Published in final edited form as:

Brain Res. 2010 December 10; 1364: 139–152. doi:10.1016/j.brainres.2010.08.038.

The neuroendocrine basis of lactation-induced suppression of GnRH: Role of kisspeptin and leptin

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Abstract

Lactation is an important physiological model of the integration of energy balance and reproduction, as it involves activation of potent appetitive neuropeptide systems coupled to a profound inhibition of pulsatile GnRH/LH secretion. There are multiple systems that contribute to the chronic hyperphagia of lactation: 1) suppression of the metabolic hormones, leptin and insulin, 2) activation of hypothalamic orexigenic neuropeptide systems NPY, AGRP, orexin (OX) and melanin concentrating hormone (MCH), 3) special induction of NPY expression in the dorsomedial hypothalamus, and 4) suppression of anorexigenic systems POMC and CART. These changes ensure adequate energy intake to meet the metabolic needs of milk production. There is significant overlap in all of the systems that regulate food intake with the regulation of GnRH, suggesting there could be several redundant factors acting to suppress GnRH/LH during lactation. In addition to an overall increase in inhibitory tone acting directly on GnRH cell bodies that is brought about by increases in orexigenic systems, there are also effects at the ARH to disrupt Kiss1/neurokinin B/dynorphin neuronal function through inhibition of Kiss1 and NKB. These changes could lead to an increase in inhibitory auto-regulation of the Kiss1 neurons and a possible disruption of pulsatile GnRH release. While the low levels of leptin and insulin contribute to the changes in ARH appetitive systems, they do not appear to contribute to the suppression of ARH Kiss1 or NKB. The inhibition of Kiss1 may be the key factor in the suppression of GnRH during lactation, although the mechanisms responsible for its inhibition are unknown.

1. Introduction

1.1. Characteristics of lactation

Lactation is an ideal physiological model to study profound adaptations in hypothalamic function brought about by naturally occurring mechanisms that serve to integrate reproductive function. There are various adaptations occurring in the lactating female, including large increases in food and water intake (Brogan et al., 1999; Malabu et al., 1994), increases in serum oxytocin and prolactin levels (Tucker, 1994; Woodside, 2007), energy sparing in response to negative energy balance brought about by the energy drain of milk production (Johnstone and Higuchi, 2003; Smith and Grove, 2002; Tucker, 1994), induction of maternal behavior (Bridges, 1994; Furuta and Bridges, 2009), suppression of responses to stress (Brunton et al., 2008; Toufexis et al., 1998), and inhibition of cyclic ovarian function

(Smith and Grove, 2002; Tsukamura and Maeda, 2001). Most of the suckling-induced changes in neuronal and hormonal activity that are responsible for these alterations in brain function remain incompletely defined. With changes in so many systems occurring during lactation, it is difficult to determine which may be directly related to the inhibition of cyclic ovarian function (Smith and Grove, 2002). Pulsatile LH secretion is greatly suppressed in both ovarian intact and ovariectomized lactators (Brogan et al., 1999; Ordog et al., 1998; Taya and Sasamoto, 1991; Xu et al., 2009b). Therefore, even though the ovarian steroid milieu during lactation varies among mammalian species, it does not directly contribute to the suppression of LH secretion. The most likely cause of the suppression of LH secretion is the inhibition of GnRH neuronal activity (Smith and Grove, 2002; Xu et al., 2009a). In considering which changes in hypothalamic function may be involved in the suppression of GnRH/LH, it is well recognized that there are overlapping and coordinated neurobiological systems whose activities are altered during lactation, such as stress and reproduction or energy balance and reproduction. Therefore, it is reasonable to hypothesize that there is redundancy in the multiple hypothalamic systems that are involved in the suppression of GnRH. These redundancies underscore the importance of the integration of pathways regulating energy balance and reproduction.

1.2. Relationship between energy balance and reproduction

The interrelationship between reproductive function and the status of energy balance is well established, such that negative energy balance is associated with a suppression of reproductive function and ovarian cyclicity (see recent reviews, (Castellano et al., 2009; Crown et al., 2007; Hill et al., 2008; Tena-Sempere, 2006). Fasting, anorexia nervosa, cachexia and bulimia are examples of negative energy balance due to hypophagia, whereas lactation and exercise-induced amenorrhea are examples of negative energy balance due to excessive energy expenditure relative to the amount of food intake (Klentrou and Plyley, 2003; Loucks et al., 1998; Marks et al., 2003; Martin et al., 2007; Smith and Grove, 2002). In most examples of negative energy balance, decreased levels of leptin and insulin signal the depleted energy stores in peripheral tissues. There is general agreement that low levels of leptin and insulin are critical signals acting on various sites in the brain to increase the drive to eat (Elmquist and Flier, 2004; Friedman, 2002; Morton et al., 2006; Seeley et al., 2004). An important site of leptin and insulin signaling is at the arcuate nucleus (ARH) through direct actions via their respective receptors on neuropeptide Y (NPY), agouti related peptide (AGRP) and proopiomelanocortin (POMC) neurons (Benoit et al., 2004; Cowley et al., 2001; Cowley, 2003; Schwartz, 2001). The modulatory roles of leptin and insulin on reproductive function are also well established, as animals that lack either leptin or insulin receptor signaling fail to develop normal reproductive function (Barash et al., 1996; Bruning et al., 2000). The central actions of leptin and insulin on reproductive function are thought to occur primarily at the ARH (Bruning et al., 2000; Cheung et al., 2000; Crown et al., 2007; Hill et al., 2008). Therefore, the low levels of leptin and insulin associated with negative energy balance may be critical factors in the adaptive mechanisms to conserve energy: increased feeding behavior, a slowed rate of metabolism and suppression of energy intensive reproductive states (Crown et al., 2007).

With respect to lactation, the relationships among peripheral energy stores, suppressed leptin and insulin, and the increased drive to eat appear to be more complicated. For example, the lactating rat has extremely low levels of leptin and insulin despite increased body weight compared to similarly aged cycling controls ((Xu et al., 2009b) and increased body fat stores during much of lactation. It is interesting that removal of the suckling pups during mid-lactation results in a rapid rise in leptin to above normal levels, perhaps reflecting the elevated body fat stores (Brogan et al., 1999; Xu et al., 2009b). Subsequent reinitiation of the suckling stimulus results in a rapid suppression of leptin to very low levels within 24 h;

however, the suppression only occurs if milk withdrawal is permitted (Brogan et al., 1999). These data suggest that during lactation leptin production is actively being inhibited by factors associated with the energy drain of milk withdrawal, but not by depleted fat stores (Vernon et al., 2002; Woodside et al., 2000). One unique aspect of lactation that could play a role in the suppression of leptin is the suckling-induced activation of the dorsomedial hypothalamus (DMH) (see Section 3.1. for more information about DMH NPY activation); this activation is associated with a suppression of sympathetic drive to the periphery (Chen et al., 2004), most likely through DMH projections to the raphe pallidus area in the brainstem, an area known for integrating sympathetic responses. The decrease in sympathetic drive could result in an inhibition of leptin production by fat tissue. However, at this time, the mechanisms responsible for the active suppression of leptin are unknown. The low levels of leptin may be important in allowing the mother to adapt to the lactating condition, by removing a signal for satiety and facilitating the chronic hyperphagia. In addition to the suppressed levels of leptin and insulin, the physical suckling stimulus itself is a potent stimulus for the increase in food intake. Within hours of initiating suckling, hypothalamic orexigenic neuropeptide systems are activated and food intake is greatly increased, before any significant milk withdrawal or change in energy balance (Chen et al., 2004; Li et al., 1999c; Woodside and Popeski, 1999). It is as if the suckling stimulus initiates homeostatic defense mechanisms in anticipation of an energy drain due to milk production. This hypothalamic anticipatory phenomenon during lactation may be the metabolic equivalent of the “fight or flight” syndrome (Smith and Grove, 2002).

It is likely that many of the signals causing the hyperphagia of lactation are involved in the suppression of cyclic reproductive function (Smith and Grove, 2002; Tsukamura and Maeda, 2001). This review will focus on how the hypothalamus integrates sensory signals from the suckling stimulus and metabolic signals (leptin, insulin) to alter hypothalamic neuronal function regulating energy balance and reproduction.

2. Concepts on the regulation of GnRH neurons—Role of Kisspeptin

Kisspeptin (Kiss1) is considered to be the primary gatekeeper in governing reproductive function through direct control of GnRH activity (see recent reviews: (Kauffman et al., 2007; Oakley et al., 2009; Ohkura et al., 2009b; Roa et al., 2009; Roseweir and Millar, 2009; Tena-Sempere, 2010; Uenoyama et al., 2009). The emerging view of Kiss1 signaling is that it is responsible for the two modes of GnRH secretion: the estrogen-induced ovulatory surge of GnRH/LH and basal, pulsatile GnRH/LH release (Dungan et al., 2007; Roa et al., 2009; Uenoyama et al., 2009). The model is most well developed in the rodent where there are two populations of Kiss1 neurons, one in the anteroventral periventricular nucleus (AVPV) and the other in the ARH. Kiss1 neurons in the AVPV are directly stimulated by the effects of estrogen acting through estrogen receptor α (ER α). These neurons in turn directly activate GnRH neurons through the Kiss1 receptor (GPR54) expressed on the cell bodies. This positive feedback effect of estrogen on AVPV Kiss1 neurons culminates in the GnRH/LH surge. The AVPV Kiss1 neurons are sexually differentiated and largely absent in the male, consistent with the inability of male rodents to mount an LH surge (Kauffman, 2009). In contrast to the positive effects of estrogen on the AVPV Kiss1 neurons, estrogen negatively regulates ARH Kiss1 neurons, also through ER α . ARH Kiss1 neurons are thought to control the negative feedback effects of estrogen on GnRH/LH pulsatile release. Recent studies have shown that the ARH Kiss1 neurons have an interesting complexity due to important co-neuromodulators synthesized and released from these cells. There is now data from several species showing that the ARH Kiss1 cells coexpress neurokinin B (NKB) and dynorphin (DYN) (Foradori et al., 2006; Goodman et al., 2007; Navarro et al., 2009; Wakabayashi et al., 2010), and are referred to as ARH KNDy neurons. The coexpression of Kiss1 with NKB and DYN allows the ARH KNDy population to be differentiated from the AVPV Kiss1

population, so that targets of these neuronal populations can be determined. Neuroanatomical evidence supports the idea that Kiss1 neurons from the AVPV project primarily to the area of GnRH cell bodies, whereas the ARH KNDy population sends major projections into the median eminence to regulate GnRH release (Clarkson and Herbison, 2006; Clarkson et al., 2009; Krajewski et al., 2005; Oakley et al., 2009; Wakabayashi et al., 2010). Therefore, it appears that positive feedback occurs at the level of GnRH cell bodies, whereas negative feedback occurs primarily at the level of GnRH terminals (Kauffman et al., 2007; Oakley et al., 2009; Ohkura et al., 2009b; Roa et al., 2009; Roseweir and Millar, 2009; Tena-Sempere, 2010; Uenoyama et al., 2009).

2.1. Lactation suppresses Kiss1 expression in the ARH and AVPV

Kiss1 mRNA levels are greatly decreased in the ARH and AVPV during lactation in the rat (Xu et al., 2009b; Yamada et al., 2007); the suppression in both these nuclei could be key factors in the inhibition of GnRH. The suppression of *Kiss1* during lactation is similar to findings of suppressed *Kiss1* in the fasted model (Kalamatianos et al., 2008), suggesting that *Kiss1* neurons may be particularly sensitive to metabolic signals. In the ARH KNDy neurons, NKB mRNA is also greatly inhibited during lactation whereas DYN mRNA levels are unaffected (Xu et al., 2009b). The decrease in mRNA levels is accompanied by decreases in *Kiss1* and NKB neuropeptide content in the ARH. Surprisingly, the decrease in *Kiss1* mRNA in the AVPV is associated with *increased* neuropeptide content, suggesting an inhibition of *Kiss1* release (True et al., 2010). The AVPV *Kiss1* data stress the importance of studying changes in both mRNA and peptide levels. The factors associated with lactation that may play a role in the suppression of *Kiss1* neurons will be discussed in Section 6, as will the potential functional significance of the differential regulation of NKB and DYN in the ARH KNDy neurons.

3. Hypothalamic neuropeptide systems involved in the hyperphagia of lactation

The regulation of appetite is derived through an elaborate array of hypothalamic networks containing orexigenic and anorexigenic signals and has been thoroughly reviewed by others (Badman and Flier, 2007; Flier, 2004; Grayson et al., 2010; Schwartz, 2001; Seeley et al., 2004; Stefater and Seeley, 2010; Woods, 2009). The ARH is considered to be a key “feeding center” and the paraventricular nucleus (PVH) is the major downstream “integration center” (Flier, 2004; Li et al., 2000; Morton et al., 2006; Seeley et al., 2004). The ARH also serves to integrate peripheral signals, such as leptin, insulin, ghrelin and PYY (Cowley and Grove, 2004), that reflect the status of energy balance. Lactation alters the activity of most of the currently identified appetitive neuropeptide systems, as reviewed previously (Smith and Grove, 2002). A brief summary of the systems involved in the hyperphagia of lactation is provided below.

3.1. Alterations in orexigenic and anorexigenic neuropeptide systems during lactation

During lactation in the rat, the energy demand due to milk production is met by a 3–4-fold increase in food and water intake (Malabu et al., 1994; Smith and Grove, 2002; Xu et al., 2009b). In some ways, the hyperphagia of lactation resembles genetic models of obesity (Kesterson et al., 1997) that are also characterized by hyperphagia, but differs in that the hyperphagia is coupled with an extreme energy drain due to milk production, resulting in an overall perceived negative energy balance. The diagram in Fig. 1 summarizes the changes in the various appetitive hypothalamic neuropeptide systems during lactation and their integration at the PVH to signal increased food intake. All key orexigenic systems are activated, including NPY and AGRP in the ARH (Chen et al., 1999; Li et al., 1998; Xu et al., 2009b), and MCH and OX (orexin-A) in the lateral hypothalamic area (LHA) (Rondini

et al., 2010; Sun et al., 2003; Sun et al., 2004). A special adaptation of lactation is the induction of NPY expression in the noncompact zone of the DMH (Chen and Smith, 2003; Chen et al., 2004; Li et al., 1998; Xu et al., 2009b), a region also associated with the regulation of food intake and obesity syndromes (Bellinger and Bernardis, 2002; Guan et al., 1998; Kesterson et al., 1997). Suppression of DMH NPY during lactation is associated with a parallel suppression of the suckling-induced increase in food intake, suggesting a key role for DMH NPY in the sustained hyperphagia (Chen et al., 2004). Taken together, the increase in NPY tone in the PVH, which would result from activated NPY neurons in the ARH and DMH that project to the PVH, likely contributes to the suppression of corticotropin-releasing hormone (CRH) activity during lactation that would signal hyperphagia (Fischer et al., 1995; Hwang and Guntz, 1997) (Fig. 1). Anorexigenic neuropeptide systems are all decreased during lactation, including melanocortin signaling from the ARH, reflecting the decrease in ARH POMC, and hence, α -MSH, coupled with the increase in ARH AGRP (Chen et al., 1999; Kim et al., 1997; Smith, 1993; Xu et al., 2009b), and a decrease in ARH CART (Sanchez et al., 2007; Xiao et al., 2005). Taken together, changes in all these systems likely contribute to the sustained hyperphagia (Fig. 1). There may be other as yet unidentified systems that contribute to the hyperphagic drive of lactation, including systems in the brainstem that are activated by suckling and project to hypothalamic areas (Li et al., 1999a; Li et al., 1999b). One activated area is the nucleus of the solitary tract; this area receives input from gut peptides and the vagus and conveys information about satiety (Grill, 2006; Myers et al., 2009). It also expresses leptin receptors and is thought to be a major contributor to regulation of satiety by leptin (Elmquist et al., 1998; Myers et al., 2009). Another activated area is the ventrolateral medulla (VLM), a key hindbrain glucosensing area (Ohkura et al., 2000; Ritter et al., 2006) that conveys information to the ARH and PVH in response to glucoprivation (Fraley et al., 2002; Ritter et al., 2001). Establishing a comprehensive view of the hyperphagia of lactation will require an understanding of the contributions of the brainstem areas to the regulation of the hypothalamic appetitive neuropeptide systems.

4. Hypothalamic appetitive neuropeptide systems regulated by lactation overlap with systems of GnRH regulation

All of the neuropeptide systems involved in the regulation of food intake and energy balance that are altered during lactation in the ARH, LHA and DMH have also been shown to have overlapping functions in the regulation of GnRH neuronal activity (Fig. 1, see the text below for a more detailed discussion). In addition, changes in peripheral signals denoting energy balance, such as leptin and insulin, are transmitted into the hypothalamus to alter these neuropeptide systems. The key question is how information from these various neuropeptide systems is transmitted to GnRH neurons. Information could be transmitted through direct effects on GnRH cell bodies to alter their activity or through contacts on GnRH fibers or terminals in the median eminence to alter their release. There could also be effects on key upstream regulators of GnRH, such as Kiss1 neurons.

4.1. Actions of hypothalamic appetitive neuropeptide systems on GnRH neuronal function

The suppression of GnRH during lactation most likely results from multiple inhibitory effects. GnRH cell bodies express receptors for the majority of the appetitive neuropeptide systems that are altered during lactation. The integrated effects of these alterations could create a profound inhibitory tone acting on GnRH neurons. Fig. 2 summarizes these alterations at the level of GnRH cell bodies.

4.1.2. Effects of increased NPY tone on GnRH activity—In addition to its key role in the regulation of food intake, NPY is one of the essential players in modulating

reproductive function. NPY's effects are estrogen sensitive and are inhibitory to LH secretion in the presence of low levels of estrogen (Crowley et al., 2007; Estrada et al., 2003; Kalra et al., 1990; Kalra and Kalra, 2004; Xu et al., 2000) or in response to continuously elevated levels of NPY (Catzeflis et al., 1993). These are the conditions present during lactation or other states of negative energy balance, supporting an inhibitory role for NPY on GnRH neuronal activity (Smith and Grove, 2002; Woodside et al., 2002). The majority of GnRH cells are contacted by NPY neurons, including NPY/AGRP fibers from the ARH (Li et al., 1999d; Turi et al., 2003), and the GnRH cells express the Y5 receptor subtype (Campbell et al., 2001). This receptor subtype has been shown to be inhibitory to LH secretion (Raposinho et al., 1999), reflecting its postsynaptic actions through activation of G-protein-coupled inwardly rectifying K⁺ channels and voltage-dependent inhibition of Ca²⁺ channels (Sun and Miller, 1999). Therefore, NPY provides a neurocircuitry for information about food intake/energy balance to be directly transmitted to GnRH neurons. The use of electrophysiological techniques and recording from GFP-identified GnRH neurons has provided new information about GnRH activity during states of negative energy balance, such as lactation and fasting (Sullivan et al., 2003; Sullivan and Moenter, 2004; Xu et al., 2009a). NPY's direct effects are to hyperpolarize GnRH neurons via postsynaptic Y5R. Basal spontaneous GnRH neuronal activity is suppressed during lactation, and a component of this suppression is the increased endogenous inhibitory NPY tone, reflecting the increase in NPY in the ARH and DMH (Xu et al., 2009b). In addition to direct actions via postsynaptic Y5R, NPY can also affect GnRH neuronal activity through presynaptic Y1R (Li et al., 1999d; Sullivan and Moenter, 2004). In response to fasting, there are increased inhibitory effects of NPY, acting through Y1R, to alter GABA tone and inhibit GnRH activity (Sullivan et al., 2003; Sullivan and Moenter, 2004). Thus, the increase in inhibitory NPY tone during lactation appears to be a significant contributor to the suppression in GnRH neuronal activity (Fig. 2).

4.1.3. Effects of increased OX and MCH on GnRH activity—There are numerous reports showing that both OX and MCH have effects on LH secretion that are dependent on the presence or absence of ovarian steroids; their effects are inhibitory in the presence of low levels of estrogen, similar to what has been observed for NPY (Chicocchio et al., 2001; Kohsaka et al., 2001; Murray et al., 2000a,b; Pu et al., 1998; Rondini et al., 2010; Small et al., 2003; Tamura et al., 1999; Tsukamura et al., 2000). Of interest, the NPY Y4 receptor subtype has also been shown to modulate GnRH/LH secretion; its effects are likely indirect through actions on OX neurons that express the Y4R (Campbell et al., 2003b). There is a functional link between OX or MCH neurons and GnRH neurons as the vast majority of GnRH neurons are contacted by orexin and MCH fibers, and GnRH cell bodies coexpress the respective receptors, OX1 and MCHR1 (Campbell et al., 2003a; Iqbal et al., 2001; Small et al., 2003; Williamson-Hughes et al., 2005). These data provide the critical neurocircuitry by which OX and MCH have direct connections to GnRH neurons and could serve as additional integrating links between the regulation of food intake and reproductive function (Fig. 2). At this time, the effects of OX acting through OX1 receptors on GnRH neurons are unknown as to whether they are stimulatory or inhibitory. However, there is considerable information suggesting an inhibitory role for MCH in GnRH regulation. Again, using electrophysiological recordings, MCH has been shown to have a strong inhibitory effect through direct actions on the MCR1R that is linked to a Ba²⁺-sensitive potassium channel (Wu et al., 2009). Furthermore, MCH is capable of completely blocking the excitatory effects of Kiss1. Therefore, increases in MCH signaling at GnRH cells could provide an important inhibitory input. Of particular interest is a recent report that lactation induces expression of MCH in neurons in the median preoptic nucleus (Rondini et al., 2010). These MCH cells also express GABA, and they may be induced by changing levels of AGRP, CART and α -MSH in the ARH (Rondini et al., 2010). These MCH neurons project to a

number of sites involved in the regulation of reproduction and may contribute to the suppression of GnRH activity. It is unknown at this time whether this population of MCH cells contributes to the orexigenic drive during lactation. The finding of induction of a special population of MCH cells in the median preoptic nucleus is reminiscent of the induction of NPY expression in the DMH during lactation and suggests that special adaptive mechanisms may have evolved to satisfy the metabolic demands of lactation and to ensure their coupling to the suppression of GnRH/LH.

5. Importance of low levels of leptin and insulin, metabolic signals denoting negative energy balance, on the hyperphagia and suppression of GnRH activity during lactation

The changes in appetitive neuropeptide systems during lactation provide a link between the chronic hyperphagia and the suppression of GnRH (Figs. 1 and 2). However, the signals that bring about these changes in the appetitive systems are unclear. During most states of negative energy balance, changes in metabolic hormones acting at the level of the ARH are the most likely candidates. As discussed in Section 1, it is a commonly held view that changes in leptin levels are the key signal to the brain denoting depleted energy stores in the body (Elmquist and Flier, 2004; Friedman, 2002; Morton et al., 2006; Seeley et al., 2004). Although this discussion will focus on leptin and insulin, it is likely that more than just decreased leptin and insulin signal negative energy balance. There are changes in other hormones, such as thyroid hormone, ghrelin, and steroids (decreased estrogen and progesterone because of decreased pulsatile LH) that could be involved in regulating food intake and GnRH/LH (Crown et al., 2007; Hill et al., 2008; Oakley et al., 2009; Tena-Sempere, 2007). During lactation, there are likely other signals associated with the suckling stimulus that are responsible for or contribute to the hyperphagia and suppression of GnRH.

5.1. Effects of restoring leptin and insulin to normal physiological levels on the hyperphagia of lactation

A compelling argument can be made that the suppression of leptin plays a pivotal role in the changes in ARH neuropeptides during lactation, since the NPY/ARGP, POMC and KNDy neurons all express leptin receptors and have been shown to respond to leptin stimulation (Benoit et al., 2004; Cowley, 2003; Smith et al., 2006). To test this idea, studies have been performed during lactation in which serum leptin and insulin were restored to normal physiological levels and effects on food intake and hypothalamic neuropeptide systems were examined (Crowley et al., 2007, 2004; Xu et al., 2009b). It is important to note in these studies that leptin was restored to physiological levels through use of minipumps, which contrasts to the typical dosing regimen consisting of intraperitoneal injections of extremely large pharmacological doses, or of large intracerebroventricular doses. The serum levels of leptin achieved with the minipumps were within the physiological range and similar to those observed during the 48-h period after pup removal when food intake quickly reverts to normal and hypothalamic appetitive systems return to control levels. In general, the results showed that restoration of leptin and insulin together reversed much of the orexigenic drive provided by the ARH; that is, by decreasing NPY and AGRP and increasing POMC (Crowley et al., 2007, 2004; Xu et al., 2009b). In one study (Xu et al., 2009b), leptin and insulin alone had differential effects on the NPY/ARGP and POMC systems, with leptin restoration affecting only POMC neurons and insulin affecting only NPY/ARGP neurons (Xu et al., 2009b). Others have shown that higher doses of leptin can clearly inhibit NPY and AGRP expression (Morrison et al., 2005). In any case, these results suggest that physiological levels of leptin and insulin can bring about changes in the ARH NPY/ARGP and POMC systems. The key finding in these studies is that despite the normalization of the orexigenic signals provided by the ARH, the lactating animals were still hyperphagic

(Crowley et al., 2007, 2004; Xu et al., 2009b), suggesting that other hypothalamic areas are critical for the hyperphagia of lactation. A prime candidate is the DMH, since the induction of NPY was not affected by the restoration of leptin and insulin (Xu et al., 2009b). We and others have demonstrated that the DMH NPY is required for the hyperphagia of lactation and other hyperphagic states (see Section 3.1 and Fig. 1). Recent studies suggest that the DMH NPY neurons are GABAergic and are differentially regulated compared to ARH NPY neurons (Draper et al., 2010). Although the factors associated with negative energy balance do not appear to be required for the induction of DMH NPY, the mechanisms responsible for its induction remain unclear.

5.2. Effects of restoring leptin and insulin to normal physiological levels on the regulation of GnRH/LH during lactation

The study described above (Xu et al., 2009b) also examined the effects of restoration of leptin and insulin on ARH KNDy mRNA and on serum LH levels. Normalizing serum leptin and insulin had little effect to restore the suppressed levels of ARH Kiss1 or NKB or of serum LH. DYN levels remained unchanged and were not affected by lactation. An interesting side note of this experiment is that the elevated levels of ARH NPY do not appear to contribute to the suppression of Kiss1 or GnRH/LH during lactation, since concomitant normalization of NPY to low levels following leptin and insulin treatment had no effect to reverse the suppression. These results seem to contradict the earlier discussion in Section 4.1.2. regarding the importance of inhibitory NPY tone in the suppression of GnRH neuronal activity at the level of cell bodies (Xu et al., 2009a). They also suggest that ARH NPY projections to the median eminence to contact GnRH fibers and terminals are not providing critical inhibitory influences (Li et al., 1999d). While these findings may point to a diminished importance of ARH NPY for GnRH regulation, it is also possible that multiple inhibitory systems are in place during lactation to ensure reproductive inhibition and energy conservation. Specifically, it is likely that the continued increase in DMH NPY and the inhibition of Kiss1 and NKB after leptin and insulin restoration provide sufficient redundant inhibitory tone to maintain the suppression of GnRH despite normalization of ARH NPY.

There are several interpretations of the leptin and insulin restoration study (Xu et al., 2009b); one is that the primary signals of negative energy balance do not appear to be a prerequisite for the suppression of GnRH/LH during lactation. It should be noted that these experiments were conducted during mid-lactation, so it is possible that during later stages of lactation, the role of negative energy balance may play a prominent role, as the intensity of the suckling stimulus wanes (Tsukamura and Maeda, 2001). The apparent lack of involvement of leptin and insulin in the suppression of GnRH/LH during lactation in the rat is in agreement with data from humans. Leptin levels are not altered during lactation in women (Sir-Petermann et al., 2001). Instead, the length of lactational amenorrhea is related to the strength of the suckling stimulus (McNeilly, 2001; McNeilly, 2002), suggesting that it is the suckling stimulus, itself, that is responsible for the suppression of GnRH/LH. Another possible interpretation of the insulin restoration study (Xu et al., 2009b) is that the stimulatory roles of leptin and insulin are being masked by redundant inhibitory signals associated with the suckling stimulus, making it impossible to completely rule out a contributing role for the hypoleptinemia and hypoinsulinemia in the suppression of GnRH/LH. This possibility is supported by data showing that leptin restoration during other states of negative energy balance, such as fasting, does stimulate LH secretion (Ahima et al., 1996; Castellano et al., 2005; Castellano et al., 2009; Nagatani et al., 1998, 2000). However, fasting and lactation are quite different models of negative energy balance, in terms of acute and chronic effects. In addition, in the case of fasting, leptin is given at the start of the fast to prevent the suppression of LH, whereas with lactation, leptin is given to overcome an ongoing suppression of LH. There are also differences in the dosing regimen of leptin that

may be a contributing factor to the different results; in the fasting studies, leptin was administered in large pharmacological doses that result in peak serum levels many fold higher than normal physiological levels, even though leptin levels were low several hours after the injection (Ahima et al., 1996). There is no published information about the ability of physiological levels of leptin to prevent the suppression of LH during fasting. We have begun to use a caloric restriction model of negative energy balance that more closely mimics the time frame and severity of the negative energy balance experienced during lactation. During caloric restriction there is a significant suppression of ARH Kiss1 mRNA and serum LH. Similar to findings in the lactation model, restoration of serum leptin to control physiological levels had no effect to recover ARH Kiss1 or serum LH (unpublished data). Similar data have been reported for a chronically food-restricted lamb model (Morrison et al., 1991). Perhaps, leptin is capable of preventing the inhibition of LH during an acute fast, but is incapable of overcoming the inhibition after chronic imposition of the condition, such as during lactation or caloric restriction (Morrison et al., 1991).

The question as to the importance of leptin as the signal linking negative energy balance and suppressed GnRH/LH remains unresolved at this time. There is also conflicting data as to whether Kiss1 is suppressed in states of negative energy balance, as some studies using fasting as a model have reported suppression of Kiss1 in just the AVPV (Kalamatianos et al., 2008) or no suppression in either the ARH or the AVPV (Donato et al., 2009). Part of the difficulty in understanding these apparently conflicting results is that different sexes and models of negative energy balance are being used or varying degrees of estradiol replacement (high, low or none) are given to the ovariectomized animals. Added to this is the variation in mode of leptin administration, peripheral or central, and at varying doses. Therefore, a word of caution must be raised in assuming that leptin is the key signal providing information linking energy balance to reproduction. Under normal conditions of restoring LH secretion following negative energy balance, leptin levels rise from very low levels to normal physiological levels; there is never an influence of very high leptin levels. Therefore, the results obtained with pharmacological levels of leptin may be misleading. There is also a question of whether the ARH is the primary site that transmits leptin signaling, since the majority of leptin receptors are expressed in other brain regions known to regulate energy balance (Myers et al., 2009). Recent studies provide convincing evidence that the ventral premammillary nucleus (PMV) may be a key site for transmitting the effects of leptin to neurons controlling reproductive function (Donato et al., 2009; Leshan et al., 2009). The PMV contains a very high concentration of leptin receptors, is involved in reciprocal innervations with the ARH and AVPV (Donato et al., 2009; Elias et al., 2000; Rondini et al., 2004), and sends direct projections to GnRH neurons in the POA (Leshan et al., 2009). Furthermore, lesions of the PMV prevent leptin stimulation of LH in fasted rats (Donato et al., 2009). Thus, the PMV appears to play a unappreciated role in regulating reproductive function.

6. Decreased ARH and AVPV Kiss1 and their relationship to the suppression of GnRH activity during lactation

The suppression of Kiss1 during lactation may be key to the suppression of GnRH, since Kiss1 is considered to be the primary gatekeeper in governing GnRH activity. This assumption is given credence by data showing that central administration of Kiss1 during lactation results in increased LH secretion (Yamada et al., 2007). Furthermore, the restoration of LH secretion following pup removal is accompanied by increases in ARH Kiss1 and NKB (Xu et al., 2009b), supporting the idea of a relationship between restoring Kiss1 and NKB and LH. At this time, the causes of the suppression of AVPV or ARH Kiss1 are unknown. In the case of the AVPV Kiss1 population, the extremely low levels of estradiol associated with lactation could play a role. It is also not known whether AVPV

Kiss1 neurons receive inputs from appetitive neuropeptide systems whose activities are altered during lactation. In addition, there may be decreased stimulatory input from the PMV to the AVPV (Donato et al., 2009). The PMV appears to play a critical role in the positive feedback effects of estrogen on the AVPV Kiss1 neurons (Donato et al., 2009). With regard to the ARH KNDy population, the low levels of leptin and insulin do not appear to be critical factors in the regulation of these neurons, nor do changes in other ARH appetitive neuropeptide systems. In the studies discussed above in which leptin and insulin were restored to normal during lactation (Xu et al., 2009b), there was no recovery of ARH Kiss1 or NKB mRNAs even though ARH levels of NPY, AGRP and POMC were nearly normalized. LH levels were also not restored with leptin or insulin treatment during lactation, consistent with Kiss1 being a critical regulator of GnRH release. It is possible that suckling-activated brainstem neurons that project to the ARH may participate in the active suppression of Kiss1 and NKB (Li et al., 1999a,b). As mentioned in Section 3.1, activation of the VLM by the suckling stimulus, in addition to playing a possible role in the hyperphagia of lactation, may also be involved in the inhibition of Kiss1 neurons in the ARH. The activated noradrenergic neurons (A1) in the VLM are glucosensing neurons (Ritter et al., 2006) and project to the ARH (Li et al., 1999b). Numerous studies have demonstrated that glucoprivation is a potent inhibitor of estrous cyclicity and LH secretion; however, this inhibition is blocked when the neural projections from A1 are eliminated (T'Anson et al., 2003; Kinoshita et al., 2003; Nagatani et al., 1996; Ohkura et al., 2000). Thus, the lactation model provides an opportunity to discover possible new mechanisms for regulation of the ARH Kiss1 system that governs reproduction.

6.1. Possible model for the ARH Kiss/NKB/DYN neurons in the regulation of GnRH release

Recently, a model has been developed describing the generation of GnRH pulses by KNDy neurons (Navarro et al., 2009; Ohkura et al., 2009a; Wakabayashi et al., 2010). This model has hypothesized that while Kiss1, and potentially NKB, directly regulate GnRH release at terminals, NKB and DYN may also act in an autoregulatory manner at the cell body to precisely time pulses of Kiss1 release (Fig. 2). First, for direct regulation of GnRH release from terminals, Kiss1 release into the ME is hypothesized to stimulate GnRH release by acting at terminals. The role of NKB in the release of GnRH is less clear. There is considerable evidence that NKB regulates GnRH release directly, since ARH NKB fibers are found near GnRH fibers in the ME, and GnRH fibers in this area express the NKB receptor, NK3 (Krajewski et al., 2005). Studies have demonstrated decreased LH release in response to central NK3 activation, suggesting NKB may directly inhibit GnRH release (Sandoval-Guzman and Rance, 2004). However, more recent evidence suggests that NKB may be stimulatory to GnRH release (Billings et al., 2010; Ramaswamy et al., 2010; Wakabayashi et al., 2010), consistent with the hypogonadotropic hypogonadism seen in humans with mutations in genes encoding NKB and NK3 (Topaloglu et al., 2009). Data from the nonhuman primate has demonstrated that while administration of a single NKB bolus is stimulatory for LH, repeated administration is not (Ramaswamy et al., 2010). This acute and desensitizing effect of NKB upon LH release may explain the apparent discrepancies in the literature, but nevertheless it remains unclear how direct actions of NKB contribute to GnRH release from terminals (Fig. 2). There is also no data on the time course or pattern of Kiss1 or NKB stimulated GnRH release in the ME.

ARH KNDy cells may also contribute to basal pulsatile GnRH secretion through autoregulatory mechanisms. This model is based on observations showing that ARH KNDy neurons also express the NKB and DYN receptors, NK3 and KOR, respectively (Navarro et al., 2009), and fibers containing NKB and DYN form synapses on Kiss1/NKB/DYN neurons (Navarro et al., 2009; Wakabayashi et al., 2010). In this model, NKB/NK3 signaling is stimulatory to the KNDy neurons, whereas DYN/KOR signaling is inhibitory. The

autoregulatory control would result in release of pulses of Kiss1 that provide the pacemaker drive for pulsatile GnRH release. Any dysfunction in NKB/DYN signaling would disrupt pulsatile GnRH release (Topaloglu et al., 2009; Wakabayashi et al., 2010). Applying this model to lactation, it is reasonable to speculate that the inhibition of NKB, in the presence of normal levels of DYN, would provide increased inhibitory drive to the KNDy neurons and lead to an inhibition of Kiss1 and the complete disruption of pulsatile GnRH release. Fig. 2 presents a schematic representation of the autoregulatory control of ARH KNDy neurons that would result in decreased Kiss1 and GnRH release during lactation.

6.2. Effects of decreased AVPV Kiss1 on GnRH activity

In addition to the effects of the various hypothalamic appetitive neuropeptide systems at the level of GnRH cell bodies (Fig. 2), it would be remiss to ignore the potential effects of the decrease in Kiss1 in the AVPV, given its direct input to GnRH cells through postsynaptic actions on Kiss1R (Irwig et al., 2004; Navarro et al., 2009; Oakley et al., 2009; Pielecka-Fortuna et al., 2008). Although this population of Kiss1 neurons is thought to be involved in the positive-feedback effects of estrogen and the ovulatory GnRH/LH surge, it is unclear whether AVPV Kiss1 neurons also contribute to basal GnRH secretion. It is possible that Kiss1 tone on GnRH cell bodies may be important for their overall synthetic and excitatory capacity and their responses to other excitatory input (Fig. 2).

7. Conclusions and future directions

Lactation is an important physiological model of negative energy balance that involves activation of potent appetitive neuropeptide systems coupled to a profound inhibition of pulsatile GnRH/LH secretion. There appear to be multiple systems that contribute to the chronic hyperphagia: suppression of the metabolic hormones, leptin and insulin, activation of systems in the ARH and LHA, and induction of NPY in the DMH. These changes ensure adequate energy intake to meet the metabolic needs of milk production (Fig. 1). The overlap in these systems with the regulation of GnRH could provide several redundancies in this regulation as well (Fig. 1). In addition to an overall increase in inhibitory tone acting on GnRH cell bodies that is brought about by alterations in NPY, MCH, OX and Kiss1 input, there are also effects at the ARH to disrupt KNDy neuronal function, leading to a possible disruption of pulsatile GnRH release (Fig. 2). While the low levels of leptin and insulin are important to the changes in ARH appetitive systems, they do not appear to be necessary for the suppression of ARH Kiss1 or NKB. It is a reasonable hypothesis that the inhibition of Kiss1 may be the key factor in the suppression of GnRH during lactation, although the mechanisms responsible for its inhibition are unknown.

Although the study of kisspeptin has greatly advanced our understanding of the regulation of GnRH, there are important questions that remain to be answered about how energy balance and reproduction are integrated. What is the importance of the special adaptations of lactation, such as induction of NPY in the DMH and MCH in the median preoptic nucleus? Do they function as redundant systems or are they critically important? How are changes in the activity of the various neuropeptide systems (NPY, MCH, OX) acting directly on GnRH cell bodies integrated to affect function? Does Kiss1 input from the AVPV to GnRH cell bodies play a role in basal pulsatile GnRH release? What are the factors responsible for the inhibition of Kiss1 in the AVPV and ARH? How is the ARH KNDy neuron differentially regulated such that Kiss1 and NKB are inhibited but DYN is unaffected? What role do suckling-activated brainstem populations play in driving hyperphagia and GnRH inhibition? What are the factors responsible for the active suppression of leptin during lactation? Since leptin plays a role in both brain and peripheral adaptations (promotes fuel sparing for milk production) to lactation, its suppression seems to be a critical factor. What is the role of leptin in linking energy balance and reproduction; is it a key factor or a modulatory one? Is

the role of leptin the same for the various models of negative energy balance, such as lactation, fasting, or caloric restriction? To resolve these issues will require using the same animal model and comparing effects of restoring leptin and insulin to physiological levels in the various states of negative energy balance. Are our views too “leptin-centric” and “ARH-centric”, such that we ignore other possibly important areas, such as the brainstem? Given our current understanding, it is reasonable to propose that brainstem pathways activated by the suckling stimulus are the primary determinants of the changes in hypothalamic targets, such as the ARH, DMH, LHA and PMV, that drive the hyperphagia and suppression of gonadotropin secretion during lactation.

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Fig. 1.

Summary of the changes in hypothalamic appetitive neuropeptide systems during lactation. Lactation causes an increase in the activity of orexigenic systems (NPY, AGRP, MCH, OX) and a decrease in anorexigenic systems (POMC and CART). The integration of these systems results in a sustained hyperphagic drive at the level of the PVH. The physical suckling stimulus can activate some of these systems and is responsible for the induction of NPY in the DMH, an adaptation of lactation that may be critical for the sustained hyperphagia. The low levels of serum leptin and insulin also provide signals for the alterations in ARH NPY/AGRP and POMC systems. The combination of the suckling stimulus plus low levels of leptin and insulin ensure that a sustained hyperphagia is maintained to meet the energy demands of milk production. The appetitive neuropeptide systems also provide overlapping regulation of GnRH neuronal activity, thus serving as integrating links between energy balance and reproduction.

Fig. 2.

Schematic representation of the regulation of GnRH during lactation. GnRH cell bodies coexpress receptors for several of the orexigenic appetitive neuropeptide systems (MCH/MCHR1, NPY/Y5R, OX/OX1) that are increased during lactation. The MCH and NPY inputs have direct inhibitory effects on GnRH activity through actions on postsynaptic receptors (the effects of the OX/OX1 system is unknown). The decrease in Kiss1 drive from the AVPV could also lead to a decrease in excitatory tone on the neurons. Together, these effects could result in a significant inhibitory tone and result in a decrease in basal GnRH neuronal activity. It is unclear whether these same neuropeptide systems also affect GnRH fibers or terminals to inhibit GnRH release. The alterations in ARH KNDy neuronal function could lead to a suppression of Kiss1 and GnRH release from the median eminence. The diagram of the ARH is a representation of the model published by Wakabayashi et al. (2010) and Navarro et al. (2009), in which the changes in Kiss1 and NKB during lactation are depicted. The suppression of NKB mRNA and peptide levels, coupled to no change in DYN levels, would result in an increased inhibitory autofeedback on the KNDy neurons. The decrease in Kiss1 and NKB release on GnRH fibers and terminals could lead to an inhibition of GnRH pulsatile release (Navarro et al., 2009; Wakabayashi et al., 2010). The factors responsible for the suppression of Kiss1 and NKB are unknown, although the low levels of leptin and insulin do not appear to be contributing factors.