M., de Oliveira, C.V., Jafarzadehshirazi, M.R., Pereira, A., Iqbal, J., Caraty, A., Ciofi, P., Clarke, I.J., 2007. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology* 148, 5752–5760.
- Gottsch, M.L., Clifton, D.K., Steiner, R.A., 2009. From KiSS1 to kisspeptins: an historical perspective and suggested nomenclature. *Peptides* 30, 4–9.
- Han, S.K., Gottsch, M.L., Lee, K.J., Popa, S.M., Smith, J.T., Jakawich, S.K., Clifton, D.K., Steiner, R.A., Herbison, A.E., 2005. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J. Neurosci.* 25, 11349–11356.
- Herbison, A.E., 2008. Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V). *Brain Res. Rev.* 57, 277–287.
- Hiney, J.K., Srivastava, V.K., Pine, M.D., Les Dees, W., 2009. Insulin-like growth factor-I activates KiSS-1 gene expression in the brain of the prepubertal female rat. *Endocrinology* 150, 376–384.
- Irwig, M.S., Fraley, G.S., Smith, J.T., Acohido, B.V., Popa, S.M., Cunningham, M.J., Gottsch, M.L., Clifton, D.K., Steiner, R.A., 2004. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology* 80, 264–272.
- Kim, W., Jessen, H.M., Auger, A.P., Terasawa, E., 2009. Postmenopausal increase in KiSS-1, GPR54, and luteinizing hormone releasing hormone (LHRH-1) mRNA in the basal hypothalamus of female rhesus monkeys. *Peptides* 30, 103–110.
- Kotani, M., Dethoux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J.M., Le Poul, E., Brezillon, S., Tyldesley, R., Suarez-Huerta, N., Vandeput, F., Blanpain, C., Schiffmann, S.N., Vassart, G., Parmentier, M., 2001. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J. Biol. Chem.* 276, 34631–34636.
- Kriegsfeld, L.J., 2006. Driving reproduction: RFamide peptides behind the wheel. *Horm. Behav.* 50, 655–666.
- Li, D., Mitchell, D., Luo, J., Yi, Z., Cho, S.G., Guo, J., Li, X., Ning, G., Wu, X., Liu, M., 2007. Estrogen regulates KiSS1 gene expression through estrogen receptor α and SP protein complexes. *Endocrinology* 148, 4821–4828.
- Liu, X., Lee, K., Herbison, A.E., 2008. Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. *Endocrinology* 149, 4605–4614.
- Luque, R.M., Kineman, R.D., Tena-Sempere, M., 2007. Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. *Endocrinology* 148, 4601–4611.
- Mikkelsen, J.D., Simonneaux, V., 2009. The neuroanatomy of the kisspeptin system in the mammalian brain. *Peptides* 30, 26–33.
- Muir, A.I., Chamberlain, L., Elshourbagy, N.A., Michalovich, D., Moore, D.J., Calamari, A., Szekeres, P.G., Sarau, H.M., Chambers, J.K., Murdock, P., Steplewski, K., Shabon, U., Miller, J.E., Middleton, S.E., Darker, J.G., Larminie, C.G., Wilson, S., Bergsma, D.J., Emson, P., Faull, R., Philpott, K.L., Harrison, D.C., 2001. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J. Biol. Chem.* 276, 28969–28975.
- Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., Terao, Y., Kumano, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Onda, H., Nishimura, O., Fujino, M., 2001. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 411, 613–617.
- Pielecka-Fortuna, J., Chu, Z., Moenter, S.M., 2008. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. *Endocrinology* 149, 1979–1986.
- Popa, S.M., Clifton, D.K., Steiner, R.A., 2008. The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. *Annu. Rev. Physiol.* 70, 213–238.
- Quaynor, S., Hu, L., Leung, P.K., Feng, H., Mores, N., Krsmanovic, L.Z., Catt, K.J., 2007. Expression of a functional G protein-coupled receptor 54-kisspeptin autoregulatory system in hypothalamic gonadotropin-releasing hormone neurons. *Mol. Endocrinol.* 21, 3062–3070.
- Revel, F.G., Saboureaux, M., Masson-Pevet, M., Pevet, P., Mikkelsen, J.D., Simonneaux, V., 2006. Kisspeptin mediates the photoperiodic control of reproduction in hamsters. *Curr. Biol.* 16, 1730–1735.
- Roa, J., Tena-Sempere, M., 2007. KiSS-1 system and reproduction: comparative aspects and roles in the control of female gonadotropic axis in mammals. *Gen. Comp. Endocrinol.* 153, 132–140.
- Roa, J., Aguilar, E., Dieguez, C., Pinilla, L., Tena-Sempere, M., 2008. New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. *Front. Neuroendocrinol.* 29, 48–69.
- Roa, J., Castellano, J.M., Navarro, V.M., Handelsman, D.J., Pinilla, L., Tena-Sempere, M., 2009. Kisspeptins and the control of gonadotropin secretion in male and female rodents. *Peptides* 30, 57–66.
- Rometo, A.M., Krajewski, S.J., Voytko, M.L., Rance, N.E., 2007. Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. *J. Clin. Endocrinol. Metab.* 92, 2744–2750.
- Roseweir, A.K., Millar, R.P., 2009. The role of kisspeptin in the control of gonadotrophin secretion. *Hum. Reprod. Update* 15, 203–212.
- Seminara, S.B., Messenger, S., Chatzidakis, E.E., Thresher, R.R., Acierno Jr., J.S., Shagoury, J.K., Bo-Abbas, Y., Kuohung, W., Schwino, K.M., Hendrick, A.G., Zahn, D., Dixon, J., Kaiser, U.B., Slangen, S.A., Gusella, J.F., O'Rahilly, S., Carlton, M.B., Crowley Jr., W.F., Aparicio, S.A., Colledge, W.H., 2003. The GPR54 gene as a regulator of puberty. *N. Engl. J. Med.* 349, 1614–1627.
- Shibata, M., Friedman, R.L., Ramaswamy, S., Plant, T.M., 2007. Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (*Macaca mulatta*). *J. Neuroendocrinol.* 19, 432–438.
- Smith, J.T., Dungan, H.M., Stoll, E.A., Gottsch, M.L., Braun, R.E., Eacker, S.M., Clifton, D.K., Steiner, R.A., 2005a. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology* 146, 2976–2984.
- Smith, J.T., Cunningham, M.J., Rissman, E.F., Clifton, D.K., Steiner, R.A., 2005b. Regulation of KiSS1 gene expression in the brain of the female mouse. *Endocrinology* 146, 3686–3692.
- Smith, J.T., Acohido, B.V., Clifton, D.K., Steiner, R.A., 2006. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J. Neuroendocrinol.* 18, 298–303.
- Smith, J.T., Clay, C.M., Caraty, A., Clarke, I.J., 2007. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. *Endocrinology* 148, 1150–1157.
- Smith, J.T., Coolen, L.M., Kriegsfeld, L.J., Sari, I.P., Jaafarzadehshirazi, M.R., Maltby, M., Bateman, K., Goodman, R.L., Tilbrook, A.J., Ubuka, T., Bentley, G.E., Clarke, I.J., Lehman, M.N., 2008. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. *Endocrinology* 149, 5770–5782.
- Teles, M.G., Bianco, S.D., Brito, V.N., Trarbach, E.B., Kuohung, W., Xu, S., Seminara, S.B., Mendonca, B.B., Kaiser, U.B., Latronico, A.C., 2008. A GPR54-activating mutation in a patient with central precocious puberty. *N. Engl. J. Med.* 358, 709–715.
- Tena-Sempere, M., 2006. KiSS-1 and reproduction: focus on its role in the metabolic regulation of fertility. *Neuroendocrinology* 83, 275–281.
- Topaloglu, A.K., Reimann, F., Guclu, M., Yalin, A.S., Kotan, L.D., Porter, K.M., Serin, A., Mungan, N.O., Cook, J.R., Ozbek, M.N., Imamoglu, S., Akalin, N.S., Yuksel, B., O'Rahilly, S., Semple, R.K., 2009. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat. Genet.* 41, 354–358.
- Wacker, J.L., Feller, D.B., Tang, X.B., Defino, M.C., Namkung, Y., Lyssand, J.S., Mhyre, A.J., Tan, X., Jensen, J.B., Hague, C., 2008. Disease-causing mutation in GPR54 reveals the importance of the second intracellular loop for class A G-protein-coupled receptor function. *J. Biol. Chem.* 283, 31068–31078.
- Zhang, C., Roepke, T.A., Kelly, M.J., Ronnekleiv, O.K., 2008. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. *J. Neurosci.* 28, 4423–4434.