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Galanin/GALP and galanin receptors: role in central control of feeding, body weight/obesity and reproduction?

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Abstract

Scientific and commercial pharmacological interest in the role of galanin and galanin receptors in the regulation of food intake, energy balance, and obesity has waned recently, following initial enthusiasm during the 1980–1990s. It has been replaced by efforts to understand the role of newly discovered peptide systems such as the hypocretin/orexins, melanocortins and cocaine- and amphetamine-regulated transcript (CART) and their relationship to the important hormones, leptin and insulin. Thus, while numerous studies have revealed the ability of galanin to stimulate food intake via actions at sites within the hypothalamus, and shown reliable changes in hypothalamic galanin synthesis in response to food ingestion; findings including the lack of a 'body weight/obesity' phenotype in galanin transgenic mouse strains and a lack of agonists/antagonists for galanin receptor subtypes have probably served to reduce enthusiasm. However, as more is learnt about the general and galanin-related neurochemistry of brain pathways involved in feeding, metabolism and body weight control, the potential importance of galanin systems is again in focus. Studies of the newly discovered galanin *family* peptide, 'galanin-like peptide' (GALP), highlight the *likely* role of galanin peptides and receptors in the physiological coupling of body weight, adiposity and reproductive function. GALP is produced by a discrete population of neurons within the basomedial arcuate nucleus (and median eminence) that send projections to the anterior paraventricular nucleus *and* that make close contacts with leutinizing hormone-releasing hormone (LHRH) neurons in basal forebrain. Furthermore, GALP neurons express leptin receptors and respond to leptin treatment by increasing their expression of GALP mRNA. Centrally administered GALP activates LHRH-immunoreactive neurons and increases plasma LH levels. These findings suggest a direct stimulatory action of endogenous GALP on gonadotropin secretion via actions within the hypothalamus/basal forebrain, with leptin actions linking this system to body adipose levels. © 2002 Published by Elsevier Science B.V.

Keywords: GALP (galanin-like peptide); Galanin GAL1 receptor; Galanin gal2 receptor; Leptin; Insulin; POMC (pro-opiomelanocortin); GnRH (gonadotropin-releasing hormone); Hypothalamus; Reproduction

1. Introduction

Several comprehensive reviews have been published recently on the broad and complex topic of central control of appetite, body weight and obesity (e.g., Sawchenko, 1998; Baskin et al., 1999; Schwartz et al., 2000; Spiegelman and Flier, 2001), while other articles have highlighted the increasing health problem of obesity, particularly within Western society and discussed medicinal treatment strategies (Bray and Tartaglia, 2000; Spiegelman and Flier, 2001). In these articles most attention was devoted to the leptin system and its role in regulating body fat content via actions at various levels of the central nervous system. These actions involve some newly discovered peptide systems, such as the hypocretins/orexins (de Lecea et al., 1998; Sakurai et al., 1998; Sutcliffe and de Lecea, 2000); melanocortins (Sawchenko, 1998; Hagan et al., 1999; Tritos and Maratos-Flier, 1999) including agouti-related protein (AgRP; Graham et al., 1997; Ollmann et al., 1997; Shutter et al., 1997), melanin-concentrating hormone (MCH; Ludwig et al., 1998; Shimada et al., 1998); and cocaine- and amphetamine-regulated transcript (CART; Kristensen et al., 1998). Considerable data also demonstrates interactions of these new peptides with the pro-opiomelanocortin (POMC) and neuropeptide Y systems within key hypothalamic centres such as the arcuate, dorsomedial and paraventricular nuclei and the lateral hypothalamic area. Thus, while mentioned, *galanin* was far from the main focus in discussions of the central regulation of feeding/body weight.

Nonetheless, since the initial discovery that centrally administered galanin was able to stimulate food intake

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(Kyrkouli et al., 1986, 1990; Tempel et al., 1988), several groups have continued to collect data on galanin-related indices in a range of experimental models, in attempts to further clarify the role of galanin receptor systems in the regulation of food intake and body weight (e.g., Kalra and Kalra, 1996; Leibowitz et al., 1998; Kalra et al., 1999).

This article on galanin peptide and receptor systems will attempt to summarize the more important and novel data on the involvement of galanin in the neural circuitry within the hypothalamus that controls this vital autonomic regulatory system. Further in this regard, data on the newly discovered galanin family peptide, 'galanin-like peptide' (GALP) will be reviewed in relation to the leptin system—information which may help to refocus attention on galanin systems and body weight/obesity and that reveals further how leptin regulates reproductive function in mammals. Firstly however, to provide a background for these discussions, some details will be given on what is currently known about central galanin systems in general, as well as a short description of the major pathways, transmitters, peptides and hormones thought to be involved in feeding behaviour and body weight control.

2. Central galanin systems

Galanin is a 29-residue neuropeptide (30 in human) and the first 15 N-terminal amino acids, which retain the biological activity of the full-length peptide, are conserved across species (e.g., Wynick et al., 1998; Fig. 1). Galanin has a widespread distribution and is co-expressed with a number of transmitters (monoamines and amino acids) and other peptides in neurons in various brain regions (Melander et al., 1986b; Merchenthaler et al., 1993; Ryan and Gundlach, 1996). The distribution of [^{125}I]galanin-(1–29) binding sites, which generally matches the distribution of galanin-immunoreactive nerve terminals, is also well conserved amongst species including rats and primates (Skofitsch and Jacobowitz, 1985; Melander et al., 1986a, 1988; Skofitsch et al., 1986; Kohler et al., 1989; Kordower et al., 1992). There is also considerable experimental evidence that indicates galanin is an important cellular messenger within the central nervous system, mediating or modulating *diverse* physiological functions including nociception (e.g., Liu et al., 2001; Wynick et al., 2001), cognition (e.g., Steiner et al., 2001), and importantly for this review, aspects of neuroendocrine activity that are associated with feeding behaviour and reproduction (see Merchenthaler et al., 1993; Crawley, 1995; Hökfelt et al., 1998 for review).

2.1. Multiple galanin receptors

Distinct galanin receptor subtypes were initially proposed to account for the multiple and differential effects of galanin and its analogues observed experimentally (e.g., Bartfai et al., 1993; Wynick et al., 1993) and subsequently three distinct galanin receptors—GAL1 gal2 and gal3—have been cloned and characterized in rat, mouse and human (Burgevin et al., 1995; Parker et al., 1995; Fathi et al., 1997; Howard et al., 1997; Smith et al., 1997b, 1998; Wang et al., 1997a,b; see Branchek et al., 2000 for review).

All galanin receptor subtypes are known to be membrane-bound, G-protein-coupled proteins, but they differ with respect to their amino acid sequence (~ 40% homology), distribution, pharmacology, G-protein-coupling and resultant cellular signalling mechanisms. Thus, while the galanin GAL1 and gal3 receptors are strongly coupled to the inhibition of adenylate cyclase; the galanin gal2 receptor stimulates phospholipase C and inositol phosphate production and is only weakly coupled to adenylate cyclase inhibition (Parker et al., 1995; Fathi et al., 1997; Smith et al., 1997b; Wang et al., 1998). Galanin can also alter potassium and calcium ion channel function, the latter by mechanisms

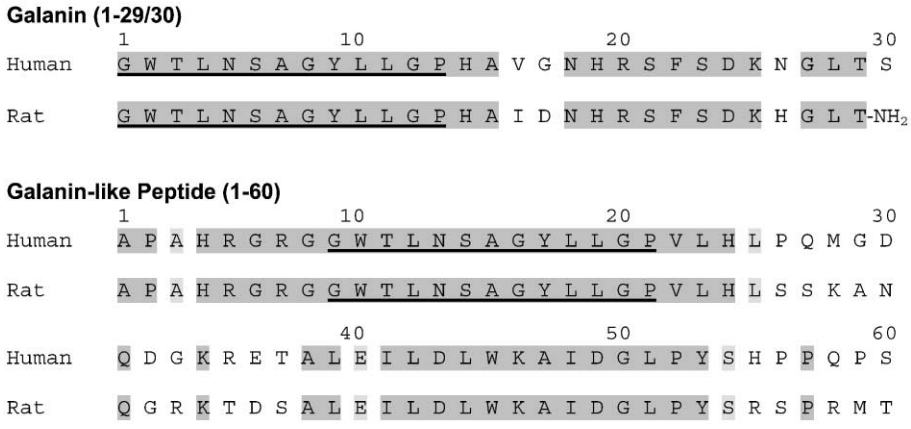


Fig. 1. Comparison of amino acid sequences for human and rat galanin and galanin-like peptide (GALP). The solid underline indicates the 13 residues shared by galanin and GALP. Darkly shaded characters are conserved residues between these two species. Lightly shaded residues in the GALP sequence are those that are different in the porcine peptide. For further details, see Ohtaki et al. (1999). A partial sequence of the mouse GALP cDNA (205 bp) has now been cloned, which demonstrates ~95% sequence homology to the rat (Juréus et al., 2001). The sequence encoding GALP residues 9–21 is identical in all species currently known, indicating its likely biological importance.

involving different G-protein subunits and protein kinase C activation (Smith et al., 1998; Simen et al., 2001; see Branchek et al., 2000 for review).

Northern blot and in situ hybridization studies have revealed the regional and cellular distribution of galanin GAL1 and gal2 receptor mRNA expression in adult and developing rat brain (Burgevin et al., 1995; Parker et al., 1995; Gustafson et al., 1996; Fathi et al., 1997; Mitchell et al., 1997, 1999a; Gundlach and Burazin, 1998; O'Donnell et al., 1999; Burazin et al., 2000). Galanin GAL1 receptor mRNA is strongly expressed in a number of regions including piriform cortex, lateral septum/diagonal band, numerous *hypothalamic* and thalamic nuclei, and key autonomic nuclei in the midbrain and brainstem. This distribution correlates reasonably well with that of [^{125}I]galanin binding sites (Skofitsch et al., 1986; Melander et al., 1988), suggesting that the galanin GAL1 receptor may be the predominant receptor in mature brain, but discrepancies that do exist could be due to the existence of other receptor subtypes with distinct binding characteristics.

In contrast to galanin GAL1 receptor mRNA, galanin gal2 receptor mRNA is widely distributed in peripheral tissues including pituitary gland, gastrointestinal tract, skeletal muscle, heart, kidney, uterus, ovary and testis, as well as being expressed in brain (Fathi et al., 1997; Howard et al., 1997; Smith et al., 1997b; Waters and Krause, 1999). Initial in situ hybridization studies (Fathi et al., 1997; Gundlach and Burazin, 1998) and more detailed, subsequent mappings (Mitchell et al., 1999a; O'Donnell et al., 1999; Burazin et al., 2000) have identified galanin gal2 receptor mRNA in several rat brain regions including hippocampus, in several *hypothalamic* nuclei, in olfactory and cortical areas, and in cerebellum, brainstem and spinal cord. Galanin gal3 receptor transcripts have been detected in brain and some peripheral tissues (Waters and Krause, 1999), but the precise and somewhat restricted distribution of galanin gal3 receptor expression within the rat brain has only recently been thoroughly described, presumably due to its relatively low abundance (Mennicken et al., 2001).

Thus, galanin GAL1, gal2 and gal3 receptor mRNAs are differentially distributed in adult rat brain, and yet share *some* overlapping patterns of distribution. Currently, no detailed reports exist on the immunohistochemical distribution of galanin receptors (proteins) in rat or other species, due to a lack of specific anti-receptor antibodies, although our laboratory has recently published data on the distribution of galanin GAL1 receptors in the hypothalamus (Burazin et al., 2001).

2.2. Galanin-like peptide (GALP)

Recently a peptide structurally related to galanin—'galanin-like peptide' (GALP)—was isolated and cloned from porcine hypothalamus and subsequent homology cloning identified GALP cDNA sequences in rat and human (Ohtaki et al., 1999). GALP is a non-C-terminally amidated peptide of 60 amino acids, in which residues (9–21) are identical to galanin-(1–13) and residues (1–24) and (41–53) are highly conserved across species (Fig. 1). Radioligand binding studies revealed that GALP-(1–60) had an ~20-fold higher affinity for galanin gal2 than for galanin GAL1 receptors. By contrast, galanin exhibited a ~5-fold higher affinity for galanin GAL1 than gal2 receptors (Ohtaki et al., 1999). In a [^{35}S]GTPγS agonist-activity binding assay, galanin displayed similar potency for both galanin GAL1 and gal2 receptors, whereas GALP-(1–60) had ~180-fold higher potency for galanin gal2 receptors. On this basis, the authors initially suggested that GALP may be the preferred ligand for the native galanin gal2 receptor (Ohtaki et al., 1999), although activity at galanin gal3 receptors was not assessed (Mennicken et al., 2001; and below).

We and others recently reported the distribution of GALP gene expression in rat brain, where it is restricted to neurons in the arcuate nucleus/median eminence (Juréus et al., 2000; Larm and Gundlach, 2000; Takatsu et al., 2001), suggesting the *possibility* of release of GALP within the hypothalamus, and/or from the median eminence to affect the *anterior* pituitary gland. Important recent studies examining the relationship between GALP neurons and their projections, with the leptin signalling system and luteinizing hormone-releasing hormone (LHRH) neurons in the basal forebrain (Juréus et al., 2000; Takatsu et al., 2001), have served to focus attention on a role for GALP in the regulation of feeding behaviour *and* reproductive function (see Section 8 for details).

3. Major pathways, neurotransmitters and hormones involved in the central regulation of feeding and body weight

Recent reviews have summarized the neuronal circuitry involved in feeding and adiposity signalling particularly in terms of leptin targets and to a lesser degree insulin targets, within the hypothalamus and autonomic centres of the brainstem (e.g., Baskin et al., 1999; Elmquist et al., 1999; Schwartz et al., 2000). In these models, leptin and insulin are thought to be key messengers in the long-term regulation of body weight by the brain. Changes in their plasma levels indicate a state of altered energy homeostasis and adiposity and the brain responds by adjusting food intake to restore adipose tissue mass.

A central theme of this rapidly developing area is that the arcuate nucleus of the hypothalamus is a *primary* target for leptin (and insulin), where the hormone(s) inhibits neuropeptide Y synthesis and increases expression of the POMC gene precursor of α-melanocyte-stimulating hormone (α-MSH). Leptin-responsive arcuate neurons are believed to project to the paraventricular nucleus and lateral hypothalamic area, where they influence the expression of peptides such as melanin concentrating hormone (MCH) and the orexins/hypocretins, which in turn are thought to stimulate feeding (see Sakurai et al., 1998; Baskin et al., 1999; Elmquist et al., 1999; Schwartz et al., 2000). Arcuate nucleus

neuropeptide Y neurons also express agouti-related protein (AgRP), which is an endogenous antagonist of the melanocortin MC_3 and MC_4 receptors. These receptors are primarily activated by α-MSH (Fan et al., 1997) and the melanocortin receptor system may be particularly important in controlling body weight, as a transgenic mouse that does not express melanocortin MC_4 receptors is severely obese (Huszar et al., 1997), as is a mouse that over-expresses AgRP (Graham et al., 1997). It is now known that the majority of arcuate POMC neurons express CART and that CART systems are also involved in central regulation of feeding and body weight. For example, CART suppresses feeding following intracerebroventricular injection and leptin administration elevates arcuate CART mRNA levels (Kristensen et al., 1998; Lambert et al., 1998).

Thus, the arcuate nucleus contains two counter-apposed populations of cells that are inhibited or activated by systemic leptin (and perhaps insulin) and in turn alter the input to the paraventricular nucleus and the lateral hypothalamic area and subsequent outputs to other brain areas to alter food intake (Elmquist et al., 1999). Several other nuclei within the hypothalamus, including subdivisions of the ventromedial and dorsomedial nuclei, and brainstem nuclei linked to the sympathetic nervous system, are also involved in this autonomic circuitry, as judged by several criteria including leptin responsiveness (activation or suppression). In fact, strong evidence exists for reciprocal effects of peptides and other transmitters on food intake/fat stores *and* sympathetic activity and energy expenditure (thermogenesis; Bray, 2000). Thus, peptides such as neuropeptide Y, MCH and *galanin* that increase food intake, reduce sympathetic activity, while a large group of peptides including α-MSH and CART reduce food intake and increase sympathetic activity. Further details of these aspects and other details can be found elsewhere in this special issue and in cited recent reviews (Baskin et al., 1999; Elmquist et al., 1999; Bray, 2000; Schwartz et al., 2000).[1]

One approach to detailing what is known about the involvement of *galanin* systems in feeding regulation and body weight control, is to describe any demonstrated or potential interaction of galanin-containing cells or terminals with the 'clearly involved' neuron populations referred to above. Thus, recent evidence for the expression of galanin receptors by the neuropeptide Y/AgRP and POMC/CART neuronal phenotypes of the arcuate nucleus and other relevant cell groups will be examined. Information about the presence of leptin receptors on relevant galanin-positive cells and leptin effects on these neurons should also provide some insight and many of these studies have been conducted by researchers over recent years.

[1] These articles provide easy-to-follow, often colourful schematics detailing all aspects of the neurochemical anatomy and signalling thought to be involved in feeding, body weight regulation and adiposity. It is recommended that readers consult these schemes when looking to integrate galanin-related findings into these current functional models.

4. Physiological and pharmacological studies of galanin in hypothalamus

High concentrations of galanin and galanin receptors are present in the hypothalamus and soon after the discovery of galanin, initial experiments were carried out to test the effect of acute intracerebroventricular administration of the peptide on feeding (Kyrkouli et al., 1986).

4.1. Effects and responses of galanin systems in the hypothalamus in feeding

These and subsequent studies reported the consistent effect of central galanin to rapidly increase food ingestion in satiated rats and potent and specific stimulatory effects of galanin on food intake via actions at sites close to the third ventricle, such as the paraventricular nucleus (Kyrkouli et al., 1986, 1990), the dorsomedial and ventromedial hypothalamic nuclei and the amygdala. Notably, repeated infusion of galanin for a 2-week period did not produce a significant change in body weight, although tachyphylaxis to the treatment was observed (Smith et al., 1994). More recent studies have described increases in galanin mRNA/peptide in several hypothalamic nuclei associated with hyperphagia and increased weight gain after inactivation of the ventromedial nucleus (Dube et al., 2000). It is thought however, that definitive studies in this area may require longer-acting galanin analogues and antagonists (see Crawley, 1999).

Early reports with pure macronutrient choice tests suggested that galanin preferably increased consumption of fat (Tempel et al., 1988). However, galanin treatment also induced large increases in consumption of mixed nutrient diets (high in fat or carbohydrate; Smith et al., 1997a), with a possible circadian pattern of nutrient preference (Tempel and Leibowitz, 1990). Subsequent reports appeared of a specific increase in galanin gene expression, peptide production and release in the anterior parvicellular region of the paraventricular nucleus and external zone of the median eminence, associated with fat ingestion, circulating glucose and body fat—with no such relationship to carbohydrate or protein ingestion (Leibowitz et al., 1998). Based on this data and the higher hypothalamic levels of galanin in obesity-prone, compared to obesity-resistant rats, these authors concluded that this galanin pathway from anterior paraventricular nucleus to the median eminence plays a role in the development of obesity produced by over consumption of fat. However, others argue that galanin increases food ingestion relatively independent of macronutrient content, under normal conditions (Crawley, 1999).

The paraventricular nucleus is also thought to play a critical role in feeding and adiposity signalling from the hypothalamus to the *brainstem*. The rich innervation of the paraventricular nucleus by neuropeptide Y and other peptide-containing terminals, including α-MSH and *galanin*, could thus influence the action or release of mediators in the brainstem (Baskin et al., 1999). Several candidate neuron

populations exist in the paraventricular nucleus including those expressing thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH) and oxytocin (see Baskin et al., 1999). With regard to the galanin system, CRH and oxytocin neurons in the parvocellular paraventricular nucleus may express either galanin GAL1 or gal2 receptors (Mitchell et al., 1997, 1999a; Gundlach and Burazin, 1998; Burazin et al., 2001), although direct double-labeling studies using appropriate gene probes or receptor antibodies, and appropriate functional studies have not yet been conducted (but see below).

4.2. Galanin receptor subtypes on hypothalamic neurons involved in feeding/body weight regulation

An important recent endeavour in establishing the position of galanin systems within the 'hypothalamic' circuitry for feeding and body weight control is the precise mapping of galanin receptor subtype expression within the hypothalamus and identification of the phenotype of galanin-receptive cells in key nuclei, such as the arcuate nucleus. For example, early studies had shown that galanin-containing fibres make synaptic contacts with POMC neurons in the arcuate nucleus (Horvath et al., 1995) and recent in situ hybridization studies have demonstrated that POMC/(CART) neurons within the arcuate express both galanin GAL1 and gal2 receptor mRNA (Bouret et al., 2000). Most of the galanin GAL1 and gal2 receptor-positive POMC neurons were located in the rostral parts of the nucleus and these cells are thought to predominantly project to the preoptic area, where LHRH (or GnRH) neurons are located (Cheung and Hammer, 1995). In this regard, good evidence exists that the POMC gene products, β-endorphin and α-MSH, are involved in regulation of reproduction (see Kalra et al., 1999).

In the studies by Bouret et al. (2000), castration was shown to decrease the level of galanin GAL1 and gal2 receptor mRNA expressed in POMC neurons, effects that could be abolished by testosterone replacement. Thus, it appears testosterone can modulate the LHRH neuroendocrine axis via regulation of galanin receptor expression in POMC neurons (Bouret et al., 2000). These findings are consistent with previous experiments demonstrating that gonadal steroids regulate arcuate POMC neuron activity (Chowen-Breed et al., 1989; Cheung and Hammer, 1995; Bouret et al., 2000). Importantly, removal of gonadal steroids by castration also decreased the number of *non-POMC* neurons expressing galanin receptor transcripts and the relative transcript abundance (Bouret et al., 2000), which has implications for the regulation of other neuronal phenotypes in the arcuate nucleus and other parts of the hypothalamus (e.g., GALP; see Section 8). In this respect, male gonadal steroids have also been shown to increase galanin neuron activity in *other* brain areas such as the medial amygdala and bed nucleus of the stria terminalis (Planas et al., 1994).

Given that POMC and other neurons in the arcuate nucleus express both galanin GAL1 and gal2 receptor mRNAs, it is possible that endogenous galanin peptides or exogenous galanin analogues could exert complex stimulatory *or* inhibitory effects on their activities. The net effect would depend on the relative affinity of the ligand for the receptor, the receptor type activated, and the signalling pathway(s) involved (Bouret et al., 2000; see Section 7). Clearly, these possibilities require further experimental examination, and should include studies on galanin gal3 receptors.

4.3. Effects of leptin on galanin and related systems

Peripheral or central administration of leptin reduces food intake in a variety of animals including rats and mice (e.g., Seeley et al., 1996; Schwartz et al., 1996). Following early studies that confirmed the importance of neuropeptide Y as a target of leptin—demonstrating decreased neuropeptide Y gene expression in obese and food-deprived animals via leptin receptors on these neurons (see Sahu, 1998a,b)—other target neurons have been systemically investigated. In studies of this kind, central administration of leptin decreased hypothalamic galanin gene expression and expression of genes encoding other 'stimulatory' peptides such as MCH and POMC, in association with a decreased food intake and reduced body weight gain in ad lib-fed rats (Sahu, 1998a). No peptide expression changes were observed in pair-fed animals and insulin levels were similar in both groups. Separate reports of the presence of leptin receptors on the cell bodies of these different peptide neurons (Håkansson et al., 1998), suggest that some of these actions of leptin could be direct. Subsequent studies that demonstrate a prior intracerebroventricular injection of leptin prevented increases in food intake produced by galanin, MCH or neuropeptide Y injection, also suggest a possible modulation by leptin of postsynaptic actions of the different peptides (Sahu, 1998b).

4.4. Effects of insulin on galanin-related parameters in hypothalamus

Much of the data on the modulation of galanin expression following the ingestion of different diets and macronutrients, and the lack of/small effect of food deprivation on galanin systems, are consistent with increases in galanin synthesis in response to food *ingestion*, rather than in response to *starvation* (see Crawley, 1999). Effects of insulin on brain galanin systems are also consistent with this idea.

High concentrations of insulin are found in the hypothalamus, mainly derived from the periphery by a saturable transport across the blood–brain barrier, and insulin receptors are present on neurons in this area. Central administration of insulin decreases galanin mRNA and peptide immunoreactivity levels in the anterior paraventricular nucleus (Wang and Leibowitz, 1997), suggesting that galanin expression may be regulated by circulating insulin/glucose levels following and between meals. Receptor responses to insulin have not been reported, although levels of galanin GAL1 receptor mRNA were increased in

the paraventricular nucleus following glucose and fat deprivation (Gorbatyuk and Hökfelt, 1998). However, these changes were strongest in lateral magnocellular regions and in the supraoptic nucleus, distinct from any actions within the anterior paraventricular nucleus.

In an interesting study of the effects of early postnatal over-nutrition, further possible links between insulin, obesity and galanin systems were identified (Plagemann et al., 1999). Postnatal pups subjected to over-nutrition from postnatal days 3–21 displayed hyperphagia, increased body weight, hyper-insulinemia and impaired glucose tolerance, throughout postnatal and adult life. From day 21 to adulthood, an increase in the number of galanin neurons in the arcuate (and paraventricular) nucleus was observed that was positively correlated with body weight. It was suggested by these authors that a form of resistance of the hypothalamic (arcuate) galanin system is acquired due to overfeeding and hyperinsulinemia during a critical period of hormone-dependent neuroendocrine system development (Plagemann et al., 1999). Interestingly these animals also display a leptin resistance, whereas leptin would normally act to decrease hypothalamic galanin (Sahu, 1998a).

In relevant clinical studies, plasma levels of galanin (and neuropeptide Y and leptin) are reported to be significantly higher in women with moderate to severe obesity and severe obesity plus non-insulin-dependent diabetes, relative to age-matched controls and patients with anorexia nervosa (Baranowska et al., 1997, 2000). However, no difference in plasma galanin levels were reported in a second study using similar methods and the precise source of these increased levels of peptide (central or peripheral) are not known. Thus, further precise and conclusive human studies are required (see Crawley, 1999).

5. Genetic models of obesity

Several naturally occurring mutations in genes related to an obese phenotype have been discovered and utilized extensively in studies of neuroanatomical, neurochemical, neuroendocrine and behavioural aspects of feeding and body weight control. The most well known of these include the *ob/ob* and *db/db* mice and the obese, *fa/fa* (fat) Zucker rats and mice, which have abnormalities of leptin signalling (e.g., Beck et al., 1993; Rovere et al., 1996; Baskin et al., 1999). In more recent years, molecular biological approaches have allowed the discovery or generation of several other genetic models in which specific genes encoding individual neuropeptides or receptors have been naturally or experimentally deleted or over-expressed (e.g., neuropeptide Y and neuropeptide Y Y2 receptor knockout mice (Erickson et al., 1996; Naveilhan et al., 1999); MCH and melanocortin-4 receptor knockout mice (Huszar et al., 1997; Shimada et al., 1998) and AgRP transgenic mice (Graham et al., 1997). Studies of the phenotype and the brain chemistry of these animals have helped to further clarify important components of the physiological pathways that regulate energy balance and body weight, often in very significant ways. Over recent years, various groups have also collected data on galanin-related parameters in the various natural models of obesity. Galanin gene knockout and galanin over-expressing mouse strains have also been generated (see Section 6).

5.1. Galanin and related systems in leptin-signalling deficit rats

Obesity in the Zucker rat is associated with hyperphagia, altered plasma insulin and corticosterone, and clear perturbations of the neuropeptide Y system. However, the response of the galanin system to physiological and 'imposed' manipulations of food intake and energy expenditure in this and other strains has been less clearly defined. Thus, while food deprivation or restriction increase neuropeptide Y gene expression (Brady et al., 1990; O'Shea and Gundlach, 1991), these manipulations do not *consistently* alter galanin mRNA levels in the hypothalamus. In normal Sprague–Dawley rats, fasting or food restriction had no effect on galanin gene expression in the arcuate or dorsomedial nuclei (Brady et al., 1990), or reduced mRNA levels in the arcuate (Brady et al., 1990; O'Shea and Gundlach, 1991). In mature Zucker rats, fasting had no effect on regional concentrations of galanin in the hypothalamus (Mercer et al., 1996), but the levels of galanin in the parvocellular paraventricular nucleus of obese Zucker rats was double that of lean littermates, while (nerve terminal) levels in the median eminence were lower (Beck et al., 1993). Galanin mRNA levels were elevated in the paraventricular nucleus of the obese Zucker (Mercer et al., 1996) in an age-dependent manner (Jhanwar-Uniyal and Chua, 1993).

With regard to macronutrient alterations, Mercer et al. (1996) showed that feeding a high-fat diet to obese Zucker rats for 4 weeks starting at 1 week post-weaning, reduced galanin gene expression and plasma insulin concentration, with a likely causal relationship between them. Notably, despite age- and phenotype-dependent changes in neuropeptide Y gene expression, no differences in CRH were found in the different age or phenotype Zucker rat groups (Mercer et al., 1996). These studies suggest that the link between galanin and fat intake may have a physiological basis, as the 'feedback' effect observed by these investigators was confined to the obese animals (Mercer et al., 1996). As mentioned, studies by Leibowitz and colleagues also strongly suggest that the paraventricular nucleus is a critical site for the effects of galanin on food intake and body weight and that levels of galanin peptide and gene expression in this region correlate with natural feeding preferences (Akabayashi et al., 1994; Leibowitz et al., 1998; see above). Again, further studies in this area would be worthwhile, including the response of different galanin receptor subtypes in subdivisions of the paraventricular nucleus and elsewhere in the hypothalamus to different macronutrients in normal and obese rat strains.

6. Genetic disruption of galanin expression in the mouse — absence of obvious feeding/body weight phenotypes

Transgenic mice that do not express or over-express galanin have now been developed (Wynick et al., 1998; Blakeman et al., 2001; Steiner et al., 2001). Despite several phenotypes that reflect other aspects of the putative galanin physiology previously established, galanin transgenic animals do not appear to display an obese or lean phenotype, or any strong alterations in feeding pattern or body weight. Thus, body weights of the null mutant mice are not significantly different from wild-type control mice during the first 8 weeks or in adulthood (Wynick et al., 1998); and no differences in body weight were reported for a galanin transgenic mouse (Crawley, 1999; Steiner et al., 2001). This contrasts with several other 'orexogenic peptide or receptor' knockouts and transgenic animals (see Section 3).

However, specific studies examining the response of these galanin mutant animals to acute or long-term changes in diet or other alterations in inputs to the hypothalamus, such as leptin or insulin treatment have not yet been reported. In addition, both mouse strains represent conventional 'whole-of-life' mutations in which compensatory genetic/biochemical effects may mask the underlying phenotype of the mutation. As pointed out recently by Crawley (1999), it may be that targeted gene mutation using 'conditional-knockout' gene technology confined to specific time periods and brain regions such as hypothalamus, will better reveal the role of endogenous galanin and other peptides in feeding and weight control.

7. Galanin systems and reproduction

The initiation and maintenance of reproductive function are governed by physiological factors associated with nutrition, adiposity and metabolic rate, and abnormalities in body weight and metabolism can alter the activity of the reproductive system. For example, severe dietary restriction and/or excessive exercise can delay the onset of puberty or produce infertility in adulthood, via hypothalamic–pituitary dysfunction. However, while the *precise* nature of the physiological mechanisms linking nutrition, metabolism and reproduction are still to be fully clarified, molecules such as leptin clearly act as metabolic signals to the reproductive axis in many species (see below). These signals in turn alter the level of key hormones such as the gonadotropins (LH/FSH) via central actions on the neural circuitry controlling LHRH secretion (see Finn et al., 1998; Magni et al., 2000 for review). The small number of LHRH neurons within the basal forebrain are vital for successful reproduction and are influenced not only by peripheral hormonal feedback mechanisms, but also directly or indirectly by many known neurotransmitters and neuromodulators.

7.1. Relationship of galanin and LHRH systems

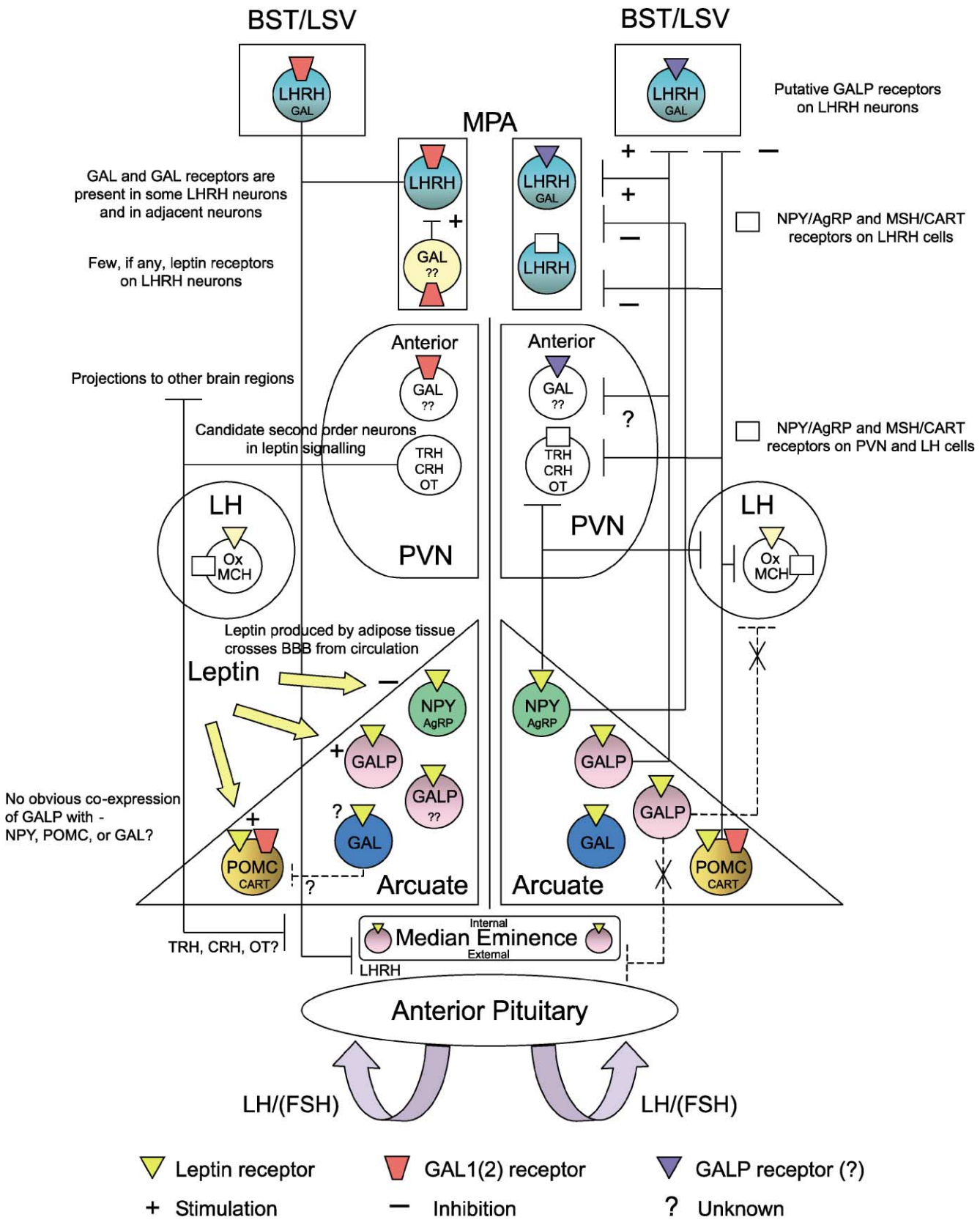
In this respect, galanin is able to modulate the release of LH and a number of LHRH neurons express galanin mRNA and immunoreactivity or receive galanin input in various species, including rat and mouse (Coen et al., 1990; Rajendren and Gibson, 1999; Rajendren and Li, 2001; Fig. 2). Furthermore, the level of galanin expression is sexually dimorphic in these species with more galanin-immunoreactive LHRH cells in intact, mature female than male animals. However, there appear to be differences between these two experimental species regarding the conditions when peptide co-expression is observed (Rajendren and Gibson, 1999). Thus, the amount of galanin in LHRH cells is negatively regulated in mice and positively regulated in rats by the gonadal hormone, oestrogen. Nonetheless, studies across the oestrus cycle and following various hormonal manipulations provide evidence that galanin expression increases in LHRH neurons during their activation (see Rajendren and Gibson, 1999). Additional studies examining the effect of exogenous galanin on LH release and on the effects of LHRH, suggest that galanin present in LHRH neurons may be released and act upon LHRH terminals to further enhance release of LHRH (see Rajendren and Gibson, 1999).

At this stage, the significance of the differences in steroid regulation of galanin co-expression in LHRH neurons in rat and mouse is unclear. But differences in the distribution and abundance of galanin are also found in other brain regions in these species (Cheung et al., 2001) and levels of GALP in the arcuate nucleus are lower in mouse than in the rat (Kerr et al., 2000; Juréus et al., 2001; unpublished observations), which may also relate to differences in the regulation of LHRH neuronal function (see Section 8).

The galanin receptor profile of LHRH cells may also be different in the two species and requires investigation. Currently there is only data on the expression in female rats of galanin GAL1 receptor mRNA by a very limited number of LHRH neurons in a restricted region of the anterior hypothalamus, relative to the abundant expression of these transcripts by near adjacent neurons of unidentified phenotype (Mitchell et al., 1999b; Fig. 2).

7.2. Effects of leptin on LHRH neurons—direct or indirect actions?

Recent studies have endeavoured to identify different populations of leptin-target cells, including LHRH neurons and others, in different regions of the hypothalamus. While LHRH neurons *may* express leptin receptors in some species such as mouse (Magni et al., 1999), few if any LHRH neurons express leptin receptors in the rat (Håkansson et al., 1998) or primate (Finn et al., 1998). Thus, while it is possible that a small population of leptin receptor-expressing LHRH neurons may mediate stimulatory actions of leptin on gonadotropin secretion in the rat, it seems more


BST/LSV
BST/LSV
LHRH
GAL
MPA
Putative GALP receptors
on LHRH neurons
GAL and GAL receptors are
present in some LHRH neurons
and in adjacent neurons
Few, if any, leptin receptors
on LHRH neurons
NPY/AgRP and MSH/CART
receptors on LHRH cells
Anterior
Projections to other brain regions
Candidate second order neurons
in leptin signalling
TRH
CRH
OT
PVN
NPY/AgRP and MSH/CART
receptors on PVN and LH cells
LH
Ox
MCH
Leptin produced by adipose tissue
crosses BBB from circulation
Leptin
NPY
AgRP
GALP
No obvious co-expression
of GALP with -
NPY, POMC, or GAL?
POMC
CART
Arcuate
TRH, CRH, OT?
Internal
Median Eminence
External
LHRH
Anterior Pituitary
LH/(FSH)
Leptin receptor
GAL1(2) receptor
GALP receptor (?)
Stimulation
Inhibition
Unknown

likely that leptin influences LHRH secretion indirectly through other intermediate neurons (Fig. 2).

A variety of arcuate nucleus neurons are thought to interact with LHRH neurons and those that synthesize/release neuropeptide Y and POMC are candidates for mediating the effects of leptin. Neuropeptide Y has been shown to influence LHRH and LH secretion in rats and other species (Kalra and Kalra, 1996) and arcuate neuropeptide Y neurons express leptin receptors. Similarly, many POMC neurons express leptin receptors (Cheung et al., 1997) and leptin regulates arcuate POMC mRNA levels in normal and *ob/ob* or *db/db* mice (Schwartz et al., 1997; Thornton et al., 1997). POMC neurons make direct synaptic contacts with LHRH neurons (Leranth et al., 1988) and peptide products of the POMC precursor are possible mediators of leptin action—β-endorphin inhibits LHRH/LH release (Kalra and Kalra, 1996) and α-MSH has been shown to modulate LH secretion in rats (Scimonelli and Celis, 1990; see above). As mentioned, arcuate POMC neurons express GAL1 *and* gal2 receptor mRNA (Bouret et al., 2000; Fig. 2).

There are several other mechanisms by which leptin could affect reproductive function, including effects on LH secretion mediated by hormones of the hypothalamo–pituitary–adrenal axis, namely CRH and/or glucocorticoids (see Finn et al., 1998). Very recent studies suggest that activation by leptin of GALP neurons in the arcuate nucleus also produces LH secretion by activation of LHRH neurons in the preoptic area (see Section 8). Overall, it appears that a major role of leptin is to sustain reproduction in animals that have adequate metabolic reserves (Finn et al., 1998; Magni et al., 2000).

8. Central GALP neurons, leptin and reproduction

8.1. GALP distribution and regulation in rat brain

Using in situ hybridization, GALP mRNA was first reported to be present in a small population of neurons within the periventricular regions of the basomedial arcuate nucleus and in the median eminence of the rat (Juréus et al., 2000; Larm and Gundlach, 2000). Subsequently, using an anti-GALP monoclonal antibody raised against rat GALP-(1–9) (a sequence not shared with galanin), the presence of GALP-positive neuronal cell bodies was confirmed within the arcuate nucleus, being particularly dense in the medial posterior part (Takatsu et al., 2001). In addition, GALP-stained cells were detected in the median eminence and the infundibular stalk, but were absent from other hypothalamic nuclei and other brain loci (Juréus et al., 2000; Kerr et al., 2000; Larm and Gundlach, 2000; Takatsu et al., 2001). Arcuate GALP cells were shown to send GALP-positive projections to various areas of the basal forebrain, including the anterior, parvicellular paraventricular nucleus, the medial preoptic area and the bed nucleus of the stria terminalis—in these latter regions they made close contacts with LHRH-expressing neuronal cell bodies (Takatsu et al., 2001; Fig. 2).

Furthermore, using double-label immunohistochemistry, the majority (90%) of GALP neurons in the arcuate nucleus were shown to express leptin receptors. Also important in advancing our knowledge of the precise circuitry involved in feeding and body weight, these authors examined whether GALP was co-expressed by other documented arcuate neuron phenotypes and compared the regional distribution of GALP immunoreactive fibres with those for known orexigenic and anorectic peptides (Takatsu et al., 2001). In these studies, GALP-positive neurons in the arcuate nucleus were reported to be different to known leptin-receptor expressing neurons located in the arcuate that express neuropeptide Y/AgRP (Håkansson et al., 1998), POMC/CART (Cheung et al., 1997; Elias et al., 1999) and galanin (Håkansson et al., 1998; Larm and Gundlach, 2000; Fig. 2).

Both GALP mRNA-containing cells and POMC/CART neurons have earlier been shown to respond to leptin treatment by increasing expression of their respective peptides, whereas neuropeptide Y/AgRP neurons are inhibited by increased leptin levels (Elias et al., 1998, 1999; Juréus et al., 2000). Thus, on the basis of the differential distribution and responses of these various neuron populations, GALP neurons are proposed to be a separate population enriched in the periventricular area of the arcuate (Takatsu et al., 2001). GALP-immunoreactive nerve fibres were detected in the paraventricular nucleus with a rostrocaudal distribution different to other cells and terminals and were not found in other feeding related areas, such as the lateral hypothalamic area

Fig. 2. Schematic illustration of the putative hypothalamic GALP system and its likely relationship with LHRH neurons in the anterior hypothalamus/medial basal forebrain and other peptide neurons in the arcuate and paraventricular nuclei. GALP neurons in the arcuate nucleus are reportedly a distinct population from adjacent neuropeptide Y/AgRP, POMC/CART and galanin neurons that also possess leptin receptors. GALP-positive fibres project to various areas of the basal forebrain, including the medial preoptic area and the bed nucleus of the stria terminalis and make close contacts with LHRH-expressing neuronal cell bodies. The external zone of the median eminence lacks GALP immunostaining, suggesting GALP is unlikely to serve as a hypophysiotropic hormone. Further studies are now required to clarify the precise role(s) of GALP in these hypothalamic systems. Importantly, galanin also modulates LH release and a percentage of LHRH neurons express galanin, or receive galanin input. The galanin receptor profile of LHRH and arcuate peptide neurons may be species-specific, but has not yet been thoroughly investigated. However, in female rats, galanin GAL1 receptor mRNA is expressed by a very limited number of LHRH neurons in a restricted region of the anterior hypothalamus, relative to the abundant expression of these transcripts by near adjacent neurons of unidentified phenotype. Also, POMC neurons in the arcuate nucleus express both galanin GAL1 *and* gal2 receptor mRNA. (See text for further details of these and other aspects and relevant citations.) Abbreviations not defined in text: BBB, blood–brain barrier; BST, bed nucleus of the stria terminalis; CRH, corticotropin-releasing hormone; GAL, galanin; LH, lateral hypothalamus; LH/(FSH), luteinizing-hormone/(follicle-stimulating hormone); LSV, lateral septum, ventral; MCH, melanin concentrating hormone; MPA, medial preoptic area; MSH, α-melanocyte-stimulating hormone; NPY, neuropeptide Y; OT, oxytocin; Ox, orexin; PVN, paraventricular nucleus; TRH, thyrotropin-releasing hormone.

(Takatsu et al., 2001) that receive many fibres positive for other leptin regulated peptides. Importantly also, no remarkable staining for GALP-positive fibres was found in the external zone of the median eminence, suggesting GALP is unlikely to serve as a hypophysiotropic hormone (Takatsu et al., 2001; see also Kerr et al., 2000; Shen et al., 2001; Fig. 2).

Very recent studies have reported the presence and distribution of GALP mRNA containing neurons within the mediobasal hypothalamus of the mouse (Juréus et al., 2001), where the distribution is near identical to that seen in the rat. The number of GALP mRNA-positive cells was significantly decreased in leptin deficient *ob/ob* mice and GALP expression was markedly increased above vehicle, control values following replacement intracerebroventricular infusion of leptin for 7 days in *ob/ob* mice (Juréus et al., 2001).

8.2. Functional effects of GALP following central administration

The possibility that GALP neuron projections making close contact with LHRH-positive perikarya and dendrites results in a direct modulation of transmitter release from these cells was investigated and revealed that centrally administered GALP (but not galanin) *activated* LHRH-immunoreactive neurons and increased plasma LH levels (Matsumoto et al., 2001). Intracerebroventricular injection of GALP (5 nmol) significantly increased plasma LH levels between 10 and 60 min post-treatment, without altering plasma levels of other anterior pituitary hormones. GALP administration was shown to increase LHRH secretion by demonstrating an attenuation of the plasma LH rises following pretreatment with an LHRH receptor antagonist and by the visualization of activated LHRH neurons, using Fos protein expression as an activation marker and double-labelling Fos-positive cells in the medial preoptic area for LHRH (Matsumoto et al., 2001). In addition GALP was shown to have no direct effect on the basal release of LH from dispersed anterior pituitary cells.

Thus, given the evidence for regulation of GALP neurons by leptin (Juréus et al., 2000, 2001), these results suggest that GALP plays an intermediary role coupling the action of leptin to endocrine control systems, in this case positive coupling to pulsatile LHRH release (Matsumoto et al., 2001), whereas neuropeptide Y may couple leptin signalling negatively to LHRH secretion. Further studies should clarify the role of GALP and galanin/GALP receptors in this system and other hypothalamic actions of GALP in rat and other species (see Fig. 2).

9. Pharmacotherapy of obesity—general principles and potential for galanin peptide systems?

Obesity is defined medically as a state of increased body weight or more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences. Body weight and composition and the storage of energy as adipose tissue are determined by the interaction of genetic, environmental and psychosocial factors with physiological systems which together act to set or alter the energy balance equation of intake and expenditure (see Spiegelman and Flier, 2001). Clear therapeutic issues and opportunities exist in the field of obesity disorders, given the enormity of the problem particularly within Western society and the apparent cascade effect that obesity can have in promoting infertility, hypertension, cardiovascular disease and even cancer (Kopelman, 2000; Spiegelman and Flier, 2001).

The present review of observations made so far on galanin systems—both galanin and GALP and their putative specific receptors—in the regulation of feeding, body weight and obesity, suggests that the role played by this peptide family is not a major or pivotal one. But nonetheless, these peptides may have the ability to modulate the activity of several key mediators, or to mediate their own relevant actions within the complex circuitry of the brain and within the neuroendocrine axis.

In particular, the apparent discrete and restricted nature of the GALP system (Juréus et al., 2000, 2001; Larm and Gundlach, 2000; Takatsu et al., 2001) and the functional evidence obtained so far (Matsumoto et al., 2001), suggest that GALP may play an important role in linking body weight signals to reproductive function. Our preliminary data on GALP expression within other key tissues, such as the testis, strengthens this putative association. Thus, in adult rats, GALP mRNA is differentially expressed within seminiferous tubules (Shen, J., Larm, J.A., O'Donnell, L., Gundlach, A.L., unpublished observations) and this could possibly be linked to leptin receptor signalling (e.g., El-Hefnawy et al., 2000).

As far as the author is aware, very little in the way of clinical or preclinical testing of galanin-based pharmaceuticals has been conducted/published, not least because of a current lack of appropriate agents. However, in light of the above observations and continuing research to further elucidate the nature of the *complete* galanin peptide family biology, aided by the recent development of some novel receptor-specific molecules (see e.g., Carpenter et al., 1999; Liu et al., 2001), such testing may become more common in the near future.

10. Future directions

10.1. Specific galanin receptor agonists/antagonists as tools for studies on obesity?

In general, research on galanin systems has lagged behind that of many other peptide systems due to the lack of a suitable range of potent and specific peptide and non-peptide antagonist molecules. Thus, early studies such as those by Crawley and others reported the ability of chimeric peptide analogues of galanin infused into the lateral ventricle to

inhibit feeding induced by galanin microinjected into the paraventricular nucleus of the hypothalamus or central nucleus of the amygdala (Bartfai et al., 1993; Crawley et al., 1993). However, these and other similar studies were conducted prior to the discovery and detailed characterization of the multiple galanin receptor subtypes (Branchek et al., 2000; see above). Now, with an increased knowledge of galanin GAL1, gal2 and gal3 receptor signalling and their distribution within the hypothalamus (Gundlach and Burazin, 1998; Mitchell et al., 1999a,b; Bouret et al., 2000; Branchek et al., 2000; Burazin et al., 2001; Mennicken et al., 2001), together with the recent development of putative, 500- to 1000-fold selective galanin GAL1 and gal2 receptor agonists and antagonists (Scott et al., 2000; Liu et al., 2001), more precise determinations of the role of galanin and galanin receptors in the control of feeding and body weight should be possible. For example, as pointed out in a recent review by Crawley (1999), 'receptor subtypes may work in opposition to each other and it may therefore be necessary to use subtype selective agonists and antagonists to determine the *precise* role of endogenous galanin peptides in feeding and energy balance'.

In addition, comprehensive analysis of transgenic animals with 'whole-of-life' or 'conditional, brain-region specific' mutations in galanin or related genes, in experimental paradigms relating to feeding and dietary constituents may reap currently unanticipated benefits. A continued focus on aspects of galanin peptide signalling associated with the reproductive cycle and adiposity levels may also be worthwhile. At this stage it is important to note that considerable attention has recently been directed towards peptides such as neuropeptide Y as potential targets for anti-obesity therapeutics (Inui, 1999; current issue) and galanin is analogous to neuropeptide Y in many ways. Thus, it is possibly only the development of appropriate pharmacological and molecular tools that prevents clarification of the role of galanin and galanin receptor subtypes in ingestive behaviour and various clinical disorders related to appetite, metabolism and body weight regulation (see Crawley, 1999).

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