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The ventral premammillary nucleus links metabolic cues and reproduction

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The amount of body fat and the energy balance are important factors that influence the timing of puberty and the normal reproductive function. Leptin is a key hormone that conveys to the central nervous system information about the individual energy reserve and modulates the hypothalamus–pituitary–gonad (HPG) axis. Recent findings suggest that the ventral premammillary nucleus (PMV) mediates the effects of leptin as a permissive factor for the onset of puberty and the coordinated secretion of luteinizing hormone during conditions of negative energy balance. In this review, we will summarize the existing literature about the potential role played by PMV neurons in the regulation of the HPG axis.

Keywords: leptin, puberty, GnRH, luteinizing hormone, hypothalamus, adiposity

INTRODUCTION

It has long been known that nutritional status is a critical factor in determining the timing of the onset of puberty (Kennedy, 1969; Frisch and McArthur, 1974). Classical studies suggested that a minimum amount of body fat is required to attain sexual maturation (Kennedy, 1969; Frisch and McArthur, 1974; Frisch, 1985). When prepubertal animals, including primates, are exposed to energy deprivation the onset of puberty is delayed or even blocked, until a favorable energy balance is achieved (Kennedy and Mitra, 1963; Kennedy, 1969; Foster and Olster, 1985). In adults, severe energy deficits are a frequent cause of hypothalamic amenorrhea (Warren et al., 1999; Welt et al., 2004; Ribeiro et al., 2007). Recent studies not only confirm the importance of the adiposity in influencing the onset of puberty, but also suggest that excess of body fat in children cause early onset of puberty (Biro et al., 2010). Epidemiological data have indicated a high prevalence of obesity among US children and adolescents (Flegal et al., 2010). Concomitantly, a higher proportion of girls have shown signs of pubertal development at earlier ages (Biro et al., 2010).

An important challenge is to understand how metabolic cues control the reproductive system. A breakthrough in the field occurred after the discovery of the gene that encodes the adipocyte-derived hormone leptin (Zhang et al., 1994). Soon after that, leptin became the missing piece that would complete the lipostatic theory, proposed several decades earlier (Kennedy, 1953). The lipostatic theory suggests that changes in fat deposition trigger a feedback system aiming to restore the balance between food intake and energy expenditure. Leptin levels reflect the body fat content in rodents and humans (Frederich et al., 1995a; Maffei et al., 1995; Considine et al., 1996). Overfeeding increases leptin levels, whereas food deprivation causes a strong decrease in the circulating concentration of leptin (Frederich et al., 1995b; Ahima et al., 1996; Considine et al., 1996). It has been proposed that the body interprets a high concentration of leptin as a signal of energy abundance, whereas falling levels of leptin signals starvation. Therefore, low leptin levels increase hunger and decrease energy expenditure, partly because energy-demanding physiological functions are suppressed in leptin-deficient states, presumably as a way to save energy and prolong survival (Ahima et al., 1996; Friedman and Halaas, 1998; Rosenbaum and Leibel, 1998). Reproduction is one of the physiological functions strongly affected by leptin (Barash et al., 1996; Ahima et al., 2000). Because lack of leptin is interpreted as a signal of starvation, leptin-deficient individuals become hyperphagic, massively obese and infertile, and exhibit a series of metabolic dysfunctions (Zhang et al., 1994; Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995; Montague et al., 1997; Strobel et al., 1998). Leptin treatment rescues the alterations in body weight, metabolism, and the reproductive system (Ahima et al., 1996; Barash et al., 1996; Chehab et al., 1996; Mounzih et al., 1997; Farooqi et al., 1999). In addition, exogenous leptin administration causes early onset of puberty in mice (Ahima et al., 1997; Chehab et al., 1997), and a similar mechanism could account for the trend observed in overweight children (Biro et al., 2010).

After the discovery of leptin, many studies focused on deciphering the mechanism by which leptin regulates the reproductive system. Although leptin receptors (LepR) are expressed in many organs, including pituitary gland and gonads (Zamorano et al., 1997), it is now clear that the main target of leptin is the brain (Cohen et al., 2001; de Luca et al., 2005). The expression of LepR is found in innumerous brain nuclei, particularly in specific populations of hypothalamic neurons (Schwartz et al., 1996; Elmquist et al., 1998; Scott et al., 2009). However, defining the key neuronal population that mediates the effects of leptin on reproduction has proven to be a challenging task (Hill et al., 2008; Castellano et al., 2010; Donato et al., 2011a; Louis et al., 2011). Recent findings suggest that the ventral premammillary nucleus (PMV)

is the long sought area in which leptin modulates the activity of the reproductive system (Donato et al., 2009, 2011b; Leshan et al., 2009; Louis et al., 2011). In this review, we will summarize the existing literature about the potential role played by PMV neurons in the regulation of the hypothalamus–pituitary–gonad (HPG) axis.

THE NEUROCHEMICAL PROFILE OF PMV NEURONS HIGHLIGHTS A KEY INTEGRATIVE FUNCTION

Ventral premammillary nucleus neurons exhibit a broad expression of receptors for hormones related to the regulation of the energy balance (**Table 1**). PMV neurons express LepR (Elmquist et al., 1998; Scott et al., 2009), the ghrelin receptor, in mice but not in rats (Zigman et al., 2006) and the insulin receptor (**Figure 1A**). Innumerous receptors for neurotransmitters involved with the regulation of energy balance are also found in the PMV, including the cannabinoid receptor 1 (**Figure 1B**), the melanocortin-4 receptor in mice (Liu et al., 2003) but not in rats (Kishi et al., 2003), the orexin receptor 1 and 2 (Marcus et al., 2001), the neuropeptide Y Y1 receptor (higher expression in mice than rats; Kishi et al., 2005), and the vasopressin receptor in hamsters (sexually dimorphic and dependent upon photoperiod length; Dubois-Dauphin et al., 1991).

Ventral premammillary nucleus neurons are potential targets of sex hormones (**Table 1**), as they express a dense amount of androgen receptor (AR) and a moderate to low amount of estrogen receptor α and β (ERα and ERβ) and progesterone receptor (Simerly et al., 1990; Yokosuka and Hayashi, 1996; Merchenthaler et al., 2004; Intlekofer and Petersen, 2011). The relatively high ratio of AR to ER and the lack of definitive data demonstrating the expression of aromatase in the PMV indicate a potential role for androgens in PMV neuronal biology (Yokosuka and Hayashi, 1996; Wu et al., 2009). Despite the strong presence of sexual steroid receptors in the PMV, it has not been determined whether changing levels of sexual hormones affect the neuronal activity or gene expression in this area.

In addition to sex steroid receptors, several neurotransmitters are expressed by PMV neurons (**Table 1**), including glutamate (Ziegler et al., 2002). The expression of glutamatergic markers, such as vesicular glutamate transporter 2 (vGluT2), can be found in the entire extension of the PMV and show a 94% colocalization with neurons that express LepR (Donato et al., 2011b). On the other hand, there is virtually no expression of GABAergic markers in the PMV, such as glutamic acid decarboxylase 67 (GAD67) or vesicular GABA transporter (vGaT; Donato et al., 2010a; Vong et al., 2011). Thus, the projections originated from PMV neurons are thought to be excitatory due to their glutamatergic component.

Peptidergic neurotransmitters are also expressed by PMV neurons (**Table 1**), including cocaine and amphetamine regulated transcript (CART), substance P (a tachykinin family member) and

Table 1 | Neurochemical characteristics of the ventral premammillary nucleus (PMV).

	Expression level	Studied species	Sexual dimorphism or specie-related differences in the expression level
NEUROTRANSMITTERS/HORMONES			
Glutamate	+++	M, R	
Nitric oxide	+++	M, R, S	
CART	++	M, R, S	Higher expression in rats and in sheep, compared to mice
Substance P	++	R	Sexually dimorphic (higher in male)
Enkephalin	++	R, Hu	
Dopamine	–	M, R, B	Described only in birds. Absent in rodents
Melatonin	–	B	Described only in birds
RECEPTORS			
Leptin receptor	+++	M, R	
Insulin receptor	++	M	
Ghrelin receptor	++	M, R	In mice but not in rats
Androgen receptor	+++	M, R	
Estrogen receptor α	++	M, R	
Estrogen receptor β	+	M	
Progesterone receptor	+	R	
Cannabinoid receptor 1	+++	M	
Melanocortin receptor 4	++	M, R	In mice but not in rats
Neuropeptide Y Y1 receptor	+++	M, R	Higher expression in mice than rats
Orexin receptor 1	++	R	
Orexin receptor 2	+++	R	
Vasopressin receptor (binding)	+++	Ha	Sexually dimorphic and dependent upon photoperiod length
Melatonin receptor (binding)	+++	S	Described only in sheep
Melanopsin (photopigment)	–	B	Described only in birds

Expression level: +++, high; ++, moderate; +, low; –, not described.
Species: B, birds; Ha, hamsters, Hu, humans; M, mouse; R, rat; S, sheep.

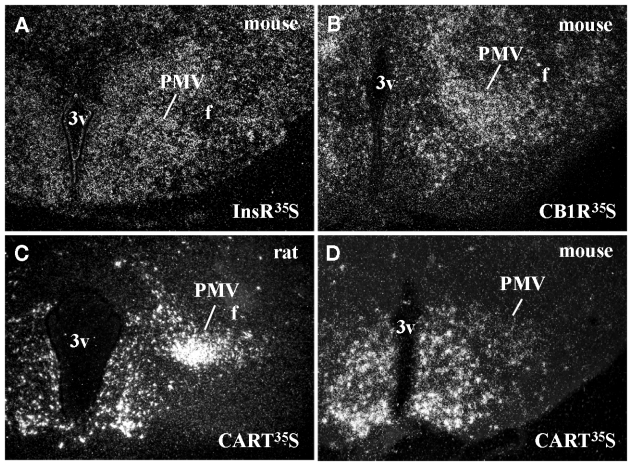

FIGURE 1 | Neurochemical characteristics of the ventral premammillary nucleus (PMV). (A,B) Darkfield photomicrographs demonstrating the distribution of insulin receptor (InsR) mRNA **(A)** and cannabinoid receptor type 1 (CB1R) mRNA **(B)** in the mouse PMV. **(C,D)** Darkfield photomicrographs demonstrating the distribution of cocaine and amphetamine regulated transcript (CART) mRNA in the rat **(C)** and in the mouse **(D)** PMV. Note the abundance of CART mRNA in the PMV of rats compared to mice.

enkephalin (Wamsley et al., 1980; Shimada et al., 1987; Larsen, 1992; Douglass et al., 1995; Sukhov et al., 1995). However, sexually dimorphic or species-related differences exist. While the PMV of rats densely expresses CART mRNA and peptide (Douglass et al., 1995; Koylu et al., 1997), the PMV of mice exhibits low CART expression (**Figures 1C–D**). In addition, the number of neurons that express tachykinin peptides in the PMV is higher in male than in female rats (Akesson, 1993). PMV neurons also densely express neuronal nitric oxide synthase (nNOS), which catalyzes the synthesis of nitric oxide (NO; Vincent and Kimura, 1992).

PMV NEURONS ARE NEUROCHEMICALLY AND NEUROANATOMICALLY WELL POSITIONED TO REGULATE REPRODUCTION

Many of the neurotransmitters expressed in the PMV are involved in the neuroendocrine regulation of reproduction. For example, glutamatergic inputs were shown to induce gonadotropin-releasing hormone (GnRH) release and subsequent activation of HPG axis (Brann and Mahesh, 1994; Gargiulo and Donoso, 1995; Dhandapani and Brann, 2000; Mahesh and Brann, 2005). Moreover, glutamate facilitates the expression of sexual behaviors (Gargiulo and Donoso, 1995) and has regulatory effects on the timing of puberty (Zamorano et al., 1998; Terasawa and Fernandez, 2001; Ojeda et al., 2006). Of note, intracerebroventricular administration of glutamate receptor agonists, such as NMDA, elicits secretion of GnRH and luteinizing hormone (LH), even in *Kiss1* and *Kiss1r* knockout mice, suggesting a role for glutamatergic neurotransmission outside the Kiss1 neuronal system (d'Anglemont de Tassigny et al., 2010). CART peptide was shown to mediate the stimulatory effects of leptin on GnRH secretion *in vitro* and *in vivo* (Lebrethon et al., 2000, 2007; Parent et al., 2000). In addition, NO has been implicated in the regulation of sexual behaviors and HPG axis (Moretto et al., 1993; Rettori et al., 1993; Mani et al., 1994; Benelli et al., 1995; Nelson et al., 1995). A complete disruption of *Nos1* gene results in hypogonadism and infertility (Gyurko et al., 2002). Furthermore, several studies found that NO is a key neurotransmitter that mediates leptin-induced GnRH/LH secretion (Yu et al., 1997; McCann et al., 1999; Watanobe and Schioth, 2001; Reynoso et al., 2007). Recently, we reported that 73% of leptin responsive cells in the PMV express NO-synthesizing enzymes (Donato et al., 2010b). Leptin does not affect the expression of *Nos1* mRNA in the PMV, but low leptin levels, as in fasting or in *ob/ob* mice, cause a reduction in the number of PMV neurons expressing the phosphorylated form of $nNOS^{S1412}$ (pnNOS). The phosphorylation of nNOS at Ser1412 increases nNOS enzymatic activity (Parkash et al., 2010) and acute injection of leptin restores the number of pnNOS neurons in the PMV of fasted mice (Donato et al., 2010b).

The projections of PMV neurons were first described in rats using the neurotracer *Phaseolus vulgaris* leucoagglutinin (Canteras et al., 1992b). It was demonstrated that PMV neurons project mainly to the periventricular zone of the hypothalamus, which is composed of nuclei involved in the regulation of anterior pituitary function. PMV neurons also project to major nuclei of the sexually dimorphic circuitry, including the ventrolateral part of the ventromedial nucleus of hypothalamus (VMH), medial preoptic nucleus, bed nuclei of the stria terminalis (BST), ventral lateral septal nucleus, posterodorsal part of the medial nucleus of the amygdala (MeA), and posterior nucleus of the amygdala (Canteras et al., 1992b). It is interesting that the major neuronal inputs to the PMV originate from neurons located in the sexually dimorphic circuitry, highlighting the intense intercommunication between this circuitry and the PMV (Simerly and Swanson, 1988; Canteras et al., 1992a,b, 1994, 1995; Coolen and Wood, 1998). For example, PMV is densely innervated by neurons located in the MeA, including cells that express urocortin 3 (Canteras et al., 1995; Coolen and Wood, 1998; Cavalcante et al., 2006b).

More recent studies in mice and in rats using genetic tools in combination with tracing techniques highlighted a putative role of the PMV in the regulation of the HPG axis. It was shown that PMV neurons project directly to GnRH perikarya in the medial preoptic area (MPA; Rondini et al., 2004; Boehm et al., 2005; Leshan et al., 2009) and to GnRH fibers in the median eminence (Donato et al., 2011b). Interestingly, among all neurons that express LepR, only those in the PMV and a subpopulation of neurons in the MPA seem to project directly to GnRH neurons (Louis et al., 2011). In addition, PMV neurons project to the anteroventral periventricular nucleus (AVPV; Canteras et al., 1992b; Rondini et al., 2004; Hahn and Coen, 2006), a key site for female reproductive function (Wiegand and Terasawa, 1982; Gottsch et al., 2004; Herbison, 2008). The AVPV contains a subpopulation of kisspeptin neurons, which is critical for the preovulatory LH surge (Smith et al., 2006; Herbison, 2008; Cravo et al., 2011). We have recently found that fibers from PMV neurons make apparent synaptic contact with kisspeptin neurons in the AVPV (Donato et al., 2011b). The arcuate nucleus (ARH) also receives a dense projection from PMV neurons (Canteras et al., 1992b), but whether kisspeptin neurons in the ARH or a specific population of ARH neurons is selectively targeted

by PMV inputs is still unknown. Overall, PMV neurons potentially regulate the reproductive system directly through inputs to GnRH neurons and also to upstream neuronal populations, such as kisspeptin cells.

PMV NEURONS ARE RESPONSIVE TO CONSPECIFIC BEHAVIORS AND SOCIALLY RELEVANT CUES

Previous studies using electrolytic lesions described a potential role for PMV neurons in odor-induced LH secretion in rats (Beltramino and Taleisnik, 1985). Olfaction is a critical sense used by rodents to discriminate socially relevant cues and to trigger social behaviors, including sexual behaviors (Romero et al., 1990; Halpern and Martinez-Marcos, 2003; Yoon et al., 2005; Brennan and Zufall, 2006). In response to conspecific odors, males and females of different species exhibit increased circulating levels of gonadotropins and sex steroids (Maruniak and Bronson, 1976; Kamel et al., 1977; Beltramino and Taleisnik, 1983; Coquelin et al., 1984). Rats and mice exposed to conspecific odors show a large number of neurons expressing Fos immunoreactivity (Fos-ir) in the PMV, which suggests that the PMV is involved in the neuronal circuitry that conveys olfactory information (Yokosuka et al., 1999; Cavalcante et al., 2006a; Leshan et al., 2009; Donato et al., 2010a). Moreover, roughly 50% of PMV neurons activated by opposite-sex odor express CART and, in male rats, CART mRNA increases after exposure to female odors (Cavalcante et al., 2006a). Most of the CART neurons in the PMV express the enzymes that synthesize NO. Besides, a parcel of nitrergic neurons is stimulated by female odors and virtually all nitrergic cells in the PMV express ARs (Yokosuka and Hayashi, 1996). Altogether, these studies indicate that PMV is apt to integrate information about circulating levels of sexual hormones and socially relevant cues (through brain areas related to pheromonal processing, such as MeA or BST) and generate appropriate neuroendocrine responses to modulate socially relevant behaviors.

The PMV may also be involved in the expression of conspecific behaviors because PMV neurons of male rats are also responsive to conspecific male odors (Donato et al., 2010a). In addition, PMV neurons express Fos-ir after mating or agonistic behavior (Kollack-Walker and Newman, 1995; Coolen et al., 1996; Pfaus and Heeb, 1997). Previous studies showed that lesions of the premammillary area increases aggression between males of the same species (Van Den Berg et al., 1983). However, these studies should be interpreted with caution due to the extension of the lesion. Restricted and/or selective lesions are required to determine the real contribution of the PMV in aggressive behaviors.

An interesting question is whether nutritional conditions may alter the responsiveness of an individual to environmental cues. PMV neurons are the target of metabolic cues and also respond to socially relevant sensory stimulation. Of note, 44% of the LepR-expressing cells in the PMV of male mice are activated by female odors, whereas in female mice, 18% of LepR cells are activated by male odors (Leshan et al., 2009). These findings suggest that food availability or energy stored affect neuronal responses to odors. However, we observed that fasting caused no changes in female odor-induced Fos-ir in the PMV and in the MeA of male rats compared to normally fed controls (Donato et al., 2010a). Although this finding may suggest a dissociation of neuronal responses to different stimuli, it is important to mention that induction of Fos protein may not be the definitive indicator of changes in neuronal activity or responsiveness. Further studies will be necessary to determine the influence of the nutritional state on the response to environmental stimulation.

PREMAMMILLARY HYPOTHALAMIC AREA MEDIATES SEASONAL REPRODUCTION IN EWES AND BIRDS

Although most of the studies about the PMV have used rats and mice as experimental models, there are several pieces of evidence that the premammillary hypothalamic area (PMH) also plays a key role in reproductive function of seasonal breeders (i.e., sheep and birds). Seasonal reproduction is a strategy used by several species to increase survival of offspring by reproducing during a period of the year when the environment offers favorable conditions. In sheep, the major environmental cue controlling reproduction is the photoperiod or day length (Duan et al., 2007). Changes in day light exposure alter the synthesis and secretion of the pineal gland hormone melatonin, which in turn binds to hypothalamic nuclei and modulates the pulsatile secretion of GnRH (Emilsson et al., 1999; Hazlerigg and Wagner, 2006; Goodman et al., 2010). The PMH of ewes is composed of the caudal ARH, the PMV and the ventral tuberomammillary nucleus. Similarly to rats, PMV neurons in ewes express CART and nNOS (Sliwowska et al., 2004). In addition, PMH of ewes is a melatonin binding site. Bilateral microimplantation of melatonin into the PMV of ewes stimulates LH secretion (Malpaux et al., 1998), indicating that in sheep, the PMV appears to play a key role in seasonal reproduction.

The reproductive cycle of a variety of avian species is regulated by circadian mechanisms driven by intrinsic oscillators (Petersen et al., 1996; Wikelski et al., 2008; Goodman et al., 2010). These mechanisms are modulated by light-sensitive neuronal populations located in the caudal hypothalamus, in a site identified as the PMH (Kang et al., 2007). In birds, a subpopulation of PMH neurons expresses dopamine, a neurotransmitter known to affect the secretion of several reproductive hormones, including LH, FSH, and prolactin. Using a complex paradigm of light-induced GnRH neuronal activation, studies identified in turkeys a photosensitive subpopulation of dopaminergic PMH neurons likely involved in GnRH secretion (Thayananuphat et al., 2007). Dopaminergic neurons (immunoreactive to tyrosine hydroxylase) in the PMH of turkeys coexpress melatonin and its synthesizing enzymes (Kang et al., 2007). Dopamine–melatonin neurons in the PMH exhibit high activity at the photosensitive phase, which was associated with higher dopaminergic neurotransmission and GnRH activation. Additionally, these neurons express the photoreceptive molecule melanopsin, which is involved in extra-retinal photoreception in birds and non-mammalian vertebrates. In hens, the expression of melanopsin mRNA in the PMH is downregulated by light in a series of models and shows a diurnal regulation; it is high during the night and low during the day (Kang et al., 2010). The PMH of turkeys also presents a distinct circadian expression of clock genes compared to the pineal gland and the brain master clock, the suprachiasmatic nucleus. In particular, *Cry1* and *Per3* seem to mediate the photic responses associated with the control of the reproductive system (Leclerc et al., 2010).

PMV NEURONS INTEGRATE METABOLIC CUES TO REGULATE REPRODUCTION RATHER THAN ENERGY BALANCE

The high expression of receptors of hormones related with the regulation of the energy balance might imply that the PMV is involved in the control of energy balance. However, bilateral excitotoxic lesions of the PMV did not affect body weight, mean food intake and circulating leptin levels in adult female rats (Donato et al., 2009). Nonetheless, PMV-lesioned rats exhibit an attenuated reduction in food intake between the proestrus and the estrus day (Donato et al., 2009). Female rats normally show a decreased food intake in the behavioral estrus that is linked with the high estrogen levels observed during the proestrus day (Drewett, 1973; Geary et al., 2001; Asarian and Geary, 2006). Therefore, the regulation of food intake across the estrous cycle by PMV neurons can be an indirect consequence of changes in sexual hormone levels after lesions of the PMV.

Leptin exerts a pivotal role in the long-term regulation of energy balance (Schwartz, 2006; Gautron and Elmquist, 2011). As mentioned, leptin administration to leptin-deficient mice (*ob/ob*) rescues all the metabolic and neuroendocrine deficits observed in these mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995; Chehab et al., 1996). Following the same paradigm, we generated *ob/ob* mice with bilateral lesions of the PMV (Donato et al., 2011b). Upon leptin treatment, these mice showed drastic reduction in food intake and body weight, indicating that leptin may restore the metabolic deficits of *ob/ob* female mice in the absence of PMV neurons (Donato et al., 2011b). To further investigate the role played by LepR in the PMV, we generated a LepR-null mouse model in which LepR is expressed selectively in PMV neurons. We found that endogenous expression of LepR only in the PMV did not affect food intake, body weight, and fat mass in male and female mice (Donato et al., 2011b). In agreement with this, a recent study found that after genetic ablation of LepR expression from all glutamatergic (vGluT2-positive) neurons, which includes PMV cells, only minor changes in body weight, food intake, and fat mass were observed in male and female mice (Vong et al., 2011). Overall, these results suggest that despite the presence of innumerous receptors involved with the regulation of energy balance, PMV neurons are not key players in the modulation of food intake and body weight. Rather, PMV neurons may function as a key integrative site conveying metabolic cues to the reproductive system. Accordingly, PMV lesions cause a temporary anestrus in rats (persistent leukocytes in the vaginal smears). However, after a few weeks PMV-lesioned rats recover their cyclicity, although vaginal cytology continues to exhibit an atypical mixed cell profile (Donato et al., 2009). Several weeks after lesions of the PMV, rats still show reduced concentration of LH and estradiol and decreased activation of AVPV and GnRH neurons at the time of the preovulatory LH surge but no changes in Kiss1 mRNA expression (Donato et al., 2009). Possibly secondary to decreased gonadotropin levels, the ovaries of PMV-lesioned rats display a lower number of antral follicles and a trend toward a reduction in the number of corpora lutea (Donato et al., 2009). These results indicate that the PMV is required for the normal activity of the HPG axis in female rats.

Following the same line, we hypothesized that the PMV would be apt to mediate the effects of leptin on the reproductive neuroendocrine axis. To test this model, we used a well-established paradigm in which leptin treatment can restore or increase LH levels in fasted rodents (Ahima et al., 1996; Nagatani et al., 1998; Gonzalez et al., 1999; Watanobe et al., 1999; Chan et al., 2003). Lesions of the PMV blocked the stimulatory effect of leptin on LH secretion in fasted rats (Donato et al., 2009). In order to investigate putative signaling pathways that mediate the acute effects of leptin on PMV neurons, patch-clamp recordings of hypothalamic slices were performed. Leptin caused a rapid depolarization of ∼75% of LepR-expressing neurons in the PMV through a putative TRPC channel (Leshan et al., 2009; Williams et al., 2011). The other 25% recorded LepR cells were hyperpolarized in response to leptin, and this response required the activation of a putative Katp channel. Importantly, pharmacological or genetic disruption of the phosphoinositide 3-kinase (PI3K) pathway prevented the leptin-induced changes in the activity of PMV LepR neurons (Williams et al., 2011). These results indicate that PI3K is required for the acute changes in biophysical properties of PMV neurons induced by leptin. Whether these changes in cellular activity underlie the physiological effects of leptin are under investigation.

We further assessed whether leptin signaling in PMV neurons is critical to induce the onset of puberty and restore fertility in leptin- or LepR-deficient mouse models. Lesions of the PMV in female *ob/ob* mice reduced the capacity of exogenous leptin to induce sexual maturation. Besides, acute injection of leptin did not increase LH and progesterone levels in PMV-lesioned *ob/ob* mice, as observed in PMV-intact *ob/ob* mice (Donato et al., 2011b). In addition, female LepR-null mice with endogenous re-expression of LepR in PMV neurons showed unambiguous signs of sexual maturation, such as vaginal opening, increased uterus weight and size, and ovaries with corpora lutea. After a period of 6 weeks of breeding tests, 50% of mice with selective reactivation of LepR in PMV neurons became pregnant, despite their obese and diabetic phenotype (Donato et al., 2011b). Notably, the improvement of the infertile phenotype of the LepR-null mice following PMV LepR reactivation was only observed in females, not in males. Additional studies will be necessary to tackle this sex-related difference. As previously mentioned, neurotransmitters found in the PMV, such as glutamate and NO, were shown to stimulate the release of GnRH. Moreover, earlier studies suggested that the lack of leptin signaling causes a deficient release of GnRH because *ob/ob* and *db/db* mice have high content of GnRH in the median eminence/medial basal hypothalamus (ME/MBH; Johnson and Sidman, 1979; Batt et al., 1982). Re-expression of LepR only in the PMV normalized the ME/MBH GnRH content in female LepR-null mice. Together, these findings have determined the PMV as a key site linking leptin action and the female reproductive physiology.

CONCLUDING REMARKS

Overall, this review highlights a series of recent data demonstrating that PMV neurons are apt to mediate the effects of leptin on GnRH secretion. The stimulatory effect of PMV neurons on GnRH release is possibly mediated by the coordinated effects of

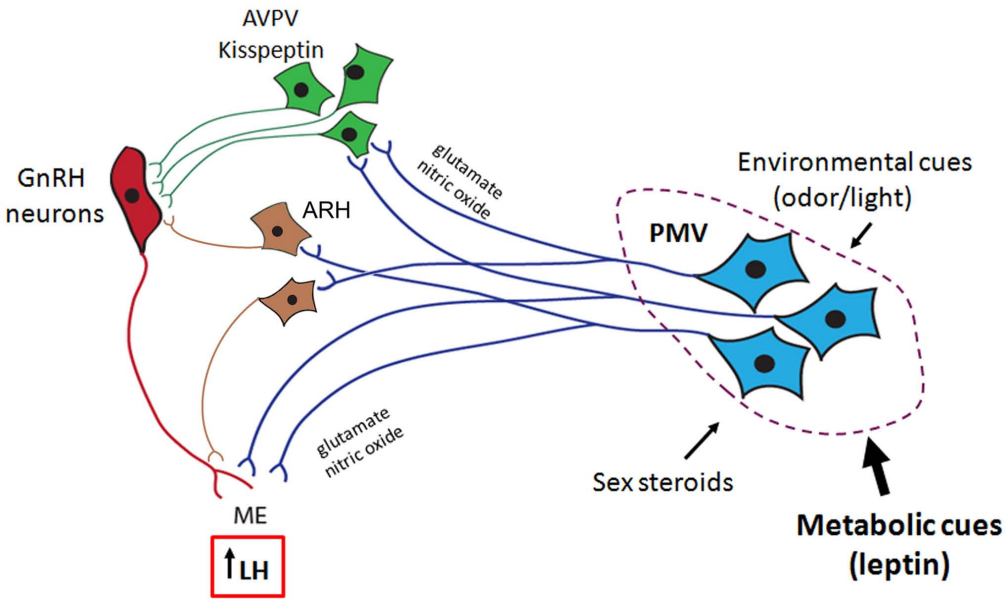

FIGURE 2 | Proposed role for the ventral premammillary nucleus (PMV) in the female reproductive physiology. The PMV integrates environmental cues (odors in rodents and daylight in seasonal breeders) and signals from the internal milieu related to the reproductive status (sex steroids) and energy store (leptin and insulin). PMV neurons express excitatory neurotransmitters (e.g., glutamate and nitric oxide) and directly project to the anteroventral periventricular nucleus (AVPV), to the arcuate nucleus (ARH), and to gonadotropin-releasing hormone (GnRH) neurons. Once stimulated, PMV neurons activate the target sites inducing GnRH release and LH secretion from the pituitary gland.

glutamate and NO on GnRH terminals in the median eminence (**Figure 2**). Although it is very likely that other neuronal populations also convey metabolic cues to modulate the HPG axis, the existing evidence suggests that the PMV is a key site relaying the effects of leptin on the reproductive neuroendocrine axis. We postulate that through PMV neurons, leptin modulates the influence of adiposity on the timing of puberty and the coordinated secretion of LH during conditions of negative energy balance. The data presented in this review provide the physiological and neuroanatomical basis underlying the effects of leptin on the HPG axis.

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