

Neuropeptide Signaling in the Integration of Metabolism and Reproduction

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Key Words

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Abstract

Fertility is gated by nutrition and the availability of stored energy reserves, but the cellular and molecular mechanisms that link energy stores and reproduction are not well understood. Neuropeptides including galanin-like peptide (GALP), neuropeptide Y (NPY), products of the proopiomelanocortin (POMC; e.g., α -MSH and β -endorphin), and kisspeptin are thought to be involved in this process for several reasons. First, the neurons that express these neuropeptides all reside in the hypothalamic arcuate nucleus, a critical site for the regulation of both metabolism and reproduction. Second, these neuropeptides are all targets for regulation by metabolic hormones, such as leptin and insulin. And third, these neuropeptides have either direct or indirect effects on feeding and metabolism, as well as on the secretion of gonadotropin-releasing hormone (GnRH) and LH. As the target for the action of metabolic hormones and sex steroids, these neuropeptides serve as molecular motifs integrating the control of metabolism and reproduction.

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Energetics and Reproduction

Species viability is predicated on reproductive success. For individual animals, the caloric demands of reproduction must be weighed against those deemed essential for immediate survival, which include thermoregulation, sensory function, and locomotor activity. Any extra energy is stored primarily as fat and glycogen, which are titered along with glucose to allow growth and reproduction. Reproducing without enough energy reserves to insure the survival of offspring would be counterproductive. To prevent this from happening, the reproductive system must monitor energy status and limit fertility to times of adequate energy reserves. Furthermore, since the time between conception and the emergence of viable offspring can take many months, some species incorporate predictive mechanisms to increase the probability of success. These mechanisms limit reproductive activity to times of the year when birth and nourishment of offspring are optimal. These strategies prevent the expenditure of energy on reproductive efforts that are risky and costly, conserving energy so that reproductive outcomes are maximized [1].

An animal's energy stores depend not only on the availability of energy sources (food), but also on energy expenditure. Conditions that lead to excessive locomotor activity drain energy reserves and generally suppress re-

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production [2]. Lactation, like locomotion, is also energetically demanding, and delivering milk to hungry offspring taxes the metabolic system. The postpartum period is calorically expensive, and the caloric requirements of lactation tap the reserves of stored adipose tissue, which are typically augmented during gestation. It is energetically disadvantageous to incur the energetic demands of pregnancy while lactating, and, although there are examples of animals becoming pregnant while nursing, these pregnancies are usually suspended in either the preimplantation or earlier embryonic stage. One reproductive strategy that resolves this conflict between the energetic demands of pregnancy and lactation is 'lactational infertility'. In most female mammals, ovulation, mating, and pregnancy are blocked during lactation [3]. Although the basic principle that reproductive activity is carefully guarded by physiological mechanisms that couple fuel availability to sexual activity is generally accepted, the molecular mechanisms that mediate this process remain poorly understood [2, 4–6].

Metabolic Hormones Linking Metabolism and Reproduction

Several metabolic hormones are recognized as important signals that link fuel reserves and reproduction. *Leptin* is an adipocyte-derived hormone, which has profound effects on feeding, thermogenesis, glucose and lipid metabolism, as well as physical activity. Plasma levels of leptin are directly proportional to fat reserves, such that declining levels of leptin trigger feeding behavior, slow metabolism, and help to conserve metabolic reserves. Animals with congenital deficiencies in either leptin or its receptor eat voraciously and become obese and hypothermic. The administration of exogenous leptin can readily reverse this phenotype. Leptin is not only important in the control of feeding and metabolism, but also appears to serve as an important signal to the reproductive system [7, 8]. In well-fed animals with normal circulating levels of leptin, the effects of additional leptin on the reproductive axis are subtle; however, in malnourished animals, the effects of leptin on reproduction can be profound [9].

Animals lacking either leptin or its receptor fail to undergo normal pubertal maturation and remain sexually infantile for their entire lives [10–12]. Leptin stimulates gonadotropin-releasing hormone (GnRH) and gonadotropin secretion, and administration of exogenous leptin to leptin-deficient animals (e.g., *ob/ob* mice), which are

reproductively incompetent, can rescue their impaired sexual function [13–15]. These findings suggest that the body interprets circulating levels of leptin as an indicator of a metabolic state, which may then act as a gate to control the activity of the reproductive axis.

Although leptin's ability to rescue metabolic and reproductive function in animals and humans with leptin deficiency is well documented [9, 13, 14], the cellular and molecular targets of leptin's action are still not completely known. Studies aimed at elucidating the targets of leptin action have shown that GnRH antiserum blocks the effect of leptin effect on LH secretion, but GnRH neurons themselves do not express the leptin receptor [16, 17]. Thus, leptin has little or no direct effect on LH secretion in the absence of GnRH, and it would appear that the action of leptin on the neuroendocrine reproductive axis is mediated by one or more populations of afferent inputs to GnRH neurons that do express the leptin receptor.

Insulin has also been directly implicated in the integration of metabolism and reproduction. Insulin is an anabolic hormone produced and secreted by the pancreas in response to glucose. Insulin promotes cellular intake and storage of energy from ingested food, while simultaneously inhibiting the utilization of stored energy. The secretion of insulin fluctuates throughout the day, with plasma levels increasing immediately following a meal and decreasing between meals. Although plasma levels of insulin vary periodically, the basal plasma level of insulin is proportional to the amount of adipose tissue. Because this steady-state level of insulin reflects the status of stored energy reserves, circulating insulin levels may be used to communicate information about long-term metabolic conditions to the reproductive axis [6]. Insulin has been shown to regulate GnRH and LH secretion. In obesity, animals become insulin-resistant, which disrupts insulin signaling. Pulsatile LH secretion is inhibited in insulin-deficient states (e.g., fasting and diabetes), and central (into the cerebral ventricle) administration of insulin can reverse the deficiency in LH under these conditions [4, 5, 18, 19].

As in the case of leptin or leptin receptor deficiency, animals that lack proper insulin signaling become obese and have disordered metabolism and reproduction [20]. Diabetic animals display a panoply of reproductive deficiencies – including delayed pubertal maturation, reduced ovulation, infertility, disrupted estrous cycles, absent or delayed LH surges (and pulsatile GnRH/LH secretion), as well as impaired sexual behaviors [21–27]. These deficits can be either reversed or ameliorated by insulin administration [23, 24, 28–30], thus testifying to the im-

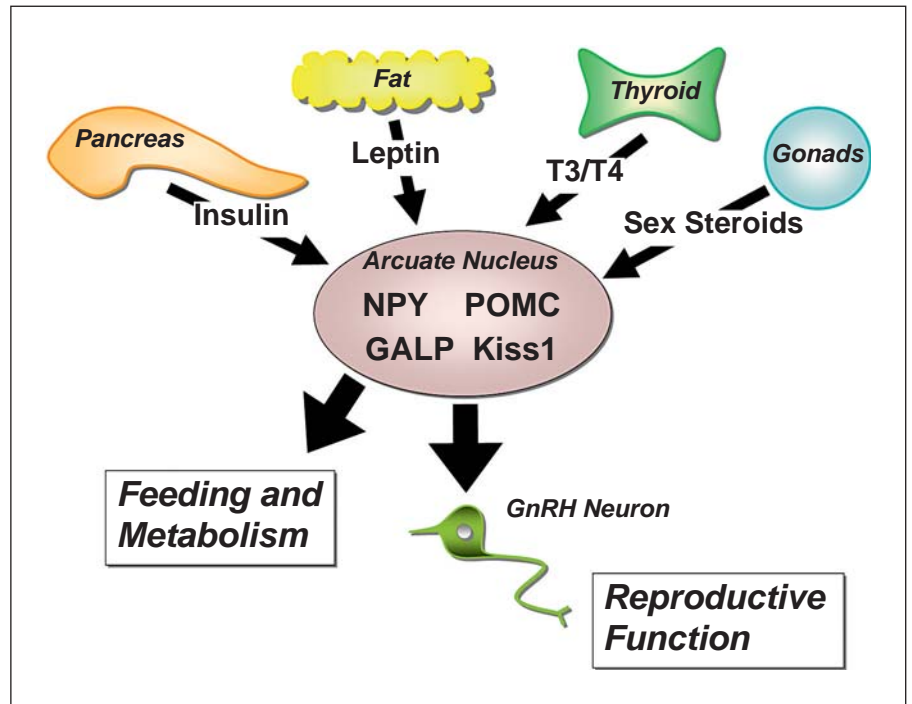


Fig. 1. Metabolic status gates reproduction to guarantee that attempts to reproduce occur only under favorable energetic conditions. Kisspeptin, NPY, GALP, and POMC neurons in the hypothalamic arcuate nucleus are targets for metabolic hormones, such as leptin and insulin, and these circuits regulate metabolism, feeding and reproduction to ensure efficiency and success.

portance of insulin signaling for normal reproductive function.

Thyroid hormone is also critical for growth, metabolism and reproduction. Abnormalities in circulating levels thyroid hormone, hyperthyroidism and hypothyroidism, are associated with metabolic and reproductive deficiencies [31–33]. Hyper- and hypothyroid individuals have reduced levels of available energy [6]. Hyperthyroidism inhibits sexual behavior in rodents [31, 32], and lambs that are hyperthyroid have stunted growth, decreased LH and testosterone secretion, and impaired gonadal function [34]. Hypothyroidism is associated with impaired menstrual cyclicity and disrupted follicular development in females [33, 35]. These conditions resulting from hypothyroidism are ameliorated by the administration of thyroid hormone, suggesting that thyroid hormone, along with leptin and insulin, should be counted among those humoral factors that relay important information about metabolism to the reproductive axis. Thyroid hormone has also been implicated in the seasonal regulation of reproduction, with thyroid hormone secretion increasing during the breeding season in some species [36] – further evidence that metabolic status and reproductive activity are inexorably intertwined.

It is also plausible that other metabolic hormones, besides leptin, insulin, and thyroid hormone – such as *adiponectin* – may influence the neuroendocrine axis, pos-

sibly through direct actions on the pituitary [37]. The complete constellation of metabolic hormones that influence the brain and pituitary has yet to be fully elucidated.

Neuropeptides as Central Processors for Integrating Metabolism and Reproduction

GnRH neurons are the final common pathway through which the brain regulates reproduction (fig. 1), although these cells are not direct targets for metabolic signals [6]. Gonadal steroids also regulate GnRH secretion through an indirect action on sex steroid-sensitive afferent inputs to GnRH neurons. Neurons that contain receptors for metabolic hormones and send afferent inputs to GnRH neurons are likely to be responsible for sensing the metabolic milieu and controlling GnRH secretion as a function of fuel availability and fat reserves. Candidates for serving this integrative function include neurons that express galanin-like peptide (GALP), neuropeptide Y (NPY), proopiomelanocortin (POMC) and its processed derivatives (e.g., α -MSH and β -endorphin), kisspeptin, and possibly orexin [38, 39], as well as catecholaminergic neurons that reside in the brainstem and project to the hypothalamus [40].

GALP is expressed in the arcuate nucleus of the hypothalamus, and its expression is regulated by leptin and insulin [41–44]. GALP neurons express the leptin receptor, ObRb, and thus would appear to be direct targets for the action of leptin. The central administration of GALP stimulates GnRH/LH secretion in the rat, mouse, and monkey, and GALP-containing fibers are found in close proximity to GnRH neurons [45, 46]. The expression of GALP mRNA is reduced by fasting and diabetes, and the deleterious effects of diabetes on reproductive function can be reversed (or attenuated) by the administration of GALP [8, 47, 48]. Thus, GALP neurons are poised to serve as cellular conduits coupling the physiological systems that regulate metabolism and reproduction.

NPY is among the most abundant peptides in the central nervous system, and it plays a key role in energy homeostasis. NPY neurons in the hypothalamus are activated by fasting, stimulate hunger and food-seeking behavior and also regulate thermogenesis, peripheral insulin secretion, and hepatic glucose output [49]. The observation that antiserum to NPY and NPY mRNA anti-sense oligodeoxynucleotides (administered centrally) diminish food consumption and reduce body weight is consistent with an orexigenic role for NPY [50, 51]. The expression of NPY mRNA in the hypothalamus is reduced by leptin [52, 53], and NPY-expressing neurons also express the leptin receptor, suggesting that they are direct targets for the action of leptin [16, 54]. Mice bearing targeted deletions of *NPY* have only subtle phenotypic abnormalities [55, 56], but leptin-deficient *ob/ob* mice also bearing targeted deletions of *NPY* eat less and are leaner than ordinary *ob/ob* mice. This testifies to the complex involvement of NPY in modulating feeding behavior. The activity of NPY neurons is also regulated by insulin, which like leptin, inhibits the expression of NPY mRNA [57, 58]. Moreover, elevations in circulating levels of insulin reverse the increase in NPY mRNA associated with fasting [57]. Thus, NPY neurons serve as an important relay center in the brain for the regulation of feeding and metabolism. NPY also influences GnRH and gonadotropin secretion, although effects depend upon the steroid milieu. For example, in intact animals and steroid-primed ovariectomized animals, the central administration of NPY administration stimulates GnRH and LH secretion [59, 60]. The expression of NPY is increased in intact female rats during the afternoon and evening of proestrus [61], and similarly, NPY gene expression is increased in ovariectomized animals just before the steroid-induced LH surge [62]. The stimulatory effects of NPY on GnRH and LH secretion are limited in scope, as

chronic administration of NPY causes delayed sexual maturity and disruption of estrous cycles [63, 64], and in some species (e.g., sheep), NPY has a predominantly negative effect on reproductive function [65].

The importance of the role of NPY in the regulation of reproduction is reaffirmed by a study of *NPY* KO mice. The pituitary glands of these animals are less responsive to GnRH than normal controls [66]. Both male and female *NPY* KO mice display reduced mating behaviors [67], but remain fertile and do not manifest abnormalities in body weight, feeding behavior, or endocrine function [68]. This apparent lack of significant metabolic and reproductive abnormalities may reflect compensatory adaptation during development of the existence of redundant pathways for controlling these complex processes [55, 56]. It is also possible that NPY plays a significant role in metabolism and reproduction *only* in states of low energetic reserves. In this regard, it is notable that, although serum levels of LH are reduced by fasting in normal animals, a similar response is not seen in *NPY* KO mice [69]. Thus, NPY neurons are likely to act as central *integrators* of metabolism and reproduction.

POMC-expressing neurons in the arcuate nucleus may also contribute to the governance of the intersection of metabolism and reproduction. The expression of *POMC* mRNA is reduced by fasting, and other leptin and insulin-deficient states, such as diabetes and impairment of leptin signaling [70–74]. Additionally, *POMC* neurons in the arcuate nucleus are direct targets for the action of leptin [7, 74], which stimulates *POMC* gene expression. The various *POMC* gene products have differential effects on the feeding and reproductive axes, but precisely how different peptide products of the *POMC* precursor are drafted into their respective functions remains unknown. For example, on the one hand, α -MSH reduces food consumption and stimulates lordosis behavior in female rats [75, 76]. On the other hand, β -endorphin stimulates food consumption and inhibits GnRH/LH secretion [77–81]. Despite the complexity, *POMC* neurons in the arcuate nucleus reside at the anatomical and physiological juncture where metabolism and reproduction are coordinated – and learning more about the processing of this complex protein could shed light on the enigmatic mechanisms by which metabolism and reproduction are controlled.

Kiss1-Kisspeptin-GPR54 Pathway as a Linchpin Coupling Metabolism and Fertility

Another possible link between metabolism and reproduction is the neuropeptide, *kisspeptin*. Kisspeptin is encoded by the *Kiss1* gene and signals through the GPR54 receptor. The kisspeptin/GPR54 signaling system is necessary for normal reproduction. Mutations in *GPR54* result in complete disruption of reproductive function in both humans and mice [82, 83]. Centrally administered kisspeptin stimulates GnRH neurons in the mouse, rat, sheep, and primate [84–86]. GPR54 is expressed by virtually all GnRH neurons, indicating that the cells are direct targets for kisspeptin [85, 87]. *Kiss1* neurons are also direct targets for the action of sex steroids, which regulate the expression of *Kiss1* mRNA [88, 89]. The activation of *Kiss1* gene expression is likely to play an important role in timing the onset of puberty, sexual differentiation of the GnRH/LH surge mechanism, and the preovulatory GnRH/LH surge itself (in females) [84, 86, 90–94]. Thus, *Kiss1*/kisspeptin/GPR54 signaling is critically involved in virtually all aspects of neuroendocrine reproductive function.

Kiss1/kisspeptin neurons also have direct links to the mechanisms that regulate metabolism. Fasting is associated with inhibition in the expression of *Kiss1* mRNA (and decreased circulating levels of leptin) [95]. Central injections of kisspeptin can reverse the fasting-induced inhibition of GnRH secretion [95]. Likewise, kisspeptin administration rescues GnRH decline in rats that are treated with leptin antibodies [96]. These findings suggest that kisspeptin is involved in relaying metabolic signals to the neuroendocrine reproductive axis.

The expression of *Kiss1* mRNA is also regulated by leptin. Leptin-deficient *ob/ob* mice have reduced levels of

Kiss1 mRNA compared to wild-type controls, and the central administration of leptin partially reduces this effect [97]. Further support of the notion that the cells are regulated by leptin is the observation that 40% of *Kiss1* cells in the arcuate nucleus express the signaling version of the leptin receptor, ObRb [97]. Kisspeptin also interacts with the insulin signaling pathway in the hypothalamus. Rats that are rendered diabetic have diminished expression of *Kiss1* mRNA, which can be reversed with insulin therapy [96]. Thus, together with GALP, POMC, and NPY neurons, *Kiss1* neurons are likely to serve as cellular conduits for relaying information about circulating levels of leptin and insulin to the neuroendocrine reproductive axis.

The cellular and molecular basis for the integration of metabolism and reproduction involves a complex interaction of hypothalamic neuropeptides with metabolic hormones, fuels, and sex steroids. Kisspeptin is unlikely to be the last of the neuropeptides discovered having relevance to both metabolic regulation and reproductive function – just as leptin, insulin, and thyroid hormone are not the full cast of metabolic hormones with actions on the neuroendocrine reproductive axis. Understanding this integrative process will require careful mapping of hypothalamic and brainstem circuitry, cataloguing of receptor expression profiles within these circuits, and a detailed analysis of the action of metabolic hormones on these pathways. There is much work ahead.

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