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Chemical identity of hypothalamic neurons engaged by leptin in reproductive control

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ABSTRACT

The adipocyte-derived hormone leptin plays a critical role as a metabolic cue for the reproductive system. Conditions of low leptin levels observed in negative energy balance and loss-of-function mutations of leptin or leptin receptor genes are characterized by decreased fertility. In recent years, advances have been made for identifying possible hypothalamic neurons relaying leptin's neuroendocrine control of reproductive function. Studies from different laboratories have demonstrated that leptin action in the hypothalamo-pituitary-gonadal (HPG) axis is exerted via hypothalamic interneurons regulating gonadotropin-releasing hormone (GnRH) cells, oppose to direct action on GnRH neurons. Following this observation, studies focused on identifying leptin responsive interneurons. Using a Cre-loxP system to re-express or delete the leptin receptor long form (LepRb) from kisspeptin neurons, our laboratory found that leptin's action on kiss1 cells is neither required nor sufficient for leptin's role in reproductive function. Endogenous re-expression of LepRb however, in glutamatergic neurons of the ventral premammilary nucleus (PMV) or ablation of agouti-related protein (AgRP) neurons from leptin signaling-deficient mice are both sufficient to induce puberty and improve fertility. Recent studies have also shown that leptin action in first order GABAergic neurons is required for fertility. Together, these studies begin to delineate key neuronal populations involved in leptin's action in reproduction. In this review, we discuss recent advances made in the field and highlight the questions yet to be answered.

Introduction

The adipocyte-derived hormone leptin, encoded by the *Lep*/*LEP* gene, circulates in plasma in free and bound forms (Zhang et al., 1994; Ahima and Flier, 2000; Elias and Purohit, 2013). Leptin levels in plasma are proportional to adipose tissue mass and therefore changing levels of leptin signal energy (in) sufficiency and function as a metabolic cue to allow adaptive physiologic responses (Maffei et al., 1995; Considine et al., 1996; Flier, 1998; Casanueva and Dieguez, 1999; Ahima et al., 2000; Chan and Mantzoros, 2005).

Reproductive function is energetically demanding due to the high energetic costs of pregnancy, lactation, and male territoriality (Schneider, 2004; Hill et al., 2008; Roa et al., 2010). States of negative energy balance has a negative impact in the reproductive physiology. Rodents and primates in negative energy balance show decreased sex steroids, pulsatile luteinizing hormone (LH) secretion and fertility (Manning and Bronson, 1989; Cagampang et al., 1990; Cameron and Nosbisch, 1991; Parfitt et al., 1991; Maffei et al., 1995; Weigle et al., 1997). Treating with leptin increases LH secretion, restores female cyclicity and improves fertility (Ahima et al., 1996; Nagatani et al., 1998; Gonzalez et al., 1999; Watanobe et al., 1999b; Donato et al., 2009). In humans with low energy stores, leptin increases LH, estradiol, and ovarian volume and the number of dominant follicles (Licinio et al., 1998; Miller et al., 1998; Warren et al., 1999; Welt et al., 2004; Chan and Mantzoros, 2005).

Leptin-deficient (*ob*/*ob*) mice are infertile, although some degree of reproductive success has been reported in young *ob*/*ob* males (Lane and Dickie, 1954). Leptin replacement in *ob*/*ob* mice induces sexual development and permits normal fertility (Barash et al., 1996; Chehab et al., 1996; Mounzih et al., 1997). It is important to note that the infertile phenotype of leptin-deficient mice is dependent on genetic background since *ob*/*ob* mice crossed onto a BALB/cJ strain have improved fertility and are leaner, suggesting the action of unknown modifier genes regulating leptin's effect in metabolism and reproduction (Qiu et al., 2001). In humans, leptin signaling deficiency caused from genetic

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mutations is rare. Nonetheless, affected individuals who are hyperphagic, morbidly obese, do not undergo a pubertal growth spurt and do not reach sexual maturation (Clement et al., 1998; Farooqi et al., 2007; Licinio et al., 2007; Mazen et al., 2009; Fischer-Posovszky et al., 2010; Galgani et al., 2010; Paz-Filho et al., 2010; Fatima et al., 2011; Mazen et al., 2011). Leptin administration to leptin-deficient subjects restores fertility (Farooqi et al., 2002; Farooqi and O'Rahilly, 2006). Therefore, it is accepted that leptin acts as a permissive signal for the onset of puberty and maintenance of reproductive function. However, the mechanisms and brain circuitry engaged by leptin regulating reproductive function are not entirely known. This review will focus on recent progress made implicating potential neuronal populations mediating leptin's regulation of reproductive function.

Search for leptin's target site(s) for reproductive control

The leptin receptor is a member of the class I cytokine receptor family and six isoforms have been identified. Of these six isoforms, the long-form (LepRb) contains JAK-STAT signaling capability and shows a high level of expression in the hypothalamus (Tartaglia et al., 1995; Chua et al., 1996; Lee et al., 1996; Ahima and Flier, 2000). LepRb expression is also seen in peripheral targets required for reproductive function. In granulosa cells, LepRa and LepRb mRNA levels increase after human chorionic gonadotropin (hCG) treatment and antagonizing leptin receptors during hCG treatment leads to a reduction in oocytes collected from oviducts (Dupuis et al., 2014). Studies using genetically modified mice however have revealed that leptin's effects on metabolism and reproduction are relayed primarily through the brain (Cohen et al., 2001; de Luca et al., 2005; Quennell et al., 2009). Thus, multiple routes do exist for leptin signaling to regulate fertility. However, LepRb signaling via neural targets alone is sufficient for reproductive function. Dense LepRb expression in the hypothalamus along with leptin's role in increasing frequency of LH pulses initially suggested that leptin acted directly on gonadotropin-releasing hormone (GnRH) neurons (Yu et al., 1997; Lebrethon et al., 2000; Parent et al., 2000; Wojcik-Gladysz et al., 2009). However, virtually no LepRb has been detected in GnRH neurons and the deletion of the *Lepr* gene from GnRH cells produces no reproductive deficit (Quennell et al., 2009; Donato et al., 2011a; Louis et al., 2011). Together, these findings suggest that leptin acts on interneurons to stimulate GnRH secretion (Quennell et al., 2009). Proteins downstream of LepRb signaling involved in regulating fertility have also been investigated. Apart from the STAT3 and STAT5 genes, whose respective phosphorylated proteins act as markers for LepRb activation and mediate gene transcription, alternative downstream signaling proteins are now believed to mediate leptin's effects in reproductive function. Studies have revealed that deletion of STAT3 and STAT5 from LepRb expressing cells in mice did not produce any reproductive abnormalities despite the severely deregulated metabolic phenotype (Singireddy et al., 2013).

Glutamate and nitric oxide from the ventral premammillary nucleus

A concentrated expression of LepRb exists in the ventral premammillary nucleus (PMV). This nucleus responds directly to leptin seen by the induction of Fos immunoreactivity and phosphorylation of STAT3 after leptin treatment. A high percentage of PMV neurons are also depolarized after leptin treatment (Elmquist et al., 1998; Elias et al., 2000; Leshan et al., 2009; Louis et al., 2011; Williams et al., 2011b). Studies in our laboratory have shown that endogenous re-expression of LepRb only in the PMV is sufficient to induce puberty and improve fertility in LepRb-null female mice (Donato et al., 2011b). In addition, bilateral lesions of the PMV impair female reproductive function and prevent the leptin-mediated increase in LH during fasting (Donato et al., 2010; Donato et al., 2011b). These studies reveal an important role the PMV may have in relaying leptin signaling to the HPG axis. However, how this nucleus integrates leptin signaling to stimulate LH secretion is not entirely known. Using standard neuroanatomical techniques and molecular mapping, several laboratories have shown that neurons within the PMV (including those expressing LepRb) project directly to GnRH cells (Rondini et al., 2004; Boehm et al., 2005; Leshan et al., 2009; Donato et al., 2011b; Louis et al., 2011). These projecting neurons express glutamate and therefore may directly activate their terminal targets including GnRH or, alternatively, kisspeptin-expressing cells (Brann and Mahesh, 1994; Mahesh and Brann, 2005; Donato et al., 2011b).

LepRb neurons within the PMV also co-express neuronal nitric oxide synthase (nNOS) and inhibiting NOS in hypothalamic explants attenuates leptin-induced LH secretion (Yu et al., 1997; Leshan et al., 2009; Donato et al., 2010; Leshan et al., 2012). Whether this leptin mediated NO signaling influencing LH secretion originates from the PMV is unknown. LepRb and nNOS co-localization however is highest in the PMV and in a recent study, LepRb deletion from nNOS expressing neurons delayed pubertal maturation in female mice (Leshan et al., 2012). It is important to point out though that removal of LepRb from other hypothalamic nuclei co-localizating LepRb and nNOS (i.e., the dorsomedial, the ventromedial, the arcuate and the posterior nuclei) may also have a role in the observed delay in pubertal maturation.

Kisspeptin neurons in the arcuate nucleus

Kisspeptins (products of the *KISS1*/*Kiss1* gene) are key regulators of reproductive function (Popa et al., 2008; Colledge, 2009; Oakley et al., 2009; Tena-Sempere, 2010; De Bond and Smith, 2014). Loss-of-function mutations in *KISS1*/*Kiss1* or kisspeptin receptor (*GPR54*/*Gpr54* also known as *KISS1R*/*Kiss1r*) genes cause infertility, prevent sexual maturation and lead to hypogonadotropic hypogonadism in mice and humans (de Roux et al., 2003; Seminara et al., 2003; d'Anglemont de Tassigny et al., 2007; Lapatto et al., 2007; Topaloglu et al., 2012). *Kiss1* mRNA expression and kisspeptin production decreases in conditions of low leptin levels as observed in *ob*/*ob* mice or wild types in fasting conditions (Castellano et al., 2005; Smith et al., 2006; Luque et al., 2007; Kalamatianos et al., 2008; Quennell et al., 2011). Furthermore, it has been shown in rodents that a subpopulation of *Kiss1* neurons in the arcuate nucleus co-express LepRb (Smith et al., 2006; Cravo et al., 2011; Louis et al., 2011; Qiu et al., 2011; True et al., 2011). Male streptozotocin (STZ) rats, which have undergone pancreatic β cells destruction, develop diabetes and show decreased circulating levels of leptin, sex steroids and LH as well as a reduction in *Kiss1* mRNA (Castellano et al., 2006). Treating with leptin reverses these effects, identifying a possible role for leptin in restoring *Kiss1* mRNA expression during states of negative energy balance and a possible pathway for activating the HPG axis. Our lab generated two genetically modified mouse models to test this idea. In one model, LepRb signaling was deleted from kisspeptin expressing cells, which lead to no reproductive deficits in male and female mice (Donato et al., 2011b). In the second model, functional LepRb was re-activated in kisspeptin cells and from this model it became clear that co-localization of LepRb and kisspeptin occurred only after pubertal maturation. This observation revealed leptin action directly on kisspeptin neurons is not required for pubertal development (Donato et al., 2011b; Cravo et al., 2013). These findings indicate leptin's permissive effect on reproduction does not require direct signaling onto kisspetin neurons, but whether kisspeptin neurons are downstream of leptin's effect still needs to be determined.