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Metabolic influences on neuroendocrine regulation of reproduction

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Abstract

Purpose of review—Reproduction is a tightly regulated function in which many mechanisms contribute to ensure the survival of the species. Among those, due to the elevated energy requirements of reproduction, metabolic factors exert a pivotal role in the control of hypothalamic-pituitary-gonadal axis. Although this control may occur at multiple levels of the axis, the majority of interactions between metabolic and reproductive systems take place in the hypothalamus. In this article, we present an overview of the state-of-the-art knowledge regarding the metabolic regulation of reproduction at the central level. We aim to identify the neuroanatomical location where both functions interconnect by discussing the likelihood of each component of the neuronal hierarchical network controlling gonadotropin-releasing hormone release to be first-order responders to metabolic cues, especially the peripheral metabolic signals leptin, insulin, and ghrelin.

Recent findings—Latest evidence suggests that the primary action of leptin, insulin, and ghrelin to regulate reproduction is located upstream of the main central elicitors of gonadotropin release, Kiss1 and gonadotropin-releasing hormone neurons, and neuroanatomically separated from their metabolic action.

Summary—The study of the neuronal interactions between the mechanisms governing metabolism and reproduction offers the platform to overcome or treat a number of prevailing metabolic and/or reproductive conditions.

Keywords

hypothalamus; kisspeptin; leptin; metabolism; reproduction

Introduction

Reproduction is one of the most energy demanding endeavors of any species and, hence, tight connections between the mechanisms controlling metabolism and reproductive integrity have developed during evolution. Metabolic cues from peripheral tissues and environmental cues translate information reflecting fuel storage and food availability to the central regulators of reproduction, thus assuring the appropriate timing of gestation and survival of the offspring. Sufficient energy stores are critical for the attainment of reproductive maturation and maintenance of fertility in adulthood [1]. Indeed, situations of

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energy depletion such as anorexia nervosa, excessive exercise, or diabetes –but also extreme energy surplus (e.g., severe obesity) – lead predominantly to delayed or absent pubertal onset in adolescents and hypogonadism in adults, which is usually characterized by hypothalamic amenorrhea [2,3]. Indeed, peripheral metabolic cues and reproductive hormones may act on different targets to regulate food intake and reproduction at multiple levels, for example, at the level of the pituitary [4,5,6]; however, there is a key central node that coordinates these two functions and that is common to both: the hypothalamus. In this article, we offer a comprehensive review of the latest advances in the identification and characterization of the neuroendocrine mechanisms that bridge metabolism and reproduction in the hypothalamus in mammals, focusing on the mechanism(s) of action of leptin, insulin, and ghrelin as the three major representatives of peripheral metabolic cues.

Transmission of the Metabolic State to the Brain

Energy storage occurs mainly at the level of white adipose tissue, where adipocytes secrete the anorexigenic adipokine leptin [7]. Circulating levels of leptin reflect body fat mass, serving as a messenger for the metabolic state to the central neuroendocrine regulatory centers of appetite, energy homeostasis, and metabolism [8]. Additionally, a number of peripheral organs secrete factors that provide further information about nutritional status and hence may impinge upon metabolic function, such as the pancreatic hormone insulin – released as a consequence of increasing glucose and, in the long term, body fat levels, and serving to increase anorexigenic inputs to the hypothalamus [9] – and gut hormones, among which ghrelin has gathered particular attention due to its orexigenic effects [10,11].

Disruption of proper metabolic signaling inflicts deleterious effects upon reproductive function [1]. For instance, humans and laboratory animals with leptin or insulin deficiency or resistance and/or increased ghrelin levels exhibit delayed or absent puberty and frequently display hypogonadotropic hypogonadism, which prevents fertility [1,9,12,13]. Ghrelin suppresses pulsatile gonadotropin-releasing hormone (GnRH) release [14,15], thus serving as a signal to suppress reproduction in times of famine [16,17]. Exactly how the energy state impinges on GnRH release remains unresolved.

Central Action of Metabolic Cues to Regulate Gonadotropin-Releasing Hormone Release

The hypothalamus is the nodal regulatory center where the mechanisms controlling metabolic state and reproductive function collide [18]. On the one hand, GnRH, the ultimate elicitor of gonadotropin function, is synthesized predominantly at the level of the hypothalamic preoptic area (POA) and is responsible for the awakening of the gonadotropic axis at puberty as well as its maintenance thereafter [19]. On the other hand, metabolic control is centered particularly in the ventromedial nucleus and the arcuate nucleus (ARC) [20]. In the ARC, two neuropeptides play crucial roles in the control of metabolic function through the melanocortin system: α -melanocyte-stimulating hormone-a [MSH; agonist of the melanocortin-3 and melanocortin-4 receptors (MC3R and MC4R)] and the agouti-related protein (AgRP; inverse agonist of MC3R and MC4R) [21,22], produced by pro-opiomelanocortin and cocaine and amphetamine-regulated transcript (POMC/CART) and neuropeptide Y (NPY)/AgRP neurons, respectively [9,20]. Neuropeptides derived from POMC/CART neurons exert a potent anorectic action, thus decreasing food intake and body weight, whereas AgRP and NPY have the opposite (orexigenic) effect, inducing food intake. A body of evidence also supports the role of these neurons as potent regulators of gonadotrope function, although, admittedly, conflicting effects have been reported regarding their action upon gonadotropin release, depending on the species and/or neuroanatomical site of action [23–26]. In the following section, we address the role of these and other

neurons as potential first-order responders to energy status in the hierarchy of neurocircuits that control gonadotropin release.

Gonadotropin-releasing hormone neurons

One possibility is that GnRH neurons themselves receive direct input from metabolic cues. In this vein, GnRH neurons have been shown to express insulin receptor mRNA and protein [27] and are activated by insulin [28]. Indeed, insulin action in the brain is essential for proper gonadotropin secretion, as observed in mice with neuronal deletion of insulin receptors (NIRKO mice) [29]. Nonetheless, recent evidence has challenged the notion of an important direct action of insulin on GnRH neurons, as mice with selective deletion of insulin receptors in GnRH neurons have normal puberty and fertility [30]. Moreover, the possibility that GnRH neurons are first-order responders to leptin or ghrelin action has been disproven due to the absence of receptors for leptin (LepR) [18] or ghrelin (growth hormone secretagogue receptor, GHSR) [31▪▪] in GnRH neurons (Fig. 1). Therefore, although a direct role of insulin on GnRH neurons cannot be fully excluded, the putative site for the metabolic control of reproduction must necessarily rest upstream of GnRH neurons, at least for these metabolic factors (although additional fine-tuning of gonadotropin release may occur directly at the level of the pituitary, as mentioned previously).

Kiss1 neurons

Kisspeptins (encoded by *KISS1*) have been identified in the last decade as the most potent secretagogues of GnRH release. Kiss1 neurons, located mainly in the ARC and the anteroventral-periventricular nucleus (AVPV) in rodents, contact GnRH neurons directly and are important for puberty onset and maintenance of reproductive function [32▪▪]. Importantly, the subpopulation of Kiss1 neurons in the ARC, referred to as KNDy neurons, coexpress the neuropeptides, dynorphin, and neurokinin B (NKB), and have been suggested to play a crucial role in the shaping of GnRH pulses [33▪]. This pivotal role of Kiss1 neurons in the neuroendocrine control of reproduction places them as likely conveyors of metabolic influences on GnRH neurons. However, mounting evidence suggests that although Kiss1 neurons may actively participate in this integrative process (see below), they are not direct targets of these metabolic cues (Fig. 1). First, although initial studies suggested the existence of LepR in a subset of Kiss1 neurons [34], recent studies using genetic models of selective ablation of LepR in Kiss1 neurons have documented that direct leptin signaling to Kiss1 neurons is not required for pubertal onset [35]. Similarly, removal of insulin receptors from Kiss1 neurons using Kiss1-specific insulin receptor knockout (KIRKO) mice [36▪▪] disclosed parallel findings, and further, Kiss1 neurons do not express ghrelin receptor GHSR [31▪▪]. Altogether, these data strongly suggest that Kiss1 neurons are not the primary conduit of the metabolic state to the reproductive axis.

Nonetheless, kisspeptin and NKB, as cotransmitters of KNDy neurons, have been suggested to participate in the transmission of information regarding energy balance to GnRH neurons. Caloric restriction significantly blunts the expression of *Kiss1* and *Tac2* (encoding NKB), leading to decreased gonadotropin release that can be reversed by administration of exogenous kisspeptin or senktide, an agonist of the NKB receptor (NK3R) [37▪▪,38,39]. Interestingly, luteinizing hormone (LH) responses to kisspeptin and senktide administration in pubertal rats are greater in conditions of energy deficit [38,40]. Furthermore, chronic administration of kisspeptin or senktide to prepubertal female rats subjected to caloric restriction induced a partial recovery of puberty onset (i.e., vaginal opening and LH secretion) [37▪▪,40]. On the contrary, excessive energy storage may also critically impinge on the activity of KNDy neurons, depending on the timing and developmental stage. Thus, prepubertal rats fed a high-fat diet displayed precocious puberty, characterized by early increases of *Kiss1* and *Tac2* expression as well as LH pulsatility [41]. In contrast, diet-

induced obesity in adult mice caused a significant inhibition of *Kiss1* mRNA in the ARC and AVPV [42]. Overall, KNDy neurons – and potentially also AVPV *Kiss1* neurons – are susceptible to regulation by metabolic factors that may modify the pattern of kisspeptin/GnRH release. An additional study supports this contention by documenting the role of KNDy neurons in the negative action exerted by estrogens on body weight [43].

Pro-opiomelanocortin and agouti-related protein neurons in the arcuate nucleus

The exclusion of GnRH and *Kiss1* neurons as first-order responders in the metabolic/reproductive regulatory circuit turns attention to other neuronal populations in the ARC as potential immediate upstream regulators of *Kiss1* and/or GnRH neurons. A plethora of neuropeptides potentially involved in the control of metabolism and reproduction are located within this nucleus. As described earlier, the melanocortin system and its two distinct populations of neurons (POMC/CART and NPY/AgRP) have been extensively documented to play regulatory roles in both metabolic and reproductive pathways [1,20]. Indeed, both groups of neurons express LepR, insulin receptor, and GHSR [44–48] (Fig. 1) and both leptin and insulin stimulate *Pomc* and inhibit *AgRP* expression [49,50], whereas ghrelin has been shown to inhibit POMC neurons and activate AgRP neurons [51,52]. Interestingly, GnRH neurons express MC3R and MC4R [53] and melanocortins and NPY can directly modify the activity of GnRH neurons [54], suggesting this as a possible route of regulation of the reproductive axis. Additionally, POMC/CART and NPY/AgRP neurons also display reciprocal connections with *Kiss1* neurons [26]; however, in this case, as leptin inhibits *Npy* expression [55] and both leptin and NPY stimulate *Kiss1* expression [25,56], it is unlikely that any stimulatory effect of leptin on *Kiss1* neurons happens through NPY action. AgRP, in turn, may play a role conveying leptin action to *Kiss1* and/or GnRH neurons. Even though AgRP neurons are not essential for reproduction [57], recent studies by Wu *et al.* [58,59] suggested that hyperstimulation of AgRP neurons – but not POMC neurons – in leptin-deficient (*ob/ob*) mice might account for the suppression of the reproductive axis in these mice, as ablation of AgRP neurons resulted in decreased body weight and recovery of fertility. As NPY/AgRP neurons have been reported to project to GnRH neurons [60] and also to *Kiss1* neurons [26], further examination of this model is required to identify their primary target(s).

In support of the ARC as a conveyor of metabolic/reproductive interactions, this nucleus hosts a dense network of neuronal fibers [61] and has access to circulating molecules outside the blood–brain barrier [62]. However, recent publications have disputed this role, at least for melanocortin neurons, as mouse models of selective deletion of LepR from POMC neurons, AgRP neurons, or both, results in minimal increases in body weight [44,63,64], suggesting that these neurons might be downstream of leptin-responsive neurons situated at a higher level.

The arcuate nucleus and beyond

Recent investigations have tried to narrow down the candidates in the search for the putative targets of leptin action in the brain to control body weight by parsing out excitatory (glutamatergic) vs. inhibitory (GABAergic) first-order leptin-responsive neurons. Deletion of LepR from GABAergic (VGAT⁺) but not glutamatergic (VGLUT2⁺) neurons resulted in loss of the antiobesity action of leptin by reducing the inhibitory tone on POMC neurons [65]. More interestingly, these mice also indicated that the facilitatory role that leptin exerts on reproduction is also mediated by the GABAergic subpopulation of LepR-expressing neurons, as they recapitulated the reproductive phenotype of *ob/ob* mice (delayed or absent puberty onset and infertility) [66]. Identifying the nature of this GABAergic population of neurons is essential for understanding the central mechanisms governing leptin action on reproductive function. It is worth mentioning that, whereas AgRP neurons are GABAergic

[65,67,68], most GABAergic neurons do not express AgRP [65] and, given the minimal metabolic phenotype of mice lacking LepR in AgRP neurons, this neuronal population is unlikely to hold this integrative role. In this regard, a recent study by Kong *et al.* [69] uncovered a role for a new population of GABAergic neurons in the ARC that express rat insulin-2 promoter in the control of energy expenditure, which may contribute to the action of leptin; however, whether these neurons are first-order leptin-responsive neurons and whether they participate in the control of reproductive function remains to be determined.

Additionally, a number of other players within the ARC may also serve as potential conveyors of metabolic control of reproduction, such as the newly identified anorexigenic peptide nesfatin-1 [70], which is coexpressed with POMC [71]. Nesfatin-1 blockade increases body weight and food intake in rats in a leptin-independent (and melanocortin-dependent) manner [70,72]. Interestingly, nesfatin-1 blockade delays puberty onset in rats [73] and reduces *Kiss1* mRNA in the ARC and AVPV, and central injection of nesfatin-1 induces LH release [74], suggesting a stimulatory role in kisspeptin release. This action however, appears to be indirect, as nesfatin-1 does not modify *Kiss1* neuronal activity directly as measured by whole-cell clamp recordings [74], but rather may be mediated by inhibition of NPY neurons in the ARC [75]. Nevertheless, these studies suggest a role for nesfatin-1 neurons in the metabolic control of reproductive function. Given the wide distribution of nesfatin-1 in the brain [70,73], precisely which subpopulation of these neurons exerts the metabolic vs. reproductive role and whether they are first-order responsive neurons remains unknown.

Additional populations of leptin-responsive neurons have been emerging in recent years, indicating that the overall action of leptin may result from the contributions of a number of neuronal circuits. In this regard, particularly relevant is the finding that nitric oxide, produced in LepR-expressing neurons of the ventral premammillary nucleus (PMV), dorsomedial nucleus (DMH), and the ARC greatly influences leptin's action. Ablation of LepR in these neurons – of which 20–30% are GABAergic – leads to hyperphagic obesity, decreased energy expenditure, and hyperglycemia [76] comparable to that of constitutive LepR-null mice. Strikingly, these mice show only a slight delay in reproductive maturation and maintain normal fertility. These findings suggest that, indeed, the metabolic vs. reproductive role of leptin may lie in distinct neuronal populations, yet all seem to be predominantly GABAergic. Then, where in the brain is the reproductive role of leptin relayed? A number of recent studies have posited the PMV as the holder of this action. Animals with a lesioned PMV have impaired reproductive function and leptin is no longer able to induce LH release [35,77]. Interestingly, LepR-expressing neurons from the PMV may exert a direct action on GnRH neurons as contacts between the two populations of neurons have been identified [78] (Fig. 1). However, the documented inhibition of *Kiss1* mRNA in congenitally leptin-deficient mice and the increased *Kiss1* mRNA in the ARC and improved gonadotropin levels following leptin administration suggest effects at the level of *Kiss1* neurons as well [34,79]. Furthermore, we have recently observed that LepR ablation from GABAergic neurons is accompanied by decreased kisspeptin input, and the hypogonadotropic state is rescued by exogenous kisspeptin [66]. These data argue against GnRH neurons as the exclusive target of the PMV neurons and strongly suggest that *Kiss1* neurons, at least the population in the ARC (i.e., KNDy neurons), convey metabolic information [78].

Conclusion

The development of new and powerful genetic tools in recent years is allowing an unprecedented increase in our knowledge of the neuronal interactions that govern energy homeostasis and its influences on reproductive maturation and function. Interestingly,

although both functions are critical for the survival of an individual and appear very tightly interconnected, mounting data suggest the existence of different neuronal circuits behind each function though, eventually, the metabolic influences on reproduction converge at the level of KNDy neurons. Admittedly, branches from both axes exist and may interact upstream of KNDy neurons to, perhaps, favor a tuned and coherent response of kisspeptins and, hence, GnRH release (Fig. 1). As new neuropeptides and neuronal interactions are being discovered, the hierarchical characterization of these routes will offer a more mechanistic view of these interactions that will, perhaps, allow us to target specific neurons to facilitate, overcome, or treat a number of prevailing metabolic and/or reproductive conditions. For instance, studies have shown that women with hypothalamic amenorrhea who are treated with leptin can have restoration of pulsatile LH secretion and menstrual cyclicity; however, this treatment may also result in further weight loss in these women [80]. In these situations, separation of the effects of metabolic pathways on energy homeostasis from those on reproductive function might allow restoration of reproductive function without causing worsening of the metabolic homeostasis. Similarly, conditions of severe obesity or insulin resistance may present with associated hypogonadotropic hypogonadism. Currently, the use of kisspeptin administration to reverse these reproductive impairments is being explored [81,82] and kisspeptin analogues are being developed to facilitate their therapeutic administration.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 363–364).

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Key Points

- Sufficient energy stores are necessary for reproductive success.
- Hypothalamic Kiss1 neurons are essential conduits of the energy status to GnRH neurons, although they do not appear to be direct targets of metabolic factors.
- Compelling evidence suggests that metabolic factors act on distinct hypothalamic centers to exert their metabolic vs. reproductive actions.

Figure 1.

Schematic representation of the neural interactions between metabolic and reproductive functions depicting the likely sites of action of leptin, insulin, and ghrelin to control gonadotropin-releasing hormone (GnRH) release. 3V, third ventricle; ARC, arcuate nucleus; ME, median eminence; PMV, ventral premammillary nucleus; POA, preoptic area.