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Galanin and the neuroendocrine axes

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Abstract. Galanin has diverse physiological functions, including nociception, arousal/sleep regulation, cognition, and many aspects of neuroendocrine activities that are associated with feeding, energy metabolism, thermoregulation, osmotic and water balance, and reproduction. This review will provide a brief overview of galanin actions in some major neuroendocrine processes. Most of the recent data are about the role of galanin in the central regulation of food intake and energy metabolism, and to some extent, in the regulation of reproduction. It seems that galanin plays a modulatory rather than regulatory role in the central and peripheral branches of the neuroendocrine systems. In the hypothalamus, it functions as a neurotransmitter/neuromodulator. In the pituitary and the peripheral endocrine glands, it acts via its receptors (GALRs) in a paracrine/autocrine fashion. The development of new, selective and potent antagonists of GALRs should keep advancing our knowledge not only in the physiology but also the pathophysiology of galanin as well. (Part of a Multi-author Review)

Keywords. Hypothalamus, pituitary, gonadotroph, lactotroph, adrecorticotroph, somatotroph, thyreotroph.

Introduction

Galanin, a 29-amino acid peptide is a cellular messenger (neurotransmitter/neuromodulator) within the central (CNS) and peripheral (PNS) nervous systems, modulating diverse physiological functions, including nociception, arousal/sleep regulation, cognition, and many aspects of neuroendocrine activities that are associated with feeding, energy metabolism, thermoregulation, osmotic and water balance, and reproduction.

To date, three galanin receptors (GALRs), referred to as GALR1–3 have been identified by molecular cloning and each has been pharmacologically characterized (for details see review by Tamas Bartfai in this issue). Recent studies have helped to establish which GALRs are involved in specific actions using newly available subtype-selective agonists and antagonists and transgenic mouse models, including galanin- and GAL-R1 and GAL-R2 gene-deletion strains. In light of the large number of putative physiological actions of galanin in the neuroendocrine regulation of the anterior pituitary and peripheral endocrine organs, providing details of all of them and the many supporting studies is beyond the scope of this review. The sections below will provide a brief overview of galanin actions in some major neuroendocrine processes. Due to space limitations, rather then listing all relevant references, when available, only review papers summarizing certain aspects of galanin action will be provided.

Parvicellular (hypophysiotropic) and magnocellular galanin systems

The parvicellular neuroendocrine neurons of the hypothalamus release specific neuropeptides (releasing and inhibiting hormones) into the hypophysial portal circulation and therefore regulate the function of the anterior pituitary (AP). These neurons are located in the paraventricular nucleus (PVN), arcuate nucleus (AN), anterior periventricular nucleus (PeN), preoptic area (POA), medial septum (MS), and diagonal band of Broca (DBB). Among these hypophysiotropic nuclei, galanin is expressed in PVN and

AN. In addition, in female rodents, galanin is also expressed in scattered neurons of the DBB, MS, and POA, where it is colocalized with luteinizing hormone-releasing hormone (LHRH; also called gonadotropin releasing-hormone or GnRH, [1]. Galanin is also expressed in magnocellular hypothalamic neurons such as those in the PVN and supraoptic nuclei (SON). Neurons from these nuclei project to the posterior lobe of the pituitary where galanin and the peptides colocalized with galanin (e.g., arginine vasopressin [AVP] and oxytocin [OT], etc.) are released into the general circulation and regulate distant targets. For a review see [2].

Pituitary galanin

In the anterior pituitary (AP), the cell-specific expression of galanin is species- and sex-dependent. In female rats, galanin is expressed in lactotrophs (prolactin, PRL) and the expression is estrogen-dependent. In male rats, galanin is present in somatotrophs (growth hormone, GH), thyrotrophs (thyroid-stimulating hormone, TSH), and corticotrophs (adrenocorticotropin hormone, ACTH). In monkeys, galanin is expressed in thyrotrophs and gonadotrophs (luteinizing hormone, LH, and follicle-stimulating hormone, FSH). In humans, galanin is present in corticotrophs. For a review see [2].

Galanin and the hypothalamic-pituitary-gonadal (HPG) axis

Galanin, made locally in the AP, may directly control pituitary LH secretion via a paracrine mechanism or galanin synthesized in the diencephalon may also reach gonadotroph cells in the pituitary via the hypophysial portal circulation. In fact, galanin release into the hypophysial portal circulation follows a pulsatile fashion necessary for biological action. Retrograde labeling studies from the median eminence confirmed these functional observations in rats by demonstrating labeled neurons in the AN and PVN following peripheral injection of the retrograde tracer fluoro-gold. Galanin, either from the hypothalamus or the AP, not only stimulates LH secretion but also enhances LHRH binding to LHRH receptors of pituitary gonadotrophs. For reviews see [2–4].

The direct action of galanin on LHRH release is supported by the presence of GAL-R1 in LHRH neurons [5] and by the observation that in humans galanin directly communicates with LHRH neurons via putative synaptic contacts (Fig. 1) [6, 7]. Interestingly, in humans, galanin contacts LHRH neurons, but LHRH cells also form direct connections with galanin-immunoreactive neurons elsewhere in the brain (Fig. 2), suggesting that LHRH may modulate galanin neuronal activity. Their presence should provide the impetus to confirm the existence and functionality of LHRH-galanin connections in animal models. The presence of reciprocal connections between galanin and LHRH suggests that these peptides may regulate the activity of each other to provide a physiologically optimal, pulsatile pattern across neuroendocrine systems.

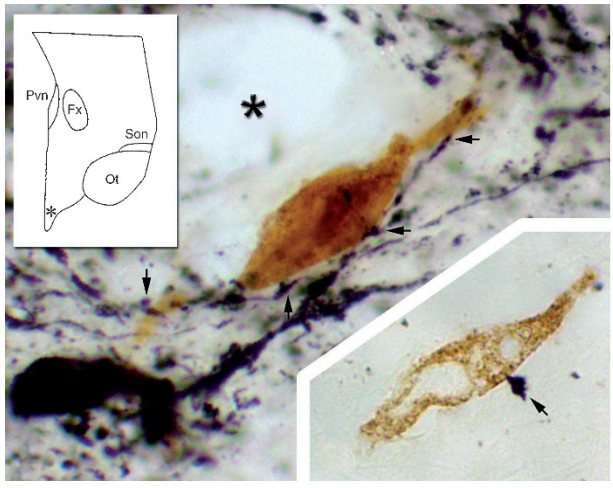

Figure 1. Juxtapositions (arrowheads) between LHRH (luteinizing hormone-releasing hormone, brown) and galanin (black) immunoreactive (IR) structures in the human hypothalamus. The position of the LHRH-IR neuron is shown by the asterisk in the upper left corner of the micrograph. The semithin section (insert) demonstrates that the LHRH- and galanin-IR elements are indeed in the same level and do not exhibit any gap between juxtaposed structures thus indicating potential synaptic connections. Pvn, paraventricular nucleus; SON, supraoptic nucleus; OT, oxytocin, Fx, Fornix. From: Dudas and Merchenthaler, Neuroscience 127 (2004) 695–707 with permission.

In addition to synaptic communication between galanin and LHRH, the fact that galanin is co-expressed with LHRH in a sexually dimorphic manner in the neurons of rat [8], mice [9], and ovine [10], but not in humans, adds an additional level of potential interaction between these systems. The level of galanin expression within LHRH neurons is sexually dimorphic in rodents [8,9], but not in sheep [10], with more co-expressing neurons in the adult female than male rats. The expression of galanin in LHRH neurons is estrogen-dependent and the stimulatory actions of estrogen are mediated via estrogen receptor-beta (ER-β) expressed in LHRH/galanin neurons [11]. Since galanin antagonists [12] or antisera against galanin [13] can block the preovulatory LH surge and ovulation in rats, it seems that galanin plays a crucial role in reproduction. Interestingly,

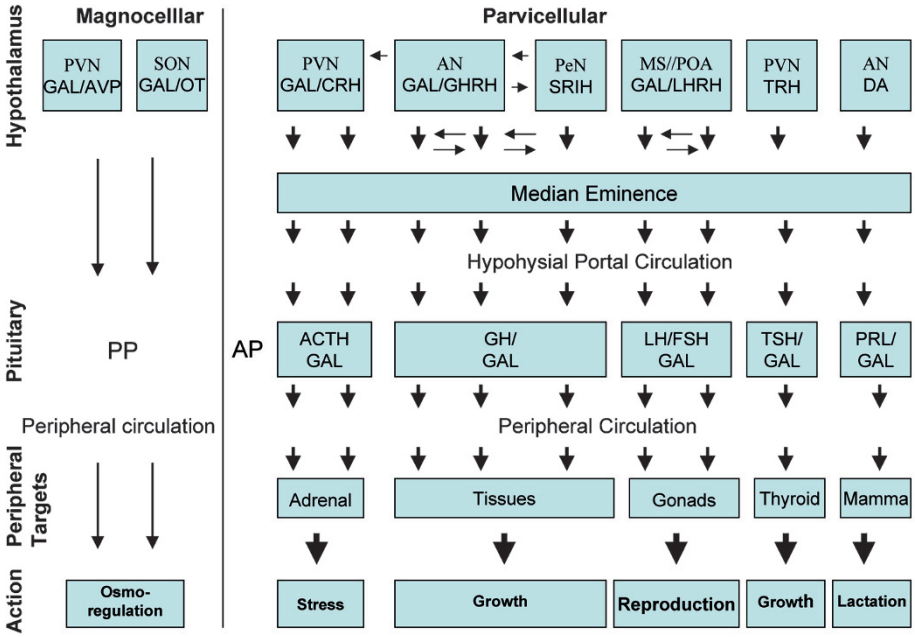

Figure 2. Schematic representation of the interactions between galanin (GAL) and hypothalamic neuropeptides and pituitary hormones. In the magnocellular hypothalamic nuclei GAL, among others, coexists with AVP in the PVN and with OT in the SON. These neuropeptides are transported to the posterior lobe of the pituitary (PP) where they are released into the general circulation by which they reach the target tissues (kidney, smooth muscle cells, etc.). In the parvicellular hypothalamic nuclei, GAL coexists with CRH in the PVN; with GH in the AN; and with LHRH in the medial septum/diagonal band of Broca/preoptic area (MS/POA). SRIH, TRH, and dopamine (DA) are synthesized in the PeN, PVN, and AN, respectively, but they are not colocalized with GAL. GAL and the other neuropeptides from the parvicellular hypothalamic nuclei in the median eminence are released into the hypophysial portal circulation by which they reach the anterior pituitary (AP). In the AP, GAL is colocalized with ACTH in corticotrophs, with GH in somatotrophs, with TSH in thyrotrophs, with LH and FSH in gonadotrophs, and with PRL in lactotrophs. The vertical arrows indicate the centrifugal flow of information. Horizontal arrows indicate interactions within the hypothalamus, nerve terminals in the median eminence, and the AP. In the hypothalamus and median eminence GAL functions as a neurotransmitter/neuromodulator while in the AP as a paracrine/autocrine messenger. AVP, vasopressin; CRH, corticotropin-releasing hormone; GH, growth hormone; AN, arcuate nucleus; SRIH, somatostatin; TRH, thyrotropin-releasing hormone; PeN, periventricular nucleus; ACTH, adrenocorticotropin hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; PRL, prolactin.

however, the amount of galanin in LHRH neurons is negatively regulated by estrogen in mice [9] and the galanin knockout mice are fertile. It has been suggested that in the rat galanin participates in the generation of LHRH and subsequent LH surges initiated by estrogen via a positive feedback mechanism during the afternoon of proestrus [14]. When the chronological patterns of LHRH, galanin, and c-fos expression within LHRH/galanin-expressing neurons and the LH surge are compared, such an action of galanin is uncertain (see below). Activation of the LHRH neuronal system occurs about 2 h before a minor elevation in LHRH expression and the preovulatory LH surge. Surprisingly, galanin expression in LHRH neurons does not begin to rise until the time of the LH surge, and it peaks 8–12 h later [15]. Since anesthetics, α-adrenergic, and an NMDA receptor blocker all prevent estrogen-induced galanin expression in LHRH neurons, neuronal activity seems to play a major role in the induction of galanin in these neurons (for a review see [4]). Thus, ER-β signaling resulting in galanin expression may require an additional neuronal signal which most likely comes from the anterior ventral periventricular nucleus (AVPV [16]).

Another caveat regardings galanin's role in the generation of the LH surge is that the principal receptor expressed in LHRH neurons is GAL-R1 [5], since its signaling pathways are inhibitory [17]. However, as suggested by the Steiner and co-workers [15], it is possible that when LHRH neuronal activity is low, galanin, co-released with LHRH in the median eminence, blocks the small amounts of LHRH released. When LHRH neuronal activity is elevated, galanin cannot suppress the release of the large bulk of LHRH, but by creating a larger potential difference in the LHRH terminal, it increases the amount of LHRH released. Via this mechanism galanin may sharpen the pulsatile pattern of LHRH release into the hypophysial portal circulation necessary for induction of an LH surge and subsequent ovulation. It is also possible that the elevated galanin mRNA seen in LHRH neurons 8–12 h after the preovulatory LH surge represents transcripts that are translated later, and therefore the

peptide prepares the LHRH neuronal system for the next cycle. The elevated levels of galanin in the LHRH neuronal axis support this hypothesis. Galanin, detected on proestrus morning, is probably the translational product of the transcripts made and stimulated by estrogen during the previous cycle.

Galanin and the hypothalamic-pituitary-adrenal axis

Although galanin and its receptors are expressed in all the anatomical components of the HPA; i.e, the hypothalamus (PVN), AP, and adrenal cortex, the data on the role of galanin in the physiology and pathophysiology of the HPA axis are still controversial.

In the hypothalamus, galanin is co-expressed with AVP and corticotrophin-releasing hormone (CRH) in the PVN and with AVP and OT in the SON. The expression of galanin, AVP and OT is modulated by various physiological stimuli, including osmotic pressure [18], and the activity of neurons expressing these neuropeptides is also regulated by noradrenergic inputs. Indeed, noradrenalin increases galanin expression in the magnocellular neurons [19]. In contrast to AVP and OT, the effects of galanin on CRH biosynthesis and release have not been thoroughly investigated. It has been shown that galanin enhances CRH secretion from perifused fetal rat hypothalamic neurons [20], and since CRH/AVP neurons in the PVN are contacted by galanin nerve terminals and GAL-R2 and -R3 are expressed in the PVN, the CRH/AVP system might be the mediator of galanin-induced suppression of ACTH secretion. For reviews see [2, 21, 22].

Acting in the AP, in humans, galanin lowers ACTH and glucocorticoid levels following its intravenous (iv) administration and lowers the ACTH response to CRH. In contrast, in the rat, galanin evokes a rise in plasma ACTH and subsequent corticosterone levels. The presence of high-affinity galanin binding sites has been detected in the AP and subsequent studies confirmed the presence of GAL-R2 and -R3 but not GAL-R1. In spite of these observations in rats and humans, the pituitary action of galanin in the regulation of ACTH is still controversial.

In the adrenal gland, galanin has been found in the medulla but not in the cortex of several species. Accordingly, splanchnic nerve stimulation increases galanin expression in chromaffin cells. Interestingly, galanin-containing nerve fibers have been detected in the zona glomerulosa of the cortex and the medulla. Among the three galanin receptors, GAL-R1 and GAL-R2 but not GAL-R3 have been found in the cortex and medulla of the adrenal gland [23].

The effect of galanin in the adrenal medulla is even more controversial than its action in the AP. However, it appears that in humans, galanin administered via iv lowers basal and insulin hypoglycemia-induced plasma norepinephrine but not epinephrine levels, and a recent study indicates that galanin has the same effects from rat adrenomedullary tissue [24, 25]. In addition to regulating the adrenal cortex centrally, galanin also exhibits direct effects in the adrenal cortex. Via GAL-R1 and GAL-R2, but not GAL-R3, galanin increases corticosterone secretion which is blocked by galantide (a galanin antagonist) or immunoneutralization of the two receptors. The stimulatory action is mediated via PKA and cAMP since both the PKA inhibitor H-89 and the adenyl cyclase inhibitor SQ-22536 abolish the corticosterone response to galanin. These findings are contradictory to the accepted signaling mechanism of GAL-R1 and GAL-R2 and suggest that even galanin signaling is tissue-and cell type selective (see the role of GAL-R1 in LHRH neurons described above). The action of galanin on corticosterone secretion is most likely indirect, involving the medullary norepinephrine system. The plasma concentrations of galanin are low (lower then those exhibiting biological effects in *in vitro* conditions; i.e., 10^{-10} vs $10^{-08}/10^{-06}$). However, the release of galanin from medullary chromaffin cells into the intercellular space may give rise to local concentrations exhibiting biological effects. At these concentrations, galanin may act via paracrine/autocrine actions and stimulate the release of norepinephrine from medullary chromaffin cells. Then, norepinephrine acting via β-adrenoreceptors in the adrenal cortex may stimulate corticosterone secretion. For a review on galanin's action in the HPA axis, see [25].

The role of galanin in the neuroendocrine regulation of growth hormone secretion

Growth hormone (GH) secretion is controlled by two antagonistic hypophysiotrophic systems: somatotropin-release inhibiting hormone (somatostatin; SRIH) and GH-releasing hormone (GHRH). The neuronal network involved in the central control of GH also includes extrahypothalamic neurons such as the noradrenergic and cholinergic systems and the intrahypothalamic neurons including those expressing galanin. These hypothalamic and extrahypothalamic systems participate in concert to regulate SRIH and GHRH neuronal activity. However, their activity is also dependent on sex steroids, glucocorticoids, activin, and GH and/or insulin-like growth factor I (IGF-I) feedback.

Among the anterior pituitary hormones, GH was the first described to be affected by galanin. Galanin increases GH levels following its central or peripheral administration, and it potentiates the effect of GHRH when the two peptides are given together. The GHRH-mediated action of centrally acting galanin was first suggested based on observations that the galanin-induced GH release was blocked by anti-GHRH serum administered prior to galanin. Moreover, galanin antiserum administered centrally dramatically alters pulsatile GH secretion, suggesting that endogenous galanin plays a role in maintaining pulsatile GH release (see below for details).

The stimulatory action of galanin on GH secretion, in addition to activating the GHRH neuronal system, may also be mediated via inhibition of SRIH [26]. Indeed, we have shown that SRIH neurons are innervated by galanin nerve terminals in the median eminence and SRIH neurons express GAL-Rs. No such connections between galanin and GHRH have been reported, although galanin is co-expressed with GHRH. Other neuropeptides (e.g., neuropeptide Y [NPY]) and neurotransmitter systems (see above) appear to mediate the actions of galanin on GH secretion as well. For instance, selective blockade of the hypothalamic epinephrine and/or norepinephrine systems blocks the stimulatory effect of galanin on GH secretion and α-adrenergic antagonists block the stimulatory effect of centrally administered galanin. However, they do not alter galanin-induced GH secretion when the peptide is administered systematically. In addition to the adrenergic system, the GABAergic and cholinergic systems also modulate galanin-stimulated GH release (for reviews on the regulation of GH secretion see [2, 27, 28].

GH, similarly to other anterior pituitary hormones, whose release is under the control of hypothalamic releasing and inhibiting hormones, also controls its own pulsatile secretion through a mechanism involving short-loop feedback regulation of the synthesis and release of GHRH. Galanin gene expression in GHRH neurons is lower in Lewis dwarf rats lacking GH compared to intact rats and in hypophysectomized rats compared to controls. These observations suggest that GH upregulates galanin expression in GHRH neurons although GHRH neurons do not express GH receptors. Therefore, the upregulation of galanin expression in GHRH neurons should be indirect [26]. As mentioned above, a subpopulation of SRIH neurons in the periventricular nucleus expresses GH and GALRs. Thus, in these neurons GH can directly regulate SRIH and galanin expression [26].

Based on the presence of GH and GALRs in SRIH neurons and the synaptic interactions among SRIH, GHRH, NPY, and galanin, Steiner and co-workers have proposed a model for the short-loop feedback control of GH secretion and the putative role of SRIH, GHRH, NPY, and galanin in affecting this interaction [26]. In this model, SRIH neurons in the PeN and NPY neurons in the AN are the primary targets for receiving feedback information about circulating levels of GH. GH stimulates the synthesis and release of both SRIH and NPY. They postulate that both NPY and SRIH act on GHRH/galanin neurons, although the precise molecular basis of their actions on these neurons is unknown. GH, acting through either SRIH or NPY neurons, inhibits the expression of GHRH and stimulates the expression of galanin. After some delay, GHRH/galanin neurons become activated and GHRH is released into the hypophysial portal circulation to stimulate GH secretion. At the same time, galanin is released at synapses on SRIH neurons in the PeN and suppresses SRIH gene expression. Once the stores of GHRH and galanin are depleted, the process shifts to enhanced SRIH secretion, whose stores have been replenished by the action of GH on SRIH synthesis. According to this model, galanin accentuates the pulsatile nature of GH secretion by inhibiting SRIH release and subsequently reducing SRIH inhibition of GH and GHRH release during pulse generation. This is in agreement with observations indicating that the administration of galanin antiserum reduces pulse amplitude in rats [29]. The pulsatile secretion of GH is sexually dimorphic; i.e., GH pulse amplitude is much greater in the male than in the female [30]. Interestingly, the level of galanin mRNA expression in GHRH neurons is also considerably higher in males than females [31]. For reviews of the role of galanin in GH secretion see [27, 28, 32–34].

The role of galanin in the neuroendocrine regulation of prolactin secretion

Similarly to GH, the regulation of prolactin (PRL) is under the dual control of the hypothalamus. PRL secretion is stimulated by yet unidentified PRL-stimulating factor(s) (PRFs) and is inhibited by PRL-inhibiting factor(s) (PIFs). The hypothalamus exerts a predominant inhibitory control on PRL release by dopamine, synthesized by the tuberoinfundibular dopaminergic (TIDA) neurons. It is generally accepted that PRL release is induced by exogenous galanin and the action of galanin is mediated via TIDA neurons. Galanin, by inhibiting dopamine release, reduces the inhibitory tone provided by this neurotransmitter and stimulates PRL secretion from the AP. However, several data indicate that galanin also

regulates PRL secretion by modulating the activity of the PRF system. Among the potential PRFs, the role of vasoactive intestinal polypeptide (VIP) seems to be convincing since passive immunoneutralization of central VIP results in suppression of galanin-induced PRL secretion and the levels of VIP in the cerebrospinal fluid and the levels of PRL in peripheral blood are elevated following galanin administration. Not only VIP but catecholamines and the opioid peptides also seem to be involved in the effects of galanin on PRL secretion (for a review see [2]). The role of endogenous galanin on PRL secretion has been indicated by studies utilizing passive immunoneutralization against galanin. In these studies, the absence of central galanin action resulted in the lack of the preovulatory PRL surge [36–38]. Galanin expression in lactotrophs is extremely sensitive to the estrogen status of the animals. A marked elevation in the expression of pituitary galanin occurs during pregnancy [39], whereas galanin is downregulated in hypothalamic magnocellular neurons during lactation [40]. Exogenous 17β-estradiol causes a 6-fold induction in the number of galanin-secreting lactotrophs [38] and a 3000-fold increase in anterior pituitary galanin mRNA content, whereas the peptide levels rise 500-fold [41]. In contrast, ovariectomy almost abolishes pituitary galanin content [42]. Wynnick and co-workers have shown that galanin exhibits mitogenic activity in a clonal lactotroph cell line acting via pituitary-specific galanin receptors [37, 38]. A number of studies in humans have confirmed these findings in rodents. Human galanin infusion significantly stimulates PRL secretion in normal female volunteers with an exaggerated response in patients with pituitary tumors [43–45].

Observations in mice carrying a loss-of-function mutation of the endogenous galanin gene have confirmed the findings mentioned above; i.e., PRL expression is reduced in adult female mice and the dams fail to lactate after pregnancy. There is an almost complete abrogation of the proliferative response of the lactotrophs to high doses of estrogen, with a failure to upregulate PRL expression or to increase pituitary cell number. Galanin, therefore, appears to act as a tonic regulator of PRL release and as a growth factor to the lactotrophs particularly in conditions where estrogen levels are elevated [46]. This hypothesis is strengthened by the observations made in galanin-overexpressing mice. Targeted overexpression of galanin to the lactotrophs and somatotrophs induces pituitary hyperplasia and adenoma formation [47].

Thus, it seems that at least in female rats, galanin participates in the physiological and pathophysiological control of PRL secretion. The mechanism of action may involve interactions at the levels of the hypothalamus (VIP, dopamine, catecholamines, opiods, etc.) as well as direct action at the levels of the AP. For reviews see [2, 48].

The role of galanin in the neuroendocrine regulation of thyroid stimulating hormone secretion

With respect to the regulation of thyroid stimulation hormone (TSH) secretion, the role of galanin, if one exists, is still controversial. Shortly after the discovery of galanin, it was shown that galanin reduces, whereas passive immunization against the peptide increases TSH secretion. The same group also showed that galanin enhances thyrotropin-releasing hormone (TRH)-induced TSH secretion from pituitary cells *in vitro*, but other studies have found that intracerebroventricularly (icv) administered galanin reduces TSH concentrations without altering pituitary responsiveness to TRH. A more recent study also found that galanin administered into the PVN significantly decreases the level of circulating TSH likely through the inhibition of TRH [35]. Thus, although 20 years have passed since the discovery of galanin, its function in the regulation of the TRH/TSH axis still remains controversial (for a review see [2]).

The role of galanin in the neuroendocrine regulation of feeding and metabolism

Galanin is an orexigenic peptide. Indeed, when galanin is injected into the PVN or the ventromedial nucleus (VMN) of the hypothalamus or the central nucleus of the amygdala, it stimulates food intake without altering feeding-associated behaviors such as drinking, grooming, and motor activity (for reviews see [3, 49, 50]. These data suggest that galanin activates acute feeding behavior rather than suppressing satiety. These effects of galanin on feeding can be abolished by the putative galanin antagonists C7 and M40 [51], and by α2-adrenoceptor antagonists [52]. Inhibition of noradrenaline synthesis also blocks galanin-induced feeding, suggesting that galanin modulates hypothalamic noradrenergic activity [52]. Galanin has been reported to increase preference for a high-fat diet given a choice between fat, carbohydrate, and protein [53], although other studies have observed no differences in macronutrient choice [54, 55]. Unlike other peptides such as NPY, chronic, central administration of galanin does not induce sustained obesity, but chronic daily administration of galanin into the PVN produces variable, complex changes in daily caloric intake, levels of obesity and regional fat deposition, depending on the fat and carbohydrate

content of the diet [56]. The observations that rats fed a high-fat diet (but not high carbohydrate or protein) display a 40 % increase in hypothalamic galanin levels [57] suggest that galanin synthesis is regulated by signals related to fatty acid metabolism.

Recent studies by Leibowitz and colleagues [58] suggest that galanin expression stimulated by dietary fat is sexually dimorphic, i.e., high-fat diet elevates galanin content not only in the PVN but also in the medial preoptic area (MPN), the median eminence and the AP in female rats only. The observations that circulating levels of ovarian hormones and galanin in PVN, MPN, median eminence, and AP are elevated with fat consumption and OVX attenuates this effect suggest that ovarian steroids are involved in the mechanism of fat-diet-induced galanin expression. Furthermore, in the same four areas affected by dietary fat, levels of galanin are stimulated by estrogen and further by progesterone replacement in estrogen-primed OVX rats and are higher in females compared to males. Because both galanin and progesterone stimulate feeding, their increase on a fat-rich diet may have functional consequences in females, possibly contributing to the increased caloric intake induced by dietary fat. This is supported by the findings that progesterone administration in estrogen-primed OVX rats reverses the inhibitory effect of estrogen on total caloric intake while increasing voluntary fat ingestion, and that female rats with higher galanin levels exhibit increased preference for fat compared to males. Thus, ovarian steroids may function together with galanin in a circuit, involving the MPN, PVN, median eminence, and AP, which coordinate feeding behavior with reproductive function to promote consumption of a fat-rich diet at times of increased energy demand [58].

Surprisingly, initial observations in GAL-R1- or GAL-R2-KO mice or galanin-KO mice did not indicate any phenotype related to differences in body weight, feeding behavior, or responses to fasting or leptin relative to littermates [59–61]. However, a recent study of GAL-R1-KO mice fed diets containing different levels of energy and fat concluded that the endogenous galanin-GAL-R1 system does play a significant role in adjusting food intake and/or metabolism to acute changes in dietary fat [62]. In response to an acute 3-day high-fat challenge, GAL-R1-KO mice display an impaired adaptation, leading to increased food intake and weight gain, compared to normal food intake and weight modulation on low-fat diets [62]. In contrast to this acute response, over a subsequent 2-week period on the high-fat diet, GAL-R1-KO mice consume less food and daily energy than when maintained on a low-fat diet and less food and energy than their heterozygous littermates. These observations suggest that overall, GAL-R1 may oppose positive energy balance or help maintain neutral balance [62]. Contrary to the role of galanin in TSH and PRL secretion, there are many excellent reviews on the role of galanin on feeding and metabolism [3, 49, 50, 63–66].

The role of galanin in osmotic regulation and water intake

Galanin is colocalized with vasopressin in the hypothalamus. Early studies on the effect of galanin in vasopressin neurons and the effects of central galanin administration revealed that galanin is involved in osmotic regulation, at the level of the PVN and SON. Vasopressin is pivotally involved in osmotic regulation and vasopressin-deficient and salt-loaded rats with increased plasma osmolarity have reduced concentrations of galanin in the neurointermediate lobe of the pituitary and in the median eminence [67], suggesting increased release of galanin. Furthermore, central administration of galanin reduces water intake [68], inhibits osmotically induced increases in vasopressin mRNA in the SON and PVN [69], and reduces vasopressin release [70] and plasma vasopressin levels [71]. Infusion of the galanin antagonist M15 increases vasopressin mRNA in normal rats, which further suggests tonic inhibition by galanin [69]. Galanin expression in the SON is altered in pharmacologically induced diabetes mellitus; and salt loading with 2 % saline as drinking water increases galanin mRNA and GAL-R1 mRNA in both the PVN and SON of the rat [72,73]. Water deprivation and salt loading also increase galanin binding and GalR1 in these neurons [72]. These data suggest that salt loading and dehydration increase both vasopressin release and the level of galanin. Galanin, in turn, acts as a negative feedback modulator of vasopressin release via GAL-R1 activation.

The subfornical organ (SFO) as a circumventricular structure plays an important role in the control of water intake and vasopressin release. Galanin nerve terminals form synapses on neurons in the SFO, suggesting that galanin could regulate the activity of these neurons. Indeed, a recent *in vitro* study utilizing SFO slice preparations indicates that galanin dose-dependently inhibits the neural activity of SFO neurons, many of which are also activated by angiotensin II. The GAL-R1 agonist M617 also inhibits SFO cells, whereas the GAL-R2/3 agonist galanin (2–11) has no effect, suggesting that galanin responses are largely mediated by GAL-R1 [74]. Consistent with this conclusion, the presence of GAL-R1 mRNA [74] and GAL-R1-like-immunoreactivity in SFO neurons

has also been reported [72]. For a recent review of galanin's involvement in osmotic regulation see [63].

Concluding remarks

Data collected in the past 2 decades on the role of galanin as a neuroendocrine regulator suggest that this peptide may play a potentially important role in the regulation of the central and peripheral components of the hypothalamo-pituitary-endocrine gland axes. However, galanin may play only a modulatory rather than regulatory role in the central and peripheral branches of the neuroendocrine systems. In the hypothalamus, it functions as a neurotransmitter/neuromodulator acting via synapses and specific receptors. In the pituitary and the peripheral endocrine glands, it acts via its receptors in a paracrine/autocrine fashion. The development of new, selective, and potent antagonists of GALRs will advance our knowledge not only of the physiology but also the pathophysiology of galanin and its receptors and thus may lead to the introduction of novel strategies in the therapy of diseases caused by alteration in galanin and its receptors.

1 Merchenthaler, I. (1998) LHRH and sexual dimorphism. Ann. N. Y. Acad. Sci. 863, 175–187.
2 Merchenthaler, I., Lopez, F. J. and Negro-Vilar, A. (1993) Anatomy and physiology of central galanin-containing pathways. Progr. Neurobiology 40, 711–769.
3 Gundlach, A. L. (2002) Galanin/GALP and galanin receptors: role in central control of feeding, body weight/obesity and reproduction? Eur. J. Pharmacol. 440, 255–268.
4 Rossmanith, W. G., Clifton, D. K. and Steiner, R. A. (1996) Galanin gene expression in hypothalamic GnRH-containing neurons of the rat: a model for autocrine regulation. Horm. Metab. Res. 28, 257–266.
5 Mitchell, V., Bouret, S., Prevot, V., Jennes, L. and Beauvillain, J. C. (1999) Evidence for expression of galanin receptor Gal-R1 mRNA in certain gonadotropin releasing hormone neurones of the rostral preoptic area. J. Neuroendocrinol. 11, 805–812.
6 Dudas, B. and Merchenthaler, I. (2004) Bi-directional associations between galanin and luteinizing hormone-releasing hormone neuronal systems in the human diencephalon. Neuroscience 127, 695–707.
7 Dudas, B. and Merchenthaler, I. (2006) Three-dimensional representation of the neurotransmitter systems of the human hypothalamus: inputs of the gonadotrophin hormone-releasing hormone neuronal system. J. Neuroendocrinol. 18, 79–95.
8 Merchenthaler, I., Lopez, F. J., Lennard, D. E. and Negro-Vilar, A. (1991) Sexual differences in the distribution of neurons coexpressing galanin and luteinizing hormone-releasing hormone in the rat brain. Endocrinology 129, 1977–1986.
9 Rajendren, G. (2002) Increased galanin synapses onto activated gonadotropin-releasing hormone neuronal cell bodies in normal female mice and in functional preoptic area grafts in hypogonadal mice. J. Neuroendocrinol. 14, 435–441.
10 Dufourny, L., Schofield, N. and Skinner, D. C. (2003) Immunoreactive galanin expression in ovine gonadotropin-releasing hormone neurones: no effects of gender or reproductive status. J. Neuroendocrinol. 15, 1062–1069.
11 Merchenthaler, I. (2005) Estrogen stimulates galanin expression within luteinizing hormone-releasing hormone-immunoreactive (LHRH-i) neurons via estrogen receptor-beta (ERbeta) in the female rat brain. Neuropeptides 39, 341–343.
12 Sahu, A., Xu, B. and Kalra, S. P. (1994) Role of galanin in stimulation of pituitary luteinizing hormone secretion as revealed by a specific receptor antagonist, galantide. Endocrinology 134, 529–536.
13 Lopez, F. J., Meade, E. H., Jr. and Negro-Vilar, A. (1993) Endogenous galanin modulates the gonadotropin and prolactin proestrous surges in the rat. Endocrinology 132, 795–800.
14 Pau, K. Y. and Spies, H. G. (1997) Neuroendocrine signals in the regulation of gonadotropin-releasing hormone secretion. Chin. J. Physiol. 40, 181–196.
15 Finn, P. D., Steiner, R. A. and Clifton, D. K. (1998) Temporal patterns of gonadotropin-releasing hormone (GnRH), c-fos, and galanin gene expression in GnRH neurons relative to the luteinizing hormone surge in the rat. J. Neurosci. 18, 713–719.
16 Herbison, A. E. (1998) Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocrine Rev. 19, 302–330.
17 Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M. and Mayaux, J. F. (1994) Molecular cloning of a functional human galanin receptor. Proc. Natl. Acad. Sci. USA 91, 9780–9783.
18 Landry, M. and Hokfelt, T. (1998) Subcellular localization of preprogalanin messenger RNA in perikarya and axons of hypothalamo-posthypophyseal magnocellular neurons: an in situ hybridization study. Neuroscience 84, 897–912.
19 Vacher, C.-M., Fretier, P., Creminon, C., Calas, A. and Hardin-Pouzet, H. (2002) Activation by serotonin and noradrenaline of vasopressin and oxytocin expression in the mouse paraventricular and supraoptic nuclei. J. Neurosci. 22, 1513–1522.
20 Bergonzelli, G. E., Pralong, F. P., Glauser, M., Cavadas, C., Grouzmann, E. and Gaillard, R. C. (2001) Interplay between galanin and leptin in the hypothalamic control of feeding via corticotropin-releasing hormone and neuropeptide Y. Diabetes 50, 2666–2672.
21 Waters, S. M. and Krause, J. E. (2000) Distribution of galanin-1, -2 and -3 receptor messenger RNAs in central and peripheral rat tissues. Neuroscience 95, 265–271.
22 Lang, R., Berger, A., Santic, R., Geisberger, R., Hermann, A., Herzog, H. and Kofler, B. (2005) Pharmacological and functional characterization of galanin-like peptide fragments as potent galanin receptor agonists. Neuropeptides 39, 179–184.
23 Andreis, P. G., Malendowicz, L. K., Rebuffat, P., Spinazzi, R., Ziolkowska, A. and Nussdorfer, G. G. (2007) Galanin enhances corticosterone secretion from dispersed rat adrenocortical cells through the activation of GAL-R1 and GAL-R2 receptors coupled to the adenylate cyclase-dependent signaling cascade. Int. J. Mol. Med. 19, 149–155.
24 Degli Uberti, E. C. Bondanelli, M., Margutti, A., Ambrosio, M. R., Valentini, A., Campo, M., Franceschetti, P., Zatelli, M. C., Pansini, R., Trasforini, G. (1996) Acute administration of human galanin in normal subjects reduces the potentiating effect of pyridostigmine-induced cholinergic enhancement on release of norepinephrine and pancreatic polypeptide. Neuroendocrinology 64, 398–404.
25 Tortorella, C., Neri, G. and Nussdorfer, G. G. (2007) Galanin in the regulation of the hypothalamic-pituitary-adrenal axis (Review). Int. J. Mol. Med. 19, 639–647.
26 Chan, Y. Y., Grafstein-Dunn, E., Delemarre-van de Waal, H. A., Burton, K. A., Clifton, D. K. and Steiner, R. A. (1996) The role of galanin and its receptor in the feedback regulation of growth hormone secretion. Endocrinology 137, 5303–5310.
27 Hohmann, J. G., Clifton, D. K. and Steiner, R. A. (1998) Galanin: analysis of its coexpression in gonadotropin-releasing hormone and growth hormone-releasing hormone neurons. Ann. N. Y. Acad. Sci. 863, 221–235.
28 Bluet-Pajot, M. T., Epelbaum, J., Gourdji, D., Hammond, C. and Kordon, C. (1998) Hypothalamic and hypophyseal regulation of growth hormone secretion. Cell. Mol. Neurobiol. 18, 101–123.

29 Maiter, D. M., Hooi, S. C., Koenig, J. I. and Martin, J. B. (1990) Galanin is a physiological regulator of spontaneous pulsatile secretion of growth hormone in the male rat. Endocrinology 126, 1216–1222.
30 Jansson, J. O., Eden, S. and Isaksson, O. (1985) Sexual dimorphism in the control of growth hormone secretion. Endocrine Rev. 6, 128–150.
31 Delemarre-van de Waal, H. A., Burton, K. A., Kabigting, E. B., Steiner, R. A. and Clifton, D. K. (1994) Expression and sexual dimorphism of galanin messenger ribonucleic acid in growth hormone-releasing hormone neurons of the rat during development. Endocrinology 134, 665–671.
32 Bertherat, J., Bluet-Pajot, M. T. and Epelbaum, J. (1995) Neuroendocrine regulation of growth hormone. Eur. J. Endocrinol. 132, 12–24.
33 Ghigo, E., Arvat, E., Bellone, J., Ramunni, J. and Camanni, F. (1993) Neurotransmitter control of growth hormone secretion in humans. J. Pediatr. Endocrinol. 6, 263–266.
34 Veldhuis, J. D. (1998) Neuroendocrine control of pulsatile growth hormone release in the human: relationship with gender. Growth Horm. IGF Res. 8 Suppl. B, 49–59.
35 Seth, A., Stanley, S., Dhillo, W., Murphy, K., Ghatei, M. and Bloom, S. (2003) Effects of galanin-like peptide on food intake and the hypothalamo-pituitary-thyroid axis. Neuroendocrinology 77, 125–131.
36 Hammond, P. J., Khandan-Nia, N., Withers, D. J., Jones, P. M., Ghatei, M. A. and Bloom, S. R. (1997) Regulation of anterior pituitary galanin and vasoactive intestinal peptide by oestrogen and prolactin status. J. Endocrinol. 152, 211–219.
37 Hammond, P. J., Smith, D. M., Akinsanya, K. O., Mufti, W. A., Wynick, D. and Bloom, S. R. (1996) Signalling pathways mediating secretory and mitogenic responses to galanin and pituitary adenylate cyclase-activating polypeptide in the 235-1 clonal rat lactotroph cell line. J. Neuroendocrinol. 8, 457–464.
38 Wynick, D., Hammond, P. J., Akinsanya, K. O. and Bloom, S. R. (1993) Galanin regulates basal and oestrogen-stimulated lactotroph function. Nature 364, 529–532.
39 Vrontakis, M. E., Schroedter, I. C., Cosby, H. and Friesen, H. G. (1992) Expression and secretion of galanin during pregnancy in the rat. Endocrinology 130, 458–464.
40 Landry, M., Roche, D., Angelova, E. and Calas, A. (1997) Expression of galanin in hypothalamic magnocellular neurones of lactating rats: co-existence with vasopressin and oxytocin. J. Endocrinol. 155, 467–481.
41 Kaplan, L. M., Gabriel, S. M., Koenig, J. I., Sunday, M. E., Spindel, E. R., Martin, J. B. and Chin, W. W. (1988) Galanin is an estrogen-inducible, secretory product of the rat anterior pituitary. Proc. Natl. Acad. Sci. USA 85, 7408–7412.
42 O'Halloran, D. J., Jones, P. M., Steel, J. H., Gon, G., Giaid, A., Ghatei, M. A., Polak, J. M. and Bloom, S. R. (1990) Effect of endocrine manipulation on anterior pituitary galanin in the rat. Endocrinology 127, 467–475.
43 Arvat, E., Gianotti, L., Ramunni, J., Grottoli, S., Brossa, P. C., Bertagna, A., Camanni, F. and Ghigo, E. (1995) Effect of galanin on basal and stimulated secretion of prolactin, gonadotropins, thyrotropin, adrenocorticotropin and cortisol in humans. Eur. J. Endocrinol. 133, 300–304.
44 Grottoli, S., Arvat, E., Gianotti, L., Ramunni, J., Di Vito, L., Maccagno, B., Ciccarelli, E., Camanni, F., Ghigo, E. (1996) Galanin positively modulates prolactin secretion in normal women. J. Endocrinol. Invest 19, 739–744.
45 Invitti, C., Pecori Giraldi, F., Tagliaferri, A., Scacchi, M., Dubini, A. and Cavagnini, F. (1993) Enhanced prolactin responsiveness to galanin in patients with Cushing's disease. Clin. Endocrinol. (Oxf) 39, 213–216.
46 Wynick, D., Small, C. J., Bacon, A., Holmes, F. E., Norman, M., Ormandy, C. J., Kilic, E., Kerr, N. C., Ghatei, M., Talamantes, F. et al. (1998) Galanin regulates prolactin release and lactotroph proliferation. Proc. Natl. Acad. Sci. USA 95, 12671–12676.
47 Vrontakis, M., Torsello, A., Leite, V., Vuille, J. C. and Zhang, H. (1996) Regulation of galanin by dexamethasone in the rat anterior pituitary and the uterus. Neuroendocrinology 64, 20–24.
48 Takahashi, S. (2004). Intrapituitary regulatory system of proliferation of mammotrophs in the pituitary gland. Zoolog. Sci. 21, 601–611.
49 Leibowitz, S. F. (2005) Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis. Neuropeptides 39, 327–332.
50 Leibowitz, S. F. and Wortley, K. E. (2004) Hypothalamic control of energy balance: different peptides, different functions. Peptides 25, 473–504.
51 Corwin, R. L., Robinson, J. K. and Crawley, J. N. (1993) Galanin antagonists block galanin-induced feeding in the hypothalamus and amygdala of the rat. European J. Neurosci. 5, 1528–1533.
52 Kyrkouli, S. E., Stanley, B. G., Hutchinson, R., Seirafi, R. D. and Leibowitz, S. F. (1990) Peptide-amine interactions in the hypothalamic paraventricular nucleus: analysis of galanin and neuropeptide Y in relation to feeding. Brain Res. 521, 185–191.
53 Tempel, D. L., Leibowitz, K. J. and Leibowitz, S. F. (1988) Effects of PVN galanin on macronutrient selection. Peptides 9, 309–314.
54 Crawley, J. N. (1999) The role of galanin in feeding behavior. Neuropeptides 33, 369–375.
55 Smith, K. E., Forray, C., Walker, M. W., Jones, K. A., Tamm, J. A., Bard, J., Branchek, T. A., Linemeyer, D. L. and Gerald, C. (1997) Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. J. Biol. Chem. 272, 24612–24616.
56 Smith, B. K., York, D. A. and Bray, G. A. (1994) Chronic cerebroventricular galanin does not induce sustained hyperphagia or obesity. Peptides 15, 1267–1272.
57 Leibowitz, S. F., Akabayashi, A. and Wang, J. (1998) Obesity on a high-fat diet: role of hypothalamic galanin in neurons of the anterior paraventricular nucleus projecting to the median eminence. J. Neurosci. 18, 2709–2719.
58 Leibowitz, S. F., Akabayashi, A., Wang, J., Alexander, J. T., Dourmashkin, J. T. and Chang, G. Q. (2007) Increased caloric intake on a fat-rich diet: role of ovarian steroids and galanin in the medial preoptic and paraventricular nuclei and anterior pituitary of female rats. J. Neuroendocrinol. 19, 753–766.
59 Gottsch, M. L., Zeng, H., Hohmann, J. G., Weinshenker, D., Clifton, D. K. and Steiner, R. A. (2005). Phenotypic analysis of mice deficient in the type 2 galanin receptor (GALR2). Mol. Cell. Biol. 25, 4804–4811.
60 Jacoby, A. S., Hort, Y. J., Constantinescu, G., Shine, J. and Iismaa, T. P. (2002) Critical role for GALR1 galanin receptor in galanin regulation of neuroendocrine function and seizure activity. Brain Res. Mol. Brain Res. 107, 195–200.
61 Wynick, D. and Bacon, A. (2002) Targeted disruption of galanin: new insights from knock-out studies. Neuropeptides 36, 132–144.
62 Zorilla, E. P., Brenman, M., Sabino, V., Lu, X., Bartfai, T. (2007) Galanin type 1 receptor knockout mice show responses to high-fat diet and glucose challenge. Physiol. Behav. 91, 479–485.
63 Lang, R., Gundlach, A. L. and Kofler, B. (2007) The galanin peptide family: receptor pharmacology, pleiotropic biological actions, and implications in health and disease. Pharmacol. Therapeutics 115, 177–207.
64 Meister, B. (2007) Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. Physiol. Behav. 92, 263–271.
65 Schioth, H. B. (2006) G protein-coupled receptors in regulation of body weight. CNS Neurol. Disord. Drug Targets 5, 241–249.
66 Volkoff, H., Canosa, L. F., Unniappan, S., Cerda-Reverter, J. M., Bernier, N. J., Kelly, S. P. and Peter, R. E. (2005) Neuropeptides and the control of food intake in fish. Gen. Comp. Endocrinol. 142, 3–19.
67 Koenig, J. I., Hooi, S., Gabriel, S. M. and Martin, J. B. (1989) Potential involvement of galanin in the regulation of fluid homeostasis in the rat. Regul. Pept. 24, 81–86.

68 Brewer, A., Echevarria, D. J., Langel, U. and Robinson, J. K. (2005) Assessment of new functional roles for galanin in the CNS. Neuropeptides 39, 323–326.
69 Landry, M., Roche, D., Vila-Porcile, E. and Calas, A. (2000) Effects of centrally administered galanin (1–16) on galanin expression in the rat hypothalamus. Peptides 21, 1725–1733.
70 Kondo, K., Murase, T., Otake, K., Ito, M. and Oiso, Y. (1991). Centrally administered galanin inhibits osmotically stimulated arginine vasopressin release in conscious rats. Neurosci. Lett. 128, 245–248.
71 Kondo, K., Murase, T., Otake, K., Ito, M., Kurimoto, F. and Oiso, Y. (1993) Galanin as a physiological neurotransmitter in hemodynamic control of arginine vasopressin release in rats. Neuroendocrinology 57, 224–249.
72 Burazin, T. C., Larm, J. A. and Gundlach, A. L. (2001) Regulation by osmotic stimuli of galanin-R1 receptor expression in magnocellular neurones of the paraventricular and supraoptic nuclei of the rat. J. Neuroendocrinol. 13, 358–370.
73 Meister, B., Cortes, R., Villar, M. J., Schalling, M. and Hokfelt, T. (1990) Peptides and transmitter enzymes in hypothalamic magnocellular neurons after administration of hyperosmotic stimuli: comparison between messenger RNA and peptide/protein levels. Cell Tissue Res. 260, 279–297.
74 Kai, A., Ono, K., Kawano, H., Honda, E., Nakanishi, O. and Inenaga, K. (2006) Galanin inhibits neural activity in the subfornical organ in rat slice preparation. Neuroscience 143, 769–777.