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Hypothalamic pathways linking energy balance and reproduction

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Hill JW, Elmquist JK, Elias CF. Hypothalamic pathways linking energy balance and reproduction. *Am J Physiol Endocrinol Metab* 294: E827–E832, 2008. First published February 19, 2008; doi:10.1152/ajpendo.00670.2007.—During periods of metabolic stress, animals must channel energy toward survival and away from processes such as reproduction. The reproductive axis, therefore, has the capacity to respond to changing levels of metabolic cues. The cellular and molecular mechanisms that link energy balance and reproduction, as well as the brain sites mediating this function, are still not well understood. This review focuses on the best characterized of the adiposity signals: leptin and insulin. We examine their reproductive role acting on the classic metabolic pathways of the arcuate nucleus, NPY/AgRP and POMC/CART neurons, and the newly identified kisspeptin network. In addition, other hypothalamic nuclei that may play a role in linking metabolic state and reproductive function are discussed. The nature of the interplay between these elements of the metabolic and reproductive systems presents a fascinating puzzle, whose pieces are just beginning to fall into place.

gonadotropin-releasing hormone; leptin; insulin; kisspeptin

SINCE ANIMALS UNDER METABOLIC STRESS must invest energy in survival first and reproduction second, the reproductive axis has the capacity to respond to changes in caloric status. Indeed, every level of the reproductive axis, the hypothalamus, pituitary gland, and gonad, has the capacity to respond to metabolic cues. In humans, anorexia, cachexia, and excessive exercise can all shut down reproductive cyclicity and the secretion of gonadal steroids that are essential for the health of many organs and tissues (28, 57). On the opposite end of the spectrum, obesity and diabetes can also negatively affect fertility (74, 92). The mechanisms regulating these processes are not well understood, but recent work has begun to yield new insights.

While long recognized, the exact nature of the relationship between energy stores and fertility has been somewhat controversial. Work in rodent models (55) and human subjects (40, 41) gave rise to the idea that a female's fat reserves must exceed a critical threshold for ovulation to occur. However, it has now become clear that the body allocates energy based on current energy balance as opposed to the absolute amount of stored adipose tissue. Ovulation is suppressed when a mammal is in negative energy balance whether that state is caused by inadequate food intake, excessive locomotor activity, or heavy thermoregulatory costs. In the mouse, ovulation occurs whenever extant energetic conditions permit, unless the process is blocked by nonmetabolic stress, social cues, or a predictive seasonal cue such as photoperiod. Mice in the wild often

continue to ovulate and become pregnant during seasons of famine despite lacking the energy reserves to carry a litter to term (12). In the human, menstrual irregularities, amenorrhea, and infertility can result from inadequate food intake to compensate for energy demands; for instance, when a severe athletic training schedule is relaxed, luteinizing hormone (LH) pulses and menstrual cycles resume without a significant increase in body fat content (2, 31). The sensitivity of the reproductive axis to current energy availability has been highlighted by recent work suggesting that even subtle declines in energy availability can produce clinically recognized menstrual disturbances (29).

The hypothalamus plays a crucial role in maintaining fertility in all mammals. The GnRH neurons within the preoptic area control the secretion of pituitary LH via the pulsatile release of GnRH from their terminals in the median eminence into the hypophysial portal blood vessels. On a minute-to-hourly basis, the GnRH pulse generator is extremely sensitive to energetic stress. GnRH pulses are readily suppressed by food restriction, high or low ambient temperature, or excessive exercise, and GnRH pulsatility returns rapidly when the energetic challenge is alleviated, usually in one to two hours. Both males and females of a large number of species exhibit this suppression despite the fact that a temporary halt to spermatogenesis is unlikely to have any long-term reproductive consequences in the male (11, 39, 73).

Circulating Leptin and Insulin Affect Fertility

Insulin. Central control of reproduction requires the hypothalamus to receive information regarding the energy status of an animal, for example by sensing hormonal signals secreted into the circulation in proportion to body adipose stores. Woods and Porte originally suggested a role for insulin in the central regulation of energy homeostasis based on the observation that insulin levels circulate in proportion to adipose tissue in most mammals (97). They demonstrated that intracerebroventricular insulin administration results in a dose-dependent reduction in food intake and body weight. Following the advent of gene targeting techniques, neuron-specific deletion of insulin receptors (NIRKO mice) was found to lead to increased body fat deposition and hypothalamic hypogonadism (infertility due to reduced GnRH release) (13), confirming that insulin sensing in the brain is required for normal reproduction. These actions may be mediated by direct insulin action on GnRH neurons or by altering input from secondary insulin sensitive neurons. One critical, unsettled question is whether GnRH neurons express insulin receptors (IRs) in vivo. To date,

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the only evidence supporting that assertion comes from conditionally immortalized GnRH-expressing cell lines that have been reported to be insulin responsive (78). Given the inherent limitations of extrapolating data from cell lines to animals, *in vivo* data must be obtained to settle this issue. While standard approaches to this question are available, genetic techniques now allow the targeted deletion of receptors from specific neuronal subtypes. It is hoped that the phenotype of mice lacking IR expression in GnRH neurons will soon be reported.

Leptin. The cloning of the *ob* gene in 1994 by Friedman and associates resulted in the discovery of another physiologically important adiposity signal secreted by fat tissue: leptin (100). Mice and humans lacking leptin (*ob/ob* mice) or the leptin receptor (*db/db* mice) develop hyperphagic morbid obesity and insulin resistant diabetes (22). Moreover, sufficient levels of leptin are a prerequisite for successful reproduction. Leptin administration blunts the fasting-induced suppression of LH secretion and fertility (44, 58, 69, 70). In women with exercise- or anorexia-induced amenorrhea, leptin treatment increases pulse frequency and levels of LH, ovarian volume, number of dominant follicles, and estradiol levels (64, 95). Furthermore, *ob/ob* mice have low LH levels and are infertile, and leptin administration, but not weight loss alone, restores their fertility (5, 19, 68, 101). Expression of leptin receptor (LepR) in the brain of *db/db* mice or mice otherwise null for LepRs restores fertility completely in males and partially in females (26, 60).

These studies support a role for central LepRs in modulating GnRH release. However, despite initial reports showing expression of LepRs in immortalized GnRH cell lines, it is now recognized that GnRH neurons do not physiologically express LepRs (14, 38, 49). Instead, leptin is believed to act indirectly via interneurons impinging on GnRH-secreting cells in the hypothalamus (24, 90), although the identity of such interneurons is unclear. A better understanding of the neural circuitry underlying leptin signaling in the hypothalamus is critical not only for the advancement of our knowledge of the connection between metabolism and reproduction but also for future development of treatment strategies for hypothalamic hypogonadism.

Leptin and Insulin Sensing in Hypothalamic Neurons

The role of the arcuate nucleus of the hypothalamus in regulating energy balance is well established. The arcuate nucleus contains proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART)-expressing neurons, whose activation suppresses feeding. In contrast, the activation of a second population of arcuate neurons, neuropeptide Y/agouti-related protein (NPY/AgRP)-expressing neurons, stimulates feeding (6, 33). The coordinated regulation of these neurons and their downstream projections to key brain regions contributes to control of energy balance. These targets include the paraventricular nucleus of the hypothalamus, the lateral hypothalamic area, and other autonomic and neuroendocrine control sites (32).

IRs are expressed in the medial portion of the arcuate nucleus where NPY/AgRP-expressing neurons are located (8). Indeed, insulin affects the expression of NPY; in fasted animals, intracerebroventricular (icv) administration of insulin decreases NPY mRNA in the arcuate nucleus and NPY peptide

in the paraventricular nucleus of the hypothalamus (79, 93). Insulin-deficient diabetic rats show increased hypothalamic levels of both NPY and its mRNA that are normalized by systemic insulin therapy (1, 80). High numbers of IRs are also found on POMC/CART neurons (9). Interestingly, no obvious metabolic or reproductive phenotype was seen in mice lacking IRs only in POMC neurons (59).

Although LepRs are expressed in many hypothalamic nuclei (34, 36, 65), significant attention has been given to neurons located in the arcuate nucleus. There, LepRs are expressed by both NPY/AgRP and POMC/CART neurons (7, 20, 34) and are required for maintaining a normal body weight (4, 67). It is important to note that insulin and leptin share some, but not all, overlapping intracellular signaling pathways and may thereby exert similar effects. Much ongoing work is dedicated to untangling the contribution of these pathways to the actions of leptin and insulin (71, 75, 98).

Evidence is accumulating that leptin has key targets outside the arcuate nucleus (47, 52). LepR is found in other hypothalamic nuclei, including the dorsomedial subdivision of the ventromedial nucleus (VMH), the caudal subdivision of the dorsomedial nucleus (DMH), the premammillary ventral nucleus (PMV), and, in a small extension, in the paraventricular nucleus (34, 36, 65). Indeed, neurons expressing LepR in the VMH also respond to glucose and insulin (15), but the pathways downstream of VMH neurons responsive to metabolic signals are not yet identified. In addition, the DMH and the PMV strongly innervate areas related to reproductive control, including the anteroventral periventricular nucleus (AVPV) and the medial preoptic area (16, 76, 88, 89). However, whether these projections originate from neurons responsive to leptin and are physiologically relevant to leptin action in reproductive control remains unsettled.

Mediobasal Hypothalamic Circuits Linking Metabolic State with Reproduction

Evidence suggests that the neurons involved in regulating energy metabolism can communicate with the hypothalamic-pituitary-gonadal (HPG) axis via interactions with GnRH neurons. NPY fibers are intimately associated with the dendrites and cell bodies of GnRH neurons in the medial preoptic area (48), and NPY fibers in the median eminence may also act on GnRH terminals (77). NPY neurons therefore have been considered good candidates to operate as neuroendocrine integrators, linking perturbations in energy balance and alterations in the activity of the reproductive axis. According to this model, NPY neurons are activated under conditions of negative energy balance, leading to an increase in NPY release from terminals in the paraventricular nucleus and preoptic area. Increased NPY release may then stimulate feeding behavior while inhibiting release of GnRH and activity in the pituitary-gonadal axis. In support of this hypothesis, a suppression of basal LH levels by fasting fails to occur in the NPY knockout female (50). In addition, *ob/ob* mice that are also NPY deficient display improved fertility compared with *ob/ob* controls (35).

However, pharmacological evidence in rats (23), rabbits (56), and monkeys (96) provides a compelling case for the existence of two mechanisms, one inhibitory and one stimulatory, through which endogenous NPY regulates the GnRH pulse generator. In numerous species, NPY has negative effects

on GnRH levels, pulse amplitude, and pulse frequency in an environment of low estradiol and progesterone, such as in unprimed, ovariectomized animals (3). In these animals, steroid replacement switches the effect of NPY to a robust positive one (10, 91). Thus, surprisingly, NPY appears to be required for GnRH surge production; LH surges are attenuated after NPY immunoneutralization or Y1 receptor blockade on the day of proestrus (48, 53). In addition, NPY knockout females show a 70% reduction in their LH surges (99). This dual effect also explains why food deprivation initiated on the day of estrus is much less effective in suppressing ovulation than fasting begun on diestrus, when estrogen levels are low (12). Exciting new findings have begun to shed light on this paradox. In hypothalamic cell lines, the ratio of estrogen receptor- α (ER α) to - β (ER β) regulates the expression of NPY gene in response to estrogen treatment. Whereas ER α favors the suppression of NPY gene, ER β stimulates peptide expression (91). The critical next step is to determine whether the ratio of ER subtypes in the hypothalamus varies across the estrous cycle. Alternatively, GABA release by a subpopulation of NPY neurons (51) may affect GnRH secretion, as all GnRH neurons express GABA_A receptors (21, 72). Interestingly, GABA release can hyperpolarize or depolarize GnRH neurons (30, 66) in agreement with opposing NPY effects on LH secretion. The origin of the GABAergic inputs to GnRH neurons is not known, and therefore further studies are necessary in order to explore NPY/GABA interaction modulating GnRH secretion.

POMC/CART neurons may also be involved in conveying metabolic status to GnRH neurons. POMC/CART-producing neurons in the arcuate nucleus project to the medial preoptic area, and terminals of POMC products (β -endorphin and α -MSH) and CART make apparent synaptic contact with GnRH-immunoreactive cells (63, 76), although the expression of melanocortin and/or opioid receptors in GnRH cell bodies is not well established. However, the various POMC gene products have differential effects on the reproductive axis; for example, α -MSH reduces food consumption and stimulates lordosis behavior in female rats (45, 81), whereas β -endorphin stimulates food consumption and inhibits GnRH/LH secretion (43, 62, 94). Further studies are needed to elucidate the precise role of these neuronal populations.

Finally, the overlapping intracellular signaling pathways of leptin and insulin raise the question of whether joint signaling by insulin and leptin in POMC and NPY cells contributes to the control of GnRH release. Although genetically ablating receptors for leptin or insulin in NPY or POMC neurons results in no obvious reproductive phenotypes (4, 59), these results may underrepresent the combined contribution of these adiposity signals to the maintenance of fertility. Double-knockout studies are needed to address this question.

Kisspeptin

Recently, the description of G protein-coupled receptor-54 (GPR54) and the cognate ligands, kisspeptins, has brought an avalanche of new findings into the reproductive field (37, 54, 83). KiSS-1 is expressed by neurons located in several hypothalamic nuclei, including the AVPV, a key site for the regulation of gonadotropin secretion, and the arcuate nucleus (46). The GPR54 receptor is expressed by GnRH neurons, and its

mutation causes hypogonadotropic hypogonadism in humans and mice (25, 27, 42, 61, 82). Fasting causes a reduction in the amount of KiSS-1 mRNA (17), which precedes the fasting-induced decline of GnRH. In the mouse, icv administration of kisspeptin evokes LH and FSH secretion at remarkably low doses (46).

In both the AVPV and arcuate nucleus, KiSS-1 is regulated by sex steroids (83–85). Estrogen and androgen receptors (ER α and AR, respectively) are found in a high percentage of KiSS-1-expressing neurons, and estradiol and testosterone increase KiSS-1 expression in the AVPV and decrease KiSS-1 expression in the arcuate nucleus. It is believed that kisspeptin has distinct actions on GnRH release depending on the steroid hormone milieu, mediating the negative feedback of sex steroids on GnRH secretion via neurons in the arcuate nucleus and the positive feedback of sex steroids via AVPV neurons (85). However, the requirement for AR and ER α in mediating the feedback effects of sex steroids on Kiss-1 expression remains to be confirmed.

Kisspeptin appears to play a role in the reproductive effects of leptin. LepRs have been found in over 40% of Kiss-1 neurons in the arcuate nucleus. Compared with wild-type mice, obese *ob/ob* male mice show decreased expression of KiSS-1 in the arcuate nucleus, which is restored by leptin treatment (85). In a recent study, investigators showed that in diabetic rats KiSS-1 mRNA is decreased in the hypothalamus. These animals exhibit low circulating levels of leptin, insulin, and LH (18). Administration of kisspeptin restored LH and testosterone secretion and icv leptin, but not insulin, and normalized KiSS-1 mRNA levels in the hypothalamus as well as circulating levels of LH. Although KiSS-1 and LepR are coexpressed in the arcuate nucleus, the specific hypothalamic sites where leptin acts to stimulate KiSS-1 in this paradigm are not known.

Thus far, the results are consistent with a model whereby leptin and perhaps other adiposity and satiety factors stimulate KiSS-1 expression, triggering production of kisspeptin and stimulation of GnRH release. It is tempting to speculate that

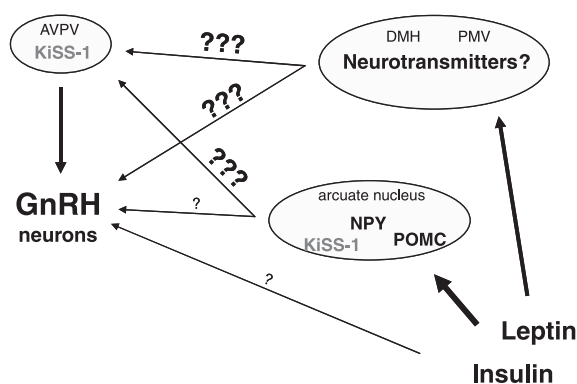


Fig. 1. Schematic representation of candidate brain pathways mediating leptin and insulin actions in reproductive control. Leptin and insulin receptors are distributed in a variety of brain nuclei, but the connections with areas related to reproductive control including the anteroventral periventricular nucleus (AVPV) and GnRH neurons are unknown. Moreover, although the arcuate nucleus neurons expressing proopiomelanocortin (POMC) or neuropeptide Y (NPY) have been extensively investigated, their projections to the AVPV are not described, and the innervation of GnRH neurons is still controversial. A few studies have also suggested the direct action of insulin in GnRH neurons, but these findings need to be replicated. DMH, dorsomedial nucleus; PMV, premammillary ventral nucleus.

reproductive deficits associated with leptin-deficient states may be attributable to diminished expression patterns of KiSS-1 or its receptor. Nevertheless, whether leptin is solely acting on KiSS-1 neurons in the central control of GnRH or whether additional peripheral regulators cooperate with leptin in the control of KiSS-1 for the integration of energy balance remains to be elucidated. The use of genetic mouse models that allow the deletion or reactivation of KiSS-1 gene in specific neurons will provide crucial access to this pathway.

Summary

We have entered an exciting era for the study of metabolic regulation of reproductive function. Our knowledge of the hypothalamic circuitry involved in monitoring energy balance and providing input to GnRH neurons continues to expand rapidly. We have discussed only a few of the neuropeptides and hormones involved, and no doubt more are waiting to be discovered. A major goal of future research should therefore be not only to discover individual players communicating energy status to the reproductive axis but also to understand how each fits within the neuronal network connecting these two critical systems. Many relationships remain to be elucidated (Fig. 1). Pursuit of these questions will yield a greater understanding of the central control of reproduction and holds out the hope of addressing the clinical impact of impaired fertility and steroid production due to metabolic causes.

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