Research report

Galanin- but not galanin-like peptide-containing axon terminals innervate hypophysiotropic TRH-synthesizing neurons in the hypothalamic paraventricular nucleus

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Abstract

Galanin and galanin-like peptide (GALP) are both orexigenic peptides involved in the regulation of food intake and energy metabolism. To determine whether these peptides may directly influence the hypophysiotropic thyrotropin-releasing hormone (TRH)-synthesizing neurons, double-labeling immunocytochemistry was performed at light and electron microscopic levels using antisera against proTRH, galanin and GALP. Galanin-IR axons densely innervated all of the major parvocellular subdivisions of the PVN where proTRH neurons were identified. The periventricular and anterior parvocellular subdivisions exhibited a prominent network of galaninergic nerve fibers, while the density of fibers was less intense in the medial parvocellular subdivision. Galanin-immunoreactive (IR) axon varicosities were juxtaposed to the majority of TRH-synthesizing neurons in the anterior, medial and periventricular subdivisions of the PVN. Ultrastucturally, galanin-IR nerve terminals established symmetric type synapses with the perikarya of proTRH-IR neurons, suggesting an inhibitory nature of these contacts. In contrast, GALP immunoreactive fibers and nerve terminals concentrated primarily in the anterior parvocellular subdivision of the PVN and were found in association with only few proTRH-IR neurons in the periventricular and medial parvocellular subdivisions.

In conclusion, the dense innervation of TRH neurons in all subdivisions of the PVN by galanin-IR axons indicates that galanin may be involved in the central regulation of the hypothalamic–pituitary–thyroid axis. In contrast, the relative paucity of GALP-containing axons in juxtaposition to TRH neurons in the anterior and periventricular parvocellular subdivisions of the PVN, the origin of hypophysiotropic TRH neurons, makes it unlikely that GALP similarly exerts direct regulatory effects on hypophysiotropic TRH neurons.

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1. Introduction

Galanin, a 30-amino acid peptide that is broadly distributed in the central nervous system and periphery, modulates a variety of physiological processes including neuroendocrine secretion, feeding behavior, nociception, cognition and reproduction [1,13,15,29]. Galanin acts via at least three recently cloned G protein-coupled receptors [34] that have differences in their amino acid sequence, distribution, pharmacology and signal transduction [42]. Galanin-like peptide (GALP), a second member of the galanin family, has recently been cloned from porcine hypothalamus [33]. This peptide is structurally related to galanin and highly conserved across species [33]. Recently, Takatsu et al. [40] showed by in situ hybridization histochemistry that GALP is synthesized exclusively in the hypothalamic arcuate nucleus (ARC) in the rat brain and demonstrated that GALP-containing nerve fibers project to the paravascular division of the hypothalamic paraventricular nucleus.
Both galanin and GALP are leptin regulated peptides [11,36] and have potent orexigenic effects [15,25]. Intracerebroventricular administration of galanin induces short term increase in food consumption, while GALP administration results in even more profound effects on food intake [25]. Furthermore, focal injections of galanin into the hypothalamic paraventricular nucleus (PVN) reduce energy expenditure [27].

An important component of the regulatory system determining energy expenditure is the hypothalamic–pituitary–thyroid (HPT) axis [17,32]. Thyroid hormones increase mitochondrial oxygen consumption and are necessary for adaptive thermogenesis in the brown adipose tissue [3]. Accordingly, during fasting, thyroid hormone levels fall, primarily due to inhibition of hypophysiotropic thyrotropin-releasing hormone (TRH) gene expression in PVN neurons, mediated by falling circulating levels of leptin [19].

We have recently demonstrated that the action of leptin on hypophysiotropic TRH is primarily indirect, mediated by at least four arcuate nucleus derived peptides, α-melanocyte stimulating hormone (α-MSH), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY) and agouti-related peptide (AGRP) [5–8]. However, it is unknown whether other peptidergic systems are also involved in adaptation of the HPT axis to fasting. Since members of the galanin peptide family are regulated by leptin [11,36] and have recently been shown to reduce the peripheral TSH levels after central administration and decrease TRH release from hypothalamic explants [38], we determined whether galanin- and GALP-containing axon terminals are in anatomical position to directly regulate hypophysiotropic TRH neurons.

2. Materials and methods

2.1. Animals

Experiments were performed on adult, male Wistar (TOXI-COOP KKT, Budapest, Hungary) and Sprague–Dawley (Taconic Farms, Germantown, NY) rats weighing between 200 and 300g. The animals were housed individually in cages under standard environmental conditions (light between 0600 and 1800 h, temperature 22 ± 1 °C, rat chow and water available ad libitum). All experimental protocols were reviewed and approved by the Animal Research Committees at the Institute of Experimental Medicine of the Hungarian Academy of Sciences and Tufts-New England Medical Center.

2.2. Tissue preparation for light microscopic immunocytochemistry

Rats were deeply anaesthetized with sodium pentobarbital (35 mg/kg of body weight, i.p.) and stereotaxically injected intracerebroventricularly with 60 µg of colchicine in 6 µl of 0.9% of saline. After 20 h of survival, the rats were perfused transcardially with 20 ml 0.01 M phosphate-buffered saline (PBS), pH 7.4, followed sequentially by 100 ml of 3% paraformaldehyde and 1% acrolein in 0.1 M phosphate buffer (PB), pH 7.4, and 30 ml 3% paraformaldehyde in the same buffer (for galanin immunocytochemistry) or 20 ml of 0.01M PBS, pH 7.4, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) (for GALP immunocytochemistry). Following perfusion, brains prepared for GALP immunocytochemistry were post-fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h at room temperature. The hypothalamic blocks were transferred into 30% sucrose in 0.01 M PBS at 4 °C overnight to promote cryoprotection. Serial 25-µm-thick coronal sections were cut in freezing microtome, collected in PBS and stored in freezing solution (30% ethylene glycol, 25% glycerol, 0.05 M PB) at −20 °C until used.

2.3. Tissue preparation for electron microscopic immunocytochemistry

For electron microscopy, 25-µm-thick coronal sections through the rostrocaudal extent of the PVN were cut on a Vibratome, collected in PBS and stored in freezing solution at −20 °C until used. The sections were then treated with 1% sodium borohydrate in 0.1 M phosphate buffer for 30 min, followed by 0.5% H2O2 in PBS for 15 min. After cryoprotection in 15% sucrose in PBS for 15 min at room temperature and 30% sucrose in PBS overnight at 4 °C, the sections were snap frozen over liquid nitrogen to improve antibody penetration. To reduce the nonspecific antibody binding, the sections were treated with 2.5% normal horse serum in PBS for 20 min.

2.4. Light microscopic double-labeling immunocytochemistry for galanin and proTRH

Every fourth section containing the PVN was treated with 1% sodium borohydrate in distilled water for 30 min followed by 0.5% Triton X-100/0.5% H2O2 in PBS for 15 min to remove endogenous peroxidase activity, and then 2% normal horse serum in PBS for 20 min to reduce nonspecific antibody binding. The sections were incubated in sheep anti-galanin antiserum (1:750,000, gift from István Merchenhaler, Women’s Health Research Institute, Wyeth Research, Collegeville, PA) [16] for 2 days at 4 °C, rinsed in PBS, and then incubated in biotinylated donkey anti-sheep IgG at 1:500 dilution (Jackson ImmunoResearch Lab, West Grove, PA) for 2 h. The sections were then immersed in streptavidin–peroxidase (Jackson Lab) at 1:1000 dilution (Jackson ImmunoResearch Lab, West Grove, PA) for 2 h, and the immunoreaction developed in 0.05% diaminobenzidine (DAB) containing 0.15% Ni-ammonium-sulfate and 0.005% H2O2 in 0.05 M Tris buffer (TB), pH 7.6. The reaction was stopped by immersion of the tissue...
sections in 0.05M TB, pH 7.6, and the reaction product further intensified by Gallyas-silver intensification technique [22] without thioglycolic acid for less than 5 min [5,23] to yield a black precipitate. After additional washes, the sections were incubated in rabbit anti-proTRH (178–199) antibody (gift from Éva Rédei, Northwestern University, Chicago, IL) at 1:20,000 dilution for 2 days at 4 °C. The sections were then washed in TBS and incubated in biotinylated mouse anti-rabbit IgG (Jackson ImmunoResearch) at 1:500 dilution for 2 h, and, after further washes in PBS, incubated in streptavidin–peroxidase at 1:1000 dilution for 2 h. After three washes in PBS, the reaction was developed in 0.025% DAB containing 0.0036% H2O2 in 0.05 M TB, pH 7.6, to yield a contrasting brown reaction product. The frequency of close appositions between galanin axon varicosities and TRH neurons was counted by direct light microscopic examination of the PVN sections at 100 × magnification. No immunoreaction product or endogenous argyrophilia was observed under these conditions if the primary antibody was omitted.

2.5. Light microscopic double-labeling immunocytochemistry for GALP and proTRH

For semiquantitative analysis of the GALP innervation of TRH neurons in the PVN, every third section through the PVN was prepared for double-labeling immunocytochemistry. Sections were initially treated with 0.5% H2O2 in PBS for 15 min, washed in PBS, and followed by treatment with 0.5% Triton-X 100 in PBS for 1 h. Following preincubation in 10% normal horse serum for 30–60 min, the sections were incubated in mouse monoclonal GALP antibody (gift from Dr. Yoshihiro Takatsu, Japan) at a dilution of 1:8000 for 2–3 days at 4 °C with continuous agitation on a rotary shaker. Primary antiserum dilutions were made in 1% normal horse serum in PBS containing 0.08% sodium azide and 0.02% Kodak Photo-Flo. After thorough rinsing in PBS, sections were incubated in biotinylated horse anti-mouse IgG (1:400, Vector labs, Burlingame, CA) for 2–3 h. The sections were then washed three times in PBS and incubated in avidin–peroxidase complex (1:100, ABC Elite

Fig. 1. (A–C) Low power photomicrographs showing the distribution of galanin- (black) and proTRH-IR (brown) neurons and fibers in different levels of the PVN. (A) anterior, (B) mid and (C) caudal levels of the PVN. While the proTRH-IR neurons are located only in the parvocellular subdivisions, galanin-IR neurons are observed in both parvo- and magnocellular subdivisions. Note the dense galanin-IR innervation of the PVN at all levels. (D–F) High power magnification of galanin-IR axon varicosities (arrows) in association with proTRH neurons in the (D) anterior, (E) periventricular and (F) medial parvocellular subdivisions of the PVN. Asterisk in (D) labels a galanin-IR neuron. Fx: Fornix, III: Third ventricle. Scale bar = 200 µm in (C) corresponds to (A–C); scale bar in (F) = 25 µm corresponds to (D–F).
Kit, Vector Labs) for 1 h followed by a 10 min incubation in tyramide signal amplification solution. The sections were rinsed in PBS and then again incubated in avidin–peroxidase complex at 1:200 dilution for 1 h. After three washes in PBS and a rinse in 0.05 M Tris buffer (pH 7.6), the color reaction was developed in 0.025% DAB containing 0.0036% H₂O₂ in 0.05 M Tris buffer, pH 7.6. After three washes, the tissues were placed into rabbit anti-preproTRH 178–199 (1:8000) for 2 days at 4 °C. After rinsing in PBS and 0.1% cold water fish gelatin (Electron Microscopy Sciences, Fort Washington, PA) 1% bovine serum albumin (BSA) in PBS, they were incubated in goat anti-rabbit IgG conjugated with 0.8 nm colloidal gold (Electron Microscopy Sciences) diluted at 1:100 in PBS containing 0.1% cold water fish gelatin and 1% BSA. The sections were then washed in the same diluent and PBS, followed by a 10-min treatment in 1.25% glutaraldehyde in PBS. After rinsing in 0.2 M sodium citrate, pH 7.5, the gold particles were silver intensified with IntenSE Kit (Amersham, Arlington Heights, IL) [2]. Sections were treated with 1% osmium tetroxide in 0.1 M PB for 30 min, dehydrated in an ascending series of ethanol followed by propylene oxide, flat embedded in Durcupan ACM epoxy resin (Fluka, Ronkonkoma, NY) on liquid release agent (Electron Microscopy Sciences) coated slides and polymerized at 56 °C for 2 days. Ultrathin 50–60 nm thin sections were cut with Leica ultracut UCT ultramicrotome (Leica Microsystems, Wetzlar, Germany), collected onto Formvar coated single slot grids, contrasted with 2% uranyl acetate and examined with a Hitachi electron microscope.

Fig. 2. Electron micrographs showing synaptic associations (arrows) between proTRH-containing neurons in the medial parvocellular subdivision of the PVN and galanin-containing axon terminals. The proTRH-IR perikarya are labeled with highly electron dense silver granules, while the galanin-IR terminals are recognized by the presence of the electron dense DAB. Note that the galanin-IR terminals form symmetric type synapses on the surface of the proTRH-IR perikarya (A, B). Scale bar = 1 μm.

Fig. 3. Distribution of GALP-IR axons in the PVN. Note the intensity GALP-IR axon terminals in the (A) anterior parvocellular subdivision in contrast to the paucity of fibers in the (B) medial parvocellular subdivision. Scale bar = 200 μm. Abbreviations: PV: periventricular parvocellular subdivision of the PVN; PVNa: anterior parvocellular subdivision of the PVN; PVNm: magnocellular parvocellular subdivision of the PVN; PVNmp: medial parvocellular subdivision of the PVN.

2.6. Electron microscopic double-labeling immunocytochemistry for galanin and proTRH

Sections processed for electron microscopy were incubated in sheep anti-galanin serum at dilution 1:60,000 for 4 days at 4 °C, followed by donkey anti-sheep IgG at 1:500 dilution (Jackson Lab) for 20 h at 4 °C and streptavidin–peroxidase (1:1000), for 1 h at room temperature. Immunoreactivity was detected with 0.025% DAB containing 0.0036% H₂O₂ in 0.05 M Tris buffer, pH 7.6. After three washes, the tissues were stained with rabbit anti-preproTRH 178–199 (1:8000) for 2 days at 4 °C. After rinsing in PBS and 0.1% cold water fish gelatin (Electron Microscopy Sciences, Fort Washington, PA) 1% bovine serum albumin (BSA) in PBS, they were incubated in goat anti-rabbit IgG conjugated with 0.8 nm colloidal gold (Electron Microscopy Sciences) diluted at 1:100 in PBS containing 0.1% cold water fish gelatin and 1% BSA. The sections were then washed in the same diluent and PBS, followed by a 10-min treatment in 1.25% glutaraldehyde in PBS. After rinsing in 0.2 M sodium citrate, pH 7.5, the gold particles were silver intensified with IntenSE Kit (Amersham, Arlington Heights, IL) [2]. Sections were treated with 1% osmium tetroxide in 0.1 M PB for 30 min, dehydrated in an ascending series of ethanol followed by propylene oxide, flat embedded in Durcupan ACM epoxy resin (Fluka, Ronkonkoma, NY) on liquid release agent (Electron Microscopy Sciences) coated slides and polymerized at 56 °C for 2 days. Ultrathin 50–60 nm thin sections were cut with Leica ultracut UCT ultramicrotome (Leica Microsystems, Wetzlar, Germany), collected onto Formvar coated single slot grids, contrasted with 2% uranyl acetate and examined with a Hitachi electron microscope.

After visualizing the GALP immunoreactivity in nerve terminals in the PVN, sections were incubated in rabbit antiserum recognizing rat proTRH (178–199) at a dilution of 1:200 dilution for 1 h. After three washes in PBS and a rinse in 0.05 M Tris buffer (pH 7.6), the color reaction was developed in 0.025% DAB containing 0.06% nickel ammonium sulfate and 0.0027% H₂O₂ for 7 min to yield a dark purple labeling.

The frequency of close appositions between GALP-containing axon terminals and TRH neurons was then counted by direct light microscopic examination of the PVN sections for each animal in the anterior, medial and periventricular subdivisions, respectively, and the mean and S.E.M. calculated for each subdivision.
3. Results

3.1. Galanin-IR innervation of proTRH-containing neurons in the PVN

Galanin-IR axons densely innervated all of the major parvocellular subdivisions of the PVN where proTRH neurons were identified (Fig. 1A–C). The periventricular and anterior parvocellular subdivisions exhibited the most prominent network of galaninergic nerve fibers, while in the medial subdivision, the innervation was less intense. Galanin-IR cell bodies were observed in both the parvocellular and magnocellular subdivisions of the PVN (Fig. 1A–C). In all parvocellular subdivisions, galanin-IR axon varicosities were in juxtaposition to the majority of TRH neurons, with the highest proportion in the anterior and periventricular parvocellular subdivisions (Fig. 1D–E). Semiquantitative analysis revealed that, in the anterior, periventricular and medial parvocellular subdivisions, 85.1 ± 2.3%, 75.8 ± 6.7% and 61.6 ± 3.4% of the proTRH containing neurons were in juxtaposition with galanin-IR axon terminals, respectively.

By ultrastructural analysis, DAB labeled galanin-IR terminals were seen to establish synapses on proTRH neurons, the latter recognized by the presence of the highly electron dense silver particles (Fig. 2). In all instances, the synapses were found to be of the symmetric type (Fig. 2).

3.2. GALP-IR innervation of proTRH-containing neurons in the PVN

Within the PVN, GALP immunoreactive fibers concentrated primarily in the anterior parvocellular subdivision of the PVN and rostral portions of the periventricular parvocellular subdivision, with moderate concentrations in more caudal portions of the periventricular parvocellular subdi-

![Image](image_url)

Fig. 4. GALP-IR innervation of proTRH-IR neurons in the PVN. (A–C) Low power micrographs showing GALP-IR fibers (black) and proTRH-IR neurons (brown) in the (A) anterior (B) mid and (C) caudal levels of the PVN. (D) Medium power magnification photomicrograph illustrates association of GALP-IR axon varicosities with two proTRH-IR neurons (arrows) in the anterior parvocellular subdivision of the PVN. (E) Medium power magnification at the mid level of the PVN demonstrate that GALP-IR axons are juxtaposed to proTRH-IR neurons only in the periventricular parvocellular subdivision, but not in the medial parvocellular subdivision. (F) Oil immersion photomicrograph of boxed in area in (E) showing GALP-IR axon varicosities in juxtaposition to two proTRH neurons in the periventricular parvocellular subdivision. Abbreviations: PV: periventricular parvocellular subdivision of the PVN; PVNa: anterior parvocellular subdivision of the PVN; PVNmp: magnocellular subdivision of the PVN; PVNmp: medial parvocellular subdivision of the PVN; Fx: Fornix; III: third ventricle. Scale bar = 200 μm in (A) corresponds to (A–C); scale bar in (E) = 250 μm corresponds to (D–E); scale bar in (F) = 50 μm.
vision and only low concentrations in the medial parvo-
cellular subdivision (Fig. 3A,B). No GALP immunoreac-
tivity was found in the perikarya and dendrites intrinsic to
the parvocellular areas of the PVN. By double-labeling
light microscopic immunocytochemistry, GALP fibers
were found in juxtaposition to only a small number of
proTRH-IR neurons in the PVN, primarily in the periven-
tricular and anterior parvocellular subdivisions (Fig. 4A–
F). Only rare associations with proTRH-IR neurons were
observed in the medial parvocellular subdivision (Fig.
4B,C,E). Semiquantitative analysis from three animals
revealed that, in the anterior parvocellular subdivision,
26.7 ± 0.3% of proTRH-IR neurons were in juxtaposition
with GALP immunoreactive fibers, whereas 9.6 ± 1.0%
proTRH-IR neurons in the periventricular and 2.1 ± 0.2%
in the medial parvocellular subdivisions of the PVN
showed close appositions.

4. Discussion

These studies demonstrate a distinct difference in the
innervation of TRH neurons of the hypothalamic PVN by
axons containing galanin vs. GALP. Whereas both substanc-
eses are present in fibers in close proximity to TRH neurons
in the anterior parvocellular subdivision of the PVN, only
galanin-containing axons establish a prominent association
with TRH neurons in the periventricular and medial parvo-
cellular subdivisions of the PVN. The significance of this
finding is inherent in the demonstration that only TRH
neurons in the periventricular and medial parvocellular
subdivisions are hypophysiotropic. Retrograde transport of
substances from axon terminals in the median eminence, the
final destination of the hypothalamic tuberoinfundibular
system, accumulate in periventricular and medial parvo-
cellular neurons of the PVN [6,14,28], and hypothyroidism
induces an increase in TRH cell size [31] and TRH gene
expression [12] only in neurons in these subdivisions. In
addition, recent studies from our laboratories have shown
that the peptide, CART, coexists with TRH in the vast
majority of periventricular and medial parvocellular neurons
but only rarely in anterior parvocellular neurons [6], further
distinguishing these two populations of TRH neurons as
distinct cell types.

Since the synapses of galanin-containing axon terminals
on TRH neurons are symmetric, and symmetric type
synapses are considered to be inhibitory [35], it may be
presumed that, if galanin has a regulatory role over
hypophysiotropic TRH neurons, it is inhibitory. This
hypothesis is further supported by the observation that
the central administration of galanin decreases circulating
levels of TSH [38] and galanin inhibits the TRH release
from hypothalamic slices in culture [38].

The origin of the galaninergic innervation of TRH
neurons in the PVN may arise from several sources. Within
the hypothalamus, the dorsomedial nucleus (DMN), arcuate
nucleus, medial preoptic area and PVN, itself, contain
populations of galanin-producing neurons that project to
the PVN [21]. The DMN contains the largest population of
galanin cells retrogradely labeled from the PVN and, there-
fore, it is believed to be the principal source for the galanin-
IR fibers in the PVN [21]. Since practically all TRH neurons
in the PVN receive innervation from neurons residing in the
DMN [30], the DMN may also be the principal source for
galanin-IR fibers in contact with TRH neurons. As hyp-
ophysiotropic TRH neurons are responsive to changes in
circulating levels of leptin [19] and DMN neurons express
leptin receptors [4], galanin originating in the DMN may
contribute to the mediation of the leptin actions on the PVN
to regulate energy homeostasis.

Galaninergic cells in the arcuate nucleus also project
directly to the PVN [21]. Indeed, the arcuate nucleus is of
major importance in the regulation of hypophysiotropic
TRH neurons via a direct arcuato-paraventricular pathway
carrying the peptides, POMC and CART, and NPY and
AGRP, from two distinct subpopulations of arcuate neu-

tors to the PVN [5,6,18,41]. Although some galaninergic
neurons in the arcuate nucleus express leptin receptors
[10], no significant increase in galanin mRNA in the
arcuate nucleus neurons has been observed in association
with food deprivation [37]. This is contrary to the other
arcuate nucleus-derived orexigenic peptides, NPY and
AGRP, in which their mRNAs are markedly increased
during fasting [9]. These observations make it less likely
that galanin-producing neurons of arcuate nucleus origin
are involved in the regulation of energy balance, and
perhaps, less likely to be responsible for the galaninergic
innervation of hypophysiotropic TRH neurons. The PVN
also contains an intrinsic population of galanin-producing
neurons [26,39]. Galanin is synthesized in parvocellular
neurons adjacent to the TRH cells [26,39], as well in the
magnocellular cells of the PVN [26,39]. Galaninergic
neurons of the PVN may be involved in the regulation
of energy homeostasis as suggested by increased galanin
expression in these neurons in overfeeding [20].

The galanin innervation to the PVN may also derive from
neuronal populations outside of the hypothalamus. Galanin
and dopamine β-hydroxylase (DBH), the synthesizing en-
zyme of noradrenaline, colocalize in neurons of the A1 and
A6 regions of the medulla, and project to the PVN [21].
Since DBH-IR axons densely innervate the TRH neurons in
the PVN [24], it is conceivable that at least a portion of the
galaninergic innervation to TRH neurons in the PVN arises
from the brainstem.

In addition to the periventricular and medial parvo-
cellular subdivisions of the PVN, galanin-containing axons
also densely innervated the TRH-producing neurons in the
anterior parvocellular subdivision. The physiological sig-
ificance of this innervation remains unclear, but may
influence brainstem vegetative centers through down-
stream projections, or the limbic system. Along these lines,
it is noteworthy that leptin-responsive neuronal popula-
tions in the arcuate nucleus including neurons producing NPY and AGRP [18,41] and α-MSH and CART [5,6], also project to TRH neurons in the anterior parvocellular subdivision of the PVN, supporting a potentially important role for the anterior parvocellular PVN in mediating the central actions of leptin.

GALP, a closely related member of galanin family with orexigenic properties [25,33], is expressed exclusively in neurons of the arcuate nucleus. The vast majority of these neurons express leptin receptors [11], indicating the potential importance of GALP as a central mediator of leptin. Contrary to the other arcuate-derived, orexigenic peptides, NPY and AGRP [9], however, GALP mRNA is increased rather than reduced by the systemic administration of leptin [11]. Furthermore, GALP containing nerve terminals are associated with very few TRH neurons in the PVN. Thus, as opposed to galanin, which may directly regulate hypophysiotropic TRH through direct synaptic associations, GALP is likely to have only indirect regulatory effects over hypophysiotropic TRH neurons. Nevertheless, Seth et al. [38] have recently reported that GALP can inhibit the release of TRH from hypothalamic explants and administration of GALP into the PVN decreases TSH levels. As GALP is an agonist at galanin receptors [33], however, it is conceivable that the exogenous administration of GALP into the PVN binds to galanin receptors and induces a response that was intended for endogenous galanin. Alternatively, exogenous GALP administration may influence the hypothalamic–pituitary–thyroid axis through an indirect mechanism.

In summary, these data demonstrate that galanin-containing axons densely innervate both hypothysiopotropic TRH neurons in the medial and parvicellular subdivisions of the PVN and non-hypophysiotropic TRH neurons of the anterior parvocellular subdivision. The establishment of synaptic contacts with TRH neurons suggests that galanin may be inhibitory to TRH neurons and contribute to the downregulation of the thyroid axis during fasting. In contrast, the paucity of associations between GALP-containing axons with TRH neurons in the periventricular and medial parvocellular subdivisions indicates that GALP-containing neurons are unlikely to exert direct regulatory effects on hypophysiotropic neurons.

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