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Galanin-like peptide as a link in the integration of metabolism and reproduction

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The arcuate nucleus is a hypothalamic center that couples energetics and reproduction. Peptide-releasing neurons in the arcuate nucleus receive and process humoral signals from the periphery and relay this information to other nuclei in the hypothalamus and preoptic area. Galanin-like peptide (GALP) is expressed in the arcuate nucleus, and GALP-containing neurons are targets for the action of leptin. GALP-containing neurons are closely apposed to gonadotropin-releasing hormone (GnRH) neurons in the preoptic area, and CNS injections of GALP stimulate GnRH-mediated secretion of luteinizing hormone. These observations indicate that GALP is a molecular signal that couples circulating indices of metabolism to the neuroendocrine reproductive system and, thus, regulates reproductive activity as a function of the energy state. In this article, we describe the involvement of GALP in metabolism and reproduction, and in the coupling between these two processes.

The brain has a remarkable ability to sense the status of metabolic fuel reserves and defend their adequacy by adjusting appetite and metabolism to maintain an appropriate body weight. Moreover, the brain governs the activity of the reproductive system as a function of these metabolic reserves and enables activation of the hypothalamic–pituitary–gonadal axis only when fuel stores are deemed adequate to support the energetic requirements of reproduction. The arcuate nucleus (Arc) in the hypothalamus is a node for this physiological integration. Recently, a newly identified peptide in the Arc has been shown to modulate both feeding behavior and reproduction, which unveils a new link in the integration of metabolism and reproduction. In this review, we discuss the emerging role of this peptide, known as galanin-like peptide (GALP), in the physiological integration of metabolism and reproduction.

The Arc integrates metabolism and reproduction

The Arc comprises the neuronal circuitry required for the neuroendocrine integration of metabolism and reproduction [1]. Two populations of neurons in the Arc play key roles in the regulation of these physiological processes. One population produces neuropeptide Y (NPY) and agouti-related peptide (AgRP), whereas the other produces α-melanocyte-stimulating hormone (α-MSH) and β-endorphin from a common precursor, proopiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART). These two cell groups (NPY/AgRP and POMC/CART) are regulated differentially by metabolic hormones such as leptin and insulin, and act in opposition to control appetite, body weight and metabolic rate [1–4]. NPY/AgRP and POMC/CART neurons send axonal projections to the paraventricular nucleus (PVN), lateral hypothalamus, and areas in the midbrain and hindbrain to regulate the circuitry that controls complex behaviors, such as feeding, and adjust the metabolic rate to maintain body weight homeostasis [3,5–7]. Disruption of these networks leads to the dysregulation of body weight and disturbances in metabolism [8]. The Arc is also an important center for the neuroendocrine control of reproduction [9]. NPY and the products of POMC (α-MSH and β-endorphin) have important effects on the secretion of gonadotropin-releasing hormone (GnRH) and gonadotropins [8,10,11], and they are regulated by sex steroids [12,13]. Thus, NPY/AgRP and POMC/CART cells have well established roles in the control of both body weight and reproduction. Recently, evidence has accumulated indicating that GALP might also be an important regulator of metabolism and reproduction.

Galanin and its receptors

Galanin was discovered in 1983 [14]. This neuropeptide comprises 29–30 amino acids (depending on species) and has a highly conserved N-terminal (amino acids 1–13) that confers its biological activity [15]. Galanin is distributed widely throughout the brain (including the Arc), spinal cord and gut [16], and is coreleased with other neuropeptides such as GnRH, growth hormone-releasing hormone (GHRH) and vasopressin, and classical neurotransmitters such as noradrenaline [17–20]. Galanin is implicated in many physiological processes, including feeding, nociception, nerve regeneration, memory, neuroendocrine function, and the regulation of secretion and contractility in the gut [21–24]. The three known subtypes of galanin receptor are membrane-bound G-protein-coupled receptors [25]. These

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Table 1. Comparison of galanin and GALP[a]

	Galanin	GALP	Refs
Length	29–30 residues	60 residues	[15,26]
Receptors	GalR1	GalR1	[25]
	GalR2	GalR2 (high affinity)	[21]
	GalR3	GALP receptor?	
		Other receptors?	
Functions	Feeding, nociception, nerve regeneration, memory, neuroendocrine secretion, gut secretion and motility and reproduction	Regulates FI, BW and reproduction	[22,24,25]
CNS location	Several regions in the brain and spinal cord	Arc and ME	[16,35,37]
PNS location	Gut	Neurohypophysis	[14,39]
Chromosomal loci	Human, 11q13.3	Human, 19q13.43	[27,35]
	Mouse, 19 2.0	Mouse, 7A1	
	Rat, 1q42	Rat, 1q12	
Fos induction after i.c.v. injection	mPOA, PVN, Arc	mPOA, PVN, Arc, hDBB, ME	[30,31]
Effects on food intake and body weight	Stimulates feeding in satiated rats	In rats, initially stimulates FI, followed by a decrease in FI and BW. In mice, inhibits FI and BW	[50,51]
Effect of leptin	Inhibits	Induces	[29]
Effect of insulin	Inhibits	Induces	[62]
Sexual dimorphism	Higher in females. Responds to estradiol	Unresponsive to sex steroids	[28,69]
Association with GnRH-positive neurons	In rats, GnRH-positive neurons contain galanin	In rats, GALP-positive neurons project to GnRH-positive neurons	[46,69]
Effects on sex behavior	Inhibits	Stimulates	[62]

[a]Abbreviations: Arc, arcuate nucleus; BW, body weight; FI, food intake; GALP, Galanin-like peptide; GnRH, gonadotropin-releasing hormone; hDBB, horizontal limb of the diagonal band of Broca; i.c.v. intracerebroventricular; ME, median eminence; mPOA, medial preoptic area; PVN, paraventricular nucleus.

share partial sequence identity but differ in their distribution, pharmacology, specific G-protein-coupling and second messenger systems [21,25].

Until recently, galanin was the only known member of this unique family of neuropeptides. However, several reports of the interaction of unidentified molecules with galanin receptor subtypes, caused speculation about the existence of additional 'galanin-like peptides' [26]. In 1999, GALP, a galanin-like molecule was discovered by scientists in Japan [26].

GALP and its binding to galanin receptors

Ohtaki *et al.* discovered GALP while searching for unique molecules that bind and activate known galanin receptors. They named the new molecule GALP because of its shared partial sequence identity with galanin and its binding *in vitro* to two of the known galanin receptors [26]. Despite the partially shared sequence, GALP and galanin are encoded by different genes [27,28]. Full-length GALP is cleaved from a larger precursor and comprises 60 amino acids. Residues 9–21 of GALP are identical to the biologically active N-terminal portion (1–13) of galanin [29] (Figure 1). Unlike galanin, GALP has a non-amidated C-terminus. GALP binds *in vitro* to two subtypes of galanin receptor, GALR1 and GALR2 [25]. GALP binds to GALR1 with a lower affinity than galanin, but binds to GALR2 with a higher affinity than galanin [25]. The affinity of GALP for GALR3 is not known. It is plausible that one or more of these galanin receptors represent the endogenous physiological receptor for GALP; however, it is also conceivable that GALP has its own unique receptor(s).

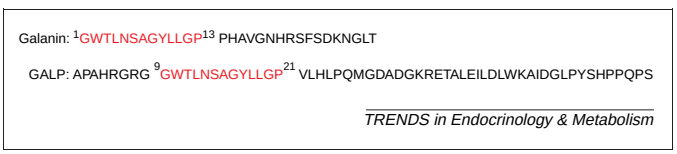

Figure 1. Amino acid sequences of galanin [16] and galanin-like peptide (GALP) [26]. Residues 1–13 of galanin and 9–21 of GALP (red) are identical. Residues 1–13 are thought to contain the biologically active portion of galanin. It remains to be determined whether this shared sequence is responsible for biological activity in GALP.

Indirect evidence indicates that GALP is likely to have its own receptor. First, centrally administered galanin and GALP produce different patterns of Fos induction throughout the brain [30,31]. This observation indicates that galanin and GALP act through different targets and receptor-mediated processes in the brain to effect their biological actions. Second, centrally administered galanin and GALP produce widely disparate effects on several physiological systems, including differential effects on feeding, luteinizing hormone (LH) secretion and sexual behavior (Table 1), which indicates that these neuropeptides act through different receptors to produce their biological actions.

Expression of GALP in the brain

GALP is localized in the brain and its gene has been partially characterized in mice, rats, macaques and humans [32–36]. Cells in the Arc and median eminence of the rat, mouse and monkey express *GALP* mRNA [35,37]. In the Arc, GALP-containing neurons are distinct from other major populations of peptide-releasing cells and do not colocalize with NPY, galanin, somatostatin or GHRH [25,33,34,38]; however a few (<10%) GALP neurons express α-MSH [38]. The anatomical distribution of cells that contain *GALP* mRNA in the Arc in mice differs slightly from that in rats. In the mouse Arc, GALP cells are located more laterally and ventrally than in the rat. Careful, detailed studies of the distribution of *GALP* mRNA and GALP peptide are necessary to make definitive

statements about their presence (or absence) in other regions of the nervous system.

Expression of GALP in the posterior pituitary

In the rat, GALP is expressed in the posterior pituitary (neurohypophysis) [39,40]. The discovery of GALP in the neurohypophysis and its apparent absence from the anterior pituitary is surprising because mRNAs that encode galanin, GALR1 and GALR2 are expressed in the anterior pituitary but not the neurohypophysis. In the latter, GALP is expressed in pituicytes. These are glial cells whose processes come in close contact with the nerve terminals of magnocellular neurons that project from the hypothalamic paraventricular and supraoptic nuclei. Expression of *GALP* mRNA in the rat neurohypophysis is induced dramatically following either salt-loading or water deprivation, which could indicate an important role for GALP in the regulation of vasopressin release and fluid balance [39]. Expression of *GALP* mRNA is also induced during lactation, which indicates that GALP regulates the secretion of oxytocin as well as vasopressin [28]. Notably, neither lactation nor salt-loading (or water deprivation) alters the expression of *GALP* mRNA in the Arc [28].

Regulation of *GALP* mRNA in brain

Metabolic hormones and fuels regulate the expression of GALP in the brain. Fasting reduces the expression of *GALP* mRNA in the Arc and leptin treatment during a fast can reverse this effect [29,32,41]. Leptin-deficient *ob*/*ob* mice have profoundly reduced levels of *GALP* mRNA in the hypothalamus [32,42], but treating these animals centrally (i.c.v) with leptin completely restores the expression of *GALP* mRNA to levels found in wild-type mice [29]. Rats and mice with dysfunctional leptin receptors (*fa*/*fa* and *db*/*db*, respectively) also have reduced expression of *GALP* mRNA [38]. Moreover, in both the rat and macaque, GALP-containing neurons express the leptin receptor [33,35]. These observations indicate that expression of *GALP* mRNA is regulated by direct actions of leptin on GALP-containing neurons in the brain.

Insulin also regulates the expression of *GALP* mRNA. Rats with streptozotocin-induced diabetes have greatly diminished expression of *GALP* mRNA in the Arc, which can be corrected with insulin treatment [43]. In addition, the administration of insulin directly into the brain during a fast stimulates the expression of *GALP* mRNA [43], which indicates that insulin also acts directly in the brain to induce the expression of *GALP* mRNA. Leptin and insulin share a common signaling pathway in the brain (phosphatidylinositol 3-kinase) [44,45], and it is conceivable that this is responsible for the common actions of leptin and insulin on the expression of *GALP* mRNA [43]. These studies demonstrate that metabolic hormones and metabolic fuels regulate *GALP* gene expression.

Thyroid hormones also influence the expression of *GALP* mRNA. Reduced circulating concentrations of thyroid hormones (caused by thyroidectomy) decrease hypothalamic levels of *GALP* mRNA, and replacement of thyroxine in thyroidectomized rats partially reverses this effect [28]. In addition, GALP delivered directly into the brain reduces the secretion of thyroid-stimulating hormone [46], again indicating that GALP plays a role in the neuroendocrine regulation of the hypothalamo–pituitary–thyroid axis. It is noteworthy that the expression of GALP is not influenced by circulating concentrations of glucocorticoids, sex steroids and growth hormone [28]; rather, its expression appears to be influenced predominantly by factors that reflect metabolic state.

The effect of GALP on feeding, body weight and metabolism

Centrally administered GALP has potent effects on feeding and body weight in rodents; however, the nature of these effects depends on the time-course and species. Within two hours of central administration to rats, GALP stimulates feeding in a manner similar to galanin [47]. This observation has been confirmed by several laboratories using GALP from different sources [48–50]. However, studies by Lawrence *et al.* [48] and Krasnow *et al.* [50] also document that GALP inhibits feeding and reduces body weight in rats 24 h after central injection. Together, these observations demonstrate that in the rat, centrally administered GALP exerts a biphasic effect on feeding, initially stimulating and later inhibiting this behavior. It has been argued that the biphasic effects of GALP might reflect the differential time-course of effects on the several receptor types that are activated by exogenously administered GALP [50]. For example, the initial stimulatory effect of GALP on feeding, which is reminiscent of that of galanin [51], might be attributable to the interaction of GALP with one or more galanin receptors, whereas the subsequent inhibition of feeding 24 h after injection of GALP reflects activity at its own, yet-to-be-identified receptor.

In mice, GALP elicits a dose-dependent suppression of feeding and body weight, both acutely (within 2 h) and 24 h after a single central injection [50]. However, mice quickly become refractory to continued injections of GALP (twice-daily injections over four days), so that beyond the first 24 h period, food intake and body weight are not different between GALP- and vehicle-treated animals. Krasnow *et al.* [50] also demonstrated that the feeding response to central GALP treatment in mice is accompanied by significant changes in locomotor activity, which do not occur in the rat. Mice treated with nine injections of GALP over four days become quiet and unresponsive to stimulation immediately after each injection. However, they recover within a few hours and then seem to behave normally. This occurs with each subsequent injection, although the period of reduced locomotion that follows immediately after each injection appears to diminish after the ninth injection. By the fourth day of twice-daily injections of GALP, in the hours following the acute 'sedation'; GALP-treated mice become dramatically hyperactive relative to their vehicle-treated controls. This hyperactivity is reminiscent of the effect of amphetamines on motor function [52], and suggests activation of dopamine-containing pathways in the brain. It is also notable that GALP produces a conditioned taste aversion, which might also contribute to its inhibitory effect on feeding and body weight [50].

Administering GALP into the brain of *ob*/*ob* mice for 14 days inhibits feeding and reduces body weight within

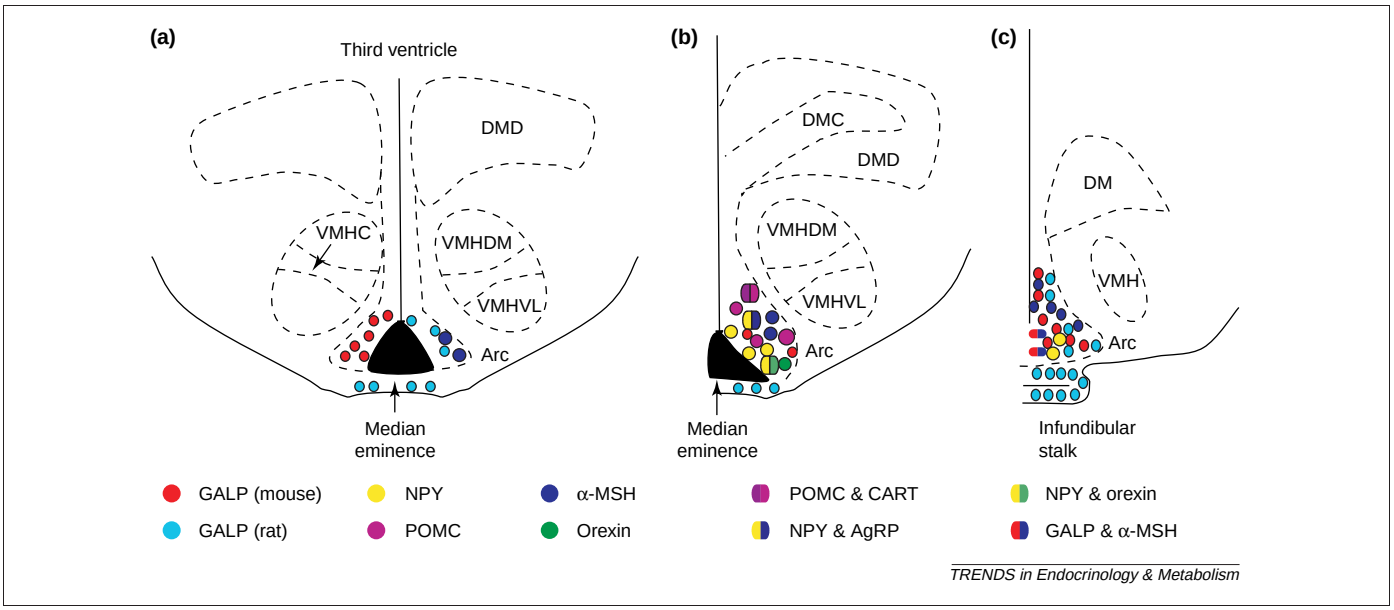

Figure 2. Localization of neuropeptides in the Arc. Each panel depicts a hypothalamic section (panel A is most rostral and panel C is most caudal). Colored dots demonstrate the approximate location of several neuropeptides. GALP is located in the median eminence and infundibular stalk of rats [33] but not mice [32]. GALP is located near several neuropeptides but is thought to colocalize only with α-MSH [38]. GALP neurons do, however, express receptors for neuropeptides such as NPY [38] and orexin [63]. Several peptides colocalize, including CART and POMC, NPY and AgRP, and NPY and galanin (bicolored dots). Others are in close proximity to one another, such as POMC and NPY. [3–6]. Abbreviations: AgRP, agouti-related peptide; α-MSH, α-melanocyte stimulating hormone; Arc, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; DM, dorsomedial hypothalamus; DMC, dorsomedial hypothalamus (compact); DMD, dorsomedial hypothalamus (diffuse); GALP, galanin-like peptide; NPY, neuropeptide Y; POMC, proopiomelanocortin; VMH, ventromedial hypothalamus; VMHC, ventromedial hypothalamus (central); VMHDM, ventromedial hypothalamus (medial); VMHDM, ventromedial hypothalamus (dorsomedial); VMHVL, ventromedial hypothalamus (ventrolateral).

24 h of the initial injection [53]. However, the feeding response to GALP does not adapt as quickly in *ob*/*ob* as in wild-type mice. Leptin-deficient animals require considerably more time (~10 days) for feeding to be restored to the levels observed in animals that receive vehicle alone, but eventually they resume feeding to levels that are indistinguishable from that of controls. However, the body weight of *ob*/*ob* mice does not fully recover during chronic treatment with GALP, and remains ~12% lower than in vehicle-treated controls despite consumption of nearly identical amounts of food. This indicates that GALP has important effects on metabolism in addition to regulating of appetite and feeding.

GALP might also activate the sympathetic nervous system [53]. Chronic treatment of *ob*/*ob* mice with GALP increases body temperature and stimulates downstream targets of sympathetic activation. Uncoupling protein 1 (UCP-1) acts in mitochondria to uncouple oxidative phosphorylation, so that fuel is oxidized to generate heat instead of ATP. Chronic treatment of *ob*/*ob* mice with GALP increases the concentration of UCP-1 and its mRNA in brown adipose tissue, with an accompanying increase in multilocular brown adipocytes. The sustained reduction of body weight in animals receiving chronic GALP treatment is likely to be attributable to the effects of GALP on the sympathetic nervous system [53].

GALP and neuroendocrine regulation of the reproductive axis

Reproduction is gated by physiological factors associated with nutrition, energy reserves and metabolic rate; however, the cellular and molecular mechanisms that link metabolism and reproduction are not fully understood. The activity of GnRH-containing neurons is influenced by metabolic hormones, including leptin and insulin [54–59]. Centrally administered GALP stimulates LH secretion through a GnRH-dependent mechanism in the rat [46] and monkey [60]. GALP-containing fibers are found in close apposition to a subset of GnRH neurons in the medial preoptic area (mPOA) and the bed nucleus of the stria terminalis (BST) [33,61]. Moreover, centrally administered GALP induces Fos expression in a subset of GnRH neurons [61]. Likewise, in the mouse, centrally administered GALP stimulates LH and testosterone secretion, demonstrating a similar effect of GALP on the neuroendocrine reproductive axis [50]. Other recent studies demonstrate that leptin stimulates the release of GALP and GnRH from hypothalamic explants *in vitro,* and that GALP antiserum blocks the stimulatory effects of leptin on GnRH secretion [46], thus, corroborating earlier suggestions that GALP plays an important role in mediating the effects of leptin on the neuroendocrine reproductive axis. The effects of GALP on GnRH secretion *in vitro* are attenuated only partially by a potent galanin receptor antagonist, galantide [46], which bolsters the suggestion that GALP has its own unique receptor. GALP might also play a role in sexual behavior because centrally administered GALP increases mounting, intromissions and ejaculatory function in male rats [62]. Taken together, these observations demonstrate that GALP exerts a direct and powerful activational effect on the reproductive system.

Hypothalamic circuitry linked to GALP-positive neurons

The afferent and efferent connections of GALP-positive neurons are yet to be fully elucidated. Immunocytochemistry reveals that GALP-positive neurons in the Arc project

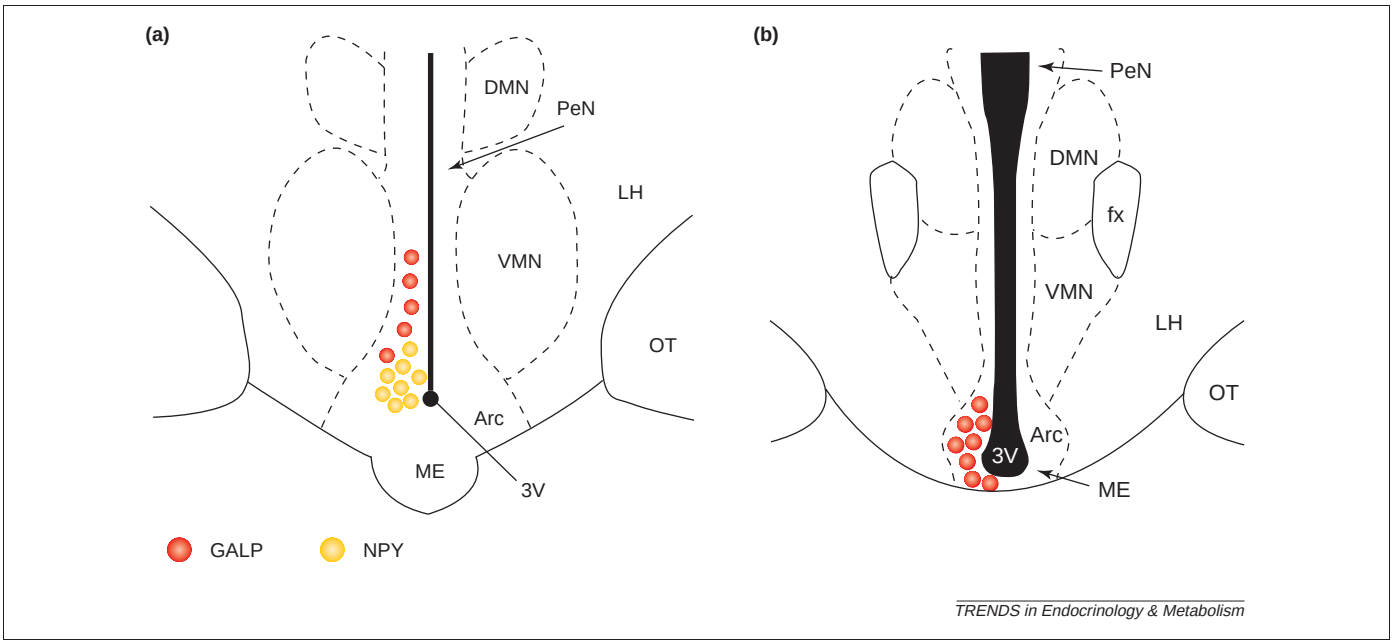

Figure 3. Localization of GALP and NPY in the macaque. Panels depict rostral (a) and caudal (b) hypothalamic sections to demonstrate the approximate location of GALP (red circles) and NPY (yellow circles). Although they localize in close proximity to each other, they represent separate and distinct cell groups in the Arc: GALP-positive neurons are localized more dorsal-medially, whereas NPY-positive neurons are localized more ventral-medially. The number of NPY-positive neurons reduces with caudal progression through the Arc. GALP-positive neurons do express NPY receptors [60]. Abbreviations: 3V, third ventricle, Arc, arcuate nucleus; DMN, dorsomedial nucleus; fx, fornix; GALP, galanin-like peptide; LH, lateral hypothalamus; ME, median eminence; NPY, neuropeptide Y; OT, optic tract; PeN, periventricular nucleus; VMN, ventromedial nucleus; VMH, ventromedial hypothalamus.

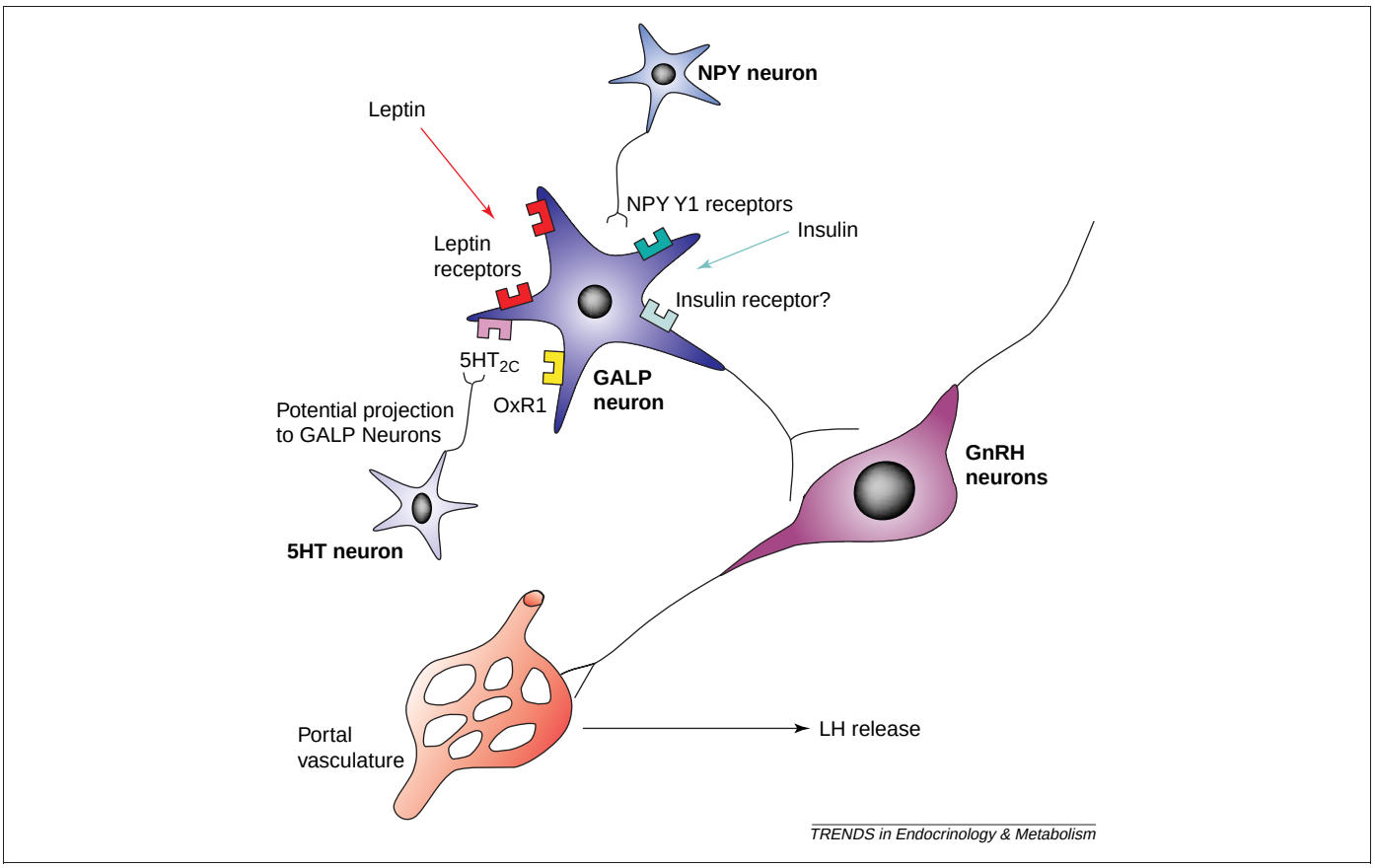

Figure 4. Proposed interactions between galanin-like peptide (GALP)-positive neurons and other neuroendocrine mediators. The GALP-positive neuron in this figure expresses receptors for leptin [29], neuropeptide Y (NPY Y1) [38], 5-hydroxytryptamine (5-HT_{2C}) [M. Cunningham, PhD Thesis, University of Washington, 2003] and orexin (OxR1) [63]. The majority (>85%) of GALP-positive neurons express leptin receptors [25], fewer express receptors for NPY, orexin and 5-HT. Although insulin also alters expression of the gene that encodes GALP [43], no insulin receptors have been identified on GALP-positive neurons. Leptin is shown with a red arrow because most GALP-positve neurons express leptin receptors and it is likely to have a large influence on the activity of GALP-positive neurons. Projections from GALP-positive neurons to neurons that contain gonadotropin-releasing hormone (GnRH) [46,61] stimulate the release of GnRH into the portal vasculature, which, in turn, stimulates the release of luteinizing hormone (LH) from the pituitary. As a result of the neuronal contacts between GALP and other factors, it is likely that GALP is a link in the integration of energy balance and reproductive function.

to several areas of the basal forebrain, including the PVN, lateral septal nucleus, BST and mPOA [33]. Following the central administration of GALP, Fos activation has been described in the horizontal limb of the diagonal band of Broca, caudal preoptic area, Arc and median eminence [30,31]. These regions could be direct targets of GALP projections; however, it is also conceivable that they reflect second-order neurons in the downstream signaling pathway of GALP. The phenotypic identity of neurons that are targets for GALP are yet to be elucidated.

Afferent inputs to GALP-positive neurons are only partially known. In the monkey, it appears that many (>40%) GALP neurons express the NPY Y1 receptor [60] and some (~25%) GALP-positive neurons express the 5-hydroxytryptamine 5-HT_{2C} receptor [60]. Fibers from orexin-positive neurons appear to be in close apposition to GALP cell bodies in the Arc, and a few (<15%) GALP-positive neurons appear to express the orexin type 1 receptor [63]. These observations indicate that GALP-positive neurons receive input from a variety of neurotransmitter systems in the brain (Figure 2 and Figure 3), notably those that are implicated in the neuroendocrine regulation of metabolism and reproduction [25].

GALP is a molecular link between metabolism and reproduction

GALP-positive neurons are attractive candidates for mediating the effects of metabolism on the neuroendocrine reproductive axis. First, these cells are direct targets for regulation by metabolic hormones (leptin, insulin and thyroid hormone), whose signaling is essential to support normal reproductive function [55,56,64–68]. Second, GALP-positive neurons, whose cell bodies reside in the Arc, send projections that terminate in the immediate vicinity of GnRH-positive neurons in the mPOA. Finally, GALP itself can stimulate GnRH secretion and sexual behavior. Thus, metabolically sensitive, GALP-containing neurons could be a conduit that relays important information about the status of fuel reserves to GnRH-positive neurons and thereby governs the activity of the reproductive axis as a function of metabolic state (Figure 4).

Acknowledgements

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