REVIEW

Novel insights into the metabolic action of Kiss1 neurons

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Abstract

Kiss1 neurons are essential regulators of the hypothalamic–pituitary–gonadal (HPG) axis by regulating gonadotropin-releasing hormone (GnRH) release. Compelling evidence suggests that Kiss1 neurons of the arcuate nucleus (Kiss1[ARC]), recently identified as the hypothalamic GnRH pulse generator driving fertility, also participate in the regulation of metabolism through kisspeptinergic and glutamatergic interactions with, at least, proopiomelanocortin (POMC) and agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons, located in close apposition with Kiss1[ARC]. This review offers a comprehensive overview of the recent developments, mainly derived from animal models, on the role of Kiss1 neurons in the regulation of energy balance, including food intake, energy expenditure and the influence of circadian rhythms on this role. Furthermore, the possible neuroendocrine pathways underlying this effect, and the existing controversies related to the anorexigenic action of kisspeptin in the different experimental models, are also discussed.

Introduction

Reproduction is a fundamental function that ensures the perpetuation of the species. As such, reproductive activity is under the regulation of a complex central and peripheral network that forms the hypothalamic–pituitary–gonadal (HPG) axis. Within the HPG axis, reproductive function is regulated by gonadotropin-releasing hormone (GnRH) neurons located in the hypothalamus, which control the production and release of gonadotropins from the pituitary to regulate gonadal function. Major progress has been made in the understanding of the central mechanisms regulating reproductive activity with the finding that GnRH synthesis and release require the stimulatory action of kisspeptin (Kp, formerly known as metastin), a hypothalamic peptide encoded by the *Kiss1* gene and produced by Kiss1 neurons. Kisspeptin signaling is of paramount importance, as humans and mice bearing mutations in *Kiss1* or its receptor (*Kiss1r*, formerly termed GPR54) display hypogonadotropic hypogonadism, pubertal failure and are infertile (1, 2).

In rodents, Kiss1 neurons are primarily located in the arcuate nucleus (Kiss1[ARC]) and the anteroventral periventricular/periventricular nucleus (Kiss1[AVPV/PeN]) and are deferentially regulated by sex steroids leading to complementary roles in maintaining reproductive success. Kiss1[AVPV/PeN] neurons, which are vestigial in males, have been shown to play a major role in driving the preovulatory LH surge in females in response to the positive feedback of estradiol (E2) under the control of the suprachiasmatic nucleus (SCN) (3, 4, 5, 6). In contrast, in response to the negative feedback of sex steroids, Kiss1[ARC] neurons regulate the tonic release of GnRH/LH, thus relaying information about the hormonal and neuroendocrine milieu (including metabolic cues) (7). In this context, the Kiss1[ARC] neuronal population co-expresses the neuropeptides neurokinin B (NKB) and dynorphin A (Dyn A), thereafter named KNDy neurons, which hold the hypothalamic GnRH pulse generator, essential for reproductive function (8). Moreover, Kiss1[ARC] neurons are predominantly glutamatergic (9, 10) and

have the potential to release the fast-acting transmitter glutamate onto (1) neighboring neurons in the ARC to potentially regulate metabolism (discussed in later sections), and (2) Kiss1$^{AVPV/PeN}$ neurons to regulate fertility (10, 11). An additional population of Kiss1 neurons has been recently identified in the posterodorsal part of the medial amygdala (Kiss1^{MePD} (12)), and a number of chemogenetic (13) and optogenetic (14) studies by our lab and others suggest an important role of Kiss1^{MePD} signaling in the regulation of the GnRH pulse generator, besides a potential role in driving emotional and sexual behavior, pubertal timing and ovulation – at least in mice (15, 16).

Reproductive function is energy costly and requires a threshold of energy reserves, as situations of negative energy balance (e.g. anorexia nervosa) or excessive energy deposits (e.g. obesity, diabetes) may impair the reproductive axis. During the last decade, major progress has been made in the understanding of a wide variety of reproductive disorders that are a direct result of metabolic abnormalities. Women suffering from undernutrition display hypothalamic amenorrhea, characterized by low gonadotropin secretion and infertility (17), while obese women display high risk of miscarriage, pregnancy complications, anovulation and infertility resulting from the increased negative feedback on gonadotrophin secretion, due to the peripheral conversion of androgens to estrogen (18). In this line, women suffering from polycystic ovary syndrome (PCOS, leading to hyper activation of the gonadotropic axis) are frequently obese (17), although the causative relationship between the metabolic and the reproductive phenotypes of PCOS patients is not completely understood. Overall, it is well established that energy imbalance has severe repercussions on reproductive fitness. However, the mechanisms mediating the interaction between reproductive function and energy balance are still largely unexplored. This review provides a summary of the action of Kiss1 neurons in the bidirectional interaction between reproduction and metabolism.

Kiss1 neurons as metabolic gates of fertility

Kiss1 neurons are critical regulatory nodes that integrate metabolic cues in order to adjust reproductive function to energy stores. A number of studies have characterized the inhibitory effect that metabolic stress (e.g. food deprivation) exerts on *Kiss1* gene expression, leading to the decrease in LH levels and, therefore, reproductive success, in rodents and primates, which frequently

manifest with delayed or absent puberty onset and infertility (19, 20, 21). Importantly, Kiss1 neurons are direct targets of peripheral metabolic hormones (e.g. leptin, insulin); however, the relevance of Kiss1 neurons as first order metabolic responders is questioned, given that the selective deletion of receptors for metabolic cues (e.g. leptin and insulin receptors) do not appear to impinge reproductive or metabolic functions (22, 23). Nonetheless, a remarkable feature of Kiss1^{ARC} neurons is their ability to directly communicate with neurons in the ARC that regulate hunger and satiety, suggesting the existence of a bidirectional metabolic-reproductive loop (discussed subsequently). The anorexigenic proopiomelanocortin (POMC) and orexigenic agouti-related peptide (AgRP)/ neuropeptide Y (NPY) neurons are fundamental players that regulate energy balance (24, 25) and also play a critical role in regulating fertility (26, 27). On one hand, POMC, a precursor polypeptide, is expressed in the ARC, the nucleus tractus solitarius (NTS) of the brainstem and the pituitary. POMC is cleaved into different biologically active peptides, including the alpha melanocyte-stimulating hormone (α-MSH), which drives satiety and increases energy expenditure through its selective binding to the melanocortin receptors, MC3R and MC4R, with MC4R being the primary receptor mediating the metabolic action of melanocortins (28, 29). On the other hand, the orexigenic AgRP and NPY peptides – both produced in AgRP neurons – increase food intake and decrease energy expenditure (30, 31, 32), thus termed hunger neurons. AgRP selectively binds MC4R to prevent the anorexigenic effect of α-MSH, while NPY binds preferentially Y1 and Y5 receptors to induce its orexigenic effect (25).

POMC and AgRP neurons have been extensively studied for their role as conveyors of the metabolic state to regulate fertility. In female mice and women, MC4R deficiency is associated with a number of reproductive disorders related to advanced puberty onset, irregular ovarian cyclicity and reduced number of developed corpora lutea in ovaries (33, 34, 35), while the activation of MC4R stimulates their libido (36). In males, MC4R does not appear to be involved in gametogenesis or gonadal steroidogenesis; however, it is involved in erectile function and sexual behavior (37, 38). However, despite the documented action of melanocortins on reproduction, including sexual behavior, the precise site/s of action underlying this effect remain ill-defined. In this context, 50% of GnRH neurons, located mostly in the medial preoptic area (POA), express MC4R (39) and 70% of these GnRH cells are excited by α-MSH, acting through both MC3R and MC4R (40). However, most of

the melanocortin actions on the HPG axis appear to be mediated through Kiss1 neurons based on the fact that: (1) Kiss1 neurons receive direct projections from α-MSH fibers, (2) *Kiss1* mRNA expression decreases in the ARC of pubertal female mice subjected to chronic inhibition of MC3R/MC4R, and (3) the stimulatory action of melanocortins on the HPG axis is blunted in kisspeptin signaling deficient mice (41). These results are in line with the presence of MC4R in a subset of Kiss1 neurons (9), suggesting a likely direct regulation of Kiss1 neurons by melanocortins.

Direct regulation of Kiss1 neurons by AgRP neurons have also been described through, at least, direct inhibitory GABAergic post-synaptic inputs (42). The relevance of this regulatory pathway is further evidenced by compelling studies in leptin deficient mouse models, which display an infertile phenotype largely derived from the increase in the activity of AgRP neurons and the subsequent reversal of the infertile phenotype after the ablation of these neurons (i.e. AgRP neurons) (42). While direct connections from AgRP to Kiss1 neurons have been demonstrated, no evidence of direct interaction between AgRP and GnRH neurons has been found, suggesting that under negative energy balance, the inhibitory action on the HPG axis exerted by AgRP neurons occurs at the level of Kiss1 neurons (and/or on upstream neuroendocrine networks), further emphasizing the critical role of Kiss1 neurons in mediating the transmission of metabolic cues onto the reproductive axis. Altogether, these data suggest that the metabolic regulation of fertility by POMC and AgRP neurons occurs mostly through their action on Kiss1[ARC] neurons, which act as gatekeepers in the metabolic regulation of reproductive activity.

Kiss1 neurons as active metabolic players

Since their characterization in 2003, the neuroendocrine community has focused mostly on the neuroanatomical characterization and functional role of kisspeptin in reproduction, while the metabolic facets of this neuropeptide remained underexamined. Recent studies have aimed at closing this gap to elucidate the potential metabolic role of Kiss1 neurons. Thus, it has been demonstrated that the absence of kisspeptin signaling (*Kiss1r*KO mice) results in increased body weight in a sex-dependent manner and that this effect is partly independent of the presence of circulating sex steroids. Adult *Kiss1r*KO females, but not males, displayed decreased food intake but higher body weight as a consequence of

reduced energy expenditure and locomotor activity, as well as impaired glucose homeostasis (43) and elevated plasma leptin levels (44). Importantly, these metabolic impairments were restored in females, but not males, in a mouse model of selective reinsertion of Kiss1r only in GnRH neurons, which prevented any changes in BW of any sex during adulthood but retained the BW changes observed in prepubertal and young adult *Kiss1r*KO mice. This indicates that a large component of the phenotype observed in adult *Kiss1r*KO mice is sex-steroid dependent. However, these mice still displayed increased insulin resistance at any age compared to controls suggesting the existence of kisspeptin-dependent mechanism in glucose homeostasis (45).

Of note, while studies in rodents support a role of Kiss1 neurons in metabolism, to date, there is no evidence of metabolic impairments in patients with kisspeptin deficiency (1, 2, 46); however, a detailed metabolic characterization of these patients has not been performed.

Overall, an active role of Kiss1 neurons in the control of energy balance has been recently demonstrated in a number of rodent studies. This effect is partly indirect through the regulation of the circulating levels of sex steroids and partly direct through the action of kisspeptin and additional co-transmitters. However, whether this direct action is mediated by the control of food intake (anorexigenic action), increase in energy expenditure, or both, remains a matter of debate. Nonetheless, this action of kisspeptin (and/or co-transmitters) as satiety signals while stimulating the reproductive axis is in line with the classic action of satiety signals, for example, melanocortins and NUCB2/Nesfatin-1 (47, 48), which get activated in situations of energetic surplus to signal the organism that enough resources for reproduction are present and it is therefore safe to shift the focus from food-seeking behaviors to reproduction.

Kiss1 neurons in the regulation of food intake

The anorexigenic effects of kisspeptin were first described in mice, as the central pharmacological administration of kisspeptin during the light cycle significantly suppressed food intake (49). However, this effect was only present in overnight fasted mice, which exhibited decreased meal frequency and total meal time during refeeding. The same kisspeptin dose was ineffective to alter food intake when tested in *ad libitum* fed animals (49). These data revealed, for the first time, a potentially anorexigenic role of kisspeptin in mice under negative energy balance. Similar data have also been described in the wild jerboa,

where exogenous kisspeptin injections significantly inhibited food intake during refeeding in females after 48 h of food deprivation, but not in *ad libitum* fed animals (50). Importantly, this effect was present in female jerboa but not in males under any of these conditions (50), revealing that not only the anorexigenic effect of kisspeptin is dependent on the energetic state, in line with the mouse data (49), but also that this effect is sexually dimorphic. These studies go in parallel with a recent work in 24 h fasted rats, where kisspeptin was able to suppress food intake (51), while this effect was not observed in *ad libitum* fed or 12 h fasted animals (19, 52). Along this line, kisspeptin does not appear to exhibit an anorexigenic action in humans, as kisspeptin infusion in overnight fasted men did not alter appetite (53). Whether the fasted period in men was not long enough for the anorexigenic effect of kisspeptin to be evident or men are not responsive to the metabolic action of kisspeptin after acute delivery is unknown. However, given the sexual dimorphism in the metabolic effect of kisspeptin described, studies in women will be required in order to evaluate whether this response in also sexually dimorphic in humans.

In contrast to these studies described, a recent study in mice suggests that i.p. injections of kisspeptin in *ad libitum* fed animals suppresses food intake within the first 4 h following a single administration of kisspeptin and for up to 24 h (54). However, not only these results contradict a number of previous studies in different species suggesting that the anorexigenic action of kisspeptin, in order for it to be revealed, requires a negative energetic state but, strikingly, the long action (up to 24 h) of a small peptidic compound (only ten amino-acids in kisspeptin-10) after peripheral administration is unprecedented, given that the stimulatory effect on LH is lost within 60 min of i.p. administration of high doses of kisspeptin (55). Whether Kiss1 neurons are able to elicit a cascade of events through other neurons to exert this role or there are technical aspects that affected this study requires further investigation.

While evidence on the metabolic actions of kisspeptin is mounting, the contribution of kisspeptin's partners, that is, co-transmitters released from Kiss1 neurons such as glutamate or NKB, may enhance the overall metabolic role of Kiss1 neurons. In this context, and in line with the absence of an anorexigenic action of kisspeptin in normal fed conditions in several experimental models, a recent study of chemogenetic activation of Kiss1^{ARC} neurons in fed mice failed to suppress feeding during the dark cycle over a period of 4 h (56).

This study suggests that none of the potential kisspeptin co-transmitters play a role in regulating food intake, at least not acutely in fed conditions. However, chronic silencing of synaptic transmission from Kiss1^{ARC} neurons in female mice results in significant weight gain derived, in part, from feeding impairments (57). While the overall food consumed was similar to controls, the circadian feeding behavior was severely impaired, losing the nocturnal pattern of feeding observed in control mice (57). This change in the circadian rhythm of feeding correlated with similar changes in physical activity and energy expenditure. These results suggested a critical role for Kiss1^{ARC} neurons in the regulation of circadian rhythms, at least for feeding and physical activity. However, the SCN in the Kiss1^{ARC} silenced mice appears to be intact and Kiss1^{ARC} neurons do not project directly to the SCN (57), suggesting that Kiss1^{ARC} neurons may serve as conduits for upstream signals from the SCN and/or that Kiss1^{ARC} neurons control downstream targets of the SCN to modulate circadian rhythms. The latter is supported by the presence of projections from Kiss1^{ARC} neurons to SCN targets involved in the regulation of circadian rhythms (i.e. subparaventricular zone (SPZ) and the dorsal medial hypothalamus (DMH)) (57, 58). These findings support a role for Kiss1 neurons in the regulation of the daily timing of food intake in a circadian-dependent manner, probably contributing to the overall reduced feeding in mice during light hours. This effect is supported by the suppression of food intake after kisspeptin administration during light hours (49, 50) and absent during dark hours (49, 56). Therefore, in the face of these studies, caution should be exercised when interpreting data of kisspeptin studies on feeding behavior if time of day and basal energetic status are not properly controlled.

Kiss1 neurons display bidirectional interactions with AgRP and POMC neurons

Kiss1^{ARC} neurons project to a wide variety of brain nuclei including central metabolic centers known to regulate food intake, body weight and thermogenesis: the median preoptic nucleus (MePO), bed nucleus of the stria terminalis (BnST), paraventricular nucleus of the hypothalamus (PVN), DMH and the lateral hypothalamus (LH) (58). A significant number of these Kiss1^{ARC} projections also target the ARC, where hunger (AgRP) and satiety (POMC) neurons are located. In this context, initial studies supported the idea that

Kiss1^{ARC} neurons directly contact neighboring POMC and AgRP neurons. First, kisspeptin can modulate the activity of POMC neurons based on the fact that (1) POMC neurons express *Kiss1r* (59), (2) kisspeptin is able to excite POMC neurons through sodium/calcium exchanger activation (60), and (3) kisspeptin increases the expression of *Pomc* in the ARC (50, 61). Second, in addition to kisspeptin input, POMC and AgRP neurons receive direct glutamatergic inputs from Kiss1^{ARC} neurons that differentially regulate them in a sex-steroid-dependent manner through the activation of distinct metabotropic glutamate receptors (10). These data support a direct action of Kiss1 neurons in the regulation of essential central metabolic centers. However, the satiety inducing effect of the activation of POMC neuron activation is remarkably slow and usually requires 24 to 48 h to inhibit food intake (through the action of α-MSH on MC4R) (56, 62). In the studies documenting the anorexigenic action of kisspeptin, the effect was observed within 1 h following administration (49, 50, 51), suggesting that the anorexigenic effect is not mediated through the activation of POMC neurons. Given the fact that Kiss1^{ARC} neurons are not within the glutamatergic pool of ARC neurons that targets the PVN to rapidly induce satiety (56), we can infer that kisspeptin must be acting through other 'fast acting' satiety inducing neurons as an indirect mechanism to suppress food intake. Nonetheless, the potential anorexigenic effect of kisspeptin acting through POMC neurons at a larger time scale cannot be excluded.

An important factor in the metabolic role of Kiss1 neurons relates to how much of the metabolic phenotype described to date in the different animal models is mediated by kisspeptin vs kisspeptin co-transmitters, for example, glutamate or NKB. In this context, it is worth noting that the degree of obesity observed in the Kiss1^{ARC}-silenced female mice (57) is significantly greater, and develops faster, than that reported in *Kiss1r*KO mice (43, 44, 45, 63), supporting a role for additional factors from Kiss1^{ARC} neurons in the control of metabolism. Importantly, glutamate has been described to mediate the effect (excitation) that Kiss1^{ARC} neurons exert on POMC neurons, and to selectively inhibit AgRP neurons (10, 64), which strongly supports a glutamatergic component in the phenotype of the Kiss1^{ARC} silenced mouse model (57). Moreover, the glutamatergic action of Kiss1 neurons onto neighboring POMC and AgRP neurons is gonadal hormone dependent, as it is enhanced by E2 in females (64, 65), in line with the sex-dependent effect of kisspeptin on food intake in

jerboa (50) and BW in *Kiss1rKO* mice (43), restricted to females (Fig. 1).

Overall, controversial data exist related to the action of kisspeptin on food intake. While the anorexigenic action of Kiss1 neurons in general, and kisspeptin in particular, cannot be ruled out under specific physiological conditions and over long periods of time, mounting evidence points to the regulation of energy expenditure, either directly or through the control of
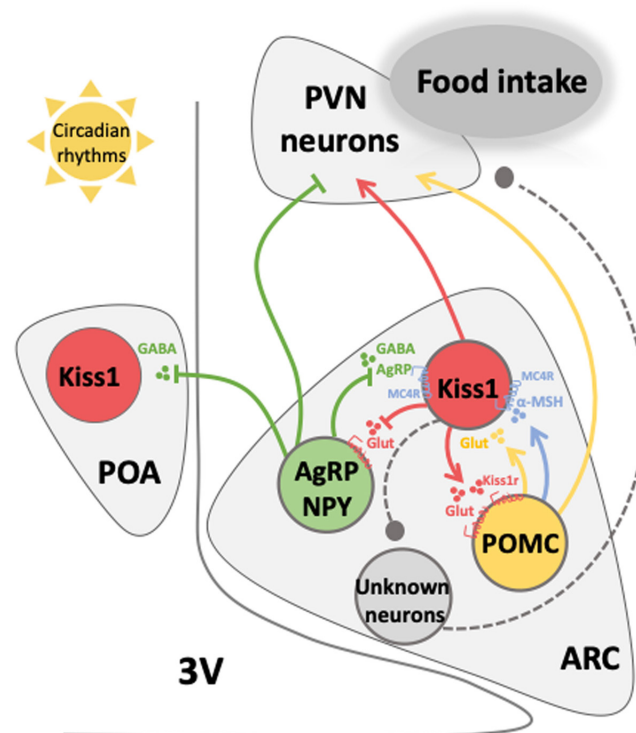

Figure 1

Schematic representation of the suggested hypothalamic neuronal network regulating food intake comprising Kiss1^{ARC} neurons interactions with POMC and AgRP neurons. Neurons located in the PVN, critical for food intake regulation, receive direct projections from the ARC Kiss1, POMC and AgRP neurons. Within the ARC, Kiss1 neurons directly contact neighboring AgRP neurons to inhibit their activity through the activation of glutamate receptors on these neurons. POMC neurons, which express Kiss1r, are directly stimulated by Kiss1^{ARC} neurons through glutamate and kisspeptin. In turn, both POMC neurons (through glutamate and αMSH) and AgRP neurons (through GABA and AgRP) send direct projections to Kiss1^{ARC} neurons, respectively, to activate and inhibit their activity; AgRP neurons also send inhibitory GABAergic projections to Kiss1^{AVPV} neurons in the preoptic area. Under the influence of circadian rhythms, Kiss1^{ARC} neurons also contribute to food intake regulation through yet unknown neuroendocrine circuits. The anorexigenic effect of Kiss1 neurons might be mediated either by: (1) direct projections to PVN neurons, (2) direct stimulation to POMC neurons which, in turn, (3) inhibit food intake at the level of the PVN, and/or (4) through indirect actions on other (unknown) fast acting satiety neurons. PVN, paraventricular nucleus of the hypothalamus; 3V, third ventricle; POA, preoptic area; Glut, glutamate; MC4R, melanocortin-receptor 4. The pointed arrows represent activations, while the flat arrows represent inhibitions.

the rhythm of feeding behavior and locomotion as the main contributor to the overall metabolic phenotype of kisspeptin deficient models.

Kiss1 neurons in the regulation of energy expenditure

While the demonstration of an action of kisspeptin on food intake has not been consistent across the different experimental models, a critical feature shared by those models that include kisspeptin signaling deficiency (*Kiss1r*KO mice) or Kiss1[ARC] silencing (43, 57, 63) has consistently been an impairment (decrease) at the energy expenditure level. In both cases, mice were less physically active, which may contribute to the loss of lean mass and increase in body fat. While this is a plausible mechanism, a direct role of Kiss1[ARC] neurons in the regulation of energy expenditure through the action on central nuclei involved in this metabolic process, for example, MePO, PVN, DMH and NTS ((66), for review), which receive projections from Kiss1[ARC] neurons (58), cannot be excluded.

The contention that kisspeptin can control energy expenditure is further supported by the identification of *Kiss1r* in brown adipose tissue (BAT) – critical for the regulation of energy expenditure through the induction of thermogenesis – and the decrease in the activation of the BAT observed in obese *Kiss1r*KO females (67). However, the complexity of the metabolic role of kisspeptin is further evidenced by the fact that the removal of kisspeptin signaling from the BAT increases metabolic rate and body temperature, thus decreasing body weight, which suggests that the obesity seen in *Kiss1r*KO and Kiss1[ARC] silenced female mice is not due to the direct action of kisspeptin signaling on BAT, but rather the consequence of impaired kisspeptin signaling in other tissues involved in the regulation of energy balance. Furthermore, the source of kisspeptin that activates Kiss1r in the BAT in unknown, and since the majority of the neuronal innervation of the BAT converges into the intermediolateral nucleus of the spinal cord (IML) before reaching the BAT, the direct projection of Kiss1[ARC] neurons to this tissue is unlikely and suggests that kisspeptin acting on the BAT does not have a central origin (Fig. 2).

Glucose homeostasis regulation through kisspeptin pathways

Glucose homeostasis is maintained by the balance of insulin and glucagon secretion to control blood glucose levels. As previously mentioned, a number of studies
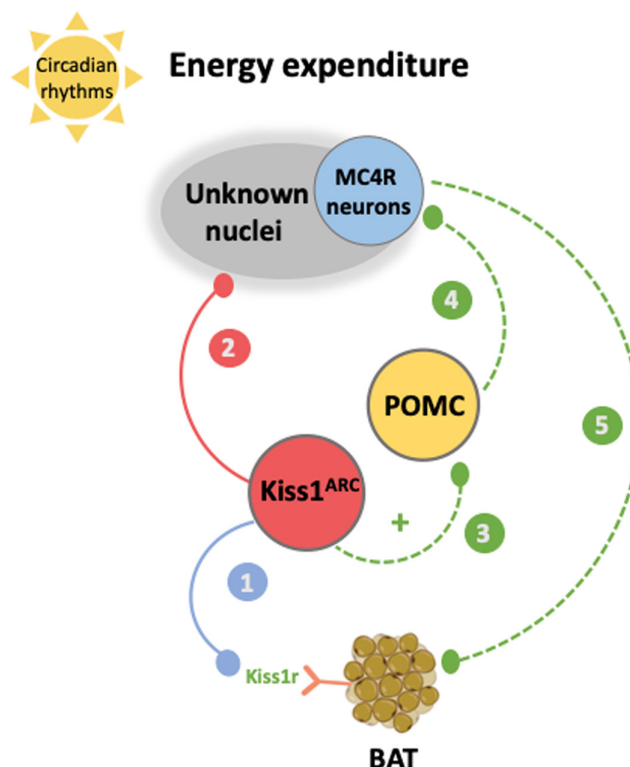

Figure 2
Hypothetical working model of the suggested hypothalamic neuronal network regulating energy expenditure comprising direct and indirect actions of Kiss1 neurons. Under the influence of circadian rhythms, Kiss1[ARC] neurons may participate in the regulation of energy expenditure through: (1) the direct action on the BAT, which expresses Kiss1r; (2) the direct action on unknown nuclei directly involved in the control of energy expenditure; or (3) through indirect actions on POMC neurons in the ARC, which (4) project onto MC4R expressing neurons in unknown nuclei to (5) regulate thermogenesis via BAT activation. BAT: brown adipose tissue. The solid lines represent Kiss1 neurons direct actions; dashed lines represent Kiss1 neurons indirect actions through intermediate neurons.

documented that mice lacking kisspeptin signaling present impaired glucose tolerance that is not exclusively derived from their increased body weight. Both standard chow fed and high fat diet (HFD) fed *Kiss1r*KO female mice displayed significant glucose intolerance after a glucose tolerance test (GTT) (43, 45). This effect was (1) age dependent as adult, but not young 6-week-old, *Kiss1r*KO females displayed glucose intolerance (63), and (2) sexually dimorphic, as *Kiss1r*KO males displayed normal basal glucose levels and normal glucose tolerance both under standard chow and HFD (43, 45). Moreover, this effect in females is exacerbated in the absence of sex steroids, that is, in OVX *Kiss1r*KO females (43). Gonadal steroids (or lack thereof in *Kiss1r*KO mice) play a critical role in the glucose intolerance observed in the absence of kisspeptin, based on the improvement of this condition after selective restoration of Kiss1r in GnRH neurons, which normalizes

the circulating levels of sex steroids. However, this model did not rescue glucose levels completely, supporting a role of kisspeptin in additional tissues, likely at the level of the liver and/or pancreatic cells (68). Indeed, increasing evidence supports a role of kisspeptin signaling in pancreatic function based on (1) the presence of Kiss1 and Kiss1r in pancreatic β and α cells (68), and (2) the *in vitro* exposure of monkey (21) and human (53) pancreatic islets to kisspeptin stimulates glucose-induced insulin secretion. Nonetheless, contradictory findings have been found in mice that suggest that Kiss1r signaling in β-cells suppresses glucose-induced insulin secretion (69). In addition to the pancreatic and hepatic action of kisspeptin, we cannot rule out that the decrease in lean mass observed in kisspeptin signaling deficient mice contributes to the different glucose tolerance compared with their WT counterparts. In addition to peripheral actions, a central effect of kisspeptin in glucose homeostasis is also possible, given that Kiss1 neurons co-express insulin receptors (22, 23, 70); however, the specific deletion of insulin receptor (alone or jointly with leptin receptor, which share common intracellular pathways) does not affect glucose homeostasis (23). Nevertheless, whether Kiss1 neurons modulate the activity of other insulin sensitive neurons cannot be excluded.

Conclusions and perspectives

Reproduction is energy demanding, therefore, the neuroendocrine mechanisms regulating reproductive function and energy balance are reciprocally linked. During the last decade, major advance has been made in the understanding of the central mechanisms synchronizing these two functions and the progress in the generation of genetic mouse models and viral techniques has greatly advanced our knowledge of the neuroendocrine circuitries underlying this effect. However, the complexity of these networks and the controversial findings in the field prevented the complete understanding of the neuronal pathways synchronizing reproductive activity with energy reserves. The role of Kiss1 neurons in the regulation of the HPG axis has been well depicted and the role of Kiss1 neurons as metabolic gatekeepers for reproductive success thoroughly characterized. In this review, we summarized the current evidence supporting a bidirectional role of Kiss1 neurons in the control of reproduction and metabolism. On one hand, Kiss1[ARC] neurons are direct targets of central and peripheral metabolic cues, which form an essential regulatory element of the HPG axis

from early stages of development. On the other hand, they actively control metabolic function through, at least, direct connections with AgRP and POMC neurons in the ARC, although additional (yet unidentified) actions onto neuronal networks that regulate energy balance are likely, given the widespread location of Kiss1 projections throughout the brain.

Interestingly, a recently identified role of Kiss1[ARC] neurons in the transmission of circadian rhythms has also been suggested to impinge energy balance due to the loss of the circadian (nocturnal) pattern of feeding behavior. A number of studies in rodent models have documented the importance of time restricted feeding, where the same number of calories can lead to normal BW if consumed over a short period of time or to obesity if consumed spread throughout the day. The remaining question in this action relates to whether Kiss1 neurons receive direct regulation from the SCN, control downstream targets of the SCN or have their own circadian pattern that regulate feeding (and locomotion).

As described in this review, the nature of the predominant mechanism underlying the metabolic role of Kiss1 neurons is also a matter of debate. While some studies point to an effect on food intake, negative data have also been described in several species, including the human, which leads to the speculation that this effect is highly dependent on age, sex and hormonal milieu. This may suggest that the control of energy expenditure, which is consistently decreased in the absence of kisspeptin signaling and Kiss1 neuron silencing, is the predominant pathway in the metabolic influence of Kiss1 neurons. Whether this is an indirect consequence of decreased locomotion activity in the absence of functional Kiss1 neurons, or a direct action on specific brain areas regulating the activation of the BAT, will require further investigation.

Finally, one of the most important unresolved questions in the metabolic action of Kiss1 neurons relates to the contribution of kisspeptin *per se* vs its co-transmitters in this action. The fact that the expression of Kiss1r in the brain is limited and highly specific to some neuronal groups (i.e. GnRH neurons) and the greater metabolic phenotype after Kiss1[ARC] silencing compared with *Kiss1r*KO mice strongly suggests that additional factors are at play in the metabolic role of Kiss1 neurons.

Overall, compelling evidence is mounting supporting a metabolic role of kisspeptin (and Kiss1 neurons). Besides increasing our understanding of the central mechanisms that govern energy balance, characterizing this effect in detail will be of critical importance in order to assess

possible metabolic risks in patients undergoing kisspeptin treatments, as *in vitro* fertilization techniques and novel approaches to increase sexual drive are successfully using kisspeptin as the main elicitor of the activation of the HPG axis in humans. To date, no metabolic alterations have been described in clinical and preclinical kisspeptin-based treatments; however, as the field progresses, there is a high probability that the description of metabolic implications will occur.

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