Published in final edited form as:
Peptides. 2007 February ; 28(2): 447–452. doi:10.1016/j.peptides.2006.09.025.

NEUROENDOCRINE ACTIONS AND REGULATION OF HYPOTHALAMIC NEUROPEPTIDE Y DURING LACTATION

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Abstract

The expression of neuropeptide Y (NPY) and its co-messenger, agouti-related peptide (AgRP), in arcuate neurons of the hypothalamus is increased during lactation in rats. Our research has been addressing the questions of the physiological actions of these peptides during lactation and the physiological signals associated with lactation that result in increased expression of their genes. Our studies indicate that NPY and AgRP exert pleiotropic actions during lactation that help integrate neuroendocrine regulation of energy balance with controls over anterior and posterior pituitary hormone secretion. Further, reciprocal signaling to the NPY/AgRP system by leptin and ghrelin is responsible for the changes in expression of these hypothalamic peptides in lactating animals, and thus, may contribute to regulation of food intake and the various neuroendocrine adaptations of lactation.

Keywords

Agouti-related peptide; Lactation; Leptin; Neuropeptide Y; Oxytocin; Prolactin

1.0 Introduction: NPY and AgRP in Lactation

Lactation is a complex, and in many respects, unique, physiological state characterized by a number of behavioral and neuroendocrine adaptations (see [15,43,45] for reviews). Behavioral adaptations in laboratory animals such as rats and mice include the abrupt onset of parental behavior and a dramatic hyperphagia. Evidence suggests that in species such as rats and mice, the energy demands associated with synthesis and secretion of milk are greater than the combined energy needs of other necessary physiological processes; strategies to meet this need include increasing daily food intake and reducing energy expenditure in other physiological processes, such as growth and reproduction [45,46]. Despite the markedly increased food

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intake, lactating rats appear to be in modest negative energy balance, and display some, but not all, neuroendocrine and metabolic adaptations associated with this condition [43,45,46].

Neuroendocrine adaptations include the well known neuroendocrine reflexes of suckling-induced release of prolactin (PRL), oxytocin (OT) and growth hormone, but also changes in the hypothalamic-pituitary-ovarian (decreased), thyroid (decreased) and adrenal axes (increased) [15,21,36,43,48]; these latter changes are also characteristic of negative energy balance [2–4,43], and may function to reduce energy expenditure to spare milk synthesis. It is interesting to note that despite the increased basal secretion of adrenocorticosteroids and the suckling-induced release of PRL and OT, stress-induced release of these hormones is selectively blunted during lactation [33]. Metabolic adaptations include hypoglycemia, and the associated hypo-leptinemia and hypo-insulinemia [9,15,18,41,43], which are also characteristic of non-lactating animals in negative energy balance [6,7,28,30]. One dissociation concerns the gastric hormone ghrelin, circulating levels of which are increased during food deprivation [6,7,28], but not during lactation (unpublished observations).

A number of studies have shown that one hallmark of lactation in rats and mice is increased expression of the genes encoding NPY and its co-messenger AgRP in arcuate neurons of the hypothalamus; concomitantly, there is increased content of NPY peptide in important nerve terminal regions, including the paraventricular nucleus and median eminence [11–13,32,34, 40,42,47,49]. NPY is also expressed in dorsomedial hypothalamic neurons specifically in lactating rats [32]. Our laboratories have been addressing the interrelated issues of 1) characterizing the physiological actions exerted by NPY and AgRP during lactation; and 2) identifying the physiological signals associated with lactation that evoke the up-regulation of these neuropeptides in the arcuate nucleus of the hypothalamus

2.0Physiological actions of NPY and AgRP during lactation

2.1 Feeding behavior

Because lactation is a condition of negative energy balance and hyperphagia, and because NPY and AgRP, whose expression is increased during lactation, are highly effective orexigenic neuromessengers, one obvious role for the up-regulated NPY/AgRP system in lactation would be in mediating the hyperphagia. However, our studies indicate that the elevated level of food intake in lactating rats is very robust and highly resistant to pharmacological agents; in particular, antagonists of NPY that are highly effective in non-lactating animals are ineffective in lactating rats. For example, central administration of antiNPY IgG, which is effective in decreasing 24-hr food intake in male rats and in inhibiting the hyperphagia associated with lesions of the ventromedial hypothalamus [19,20], had no effect on food intake in lactating rats (unpublished observations). Similarly, the antagonist NPY analogue [D-tyr(27,36), D-thr(32)] NPY(27–36), which inhibits NPY-induced and deprivation-induced feeding behavior [35], did not affect food intake when infused alone into the third ventricle of lactating rats [16]. Also ineffective in lowering food intake in these rats was the anorexigenic peptide, α-melanocyte-stimulating hormone (MSH), in a regimen that was clearly effective in non-lactating rats [16]. However, a combination of the NPY antagonist analogue plus α-MSH significantly reduced food intake when infused centrally in lactating rats over several days, but even this combined treatment did not return food intake to the non-lactating level, and the inhibitory effect waned by the fourth day of infusion [16]. We view the effect of α-MSH as functionally antagonistic to AgRP at the central MC4 receptor, consistent with current concepts on their respective agonist and inverse agonist actions at this receptor [1,24.37]. While these data suggest that NPY and AgRP do contribute to the hyperphagia of lactation, it is obvious that other systems must be involved. This is consistent with observations made in lactating NPY “knockout” mice, whose food intake is normal [26].

2.2 Prolactin (PRL) and oxytocin (OT) secretion

Early reports from Ciofi and coworkers [12,13] indicated that some of the increased NPY expression in the hypothalamic arcuate nucleus was due to a novel expression of NPY in the tuberoinfundibular dopamine (TIDA) neurons, a phenomenon they observed only in lactating rats and mice. Because this system is the major hypothalamic hormonal regulator of PRL secretion [8], we have tested whether NPY affects PRL secretion and interacts with other physiological regulators of this neuroendocrine system. In a series of experiments employing several *in vitro* models that measure PRL secretion from cultured rat anterior pituitary (AP) cells [47], we found that NPY is equipotent and equieffective with DA in inhibiting basal PRL secretion, using physiologically relevant concentrations of 10-100 nM; when given together in a submaximal concentration of 10 nM, NPY plus DA inhibits PRL secretion to a greater extent than each alone. When cultured AP cells are first exposed to DA, and then the DA is removed, there is a prominent rebound stimulation of PRL secretion. Removal of NPY after initial exposure similarly results in increased PRL secretion from AP cells; further, removal of both DA and NPY after initial exposure to both leads to a greater release of PRL than removal of only one of these factors. Using cultured AP cells obtained from mid-lactating rats in a perfusion system, the TRH-stimulated release of PRL is also significantly inhibited by either DA or NPY, and their combination (at 100 nM each) abolishes TRH-stimulated PRL release.

TRH is a classic activator of the inositol phosphate/Ca^{2+} messenger system, and produces an increase in cytosolic Ca^{2+} that is characterized by an initial “spike” resulting from an action of inositol 1,4,5 trisphosphate produced from the action of phospholipase C on membrane phospholipids, followed by a lower “plateau”, which is mediated by entry of extracellular Ca^{2+} via voltage-regulated Ca^{2+} channels [23]. The latter change may result from an action of diacylglycerol-mediated effects on protein kinase C, and/or from lipoxygenase metabolites of arachidonic acid. We have studied these mechanisms ([47] and unpublished observations) using cultured AP cells from lactating rats that were loaded with the fluorescent Ca^{2+} probe fura-2. Our findings indicate that, as for PRL release, either DA or NPY will inhibit the cytosolic Ca^{2+} increase induced by TRH, and the combination of DA and NPY produces an additive inhibition. However, while DA inhibits both intracellular Ca^{2+} mobilization, as well as entry of extracellular Ca^{2+}, the inhibitory effect of NPY on PRL is more selective, being limited to inhibition of the influx of extracellular Ca^{2+}. Table 1 summarizes the results from a large number of pharmacological studies conducted in our laboratory to characterize the inhibitory actions of NPY as compared with DA.

It has not been possible to conclusively identify a specific NPY receptor subtype mediating the inhibition of PRL secretion by NPY. As summarized in Table 2, agonists having activity at Y1, Y4 and Y5 receptors uniformly inhibit [Ca^{2+}]i and PRL responses to TRH, while a selective Y2 agonist (C2-NPY [(Cys2) NPY (1–4)-8-aminooctanoyl) D-Cys (27)] NPY (25-32), which also fails to induce feeding behavior [22], is unique in not affecting basal or TRH-induced PRL secretion. Taken together, the results of these studies suggest that the inhibitory effects of NPY on PRL secretion are mediated by a receptor coupled to inhibition of extracellular Ca^{2+} entry. While NPY also inhibits cAMP formation in the anterior pituitary, this effect does not appear important for inhibition of PRL release (unpublished observations).

Thus, another important action of NPY during lactation is to provide an additional inhibitory signal to the lactotrophe along with DA. The question may be raised about the need for such additional inhibitory signaling. Perhaps not widely appreciated, however, is that the suckling–induced release of PRL is actually episodic, characterized by large mass secretory episodes [25]. Hence, it may be the case that multiple excitatory and inhibitory signals to the lactotrophe help shape this pulsatile mode of secretion, which may be necessary to preserve receptor sensitivity at the mammary gland.

In addition to the inhibitory modulation of PRL, our pharmacological studies also demonstrate that NPY acts within the paraventricular and supraoptic nuclei to stimulate OT release in lactating rats; moreover, a synergistic action of NPY and the α1 adrenergic agonist phenylephrine could be demonstrated, as the combination of these two agonists at submaximal doses produced an elevation in OT release that was greater than additive [38]. In addition to modulating both of these neuroendocrine reflexes, it may be the case that other alterations in neuroendocrine systems during lactation are mediated in part by actions of NPY and/or AgRP. Thus, NPY and AgRP, under physiologic conditions likely to be present during lactation (e.g., low ovarian steroids), inhibit LH and TSH secretion and stimulate release of ACTH via centrally mediated actions (see [15,29] for review). It has not yet been directly demonstrated that these changes in neuroendocrine function during lactation are mediated by actions of NPY and/or AgRP, but if this is the case, then the hypothalamic NPY/AgRP system may be viewed as a system that integrates regulation of energy balance with the alterations in anterior and posterior pituitary hormone secretion as an adaptation to negative energy balance.

3.0 Regulation of NPY and AgRP expression by metabolic signals associated with lactation

The increased expression of NPY (and AgRP) mRNA in arcuate nucleus and the increased NPY-like immunoreactive content and unique innervation pattern in median eminence during lactation depend upon the continual presence of the suckling stimulus, as these neurochemical changes are reversed by removal of the litters and restored within hours of litter return [13, 32,47]. It may be the case that suckling-activated afferents that impinge upon arcuate NPY/AgRP neurons activate stimulus-transcription coupling leading to increased expression of these peptides. Alternatively, the effect of suckling could be mediated by an action of PRL, which is known to gain access to the arcuate nucleus, activating TIDA neuronal activity, for example [8]. However, neither we (unpublished) nor others [31,39] have been able to prevent the effect of suckling on NPY expression by inhibiting suckling-induced PRL secretion with the DA agonist bromocriptine or to mimic the effect of suckling in non-lactating rats by elevating PRL with the DA antagonist haloperidol.

Our recent studies suggest that the reduction in circulating leptin and perhaps insulin, which reflect negative energy balance produced by the demands of milk synthesis, may mediate the effect of suckling [17]. To test this hypothesis, we devised a repletion paradigm that restores circulating leptin or insulin to the higher circulating levels characteristic of cycling female rats by infusing these hormones via subcutaneously implanted Alzet Osmotic Minipumps ™. With leptin repletion, (400 ng recombinant rat leptin/μl; 1 μl/hr release rate), one can readily reverse the hypoleptinemia in lactating rats without affecting plasma levels of insulin. In recent studies [17], we have shown that selective leptin repletion in lactating rats 1) reverses the up-regulation of NPY mRNA and AgRP mRNA expression in arcuate neurons, 2) decreases NPY immunoreactivity in the PVN and ME, and 3) produces a small, but significant reduction in food intake. Similar effects were seen with insulin repletion, but the concomitant increase in leptin produced by this manipulation does not allow for definite conclusions to be drawn specifically regarding a role for insulin. Thus, as in other states of negative energy balance, the reduction in inhibitory leptin signaling to the arcuate NPY/AgRP neurons appears to be important for stimulating the expression of these orexigenic peptides.

Food deprivation is associated with increased secretion of the gastric hormone ghrelin [6,27, 28,44], and this peptide also contributes to the increased expression of NPY and AgRP, both of which mediate its orexigenic actions [7,10,27]. As noted above, we have observed no gross alterations in circulating levels of ghrelin in lactating rats vs. cycling female rats. However, our recent pharmacological studies with a selective ghrelin receptor antagonist [D-Lys3] growth hormone releasing peptide -6 (GRA peptide; [5]) implicate a role for ghrelin in

regulation of food intake and NPY/AgRP expression. Systemic treatment with this peptide (10 mg/kg, ip at 6-hr intervals) decreases food intake in cycling and lactating females over a 2-day treatment period. Interestingly, expression of NPY and AgRP was reduced in GRA-treated lactating rats, but not in cycling rats; proopiomelanocortin expression was not altered by the drug treatment in any group (unpublished observations).

These findings suggest a role for ghrelin in regulation of food intake and orexigenic peptide expression during lactation. Because ghrelin secretion does not appear to be altered during lactation, it may be the case that the reduction in inhibitory leptin signaling allows for the expression of orexigenic signaling by ghrelin on the NPY/AgRP network. This would be consistent with abundant evidence of reciprocal leptin/ghrelin signaling to these hypothalamic pathways regulating energy balance [7,28]. Alternatively, the GRA treatment may be antagonizing an action of the central ghrelin system on NPY and AgRP [14,27].

4.0 Summary: Pleiotropic regulation and actions of NPY and AgRP during lactation

The pleiotropic actions exerted by NPY and AgRP during lactation, e.g., orexigenesis, modulation of the suckling-induced release of OT and PRL, and possibly regulation of hypothalamic-pituitary-gonadal, thyroid and adrenal axes, may have as a common theme the integration of regulatory mechanisms that govern appetite and energy expenditure with those affecting anterior and posterior pituitary hormone secretion in response to negative energy balance. We [15] and others [43] have hypothesized that the suckling stimuli provided by the offspring evoke increased gene expression for NPY and AgRP via the neuroendocrine reflexive release of PRL and OT, responsible for milk synthesis and secretion, respectively. The resultant energy drain is signaled to the NPY/AgRP system by the reciprocal effects of leptin and ghrelin, which may not be limited to regulation of food intake, but which may also underlie the broader neuroendocrine adaptations in lactation. If so, future insights into understanding the physiology of lactation may be more profitably pursued within the overarching framework of neuroendocrine regulation of energy balance, rather than solely through the concepts and phenomena of reproductive neuroendocrine physiology.

Acknowledgements

This research was supported by NIH grants HD-13703 and HD-20074 (WRC), DK-37273 (SPK) and DA-13367 (KAK).

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Table 1

Inhibition of secretagogue-induced [Ca^{2+}]i increases and PRL secretion by NPY and DA.

	Inhibition by	
Stimulus	**DA**	**NPY**
TRH	YES	YES
TRH (Ca2+ free)	YES	NO
Ionomycin (Ca2+ free)	YES	NO
KCl	YES	YES
TRH (thapsigargin)	YES	YES

Table 2

Effects of NPY agonists on TRH-induced changes in [Ca^{2+}]i and PRL secretion from cultured AP cells from lactating rats.

Agonist	Active at NPY receptor(s)	Inhibition of TRH-Induced [Ca^{2+}]i	PRL Release
[Pro34] NPY	Y1, Y2, Y4	YES	YES
[Pro34] PYY	Y1, Y4, Y5	YES	YES
[Leu31, Pro34] NPY	Y1, Y4, Y5	YES	YES
[Leu31, Pro34] PYY	Y1, Y4, Y5	YES	YES
NPY3-36	Y2, Y5	YES	YES
PYY3-36	Y2, Y5	YES	YES
NPY13-36	Y1, Y2, Y4, Y5	YES	YES
hPP	Y1, Y4, Y5	YES	YES
C2-NPY	Y2	NO	NO