Neuroscience 117 (2003) 105–117

DISTRIBUTION AND REGULATION OF GALANIN RECEPTOR 1 MESSENGER RNA IN THE FOREBRAIN OF WILD TYPE AND GALANIN-TRANSGENIC MICE

J. G. HOHMANN,[a] A. JURÉUS,[c] D. N. TEKLEMICHAEL,[b] A. M. MATSUMOTO,[e,f] D. K. CLIFTON[b] AND R. A. STEINER[a,b,c,d]*

[a]*Neurobiology and Behavior Program, University of Washington, Seattle, WA 98195, USA*

[b]*Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, USA*

[c]*Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195, USA*

[d]*Department of Zoology, University of Washington, Seattle, WA 98195, USA*

[e]*Department of Medicine, University of Washington, Seattle, WA 98195, USA*

[f]*Geriatric Research, Education and Clinical Center, VA Puget Sound Health Care System, Seattle, WA 98108, USA*

Abstract—To learn more about molecular alterations in the brain that occur as a consequence of either the chronic excess or absence of peptide neurotransmitters, we examined the impact of genetically manipulating the neuropeptide galanin on the expression of one of its cognate receptors, galanin receptor 1. First, we examined the distribution of galanin receptor 1 messenger RNA in the mouse forebrain, and found it to be abundantly expressed in many brain regions, including in numerous hypothalamic and other forebrain regions associated with neuroendocrine function. The distribution of galanin receptor 1 messenger RNA in the mouse was similar to previous reports in the rat, with additional expression noted in the caudate putamen and in several midbrain regions. Next, using quantitative *in situ* hybridization, we measured cellular levels of galanin receptor 1 messenger RNA in the brains of mice that either overexpress galanin (galanin transgenic) or lack a functional galanin gene (galanin knockout). We report that relative to wild-type controls, the expression of galanin receptor 1 messenger RNA was increased in discrete areas of the brain in galanin-transgenic mice, but that depletion of galanin/noradrenergic innervation to the hypothalamus with the neurotoxin 6-hydroxydopamine did not alter levels of galanin receptor 1 messenger RNA. We also report that levels of galanin receptor 1 messenger RNA were not different between galanin-knockout and wild-type mice. These results suggest that compensatory adjustments in the expression of cognate receptors represent one mechanism by which the developing nervous system attempts to maintain homeostasis in response to overexpression of a peptidergic transmitter. However, the lack of significant changes in galanin receptor 1 messenger RNA in galanin-knockout mice suggests that developmentally programmed levels of receptor expression are maintained even in the complete absence of ligand.

Key words: galanin, receptor, transgenic, knockout, hypothalamus, mouse.

Overexpression or targeted ablation of some neuropeptide genes in mice has produced phenotypes that have revealed important—and sometimes surprising—physiological functions (e.g. the narcolepsy phenotype following deletion of the orexin gene; Chemelli et al., 1999). However, genetic alteration of other neuropeptides has resulted in phenotypes that are not as robust as expected. This phenomenon is exemplified by the neuropeptide Y (NPY) knockout, (Erickson et al., 1996), which has normal body weight and food intake, despite the well-described role of NPY as a potent orexigenic molecule. If mutant animals have mild or absent phenotypes compared with normal animals, one might infer that the altered gene is relatively unimportant for either development or physiological homeostasis. Alternatively, it is conceivable that in response to the congenital absence or excess of a particular ligand, compensatory alterations in the activity of other components within the neural circuit help to rectify the problem and thus sustain normal phenotypic development.

To learn more about how the nervous system adjusts to the permanent excess or absence of peptide neurotransmitters, we examined the impact of genetically manipulating the expression of the neuropeptide galanin. Galanin is expressed throughout the central and peripheral nervous systems (Cheung et al., 2001; Perez et al., 2001; Ryan and Gundlach, 1996; Skofitsch and Jacobowitz, 1985) and is a cotransmitter with several classical and peptidergic neurotransmitters (Melander et al., 1986). Galanin is highly expressed in the forebrain and hindbrain, and in the brainstem galanin regulates and is coexpressed with norepinephrine (Ma et al., 2001). Galanin is a modulator of many diverse neurophysiological processes, including the regulation of gonadotropin and growth hormone secretion, neurotrophic and neuroprotective actions, learning and memory, and nociception (Chan et al., 1996; Kerr et al., 2001; Marks et al., 1993; McDonald et al., 1998;

*Correspondence to: R. A. Steiner, Department of Physiology and Biophysics, Box 357290, University of Washington, Seattle, WA 98195-7290. Tel: +1-206-543-8712; fax: +1-206-685-0619.
E-mail address: steiner@u.washington.edu (R. Steiner).

Abbreviations: aCSF, artificial cerebral spinal fluid; bp, base pair; CA1, ammons horn of hippocampus; cRNA, complementary RNA; DHBA, 3,4-dihydroxybenzalamine; DMN, dorsomedial nucleus; EDTA, ethylenediaminetetraacetic acid; GALR1, galanin receptor 1; GALR2, galanin receptor 2; GALTG, galanin transgenic; GALKO, galanin knockout; HDB, horizontal limb of diagonal band; mRNA, messenger RNA; NPY, neuropeptide Y; PeN, periventricular nucleus; PVN, paraventricular nucleus; VMN, ventromedial nucleus; WT, wild type; 6-OHDA, 6-hydroxydopamine.

0306-4522/03$30.00+0.00
doi:10.1016/S0306-4522(02)00798-4

Wynick et al., 2001). One striking aspect of galanin physiology is its robust regulation in the nervous system, particularly in response to insult and injury, after which galanin is dramatically up-regulated in both central and peripheral neurons (Cortes et al., 1990; Zigmond and Sun, 1997). Galanin has also been implicated in the cognitive decline experienced by Alzheimer's disease patients, where it is thought to inhibit the actions of acetylcholine in degenerating memory circuits of the basal forebrain and hippocampus (Chan-Palay, 1988; Steiner et al., 2001).

Galanin signals through at least three different G protein-coupled receptor subtypes in the brain. Two of these, galanin receptor 1 (GALR1) and galanin receptor 2 (GALR2), are highly expressed in the rat brain (Burgevin et al., 1995; Gustafson et al., 1996; Landry et al., 1998; Mitchell et al., 1997; O'Donnell et al., 1999; Parker et al., 1995; Waters and Krause, 2000). While a preliminary report of the distribution of GALR2 in the mouse has been published (Holmes et al., 2001), no anatomical studies in the brain exist for GALR1 in this species. To establish the anatomical foundation for studies of gene regulation, we first mapped the distribution of GALR1 messenger RNA (mRNA) in the forebrain of the mouse. Next, we investigated the impact of genetically increasing or decreasing endogenous galanin production on the expression of GALR1 mRNA in the mouse forebrain using two models of altered galanin expression. One model was a transgenic mouse that overexpresses galanin under the control of the dopamine β-hydroxylase promoter (galanin transgenic [GALTG]) (Mazarati et al., 2000; Steiner et al., 2001). The other model was a mouse with a targeted deletion of the galanin gene (GALKO) (Wynick et al., 1998). We postulated that to maintain homeostasis in galanin signaling, the expression of GALR1 mRNA would be altered during development in compensation for the reduction or overabundance of the ligand in galanin-mutant mice.

EXPERIMENTAL PROCEDURES

Animals

All mice used in these studies were bred and maintained in the mouse colony at the University of Washington. They were maintained under constant temperature with a 14-h/10-h light/dark cycle, and were given free access to water and rodent chow. The Animal Care Committee of the School of Medicine at the University of Washington approved all experimental procedures in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and to reduce their discomfort. The GALTG and GALKO mice were developed and characterized as previously described (Holmes et al., 2000; Mazarati et al., 2000; Steiner et al., 2001; Wynick et al., 1998).

Experimental design

Experiment 1. To assess the forebrain distribution of GALR1 mRNA, young adult male C57BL6/J mice (n=6) were killed, and 20-μm coronal sections throughout the forebrain were subjected to *in situ* hybridization assays with both sense and antisense riboprobes.

Experiment 2a and 2b. To examine whether the expression of GALR1 is different between GALTG and wild-type (WT) mice, we measured and compared levels of GALR1 mRNA in young adult male (experiment 2a) or female (experiment 2b) C57BL6/J mice (n=5/6 per sex for each genotype) by *in situ* hybridization. In some sections, galanin mRNA expression was evaluated in the entorhinal cortex and piriform cortex of both WT and GALTG mice.

Experiment 3. To determine whether expression of GALR1 in GALTG mice is affected when noradrenergic/galaninergic neurons are destroyed, the neurotoxin 6-hydroxydopamine (6-OHDA) was administered to young adult male C57BL6/J WT and GALTG mice. Mice were housed individually for 1 week prior to treatment. Mice were separated into four weight-matched groups, and a single treatment was administered into the lateral ventricles by freehand injection, as previously described (Hohmann et al., 2000). The groups were as follows: WT artificial cerebral spinal fluid (aCSF) (n=5); WT 6-OHDA (n=5); GALTG aCSF (n=6); GALTG 6-OHDA (n=6). The dose of 6-OHDA (Sigma, St. Louis, MO, USA) was 50 μg in 5 μl aCSF with 0.04% ascorbic acid, and control animals were dosed with 5 μl aCSF with 0.04% ascorbic acid. Rectal temperatures were obtained each hour for the first 3 h after treatment, and then again on day 5. Body weight and food consumption were monitored daily throughout the course of the experiment. After 7 days, mice were killed under isoflurane anesthesia, brains dissected and quick-frozen to −80 °C until *in situ* hybridizations were performed. A separate group of young adult male WT mice was given either 6-OHDA (n=5) or aCSF (n=5) (*supra vide*); body temperatures, food intake and consumption were measured as described above; and then, the animals were killed for measurement of catecholamine content in the hypothalamus, hippocampus and brainstem.

Experiment 4. To determine whether the expression of GALR1 is different between GALKO and WT littermate mice, we measured and compared levels of GALR1 mRNA (n=6 for each genotype) by *in situ* hybridization.

Generation of GALR1 and galanin riboprobes

Total mRNA was extracted from mouse hypothalamus with a Totally RNA isolation kit (Ambion, Austin, TX, USA). M-MLV reverse transcriptase (Gibco, Gaithersburg, MD, USA), and oligo-dT primers (Ambion) were used for generation of total cDNA. PCR cloning was performed with the CloneAMP pAMP system (Gibco) and deoxy-UMP containing primers. PCR primers were as follows: GALR1, 5'-CAUCAUCAUCAUCTTTTCAGGCCACCGTGTATG-3' (forward primer) and 5'-CUACUACUACUAGATGAGCAGTAAGGGCAGAAGG-3' (reverse primer). For PCR, the initial melting temperature was 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, with a final extension of 72 °C for 10 min. PCR was performed with *Taq*DNA polymerase (Gibco) in a reaction volume of 50 μl. Products were visualized on a 1% agarose gel and purified with a QIAprep Miniprep kit (Qiagen, Valencia, CA, USA) and cloned into pAMP plasmids (Gibco). The insert was sequenced and verified to be a 377 base-pair (bp) cDNA, corresponding to bases 590–967 of GALR1 mRNA (Wang et al., 1997).

To make galanin riboprobe templates, the plasmid vector pGemT-Easy containing a 493-bp cDNA corresponding to the entire coding region of preprogalanin was kindly provided by Dr. James Hyde of the University of Kentucky. The plasmid was linearized with *Sac*II and transcribed with SP6 to generate a complementary RNA (cRNA) antisense probe complementary to mouse galanin mRNA. A sense probe was generated by linearizing the plasmid with *Pst*I, and transcribed with T7. To produce galanin-receptor riboprobe templates, PAMP plasmids were linearized by digestion with EcoR1 (New England Biolabs, Beverly, MA, USA) and transcribed with SP6 RNA polymerase (Roche, Indianapolis, IN, USA) for generation of antisense cRNA probes, or linearized with BamH1 (New England Biolabs) and transcribed

with T7 RNA polymerase (Roche) for generation of sense probes. The probes were treated with DNAse one (Roche) and purified on Quick Spin columns (Pharmacia, Piscataway, NJ, USA), as described previously (Cheung et al., 2001).

In situ hybridization

Single-label *in situ* hybridization was performed on coronal brain sections (20 μm) that were cut on a cryostat, collected in 1:4 series and thaw-mounted onto Superfrost Plus slides (VWR Scientific, West Chester, PA, USA), as previously described (Cheung et al., 2001). A hybridization solution containing ^{33}P-labeled (0.3 pmol/ml for GALR1) or ^{35}S-labeled (0.25 μg/ml/kb for galanin) cRNA probes and yeast tRNA (2 mg/ml) was applied on the tissue (80 μl/slide). The slides were cover-slipped and incubated in humid chambers overnight at 60 °C. Slides were then treated with RNAse A (Sigma) and washed under conditions of increasing stringency, including two hot incubations at 65 °C, followed by dehydration and air-drying. The slides were finally dipped in either Kodak NTB-3 or Kodak NTB-2 emulsion (Kodak, Rochester, NY, USA), exposed for 7–10 days, developed and counterstained with Cresyl Violet.

Image analysis

Slides were anatomically matched with the aid of a mouse brain atlas (Franklin and Paxinos, 1997) so that an equivalent number of slides from each region of interest could be analyzed for each experimental group. Analysis of mRNA expression in the hypothalamus was performed with the MCID image analysis system (Imaging Research, St. Catherines, Ontario, Canada). Sections were visualized with a Nikon Optiphot II microscope (Meridian Instruments, Kent, WA, USA) with a 10× objective, under dark-field illumination. Video images were captured with a DAGE model 65 camera (Dage-MTI, Michigan City, IN, USA) and were projected to a monitor. Each region of interest was outlined, and the average gray levels within the outline were measured to obtain an estimate of the signal. A similar-sized region devoid of specific signal was also outlined, and the average gray level of this region was measured to determine the background level. The signal-to-background ratio [SBR=(signal level)/(background level)] was calculated for each area. For the hippocampus and diagonal band measurements, individual cells were counted, and the number of silver grains in each mRNA-positive cell was quantified. This was performed with an image processing system consisting of a Scion VG-5 video acquisition board (Scion Corporation, Frederick, MD, USA) attached to a Macintosh G3 computer (Apple Computer Corporation, Cupertino, CA, USA). The sections were viewed under a Zeiss Axioscope microscope (Zeiss, New York, NY, USA) equipped with a 40× epi-illumination dark-field objective. Video images were obtained with a Cohu model 4915 CCD camera (Cohu, Inc., San Diego, CA, USA) attached to the microscope. For distribution studies of GALR1 and galanin in the mouse forebrain, each expressing region was scored qualitatively for both number of cells and intensity of labeling within cells. Cell numbers were scored from low (+) to very high (++++), based on the percentage of positively labeled cells compared with the total number of cells in each region. Intensity was scored qualitatively from weak (+) to very dense (++++) for the strength of signal per cell in each region. Representative sense probe slides for both GALR1 and galanin were examined for all analyzed brain regions to assess for specificity of labeling. No specific signal was observed in any region with either sense probe. A reader unaware of either the genotype or treatment group performed the image analysis.

Catecholamine measurements

To extract catecholamines, tissues were homogenized in the following volumes of 0.1-M perchloric acid with 0.01% cysteine: brainstem, 400 μl; hippocampus, 200 μl; hypothalamus, 200 μl; and forebrain, 600 μl. 3,4-diydroxybenzylamine (DHBA) was used as an internal standard at 10,000 pg/10 μl. A 400-μl aliquot was sonicated and spun in a centrifuge at 18,700 ×*g* for 10 min. A 100-μl aliquot of supernatant was added to 20 mg of acid-washed alumina and 400 μl of 0.5-M Tris buffer with 2% EDTA and rotated overnight. The alumina was washed twice with 1 ml of distilled water, and then catecholamines were desorbed with 100 μl of 0.1-M perchloric acid buffer with 0.01% cysteine. Norepinephrine and dopamine content was measured by electrochemical detection, as previously described (Liebmann and Matsumoto, 1990). The HPLC was run at a flow rate of 1 ml/min, and an injection volume of 20 μl was used. The working potential of the electrochemical detector was +0.8 V, and the full-scale sensitivity was 5 nA for all tissues. Quantification of catecholamines was done by comparing the peak heights of unknowns to those of known quantities of catecholamine standards and DHBA, with an HP 3393A integrator. The lower limit of detection ranged from 10 to 100 pg. Samples that were below the limit of detection were assigned a value equal to the limit of detection. All catecholamine levels were normalized per milligram of protein. Protein was determined by the Bio-Rad protein microassay (Hercules, CA, USA).

Statistical analysis

For all experiments, Student's unpaired *t*-test was used to compare differences between either genotypes or treatment groups. Results of statistical tests were considered significant at $P<0.05$. Results are expressed as mean values±S.E.M.

RESULTS

Distribution of GALR1 mRNA in the forebrain of the mouse

The distribution of cells expressing GALR1 mRNA in the forebrain of the mouse was mapped by *in situ* hybridization analysis to establish an anatomical benchmark for further studies. GALR1 mRNA-containing cells were found to be widely distributed (Table 1). The thalamus was the most densely labeled region in the forebrain. In the thalamic nuclei that expressed GALR1 mRNA, labeled cells were generally so tightly packed that they appeared as a continuum of cells covering the entire region. Thalamic areas that were densely labeled included the paraventricular nuclei (PVN), the central medial nucleus, the paracentral nucleus, and the mediodorsal nucleus (Fig. 1b, c; Fig. 2c). In the epithalamus, both the medial and lateral habenula had discrete clusters of positively labeled cells (Fig. 1c; Fig. 2c).

The hypothalamus was also a region showing extensive receptor expression, with most major nuclear groups exhibiting GALR1 mRNA signal. The most densely labeled regions were the dorsomedial nucleus (DMN), the periventricular nucleus (PeN), the ventromedial nucleus (VMN), and the medial preoptic area (Fig. 1a, c). Many other hypothalamic areas exhibited moderate numbers of GALR1 mRNA-positive cells, including the lateral anterior nucleus, lateral hypothalamic area (LH), posterior hypothalamus, and supraoptic nucleus. Another major area of GALR1 mRNA expression in the forebrain was the amygdala, with several nuclei displaying dense labeling. The highest-expressing area was a continuous band of cells

Table 1. Distribution of galanin receptor 1 (GAL-R1) messenger RNA (mRNA) in the forebrain of the mouse

Area	Cell number	Intensity	Area	Cell number	Intensity
Telencephalon			Reunions nucleus	+	++
Dorsal peduncular cortex	++	+	Xiphoid nucleus	+	++
Nucleus accumbens	+	++	Hypothalamus		
Olfactory tubercle	++	++	Anteroventral periventricular nucleus	++	++
Caudate putamen	++	++	Periventricular nucleus	++	+++
Lateral septum	++++	+++	Ventrolateral preoptic nucleus	+	++
Vertical limb of the diagonal band	++	++	Lateral preoptic nucleus	++	++
Horizontal limb of the diagonal band	++	++	Medial preoptic area	+++	++
Bed nucleus of the stria terminalis	+++	+++	Anterodorsal preoptic nucleus	++	++
Ventral palladium	++	++	Lateral hypothalamus	+++	++
Substantia innominata	++	++	Paraventricular nucleus	++	++
Basal nucleus of Meynert	++	++	Supraoptic nucleus	+	+++
Central amygdaloid nucleus	++	++	Lateroanterior hypothalamic nucleus	+++	+++
Medial amygdaloid nucleus	+++	+++	Anterior hypothalamic area posterior part	++	++
Basomedial amygdaloid nucleus	+++	+++	Dorsomedial nucleus	+++	+++
Interstitial nucleus of the posterior limb of the anterior commissure	+	++	Ventromedial nucleus	+++	++
			Arcuate nucleus	+	+
Hippocampus	+	+	Posterior hypothalamic area	++	++
Dentate gyrus (ventral)	++	++	Premammillary nucleus	++	+++
Amygdalohippocampal area	++	++	Mammillary nucleus	++	++
Retrosplenial granular cortex	+++	+	Ventral tuberomammillary nucleus	+	++
Pre-subiculum	++	++	Mesencephalon		
Subiculum	++	++	Peripeduncular nucleus	++	+++
Entorhinal cortex	+++	+++	Olivary pretectal nucleus	++	++
Diencephalon			Medial pretectal nucleus	++	++
Medial habenula	++	++	Nucleus of posterior commissure	++	++
Lateral habenula	++	++	Sub-geniculate nucleus	+	++
Parasubthalamic nucleus	+	++	Geniculate nucleus	++	++
Zona incerta	++	++	Substantia nigra (compacta and reticulata)	++	++
Thalamus			Edinger-Westphal nucleus	+	+++
Paraventricular thalamic nucleus	++++	++++	Superior colliculus	+++	++
Central medial nucleus	++++	++++	Periaqueductal gray (dorsal to aqueduct of Sylvius)	+++	++
Central lateral nucleus	+++	++++			
Mediodorsal nucleus	++++	++++	Sub-brachial nucleus	+	++
Paracentral nucleus	+++	++++	Interpeduncular nucleus	+	++
Intermediodorsal nucleus	+++	++++	Deep mesencephalic nucleus	+	++
Interanteromedial nucleus	++	++++			

GAL-R1 mRNA-positive cells were qualitatively scored for total cell count (cell number) and relative expression level per cell (intensity) in each forebrain region examined. Scoring for cell count was: + low, ++ moderate, +++ high, ++++ very high. Scoring for intensity was: + weak, ++ moderate, +++ dense, ++++ very dense. Young adult male C57BL6/J mice (n=6) were used for analysis, and results from all animals were combined for scoring.

extending from the medial amygdaloid nucleus (dorsal and ventral) to the basomedial amygdala (Fig. 1d).

In the telencephalon, the caudate putamen was labeled throughout its rostral-caudal extent with scattered GALR1 mRNA-expressing cells (Fig. 2a). All divisions of the lateral septum were densely labeled, and this region contained among the highest numbers of GALR1 mRNA-positive cells seen anywhere in the forebrain (Fig. 1a; Fig. 2a). A few scattered cells were seen in the nucleus accumbens, in both the core and shell regions. The ventral palladium exhibited a moderate clustering of labeled cells just below the anterior commissure (anterior part) (Fig. 2b). Both the vertical and horizontal limbs of the diagonal band had moderate numbers of labeled cells, but the medial septum was virtually devoid of specific signal (Fig. 1b). Distinct, but faintly labeled cells were seen in the dorsal peduncular cortex. Most divisions of the bed nucleus of the stria terminalis had moderate to high numbers of GALR1 mRNA-expressing cells, especially at the coronal level of the preoptic area. The basal nucleus of Meynert and substantia innominata also had moderate numbers of scattered cells.

Although no labeling was observed in the rostral hippocampus, the caudal hippocampus exhibited low to moderate levels of GALR1 mRNA expression, including the pyramidal cell regions of ammon's horn (CA1 and CA3) and the ventral-most part of the dentate gyrus (Fig. 2d). Groups of labeled cells were also identified in the amygdalohippocampal area and peripeduncular nucleus (Fig. 2d). In the caudal regions of the forebrain, several areas exhibited low to moderate numbers of GALR1 mRNA-expressing cells. The areas showing the most prominent

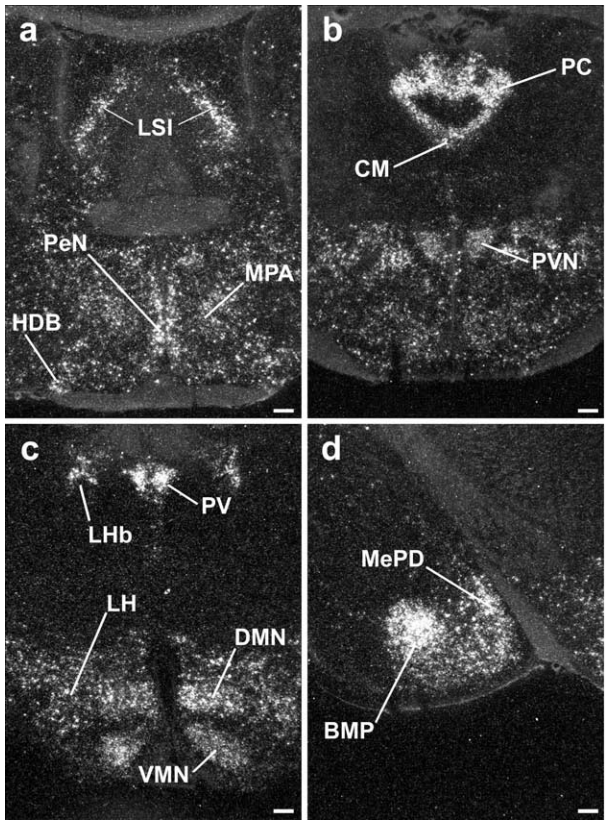

Fig. 1. Photomicrographs showing coronal sections of galanin receptor 1 (GALR1) messenger RNA (mRNA) distribution in the hypothalamus and amygdala of the wild-type mouse. (a) lateral septum (LSI), periventricular nucleus (PeN), medial preoptic area (MPA) and horizontal limb of the diagonal band (HDB); (b) paracentral thalamic nucleus (PC), central medial thalamic nucleus (CM), paraventricular hypothalamic nucleus (PVN); (c) paraventricular thalamic nucleus (PV), lateral hypothalamus (LH), lateral habenula (LHb), dorsomedial hypothalamic nucleus (DMN), ventromedial hypothalamic nucleus (VMN); (d) basomedial amygdaloid posterior nucleus (BMP), medial amygdaloid posterior dorsal nucleus (MePD). A ^{33}P-labeled riboprobe corresponding to bases 590–967 of mouse GALR1 mRNA was applied to 20-μm fresh-frozen sections, which were dipped in photographic emulsion, and exposed for 10 days. Sharpness, contrast and brightness were adjusted in Adobe Photoshop. Bar=200 μm.

expression were the various subdivisions of the mammillary nuclei, the superior colliculus (Fig. 2f), and a region of the periaqueductal gray just dorsal to the aqueduct of Sylvius. In the caudal cortex, many moderately labeled cells were seen throughout the extent of the retrosplenial granular cortex (Fig. 2f), and parts of the entorhinal cortex were densely labeled for GALR1 mRNA (Fig. 2e). These two areas were the only cortical regions of the forebrain that expressed detectable amounts of GALR1 mRNA.

Regulation of GALR1 mRNA in GALTG mice

To test the hypothesis that galanin regulates the expression of its own GALR1 receptor mRNA, we compared levels of GALR1 mRNA in various brain regions between groups of male mice that either overexpressed galanin (GALTG) or expressed galanin at normal levels (WT).

In the hypothalamus, levels of GALR1 mRNA were significantly higher in the GALTG compared with WT mice in several areas, including the PeN, DMN and VMN (Fig. 3a; Fig. 4). As galanin is a sexually dimorphic neuropeptide, we sought to determine whether GALR1 is also up-regulated in the hypothalamus of female GALTG mice. In females, as in males, levels of GALR1 mRNA were significantly higher in GALTG than WT, and this proved to be the case in the same three hypothalamic areas—the PeN, the DMN and the VMN (Table 2). Other hypothalamic regions, including the PVN did not express GALR1 differently between WT and GALTG in either male or female mice (data not shown).

We investigated whether altered expression of GALR1 occurs in other brain regions by measuring mRNA levels in two additional areas previously shown to be neurochemically altered in GALTG mice. The first target for exploration was the horizontal limb of the diagonal band (HDB), as in GALTG mice this area exhibits reductions in levels of choline acetyltransferase, the enzyme responsible for the synthesis of acetylcholine (Steiner et al., 2001). To determine whether, as in the case of the hypothalamus, we might also observe a compensatory increase in GALR1 expression in the HDB, we compared levels of GALR1 mRNA between GALTG and WT animals in this region. We had previously discovered a clear increase of galaninergic innervation into the HDB (Steiner et al., 2001), but despite this we observed no difference in the expression of GALR1 mRNA between genotypes in any part of the HDB (Fig. 3b).

We also measured GALR1 mRNA in the CA1 region of the hippocampus, and found that the number of expressing cells was significantly higher in GALTG than in WT mice (Fig. 3c). To determine whether this increase in GALR1 might result from an increase in galaninergic input to the hippocampus, we examined galanin mRNA levels in the entorhinal cortex, which provides a major hippocampal projection through the perforant path (Dolorfo and Amaral, 1998). WT mice did not express galanin at all in this region, but GALTG mice exhibited a massive ectopic expression of galanin in the entorhinal cortex, as well as in the rostral piriform cortex (Fig. 5).

Regulation of GALR1 mRNA after lesioning noradrenergic/galaninergic neurons with 6-OHDA

To test the hypothesis that the expression of GALR1 mRNA responds acutely to alterations of exposure to its ligand, the noradrenergic neurons that overexpress galanin in GALTG mice were destroyed to reduce galanin hyperinnervation to the hypothalamus, and levels of GALR1 mRNA were compared between lesioned and sham-lesioned animals. Mice were given a single injection of the neurotoxin 6-OHDA, and the effectiveness of the treatment was verified by documenting the reduction in core temperature known to occur following this lesion (Cleren et al., 1999) (Fig. 6a). In a separate group of mice, catecholamine content was measured after placement of the lesion, confirming a significant and selective decline in norepinephrine (but not dopamine) content in all areas examined (i.e. hypothalamus, hippocampus, and brainstem) following 6-OHDA treatment (Table 3). 6-OHDA-

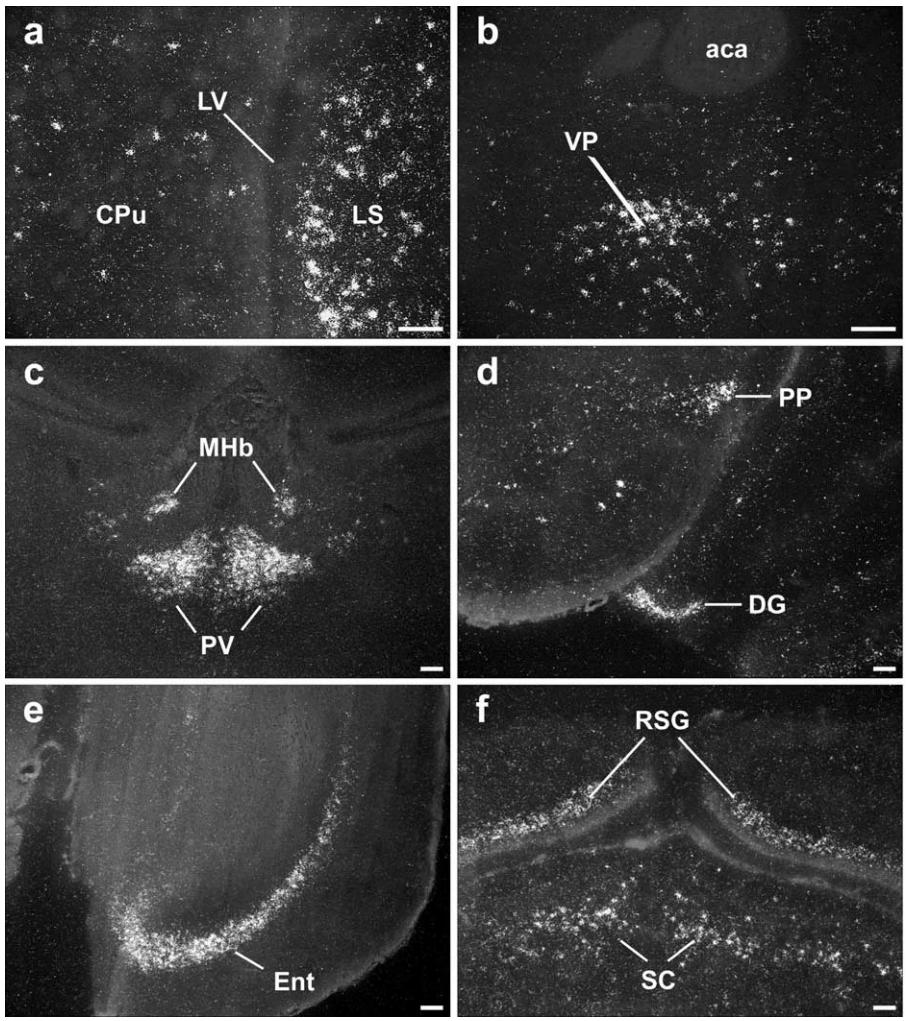

Fig. 2. Photomicrographs showing coronal sections of galanin receptor 1 (GALR1) messenger RNA (mRNA) distribution in the forebrain of the wild-type mouse. (a) caudate putamen (CPu), lateral septum (LS), lateral ventricle (LV); (b) anterior part of anterior commissure (aca), ventral palladium (VP); (c) medial habenula (MHb), paraventricular thalamic nucleus (PV); (d) dentate gyrus (DG), peripeduncular nucleus (PP); (e) entorhinal cortex (Ent); (f) retrosplenial granular cortex (RSG), superior colliculus (s.c.). A ^{33}P-labeled riboprobe corresponding to bases 590–967 of mouse GALR1 mRNA was applied to 20-μm fresh-frozen sections, which were dipped in photographic emulsion, and exposed for 10 days. Sharpness, contrast and brightness were adjusted in Adobe Photoshop. Bar=200 μm.

lesioned mice exhibited a transient loss of weight and a reduction in food intake compared with vehicle-treated animals, but these differences were significant only at the 24-h mark after treatment (data not shown). Seven days following the lesion, levels of GALR1 mRNA in the hypothalamus were measured and compared. No differences were observed between GALTG mice treated with 6-OHDA and those treated with vehicle (Fig. 6b).

Regulation of GALR1 mRNA in GALKO mice

We examined whether galanin receptor expression is altered when galanin is completely absent by comparing levels of GALR1 mRNA in the hypothalamus and hippocampus of GALKO and WT mice. The overall pattern of GALR1 gene expression in GALKO mice was very similar to that observed in WT mice, and there were no significant differences between GALKO and WT mice in levels of GALR1 mRNA in the hypothalamus (Fig. 7) or hippocampus (data not shown).

DISCUSSION

Distribution of GALR1 in the forebrain of the mouse

Several differences were evident in the distribution pattern of GALR1 mRNA between the mouse and that previously reported in the rat (Burgevin et al., 1995; Gustafson et al., 1996; Landry et al., 1998; Mitchell et al., 1997; O'Donnell et al., 1999; Parker et al., 1995; Waters and Krause, 2000). In the telencephalon, we found many GALR1 mRNA-positive cells throughout the rostral to caudal extent of the caudate putamen. In contrast, virtually no expression of GALR1 has been reported in the caudate of the rat. Also, in the rat a robust expression of GALR1 mRNA has been reported in the piriform cortex (O'Donnell et al., 1999), whereas in mice no expression of GALR1 mRNA was found in any piriform cortex region. This curious finding could be explained by the species difference in the piriform cortex distribution of galanin mRNA. In the rat, galanin

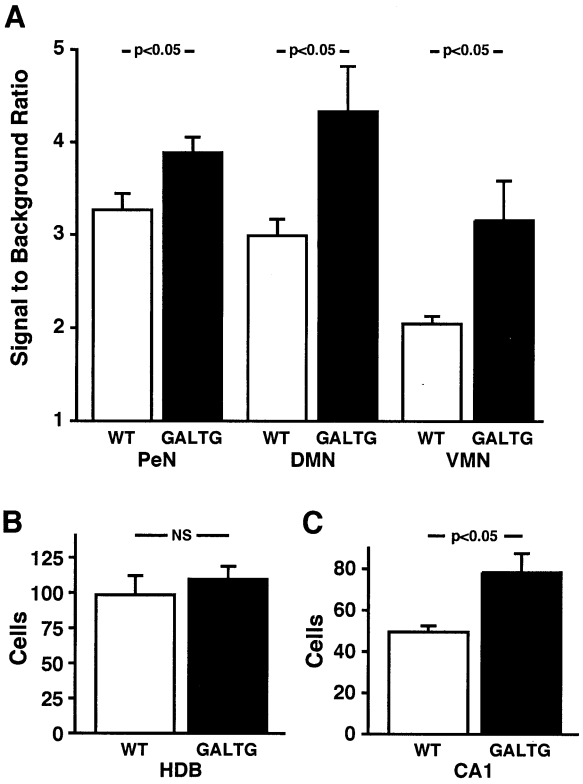

Fig. 3. Galanin receptor 1 messenger RNA levels of wild-type (WT) and galanin transgenic (GALTG) mice in (a) periventricular (PeN), dorsomedial (DMN) and ventromedial (VMN) nuclei of the hypothalamus; (b) horizontal limb of the diagonal band (HDB); (c) CA1 region of the hippocampus. Data are presented as mean signal to background ratio (hypothalamus) or mean cell counts (horizontal limb of diagonal band and CA1)±S.E.M. (n=6 animals per genotype).

mRNA is present in the piriform cortex (Ryan and Gundlach, 1996), but no galanin mRNA has been reported in the piriform cortex of the mouse (Cheung et al., 2001). It may be that in the absence of galanin production in this region, expression of the receptor is not required. GALR1 mRNA was observed in other cortical areas such as the retrosplenial granular and entorhinal cortices. Curiously, the entorhinal cortex also exhibited ectopic expression of galanin in GALTG mice, but whether the same cells that express galanin also express GALR1 in these regions is not known. In the amygdala, the regions having the highest levels of GALR1 mRNA expression were the basomedial and medial nuclei (both dorsal and ventral). These nuclei are both reciprocally connected to the hypothalamus, and to cortical regions involved in processing of emotional memories. The medial amygdaloid nucleus is considered to be an essential link between cortical circuits and regions of the hypothalamus that regulate sexual and maternal behaviors (Aggleton, 2000). The abundance of GALR1 in medial amygdala regions suggests that galanin mediates limbic system processing of neuroendocrine behaviors.

In the diencephalon, there is a remarkably good agreement in the distribution of GALR1 mRNA between the rat and mouse. The region having the densest expression of GALR1 mRNA was the thalamus, especially the midline and intralaminar nuclei, several of which project to the hypothalamus (Campeau and Watson, 2000) In the hypothalamus itself, GALR1 mRNA—like its ligand galanin—was distributed widely in all major nuclear groups. All regions of the preoptic area exhibited robust labeling for GALR1 mRNA, with highest levels in the medial preoptic area. As this region is involved in controlling sexual and reproductive behaviors, this underscores the putative role of galanin as a mediator of these processes. Nearly every nucleus associated with neuroendocrine functions had at least moderate amounts of GALR1 mRNA, including the PeN, PVN, DMN, LH and VMN. The exception to this was the arcuate nucleus, where only a few GALR1 mRNA-expressing cells were noted, which may not be surprising since in the mouse galanin is only expressed in a limited subset of cells in the arcuate nucleus (Cheung et al., 2001).

In the mesencephalon, relatively little GALR1 mRNA has been reported in the rat, except in the superior colliculus, central gray, and (in some studies) the substantia nigra (Burazin et al., 2000; Parker et al., 1995). In contrast, in the mouse we found GALR1 mRNA to be widely distributed throughout the midbrain, suggesting a more important role for galanin in these regions of this species compared with the rat. For example, the expression of GALR1 mRNA in the geniculate, pretectal and superior collicular areas implies that in the mouse, galanin has a role in visual processing, as these are all relay nuclei for input to visual cortex (Watanabe et al., 2001). Notably, a robust signal for GALR1 mRNA was observed in the peripeduncular nucleus, a region closely associated with maternal and sexual behaviors (Wedemeyer et al., 1999). Overall, the expression pattern of GALR1 mRNA in the forebrain of the mouse supports a role for this receptor as an important mediator of behavioral and physiological components of neuroendocrine circuitry.

Regulation of GALR1 expression in GALTG mice

In GALTG mice, galanin gene expression was placed under the control of the dopamine β-hydroxylase promoter, resulting in overexpression of galanin in all noradrenergic (NE) cells in the brain. Galanin is a cotransmitter with norepinephrine in the forebrain, and one expected consequence of overexpressing galanin in noradrenergic neurons is increased release of galanin in noradrenergic projection fields regions such as the hypothalamus and hippocampus (Steiner et al., 2001). The increase of GALR1 mRNA in these brain regions of GALTG mice supports the hypothesis that regulation of receptor gene expression is part of a compensatory homeostatic mechanism, which maintains normal galaninergic tone in the face of chronically elevated levels of galanin. The hypothalamic regions where both male and female GALTGs expressed elevated levels of GALR1 compared with WT (PeN, DMN and VMN) are known to be centers of neuroendocrine integration (Bernardis and Bellinger, 1998; Rohner-Jeanrenaud, 1995; Simerly, 1998). Cells in these nuclei are richly endowed with receptors for both neuropeptides and sex steroids, and neurons in these regions send and receive

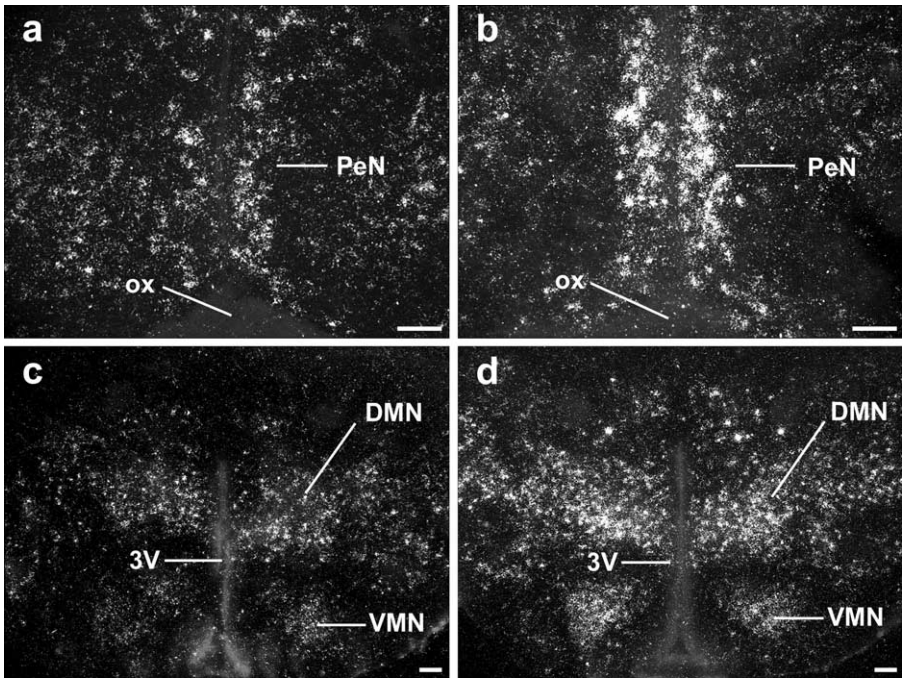

Fig. 4. Photomicrographs showing coronal sections of galanin receptor 1 (GALR1) messenger RNA (mRNA) levels in the hypothalamus between wild-type (WT) (a and c) and galanin transgenic (GALTG) mice (b and d). (a) WT periventricular nucleus (PeN); (b) GALTG PeN; (c) WT dorsomedial nucleus (DMN) and ventromedial nucleus (VMN); (d) GALTG DMN and VMN. Optic chiasm (ox), third ventricle (3V). A ^{33}P-labeled riboprobe corresponding to bases 590-967 of mouse GALR1 mRNA was applied to 20-μm fresh-frozen sections, which were dipped in photographic emulsion, and exposed for 10 days. Sharpness, contrast and brightness were adjusted in Adobe Photoshop. Bar=200 μm.

projections to and from other hypothalamic areas (Gu and Simerly, 1997; Luiten and Room, 1980; Thompson et al., 1996). If galanin signaling in these nuclei were important for maintaining proper neuroendocrine function, then mitigating the impact of galanin overexpression by altering galanin receptor expression would be beneficial as an adaptive mechanism to limit potentially harmful swings in the secretion of this neuropeptide.

If the increase in GALR1 expression in GALTGs helps to compensate for elevated galaninergic activity in these animals, then ligand-bound GALR1 should ultimately lead to an *inhibition* of galanin secretion. This inhibition could result from the direct activation of GALR1 expressed by galanin neurons themselves, or it could be mediated by GALR1 receptors located in other neurons that interact with galanin neurons. GALR1 and galanin mRNAs have overlapping patterns of expression in the mouse forebrain and midbrain (J. Hohmann, unpublished observation), which is consistent with a putative autoregulatory role for this receptor. It has also been established in the rat that galanin and GALR1 coexist in the same forebrain neurons (Landry et al., 1998; Miller et al., 1997). Thus, it is conceivable that an increase in the expression of inhibitory GALR1 autoreceptors plays a compensatory role for elevated galanin tone in GALTGs. If this were the case, we would expect levels of GALR1 mRNA to be elevated in neurons that (1) express galanin and (2) receive projections from neurons that overexpress galanin in GALTGs (i.e. NE neurons). Of the three hypothalamic nuclei in which GALTGs express elevated levels of GALR1 mRNA, the PeN and DMN have been shown to contain galanin-expressing neurons, identifying these nuclei as possible sites for GALR1 autoregulation (Cheung et al., 2001; Landry and Hokfelt, 1998).

Table 2. Galanin receptor 1 messenger RNA levels in female galanin transgenic (GALTG) mice and wild-type (WT) controls

Region	Female WT	Female GALTG
DMN	1.17±0.03	1.53±0.07*
VMN	1.16±0.03	1.34±0.07*
PeN	1.08±0.03	1.24±0.05*

* $P<0.05$ compared to female WT.

Data are expressed as mean signal to background ratio±S.E.M. ($n=6$ per group). DMN indicates dorsomedial nucleus; VMN, ventromedial nucleus; PeN, periventricular nucleus.

The DMN receives afferent input from NE neurons in the brainstem, particularly from the locus coeruleus (Palkovits, 1999) where virtually all of the NE neurons normally coexpress galanin and show dramatic overexpression of galanin in the GALTGs (Holets et al., 1988; Steiner et al., 2001). Whether galaninergic/NE efferents from brainstem neurons activate GALR1 autoreceptors in the DMN is unknown. However, it is plausible that GALR1 is up-regulated in the DMN as a presynaptic receptor in terminal fields of projections from the DMN, such as the PVN. Galanin-containing neurons provide a major input to the parvocellular PVN, where they are thought to regulate the release of hormones from the anterior pituitary (Ceccatelli et al.,

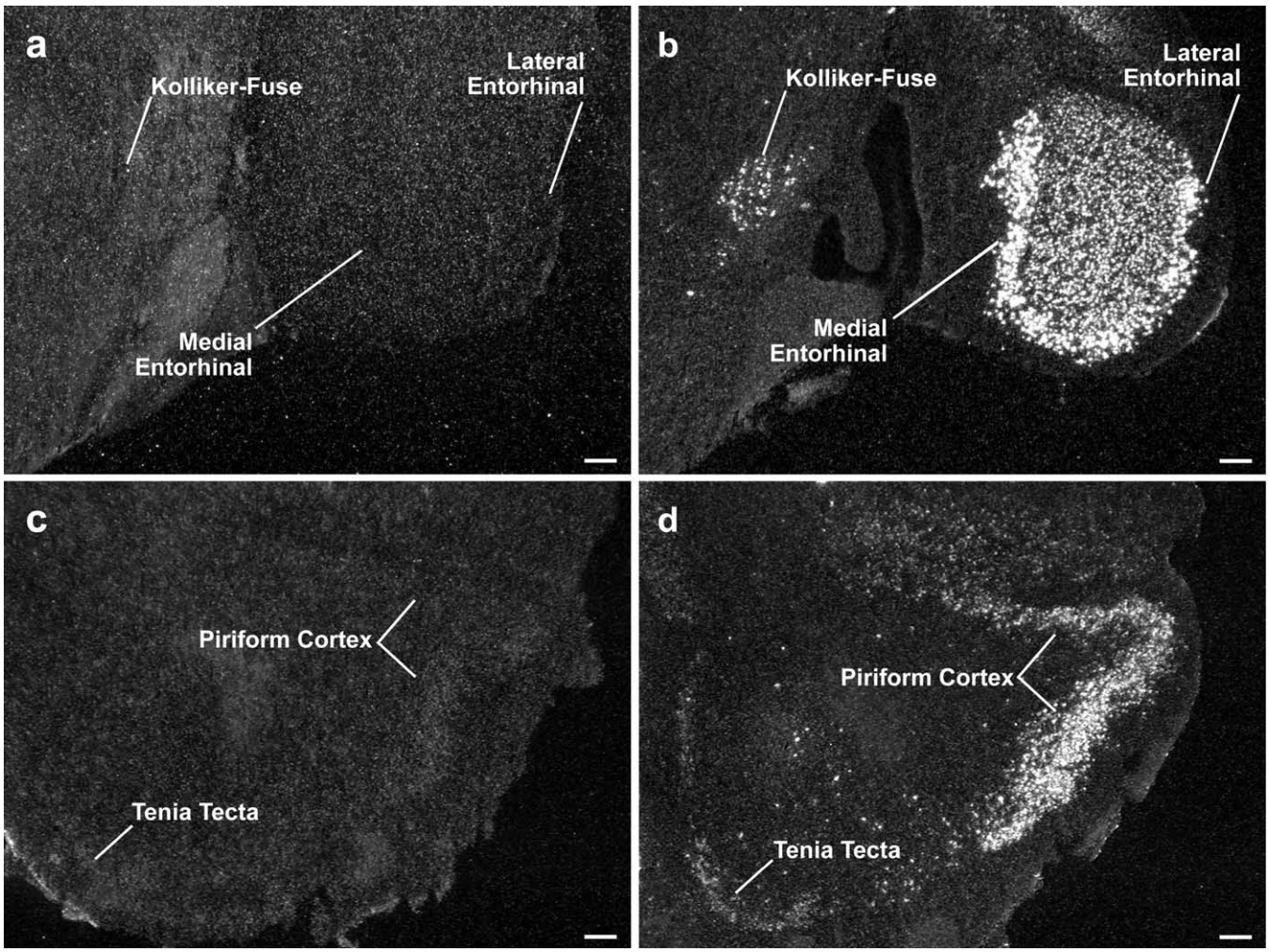

Fig. 5. Photomicrographs showing coronal sections of galanin messenger RNA in (a) the entorhinal cortex of wild-type (WT) mice; (b) the entorhinal cortex of galanin transgenic (GALTG) mice; (c) the piriform cortex of WT mice; (d) the piriform cortex of GALTG mice. A ^{35}S-labeled riboprobe corresponding to the entire coding region of preprogalanin was applied to 20-μm fresh-frozen sections, which were dipped in photographic emulsion, and exposed for 7 days. Sharpness, contrast and brightness were adjusted in Adobe Photoshop. Bar=200 μm.

1992; Levin et al., 1987). This same region of the PVN also receives afferent input from the noradrenergic brainstem (Palkovits, 1999). This being the case, it could be important for the release of galanin into the PVN from these dual afferents (the DMN and brainstem nuclei) to be tightly controlled, perhaps in part through the regulation of GALR1 expression. The lack of a difference between GALTG and WT in their expression of GALR1 mRNA in the PVN is particularly surprising, given the potentially important role of galanin in regulating the activity of this nucleus. Notwithstanding, it is conceivable that differential expression of GALR1 mRNA would become manifest between genotypes, but only following physiological or experimental challenges, such as fasting or gonadectomy.

At least some of the GALR1 that is up-regulated in GALTGs belongs to neurons that do not contain galanin. The expression of GALR1 is also influenced by genotype in the VMN, an area where little or no galanin has been detected in either the rat or the mouse (Cheung et al., 2001; Ryan and Gundlach, 1996). However, the VMN receives a direct projection from the subiculum (Fahrbach et al., 1989; Kita and Oomura, 1982). Although galanin is not produced in the subiculum of normal WT mice, it is ectopically expressed at high levels there in GALTG mice (Steiner et al., 2001). Thus, it seems reasonable to infer that GALR1 is up-regulated postsynaptically in the VMN to compensate for the anomalous effects of increased galanin input from subicular areas in the transgenic animals. A similar argument can be made for the CA1 region of the hippocampus, where GALR1 expression in GALTGs was significantly increased, possibly due to the massive ectopic increase in galaninergic input from the entorhinal cortex.

The increased expression of GALR1 mRNA in the PeN, DMN and VMN of GALTG mice occurred following *life-long* exposure to elevated levels of galanin. Several studies have demonstrated changes in GALR1 expression acutely after lesions that lead to altered expression levels of galanin (Landry and Hokfelt, 1998; Zhang et al., 1998). Often, changes in the expression of galanin mRNA and GALR1 mRNA are inversely related; for example as a

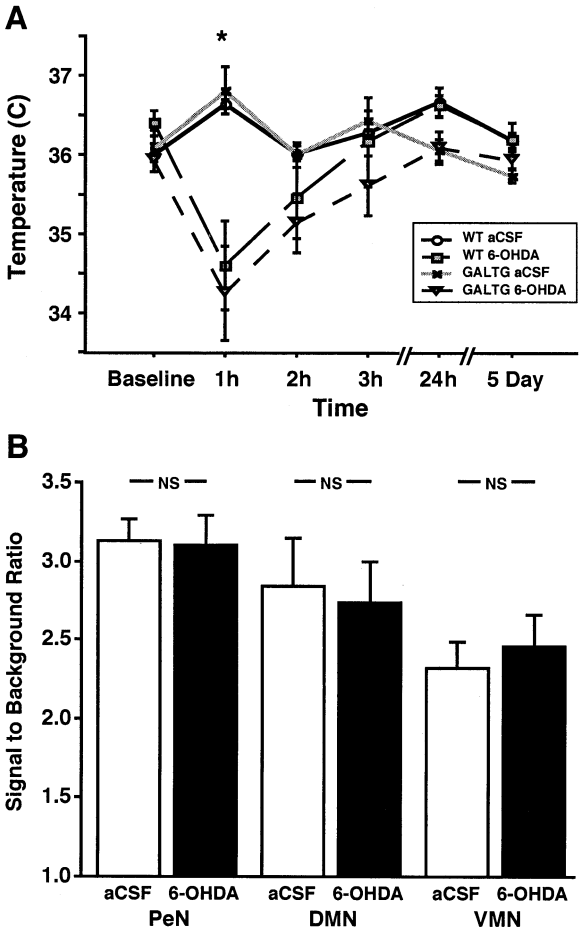

Fig. 6. (a) Body temperatures of 6-hydroxydopamine treated (6-OHDA) and sham-treated (aCSF) wild-type (WT) and galanin transgenic (GALTG) mice. Data are presented as mean temperature±S.E.M. (b) Galanin receptor 1 messenger RNA levels in 6-OHDA and sham-treated GALTG mice. Data are presented as mean signal to background ratio±S.E.M. (n=5–6 animals per group). *P<0.01 compared to WT aCSF.

result of colchicine treatment, hypophysectomy, or axotomy. To determine whether expression of GALR1 mRNA in GALTG mice can also be influenced by *acute* changes

Table 3. Levels of norepinephrine (NE) and dopamine (DA) in 6-hydroxydopamine-treated (6-OHDA) or sham-treated (aCSF) mice

Region	aCSF-treated	6-OHDA-treated
Hypothalamus		
NE	34.1±1.1	18.1±3.9*
DA	6.4±0.4	6.8±0.3
Hippocampus		
NE	12.1±0.7	1.7±0.9*
DA	0.8±0.2	0.8±0.3
Brainstem		
NE	18.4±0.3	9.2±0.9*
DA	0.8±0.1	1.0±0.1

* P<0.01 compared to aCSF-treated mice. Data are presented as mean ng/ml±S.E.M. (n=5 mice per group).

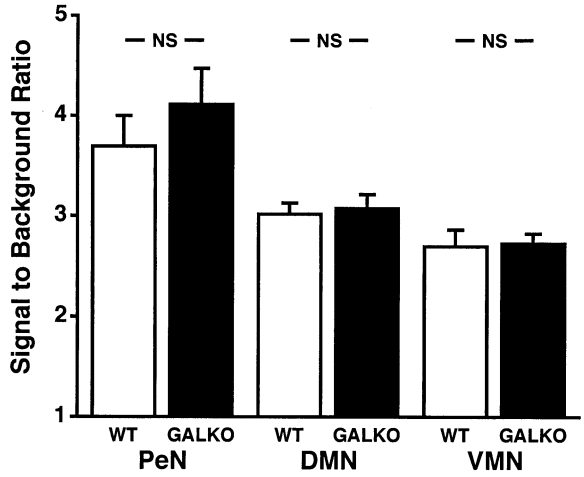

Fig. 7. Galanin receptor 1 messenger RNA levels of wild-type (WT) and galanin knockout (GALKO) mice in the periventricular (PeN), dorsomedial (DMN) and ventromedial (VMN) nuclei of the hypothalamus. Data are presented as mean signal to background ratio±S.E.M. (n=6 animals per genotype).

in galanin levels, we examined the effects of lesioning NE/galanin projections from the brainstem with 6-OHDA. We used a dose of 6-OHDA that had previously been shown to selectively deplete stores of NE in the hypothalamus (Cleren et al., 1999), and which should also reduce levels of the cotransmitter galanin. Because all available hypothalami from lesioned mice were needed for the analysis of either mRNAs or catecholamine measurements, it was not possible to directly assay galanin content or innervation after 6-OHDA treatment. However, as expected, NE content was dramatically reduced in all regions examined, demonstrating that brainstem noradrenergic/galaninergic neurons were seriously compromised by the neurotoxin. Also, body temperatures of lesioned mice were dramatically (but transiently) lower after treatment, reflecting the temporary hypothermic effect of massive release of NE into thermoregulatory regions of the hypothalamus. To our surprise, no changes in GALR1 mRNA were observed in any hypothalamic region as a result of this 6-OHDA insult, suggesting that acute changes in ligand exposure were not sufficient to alter levels of GALR1 mRNA. However, another plausible explanation is that the 6-OHDA lesion was not successful in reducing galanin levels in the hypothalamus to the degree necessary to be recognized by the GALR1 control system. This could result from the continued release into the hypothalamus of galanin ectopically produced in the subiculum and/or piriform cortex of GALTG mice, which presumably were unaffected by 6-OHDA lesions. Also, as we only measured levels of GALR1 mRNA at one time point after placement of the lesion (7 days), it is possible that sampling at earlier or later time points might have revealed changes between treatment groups. Further studies will be necessary to differentiate between the chronic and acute effects of altered galanin gene expression on GALR1 expression, but evidence from these lesion studies suggests that basal re-

ceptor mRNA levels may be primarily determined by some other developmental mechanism.

Regulation of GALR1 expression in GALKO mice

In contrast to what was observed in GALTG mice, no changes in GALR1 mRNA levels were noted in GALKO mice compared with WT mice in either the hypothalamus or hippocampus. This finding suggests that a certain level of receptor expression is genetically and developmentally programmed, and is not dependent on the presence of the ligand. This is different from the situation in GALTG mice, where brain circuits respond to the chronic "insult" of high galanin levels by activating counter-regulatory mechanisms. Thus, it would appear that in the complete absence of ligand in the GALKO, there would be nothing gained by changing levels of GALR1 receptor expression, and it would be more advantageous developmentally to alter the expression of other molecules that could functionally compensate for the loss of galanin. A potential compensatory molecule would be galanin-related peptide, a neuropeptide that is expressed in the hypothalamus and is known to activate galanin receptors (Ohtaki et al., 1999).

A caveat to these receptor studies is the fact that we examined only one component of the receptor regulatory process—control at the mRNA level, which may or may not accurately reflect the levels of protein present. It is conceivable that GALR1 activity is altered in GALKO mice by other mechanisms, such as posttranslational processing, receptor insertion into the membrane, internalization, or turnover. Notwithstanding, it is worth noting that a recent study has shown that when levels of hypothalamic galanin mRNA are experimentally induced, this is accompanied by an increase in *both* GALR1 mRNA and protein, suggesting that stimulation of GALR1 mRNA is accompanied by higher protein production (Burazin et al., 2001). Although levels of GALR1 protein were not examined in our study, levels of its mRNA certainly reflect one important aspect of functional regulation; thus, it seems reasonable to infer that transcriptional regulation of the receptor gene is not a primary mechanism for compensation in the GALKO animal.

Another potential mechanism for compensatory adjustment in galanin-mutant mice could be alterations in the expression of other galanin receptor subtypes such as GALR2, which is also broadly expressed in the mouse brain (Holmes et al., 2001). Preliminary evidence from our laboratory suggests that GALR2 mRNA is down-regulated in GALTG mice, in contrast to the up-regulation seen with GALR1 mRNA (J. G. Hohmann, unpublished observation). The apparent differential regulation of these two receptor subtypes in GALTG mice may not be too surprising considering their unique roles as mediators of galanin's actions. Based on studies involving a GALR2 specific agonist, Lui et al. have suggested that GALR1 activation subserves galanin's antinociceptive effects, whereas activation of GALR2 may actually decrease pain threshold (Liu et al., 2001). Also, GALR1 and GALR2 are oppositely regulated following manipulations that alter levels of galanin mRNA, implying distinct physiological roles for each receptor subtype (Burazin et al., 2001; Zhang et al., 1998). Conclusive evidence for alterations in neuropeptide receptor mRNA as a function of ligand overexpression is scant. However, a recent report showing differential regulation for two of the NPY receptor subtypes in NPY knockout mice suggests that remodeling of receptor circuits may be functionally important (Trivedi et al., 2001). Our results, demonstrating altered expression of GALR1 in galanin-overexpressing mice, suggest that compensatory adjustments in the expression of cognate receptors represent one mechanism by which the nervous system attempts to maintain homeostasis in the face of permanent alterations in the availability of neuropeptide transmitters.

Acknowledgements—This work was supported by grants from the NIH (R01 HD27142 and the Specialized Centers Program for Reproduction Research, U5412629, and AG05136), NSF (IBN-9720143/0110686), the Pediatric Epilepsy and Alzheimer's Research Centers at the University of Washington, the Swedish Foundation for International Cooperation in Higher Education and Research, and Veterans Administration Research Funds. We are grateful to Dr. David Wynick of the University of Bristol for providing the founding source of galanin knockout mice used in these studies. We thank Brett T. Marck and Tom Teal for technical assistance.

REFERENCES

Aggleton J (2000) The amygdala. Oxford University Press: New York.

Bernardis LL, Bellinger LL (1998) The dorsomedial hypothalamic nucleus revisited: 1998 update. Proc Soc Exp Biol Med 218:284–306.

Burazin TC, Larm JA, Gundlach AL (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.

Burazin TC, Larm JA, Ryan MC, Gundlach AL (2000) Galanin-R1 and -R2 receptor mRNA expression during the development of rat brain suggests differential subtype involvement in synaptic transmission and plasticity. Eur J Neurosci 12:2901–2917.

Burgevin M, Lonquet I, Quarteronet D, Habert-Ortoli E (1995) Cloning, pharmacological characterization, and anatomical distribution of a rat cDNA encoding for a galanin receptor. J Mol Neurosci 6:33–41.

Campeau S, Watson SJ Jr (2000) Connections of some auditory-responsive posterior thalamic nuclei putatively involved in activation of the hypothalamo-pituitary-adrenocortical axis in response to audiogenic stress in rats: an anterograde and retrograde tract tracing study combined with Fos expression. J Comp Neurol 423: 474–491.

Ceccatelli S, Giardino L, Calza L (1992) Response of hypothalamic peptide mRNAs to thyroidectomy. Neuroendocrinology 56:694–703.

Chan YY, Grafstein-Dunn E, Delemarre-van de Waal HA, Burton KA, Clifton DK, Steiner RA (1996) The role of galanin and its receptor in the feedback regulation of growth hormone secretion. Endocrinology 137:5303–5310.

Chan-Palay V (1988) Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. J Comp Neurol 273:543–557.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98:437–451.

Cheung CC, Hohmann JG, Clifton DK, Steiner RA (2001) Distribution

of galanin messenger RNA-expressing cells in murine brain and their regulation by leptin in regions of the hypothalamus. Neuroscience 103:423–432.

Cleren C, Vilpoux C, Dourmap N, Bonnet JJ, Costentin J (1999) Acute interactions between L-DOPA and the neurotoxic effects of 1-methyl-4-phenylpyridinium or 6-hydroxydopamine in mice. Brain Res 830:314–319.

Cortes R, Villar MJ, Verhofstad A, Hokfelt T (1990) Effects of central nervous system lesions on the expression of galanin: a comparative in situ hybridization and immunohistochemical study. Proc Natl Acad Sci USA 87:7742–7746.

Dolorfo CL, Amaral DG (1998) Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. J Comp Neurol 398:25–48.

Erickson JC, Clegg KE, Palmiter RD (1996) Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381:415–418.

Fahrbach SE, Morrell JI, Pfaff DW (1989) Studies of ventromedial hypothalamic afferents in the rat using three methods of HRP application. Exp Brain Res 77:221–233.

Franklin KB, Paxinos G (1997) The mouse brain in stereotaxic coordinates. Academic Press: San Diego, CA.

Gu GB, Simerly RB (1997) Projections of the sexually dimorphic anteroventral periventricular nucleus in the female rat. J Comp Neurol 384:142–164.

Gustafson EL, Smith KE, Durkin MM, Gerald C, Branchek TA (1996) Distribution of a rat galanin receptor mRNA in rat brain. Neuroreport 7:953–957.

Hohmann JG, Teal TH, Clifton DK, Davis D, Hruby VJ, Han G, Steiner RA (2000) Differential role of melanocortins in mediating leptin's central effects on feeding and reproduction. Am J Physiol 278: R50–R59.

Holets VR, Hokfelt T, Rokaeus A, Terenius L, Goldstein M (1988) Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 24: 893–906.

Holmes FE, Mahoney S, King VR, Bacon A, Kerr NC, Pachnis V, Curtis R, Priestley JV, Wynick D (2000) Targeted disruption of the galanin gene reduces the number of sensory neurons and their regenerative capacity. Proc Natl Acad Sci USA 97:11563–11568.

Holmes FE, Vanderplank P, Durkin MM, Walker MW, Branchek TA, Wynick D (2001) The distribution of the second galanin receptor (GALR2) protein in mouse brain. Soc Neurosci Abstracts 149:148.

Kerr BJ, Thompson SW, Wynick D, McMahon SB (2001) Endogenous galanin is required for the full expression of central sensitization following peripheral nerve injury. Neuroreport 12:3331–3334.

Kita H, Oomura Y (1982) An HRP study of the afferent connections to rat medial hypothalamic region. Brain Res Bull 8:53–62.

Landry M, Aman K, Hokfelt T (1998) Galanin-R1 receptor in anterior and mid-hypothalamus: distribution and regulation. J Comp Neurol 399:321–340.

Landry M, 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.

Levin MC, Sawchenko PE, Howe PR, Bloom SR, Polak JM (1987) Organization of galanin-immunoreactive inputs to the paraventricular nucleus with special reference to their relationship to catecholaminergic afferents. J Comp Neurol 261:562–582.

Liebmann JE, Matsumoto AM (1990) Acute selective withdrawal of testosterone negative feedback increases luteinizing hormone secretion without altering hypothalamic catecholaminergic neuronal activity. Endocrinology 126:555–564.

Liu HX, Brumovsky P, Schmidt R, Brown W, Payza K, Hodzic L, Pou C, Godbout C, Hokfelt T (2001) Receptor subtype-specific pronociceptive and analgesic actions of galanin in the spinal cord: selective actions via GalR1 and GalR2 receptors. Proc Natl Acad Sci USA 98:9960–9964.

Luiten PG, Room P (1980) Interrelations between lateral, dorsomedial and ventromedial hypothalamic nuclei in the rat: an HRP study. Brain Res 190:321–332.

Ma X, Tong YG, Schmidt R, Brown W, Payza K, Hodzic L, Pou C, Godbout C, Hokfelt T, Xu ZQ (2001) Effects of galanin receptor agonists on locus coeruleus neurons. Brain Res 919:169–174.

Marks DL, Smith MS, Vrontakis M, Clifton DK, Steiner RA (1993) Regulation of galanin gene expression in gondadotropin-releasing homrone neurons during the estrous cycle of the rat. Endocrinology 132:1836–1844.

Mazarati AM, Hohmann JG, Bacon A, Liu H, Sankar R, Steiner RA, Wynick D, Wasterlain CG (2000) Modulation of hippocampal excitability and seizures by galanin. J Neurosci 20:6276–6281.

McDonald MP, Gleason TC, Robinson JK, Crawley JN (1998) Galanin inhibits performance on rodent memory tasks. Ann N Y Acad Sci 863:305–322.

Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci 6:3640–3654.

Miller MA, Kolb PE, Raskind MA (1997) GALR1 galanin receptor mRNA is co-expressed by galanin neurons but not cholinergic neurons in the rat basal forebrain. Brain Res Mol Brain Res 52: 121–129.

Mitchell V, Habert-Ortoli E, Epelbaum J, Aubert JP, Beauvillain JC (1997) Semiquantitative distribution of galanin-receptor (GAL-R1) mRNA-containing cells in the male rat hypothalamus. Neuroendocrinology 66:160–172.

O'Donnell D, Ahmad S, Wahlestedt C, Walker P (1999) Expression of the novel galanin receptor subtype GALR2 in the adult rat CNS: distinct distribution from GALR1. J Comp Neurol 409:469–481.

Ohtaki T, Kumano S, Ishibashi Y, Ogi K, Matsui H, Harada M, Kitada C, Kurokawa T, Onda H, Fujino M (1999) Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J Biol Chem 274:37041–37045.

Palkovits M (1999) Interconnections between the neuroendocrine hypothalamus and the central autonomic system: Geoffrey Harris Memorial Lecture, Kitakyushu, Japan, October 1998. Front Neuroendocrinol 20:270–295.

Parker EM, Izzarelli DG, Nowak HP, Mahle CD, Iben LG, Wang J, Goldstein ME (1995) Cloning and characterization of the rat GALR1 galanin receptor from Rin14B insulinoma cells. Brain Res Mol Brain Res 34:179–189.

Perez SE, Wynick D, Steiner RA, Mufson EJ (2001) Distribution of galaninergic immunoreactivity in the brain of the mouse. J Comp Neurol 434:158–185.

Rohner-Jeanrenaud F (1995) A neuroendocrine reappraisal of the dual-centre hypothesis: its implications for obesity and insulin resistance. Int J Obes Relat Metab Disord 19:517–534.

Ryan MC, Gundlach AL (1996) Localization of preprogalanin messenger RNA in rat brain: identification of transcripts in a subpopulation of cerebellar Purkinje cells. Neuroscience 70:709–728.

Simerly RB (1998) Organization and regulation of sexually dimorphic neuroendocrine pathways. Behav Brain Res 92:195–203.

Skofitsch G, Jacobowitz DM (1985) Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 6:509–546.

Steiner RA, Hohmann JG, Holmes A, Wrenn CC, Cadd G, Jureus A, Clifton DK, Luo M, Gutshall M, Ma SY, Mufson EJ, Crawley JN (2001) Galanin transgenic mice display cognitive and neurochemical deficits characteristic of Alzheimer's disease. Proc Natl Acad Sci USA 98:4184–4189.

Thompson RH, Canteras NS, Swanson LW (1996) Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 376:143–173.

Trivedi PG, Yu H, Trumbauer M, Chen H, Van der Ploeg LH, Guan X

(2001) Differential regulation of neuropeptide Y receptors in the brains of NPY knock-out mice. Peptides 22:395–403.

Wang S, He C, Maguire MT, Clemmons AJ, Burrier RE, Guzzi MF, Strader CD, Parker EM, Bayne ML (1997) Genomic organization and functional characterization of the mouse GalR1 galanin receptor. FEBS Lett 411:225–230.

Watanabe T, Michaelis T, Frahm J (2001) Mapping of retinal projections in the living rat using high-resolution 3D gradient-echo MRI with Mn2+-induced contrast. Magn Reson Med 46:424–429.

Waters SM, Krause JE (2000) Distribution of galanin-1, -2 and -3 receptor messenger RNAs in central and peripheral rat tissues. Neuroscience 95:265–271.

Wedemeyer C, Vivas L, Carrer HF (1999) Peripeduncular lesion alters expression of c-fos induced in the female rat brain by male mounting. J Neuroendocrinol 11:221–228.

Wynick D, Small CJ, Bacon A, Holmes FE, Norman M, Ormandy CJ, Kilic E, Kerr NC, Ghatei M, Talamantes F, Bloom SR, Pachnis V (1998) Galanin regulates prolactin release and lactotroph proliferation. Proc Natl Acad Sci USA 95:12671–12676.

Wynick D, Thompson SW, McMahon SB (2001) The role of galanin as a multi-functional neuropeptide in the nervous system. Curr Opin Pharmacol 1:73–77.

Zhang X, Xu ZO, Shi TJ, Landry M, Holmberg K, Ju G, Tong YG, Bao L, Cheng XP, Wiesenfeld-Hallin Z, Lozano A, Dostrovsky J, Hokfelt T (1998) Regulation of expression of galanin and galanin receptors in dorsal root ganglia and spinal cord after axotomy and inflammation. Ann N Y Acad Sci 863:402–413.

Zigmond RE, Sun Y (1997) Regulation of neuropeptide expression in sympathetic neurons: paracrine and retrograde influences. Ann N Y Acad Sci 814:181–197.

(Accepted 14 October 2002)