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Galanin Is a Potent In Vivo Modulator of Mesencephalic Serotonergic Neurotransmission

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Neurochemical, molecular, immunohistochemical and behavioral methods were used to examine the in vivo effects of the neuropeptide galanin on central 5-HT neurotransmission and on 5-HT$_{1A}$ receptor-mediated responses. Intraventricularly infused galanin caused a long-lasting and dose-dependent reduction of basal extracellular 5-HT levels in the ventral hippocampus of awake rats as measured by microdialysis. Infusion of galanin into the dorsal raphe nucleus (DRN), but not intrahippocampally, reduced 5-HT release. The effect of i.c.v. galanin on 5-HT release was blocked by the galanin receptor antagonist M35, acting most likely via galanin receptors at the level of the DRN. Galanin also reduced the levels of tryptophanhydroxylase mRNA in the DRN. Therefore, the effects of galanin on 5-HT$_{1A}$ receptor-mediated responses were further investigated. Surprisingly, galanin significantly attenuated the reduction of hippocampal 5-HT release induced by systemic injection of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. Galanin also attenuated 8-OH-DPAT-induced hypothermia and locomotor activity in rats. These results indicate that galanin has important inhibitory actions on central 5-HT neurotransmission and on 5-HT$_{1A}$ receptor-mediated events.

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KEY WORDS: *Galanin; Serotonin; Hippocampus; Dorsal raphe nucleus; 5-HT$_{1A}$ Receptors; Microdialysis; Locomotor activity*

Galanin, a 29- or 30-amino acid peptide (Tatemoto et al. 1983), is widely distributed throughout the central nervous system (CNS) as demonstrated by immunohistochemical techniques (Skofitsch and Jacobowitz 1985, 1986; Melander et al. 1986; Rökaeus 1987). Studies during the past decade have shown that galanin is involved in a variety of physiological and behavioral functions (for review, see Crawley 1996, 1999; Hökfelt et al. 1998; Mazarati et al. 2001; Ögren et al. 1999; Wrenn and Crawley 2001). In rats pretreated with colchicine (Melander et al. 1986; Fuxe et al. 1990), as well as in naive rats (Xu et al. 1998) a moderate level of galanin immunoreactivity was observed in a subpopulation of the serotonergic neurons of the dorsal raphe nucleus (DRN), which extensively innervate the forebrain (Dahlström and Fuxe 1964). In addition, galanin mRNA was found to be localized in 5-HT-positive cells in the DRN (Priestley et al. 1993) and a relatively high [^{125}I]galanin binding was observed in the raphe nuclei (Skofitsch et al. 1986). At least three G-protein-coupled receptors (for review, see Branchek et al. 2000) mediate the actions of galanin, of those GalR1 mRNA is found exclusively in the CNS and PNS, whereas GalR2 and

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GalR3 are expressed also in other organs (Waters and Krause 2000).

Several neurochemical studies provided the first evidence for the possibility of galanin interactions with 5-HT and a modulatory action of galanin on brain 5-HT neurotransmission (Fuxe et al. 1998). An early biochemical study based on the measurements of tissue concentrations of 5-HT and 5-HIAA in the rat found a decrease in 5-HT metabolism (5-HIAA to 5-HT ratio) in the limbic forebrain 20 min after galanin administration (Fuxe et al. 1988a). This effect was suggested to be mediated by an inhibitory effect of galanin at the level of the mesencephalic 5-HT raphe neurons. Subsequent electrophysiological studies using slice preparations have shown that galanin can hyperpolarize cell membranes of dorsal raphe neurons in vitro (Xu et al. 1998). Recent studies, using quantitative receptor autoradiography and in situ hybridization, have also given evidence that galanin in vivo can affect 5-HT neuronal activity in the dorsal raphe, which is known to contain a high number of 5-HT_{1A} receptors (Pazos and Palacios 1985), as well as galanin receptors (O'Donnell et al. 1999). Thus, intracerebroventricularly (i.c.v.) administered galanin was shown to reduce 5-HT_{1A} receptor mRNA and 5-HT_{1A} receptor binding, as well as galanin mRNA in the DRN with maximal effects 2 h after galanin administration (Razani et al. 2000).

A series of in vitro binding studies using membrane preparations from ventral limbic cortex have given evidence that porcine galanin in the nanomolar range can reduce the affinity of 5-HT_{1A} agonist binding sites (Fuxe et al. 1988b, 1998; Hedlund and Fuxe 1996). The receptor binding data suggest a preferential interaction with the 5-HT_{1A} receptor subtype, because galanin did not alter 5-HT_2 receptor agonist and antagonist binding in the limbic cortex (Fuxe et al. 1990).

This finding led to the proposal of an antagonistic intramembrane galanin-5-HT_{1A} receptor interaction at the postsynaptic level (Fuxe et al. 1988b, 1990). Finally, functional evidence for a modulatory role of galanin on 5-HT_{1A} receptor-mediated behavior in the rat has also been provided (Misane et al. 1998). Thus, galanin given i.c.v., 10 min or 2 h prior training, attenuated the impairment of passive avoidance caused by systemic administration of the preferential 5-HT_{1A} receptor agonist 8-OH-DPAT (Misane et al. 1998; Razani et al. 2001). The deficit in passive avoidance by systemic 8-OH-DPAT appears to be related to activation of postsynaptic 5-HT_{1A} receptors in the limbic forebrain, e.g. the hippocampus (Carli et al. 1993; Misane et al. 1998). However, the reduction in the postsynaptic 5-HT_{1A} receptor response after i.c.v. galanin was not associated with alterations in 5-HT_{1A} receptor binding sites or mRNA levels in the hippocampus (Razani et al. 2001).

The aim of the present study was to examine whether centrally administered galanin could affect basal 5-HT release in the ventral hippocampus in awake rats using in vivo microdialysis. Of particular importance was to analyze whether the effect of galanin on 5-HT release is mediated by interactions with somatodendritic 5-HT_{1A} receptors or with 5-HT_{1A} receptors at the postsynaptic level. Further, the concomitant activation of galanin and somatodendritic 5-HT_{1A} receptors was studied to examine whether the hypothesized inhibitory effects of each receptor subgroup on 5-HT release will interact in the control of the activity of the mesencephalic 5-HT neurons.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (B&K Universal, Solentuna, Sweden) weighing 250–300 g were used in the study. The rats were maintained on a 12 h light/dark schedule (lights on at 6:00 A.M.) and a room temperature of 21°C with a 40–50% relative humidity at the animal facilities of the Department of Neuroscience. The rats had free access to standard lab chow (Ewos R36, Ewos, Sweden) and tap water. A five-day adaptation period always preceded the experiments. All experiments were performed in accordance with general recommendations of Swedish animal protection legislation. The experiments were approved by the Animal Ethics Committee of North Stockholm, Sweden.

Microdialysis

Seven days before the microdialysis experiment, the rats were anesthetized with enflurane (3% in N_2O:O_2 (1:1) at 0.8 l/min) and placed in a stereotaxic frame (David Kopf Instruments, CA, USA) using a flat skull position, incisor bar −2.7 to 3.2 mm. The body temperature of the rat was controlled by a rectal thermometer and maintained at +37°C using a CMA/105 temperature controller (CMA/Microdialysis, Stockholm, Sweden). The middle scalp incision of 2–3 cm was made and the flaps were kept aside using hemostatic forceps. After exposing the skull, a hole for a probe and two holes for the fixing screws were drilled using a fine trephine drill. The Eicom (Eicom Corp., Kyoto, Japan) or CMA/11 guide cannula with a dummy was implanted into the ventral hippocampus at the coordinates AP −5.0, L +4.8, V −4.7 mm, according to the stereotaxic atlas of Paxinos and Watson (1997). Another guide cannula (Plastic One, Roanoke, VA) for i.c.v. injections was implanted into the lateral ventricle at the coordinates AP −1.3, L +1.8, V −3.8 mm or, in a separate group of rats into the DRN at the coordinates AP −7.8, L +2.4, V −7.0 mm. Two microscrews were placed into the skull and the whole assembly was secured with dental cement (Dentalon Plus, Heraeus,

Germany). The rats recovered for seven days. During this period, the body weight was monitored and rats losing body weight were excluded from the experiment. On the day of experiment, the rats were placed into the CMA/120 system for freely moving animals (CMA/Microdialysis) in order to habituate and to initiate the intracerebral perfusion. An Eicom A-I type probe or a CMA/11 microdialysis probe with 2 mm cuprophane membrane length and the injection cannula (Plastic One) were inserted into each respective guide cannula of the operated rat. The probes were perfused at a constant flow-rate of 1.25 μl/min with the Ringer solution. Following a stabilization period of 2–3 h, the samples were collected every 20 min using a CMA/170 refrigerated fraction collector. The first three or four samples were collected for determination of basal extracellular levels of 5-HT. The concentrations of 5-HT at the time point of the drug injection (0 min in all figures) were taken as 100%. The drug (galanin, M35) in the treated groups or aCSF in the control groups were infused through the injection cannula at a rate of 0.5 μl/min (intraparenchymally) or 2 μl/min (i.c.v.). 8-OH-DPAT or saline were administered subcutaneously (s.c.) in the scruff of the neck. The fractions were collected for up to six hours.

HPLC Analysis

5-HT was determined by precolumn derivatization with benzylamine and microbore column liquid chromatography with fluorescence detection as described elsewhere (Ishida et al. 1998). Briefly, the derivatization reagent was prepared by mixing 0.3 M CAPS buffer (pH 12.0), 0.2 M benzylamine (Tokyo Kasei Kogyo, Japan), 0.1 M potassium hexacyanoferrate (III) and methanol in ratio 1:1:5:10, v/v. The final derivatization reagent (20 μl) was added to 20 μl of the microdialysis sample or a 5-HT standard solution and the mixture was allowed to stand at room temperature for 2 min before being injected onto the chromatographic column. An micro-LC pump, LC100 (BASJ, Tokyo, Japan), a CMA/260 Degasser and a CMA/200 Refrigerated Microsampler (CMA/Microdialysis, Stockholm, Sweden), equipped with a 10 μl loop and operating at +6°C, and a L-7480 Fluorescence detector (Merck/Hitachi, Germany/Japan) equipped with a 2 μl semi-micro flow-through cell were used. The mobile phase was a mixture of 40 mM sodium phosphate buffer, pH 7.5 and acetonitrile at 60:40 ratio, containing 1 mM disodium-EDTA and 50 mM 1-octane-sulfonic acid sodium salt. The eluent was pumped at a flow rate of 50 μl/min through a microbore column (100 × 1 mm I.D.) packed with C18 silica, 5 μm particle size (Chemical Insp. and Testing Inst., Hita, Japan). The detection limit (signal-to-noise ratio = 3) for 5-HT was 80 atmol in 5 μl injected onto the column.

Locomotor Activity

All surgical procedures were performed as described in the Microdialysis section with some minor modifications. Briefly, guide cannulae (26 GA; 3.8 mm and a diameter of 0.45 mm, Plastics One) were implanted bilateraly to the lateral ventricle at the following coordinates: AP −1.3 mm, L ± 1.8 mm and V −3.8 mm (Paxinos and Watson 1997). The operated rats were maintained in groups of two per cage (Macrolon© IV) separated by a transparent plastic division. They were allowed to recover for at least five days. On the day of experiment, the rats were taken to the experimental room and allowed to habituate for a period of 60 min. A CMA/100 microinfusion pump (CMA/Microdialysis) equipped with two 25 μl Hamilton syringes, was used for bilateral infusions of galanin (0.5 nmol or 3 nmol) or aCSF at 2.0 μl/side (flow rate of 2.0 μl/min). After the infusion was completed, the injection cannulae were left inside the guide cannulae for an additional 60 s before being replaced with the dummy cannulae. Eighty minutes after the infusions, the rats were placed individually into the locomotor cages (Macrolon III with 50 ml bedding), which were then transferred onto a motor activity detection system. A computerized multicage infrared-sensitive motion detection system (Motron Products, Stockholm, Sweden), (Ögren et al. 1979) was used to detect the horizontal activity (locomotion and motility) and vertical activity (rearing) in 5 min intervals. Horizontal movements were detected by 40 photosensors placed in the floor (in 4 cm squares) of the motility meters. The vertical detectors were mounted 13 cm above the floor of the measurement box. Locomotion was defined as the movement between two rows of the photosensors located at opposite ends of the cage floor. Motility was defined as any movement covering each photosensor, i.e. a distance of 4 cm. Locomotor activity was recorded during a period of 60 min, during which the rats habituated in the new environment. 140 min after galanin or aCSF treatment, the animals were injected s.c. with 8-OH-DPAT (0.3 mg/kg) and locomotor activity was recorded for a second period of 120 min, referred in the text as 8-OH-DPAT induced motor activity. After the experiment, the rats were sacrificed by exposure to CO_2 gas. Evans Blue vital dye (0.1%) was injected i.c.v. using the same injection cannulae. The brains were removed, sliced and stained for histological verification of the injection sites.

Body Temperature

Rats with i.c.v. cannulae (see Microdialysis section) were given galanin or aCSF 120 min before the systemic administration of 8-OH-DPAT (0.1 or 0.3 mg/kg s.c.). Rectal temperature of each animal was recorded in 20 min intervals using a digital thermometer.

In situ Hybridization

The injection cannulae were implanted and galanin or aCSF were injected as described in the Microdialysis section. After decapitation the brains were rapidly removed from the skull and frozen on dry ice. The tissue was kept at −70°C until sectioned in a cryostat. Coronal sections 10 μm thick were cut at the level of the mesencephalic raphe nuclei (Bregma −7.3 mm to −8.3 mm) and thaw mounted on Fisher Super Frost Plus Slides (Kebo Lab, Sweden). The tryptophanhydroxylase TrpH-1 riboprobe was prepared from a 521 bp from rat cDNA (position 1220–1741 on the cDNA) in bluescript KS^+ (Stratagene, La Jolla, CA). The plasmid was linearized with Apa I and transcribed with the T7 RNA polymerase for the antisense probe and the sense was generated with linearizing the plasmid with Sac I and the transcription was done with the T3 RNA polymerase. The cDNA was kindly provided by Dr. Michael Bader (MDC-Berlin, Germany). After labeling the probes with [^{35}S]-a-UTP (1000Ci/mmol, Amersham Buckinghamshire, UK), as described earlier (Bunnemann et al. 1992), the probe was purified on Nensorb columns (Dupont, DE) and the quality was checked on a 5% polyacrylamide/8M urea gel. The slides were brought to room temperature and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.0 for 15 min. After fixation the slides were washed for 10 min in PBS and rinsed twice in sterile water for 5 min. Deproteination was performed using 0.1 M HCl for 10 min. After two washing steps in PBS for 5 min, the slides were acetylated (to reduce the film background) in a mixture of 0.1 M triethanolamine (pH 8) and 0.25% acetic anhydride for 20 min. The sections were then washed twice in PBS for 5 min, dehydrated in graded ethanol and air-dried. The sections were prehybridized with a prehybridization buffer (50% deionized formamide, 50 mM Tris-HCl pH 7.5, 25 mM EDTA (pH 8.0), 20 mM NaCl, 0.25 mg/ml yeast tRNA, 2.5 × Denhard's solution (0.05% Ficoll, 0.05% polyvinylpyrrolidone, 0.05% bovine serum albumin) in a humidified chamber for 2 h at 37°C. After draining the prehybridization buffer off the slides, the sections were hybridized under coverslips for 16 h in 53°C with 100 μl of hybridization buffer (50% deionized formamide, 20 mM Tris-HCl pH 7.6, 1 mM EDTA pH 8.0, 0.3 M NaCl, 0.2 M dithiothreitol, 0.5 mg/ml yeast tRNA, 0.1 mg/ml polyadenylic acid, 1 × Denhardt's solution, 10% dextran sulfate) containing [^{35}S]-labeled antisense probe or sense probe for the control sections at concentrations of 10^6 cpm of the probe/100 μl. The coverslips were removed by immersing the slides in 1 × standard saline citrate (SSC) solution at 48°C for 15 min. Subsequently, the slides were washed twice in 50% formamide in SSC, twice in 1 × SSC for 20 min, treated with RNase A (10 μg/ml) for 45 min at 37°C and then washed in 1 × SSC, 0.5 × SSC and 0.25 × SSC at 48°C for 10 min. The last wash was done in 0.25 × SSC at room temperature. After the wash all sections were dehydrated in graded alcohol and air-dried. For signal detection, all sections were placed on β-max-Hyperfilm (Amersham). The exposure time on film was six weeks. The semiquantitative measurement of the TrpH mRNA levels in the film images was made using a computer-assisted image analysis system as described elsewhere (Zoli et al. 1990).

The intensity of labeling obtained with the [^{35}S] labeled antisense probe represented total labeling, whereas labeling with the sense probe was used as a measure of the non-specific labeling. The mean gray values were measured in the film images corresponding to the DRN (the sampling field was 0.09 mm^2) and the area outside the DRN, which served as a background value (Razani et al. 2000). The relative transmittance (T%) values were expressed as the ratios between the gray intensities in the DRN and the background. The optical density (OD) was then calculated using the formula OD = −log T%. The specific OD was calculated as the total OD minus non-specific OD.

Immunohistochemistry

Each rat was implanted with a guide cannula (Plastic One) for direct, intra-raphe injections of galanin following the surgery protocol described in the Microdialysis section. On the day of experiment, the rats were injected with aCSF or porcine galanin (1.5 nmol/0.5 μl) into the DRN. The rats were killed 20 or 60 min after the galanin injection or 20 min after the aCSF administration. The rats were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and killed by transcardial perfusion with 50 ml 0.9% NaCl (room temperature) followed by 150 ml of ice-cold fixative (4% paraformaldehyde (w/v) with 0.2% picric acid (w/v) in 0.1 M phosphate buffer, pH 6.9). The brains were dissected out and cut in 2 mm slices and immersed for 90 min in the fixative at +4°C. The brain slices were then placed in 10% sucrose in 0.1 M phosphate buffer with 0.01% sodium azide. The brain slices were rapidly frozen using CO_2, cut in 14 μm coronal serial sections (200 μm intervals) through the mesencephalon on a cryostat (Microm, Germany) and thaw-mounted on gelatin coated slides. The sections were rehydrated in 10 mM phosphate buffered saline (PBS) and incubated over night at +4°C with the rabbit anti-galanin antibody (IHC 7153, Peninsula Laboratories) diluted by 1:800. Then, the sections were rinsed in PBS and incubated with the streptavidine anti-rabbit antibody diluted 1:20 (Amersham) for 1 h at room temperature, rinsed in PBS again and, finally incubated with streptavidine-Texas Red diluted 1:100 (Amersham) at room temperature for 1 h. The sections were rinsed with PBS and coverslipped with Dako fluorescent mounting medium (Dako). Mi-

crophotographs were taken with Tri-X (Kodak) black and white film using a Nikon Microphot-FX microscope equipped with Hg-lamp epilumination and appropriate excitation filters. The negatives were scanned with a Polaroid Sprintscan 35/LE film scanner and the final figure was assembled in Adobe Photoshop (5.0) without further modifications and printed at 600 dpi using an Epson printer.

Peptide and Compounds

8-Hydroxy-2-(di-*n*-propyloamino)tetralin HBr (8-OH-DPAT), (RBI, Natick, MA), porcine galanin or M35 (Peninsula Lab. Europe Ltd, Merseyside, UK) were dissolved in artificial CSF (aCSF) (Härfstrand et al. 1986) containing 123.4 mM NaCl, 23.4 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2 \cdot 2H_2O$, 0.8 mM $MgCl_2 \cdot 6H_2O$, 0.5 mM Na_2SO_4, 5.8 mM glucose, pH adjusted to 7.1, and stored at the temperature of 0–8°C in aliquot vials until use (up to two days). Sodium pentobarbital (standard saline solution of 60 mg/ml) was obtained from Apoteksbolaget, Stockholm, Sweden.

5-hydroxytryptamine HCl (Serotonin) was purchased from Sigma (St. Louis, MO), all other chemicals were from Merck (Darmstadt, Germany) or from Fluka Chemie (Buchs, Switzerland).

Statistical Analysis

The data were examined using a repeated-measures 2-way analysis of variance (ANOVA). Fisher's protected least significant difference test (Fisher's PLSD-test) was used to analyze the statistical significance between the groups at different time points. A level of $p < .05$ was accepted as evidence for a statistically significant effect.

RESULTS

The Effect of Galanin on Extracellular 5-HT Levels Monitored by Microdialysis

Galanin administration into the lateral ventricle of the awake rats caused a dose-dependent and long-lasting reduction of basal extracellular 5-HT levels in the ventral hippocampus, as measured by in vivo microdialysis (Figure 1). The basal 5-HT levels in the hippocampus were 6.4 ± 0.3 fmol/20 μl (means ± SEM, n = 6 rats). The highest dose of galanin (1.5 nmol) reduced extracellular 5-HT by about 31% ($F_{3,14} = 20.064$; $p < .0001$) of the aCSF-injected rats at 120 min, whereas the lower dose of 0.5 nmol galanin reduced extracellular 5-HT levels by only 18% ($p < .01$; n = 4 rats). In both cases, the first significant decrease of 5-HT was observed 60 min following galanin injection. Galanin given at a dose of 0.15 nmol had no significant effect when compared with the aCSF injected rats. The long-lasting effect of galanin on hippocampal 5-HT release was confirmed in another series of microdialysis experiments where galanin (1.5 nmol i.c.v.) caused a similar 30% reduction of 5-HT levels, which persisted for at least 5 h, as shown in the inset of Figure 1.

To analyze whether the action of i.c.v. galanin could be mediated via 5-HT neurons in the DRN or their hippocampal terminals, galanin was infused into the DRN while measuring 5-HT release in the ventral hippocampus. In another group of rats, a combined injection/microdialysis probe was implanted into the ventral hippocampus, which allowed infusion of galanin in the vicinity of the microdialysis membrane. As shown in Figure 2, the intra-raphe injection of galanin (1.5 nmol) reduced hippocampal 5-HT levels as in the case of i.c.v. administered galanin, with a maximal reduction to 59.3 ± 1.2% (means ± SEM, n = 6 rats, $F_{1,8} = 477.38$; $p < .0001$) of the control levels at 120 min. In contrast, intrahippocampally infused galanin failed to alter basal 5-HT release (Figure 2, inset). The neuroanatomical verification of local galanin injections was done by immunohistochemical staining of galanin in the DRN, as shown in Figure 3. In sections from the aCSF injected rat (Figure 3, panel A, the injection site marked by an asterisk), only very weak endogenous galanin-immunoreactivity (GAL-IR) was found in the dorsal and ventral part of the DRN (Paxinos and Watson 1997). However, in rats injected with porcine galanin (1.5 nmol, given 20 min before the sacrifice) into the vicinity of DRN (indicated by an asterisk), the GAL-IR area covered the entire dorsal raphe nucleus and predominantly its ventral part (DRV) (Figure 3, panel B).

The Effect of Galanin on Expression of Tryptophanhydroxylase

The bilateral i.c.v. administration of galanin (2 × 1.5 nmol/rat) produced a significant decrease of tryptophanhydroxylase (TrpH) mRNA by 28% ($F_{1,12} = 7.403$; $p < .02$) in the DRN, two hours after galanin administration (Figure 4). Representative autoradiograms at the level of DRN show the decrease in the optical density of TrpH mRNA following i.c.v. galanin (Figure 5).

The Effect of Galanin Antagonist M35 on 5-HT Release

In order to demonstrate whether the effect of galanin on 5-HT neurotransmission is mediated through galanin receptors, the peptidergic galanin receptor antagonist M35 or aCSF were infused 120 min before the administration of galanin. The profiles of hippocampal 5-HT levels before and after M35 (1.5 nmol) or aCSF injection (time 0 min), followed by galanin (1.5 nmol) given in both groups at 120 min and monitored for additional 120 min are shown in Figure 6. As seen, the reducing ef-

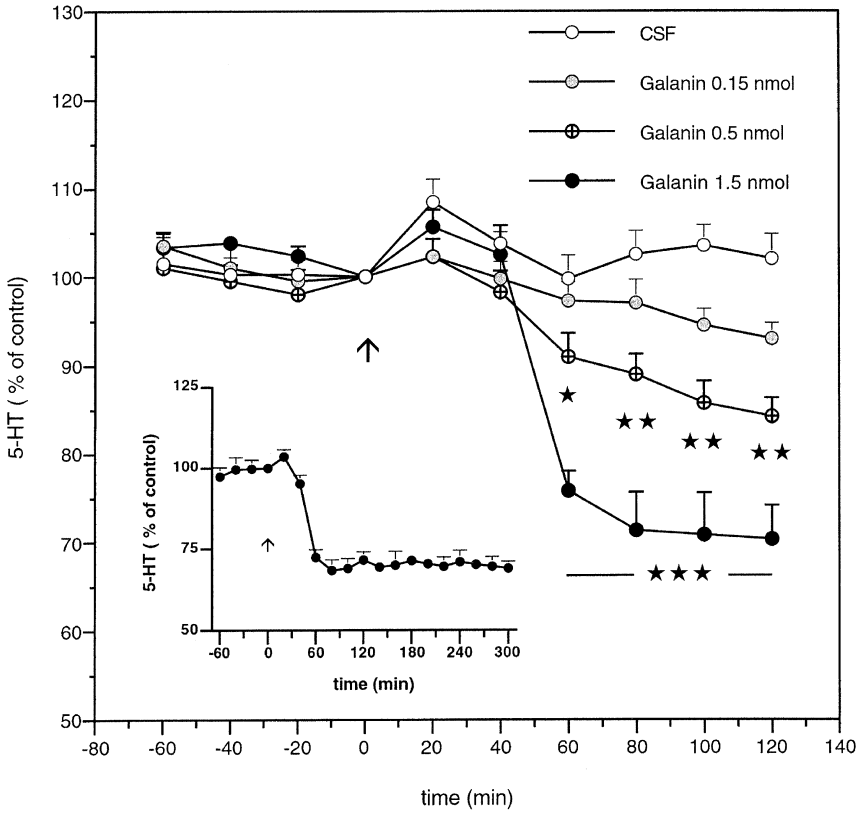

Figure 1. The dose-dependent effect of porcine galanin on basal 5-HT release in the ventral hippocampus of the awake rat. Galanin was dissolved in aCSF and injected i.c.v. at time 0 (↑). Data are expressed as means ± SEM, n (aCSF; 0.15; 0.5 nmol) = 4 rats, n (1.5 nmol) = 6 rats. ★★★ $p < .0001$, ★★ $p < .01$, ★ $p < .05$, compared with the aCSF group; ANOVA, Fisher's PLSD test. Inset: The long-lasting effect of porcine galanin on basal 5-HT release in the ventral hippocampus of the awake rat. Galanin (1.5 nmol) was dissolved in aCSF and injected i.c.v. at time 0 min. Data are expressed as means ± SEM, n = 4 rats.

fect of i.c.v. injected galanin on hippocampal 5-HT release was almost completely blocked by pre-administration of the chimeric peptide M35 (Bartfai et al. 1992). In the aCSF/galanin injected rats, galanin caused a significant reduction of extracellular 5-HT at 60–120 min after its administration (180–240 min in the graph), similar to the effect shown in Figure 1. Administration of M35 itself caused a slight but significant reduction of

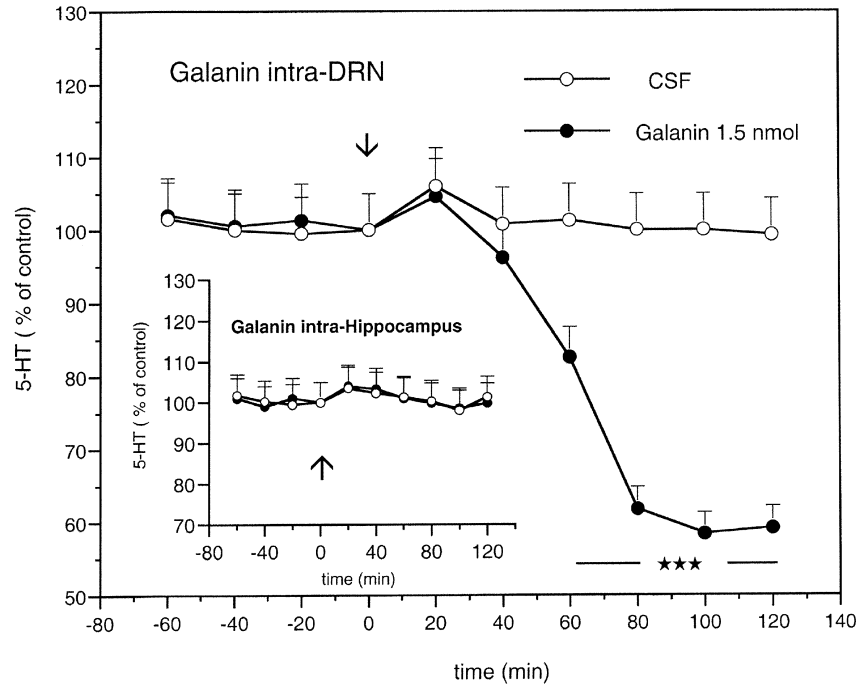

Figure 2. Modulation of basal 5-HT release in the ventral hippocampus by local injections of galanin (1.5 nmol) into the DRN or into the ventral hippocampus (Inset) in the vicinity of the microdialysis probe. The intra-raphe injection of galanin at time 0 (↑) caused the reduction of hippocampal 5-HT to 59.3 ± 1.2% (means ± SEM, n = 6 rats, $F_{1,8} = 477.38$; ★★★ $p < .0001$) of the control values at 120 min. Intra-hippocampally injected galanin at time 0 (↑) failed to alter basal hippocampal 5-HT levels.

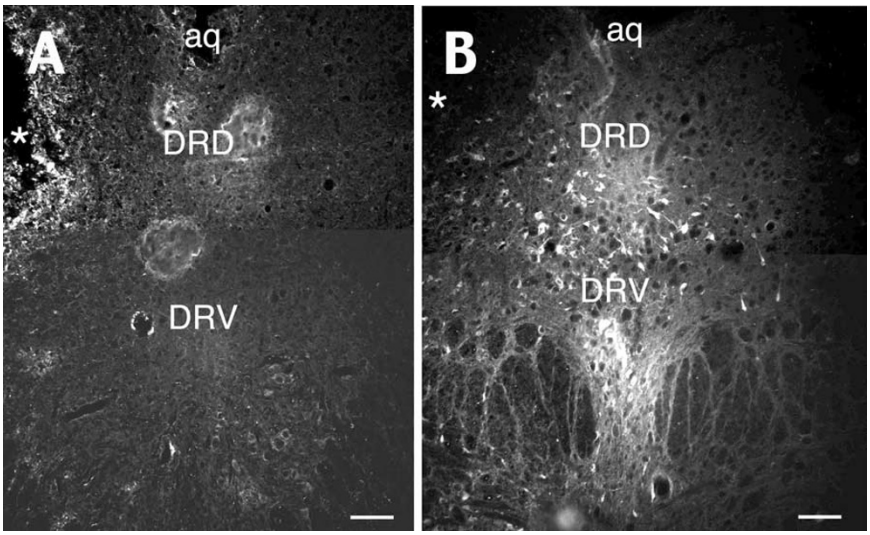

Figure 3. Neuroanatomical verification of the site and spread of galanin-immunoreactivity over the DRN induced by infusion of galanin in the vicinity of the DRN. A. In the aCSF injected rat (with the injection site marked by an asterisk), only weak endogenous galanin-immunoreactivity was observed in the dorsal and ventral part of the DRN. Note the anatomically preserved integrity of the nucleus. B. In rats injected with porcine galanin (1.5 nmol, given 20 min before the sacrifice) in the vicinity of DRN (indicated by an asterisk), galanin-positive staining of the cells was found only in the dorsal part (DRD) and the ventral part (DRV) of the dorsal raphe nucleus. aq = aqueduct, bar = 100 μm.

basal extracellular 5-HT at 120 min ($p < .05$). However, pretreatment of rats with M35 significantly attenuated the effect of galanin by about 25% ($F_{1,6} = 21.307$; $p < .005$) at the end of the sampling period (180–240 min). The basal 5-HT values at 240 min were 94 ± 8.3% of the pre-drug levels (means ± SEM, n = 4 rats).

Interactions between 5-HT$_{1A}$ and Galanin Receptors in the DRN

The interactions between galanin and 5-HT$_{1A}$ receptors were studied by use of both neurochemical (microdialysis) and behavioral (temperature, motor activity) methods.

Microdialysis Study. Galanin or aCSF (control group) were infused 120 min before the systemic administration of 8-OH-DPAT (0.3 mg/kg s.c.) in the control group (Figure 7). 8-OH-DPAT injection into the aCSF treated group significantly reduced hippocampal 5-HT release by about 55% of the basal 5-HT levels within 40 min ($F_{3,13} = 87.3$; $p < .0001$ versus aCSF group). The reduced 5-HT levels returned to the basal levels within 180 min after 8-OH-DPAT. Galanin at the dose of 0.15 nmol, which itself did not affect basal 5-HT release, significantly attenuated the reduction of hippocampal 5-HT levels caused by 8-OH-DPAT at 160 min ($p < .05$ versus aCSF/8-OH-DPAT group, n = 4). The higher dose of galanin (0.5 nmol) caused a moderate but significant ($p < .05$; and $p < .01$, n = 4) reduction of the basal 5-HT levels to about 84% of the aCSF injected group at 80–120 min. However, the systemic 8-OH-DPAT administration at 120 min in the 0.5 nmol galanin pretreated group failed to further reduce hippocampal 5-HT to the levels observed in the control, aCSF/8-OH-DPAT group during 140–180 min period ($p < .05$ and $p < .001$). The 5-HT levels in the galanin pretreated rats remained reduced at 75–82% of the initial 5-HT values during the entire post-galanin sampling period. At 280 and 300 min, the 5-HT levels were significantly lower ($p < .05$) than the recovered 5-HT concentrations in the aCSF/8-OH-DPAT treated group.

Hypothermia Study. A similar experimental protocol was used in order to test the effects of i.c.v. galanin on 8-OH-DPAT-induced hypothermia in the awake rat (Figure 8). Galanin given at a dose of 3 nmol (1.5 nmol/ventricle) or aCSF had no effect on the body temperature measured by the rectal probe. However, this dose of galanin attenuated the hypothermic effect induced by 8-OH-DPAT given at 0.1 mg/kg (Figure 8, panel A, $p < .05$ for 140–260 min interval) but not at the 0.3 mg/kg dose (Figure 8, panel B).

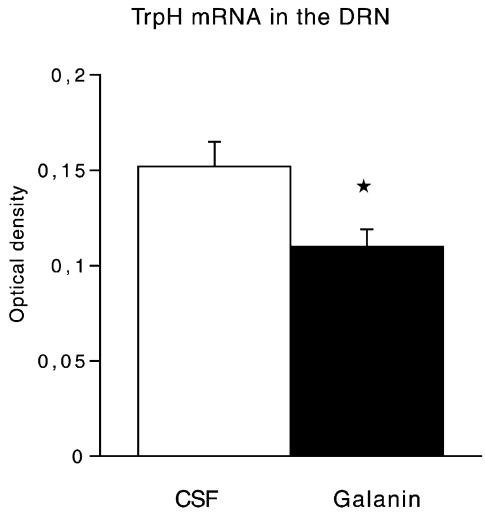

Figure 4. Effects of 3 nmol i.c.v. galanin on TrpH mRNA levels in the DRN 2 h after administration. The optical density values are shown as means ± SEM, n = 7. ★ $p < .02$ versus the aCSF group.

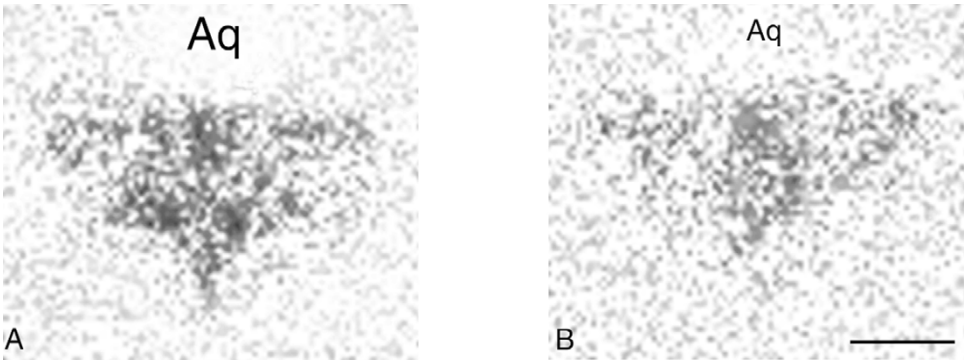

Figure 5. Representative autoradiograms of TrpH mRNA at the level of DRN showing the decrease in the optical density of TrpH mRNA two hours after i.c.v galanin administration (3 nmol/rat) (panel B) compared with the aCSF injected rat (panel A). Aq = aqueduct.

Motor Activity Study. The effects of galanin on spontaneous locomotor activity and that induced by systemic administration of 8-OH-DPAT were measured using a computerized multicage system. In non-habituated rats, the exploratory activity, quantified as the total number of rearings during the initial 20 min recording period, was significantly reduced by about 38% ($F_{1,12}$ = 7.403; $p < .02$) in the two groups pretreated with galanin (0.5 nmol and 3 nmol/rat) given bilateraly into the lateral ventricle 80 min before start of the data acquisition (Figure 9). In contrast, locomotion and motility were not significantly different form the aCSF injected animals. There were also no differences between the control and galanin-injected groups, in which the motor activity was measured 15 min after the drug/aCSF administration (data not shown). Systemic administration of 8-OH-DPAT at a dose of 0.3 mg/kg caused a powerful increase in locomotor activity consistent with previous results (Evenden and Angeby-Moller 1990). The bilateral i.c.v. injection of galanin at both doses (0.5 nmol/rat and 3 nmol/rat) 140 min before the test caused a robust and dose-dependent reduction of locomotion and motility produced by 8-OH-DPAT (0.3 mg/kg s.c.) as shown in Figure 10, panels A, B, C. For locomotion, the maximal effect of galanin was seen within the first 30 min after 8-OH-DPAT: 0.5 nmol galanin: $p < .0001$ (5, 10 min); $p < .005$ (15–50 min time points) and $p < .05$ (55–70 min intervals). Galanin at 3 nmol dose attenuated locomotion for up to 85 min following 8-OH-DPAT: $p < .0001$ (5–30 min, with maximal value $F_{2,21}$ = 44,487 at 5 min); $p < .005$ (35–70 min) and $p < .05$ (65–85 min). The motility measures showed a similar statistical differences between aCSF/8-OH-DPAT and galanin/8-OH-DPAT groups as shown in Figure 10, panel B, with maximal value $F_{2,21}$ = 23.778 at 5 min after 8-OH-DPAT. The rearing activity induced by 8-OH-DPAT was attenuated only at a higher dose of galanin within the first 40 min (maximal value $F_{2,21}$ = 9.374 at 10 min), whereas the lower dose did not differ from the effect of 8-OH-DPAT alone.

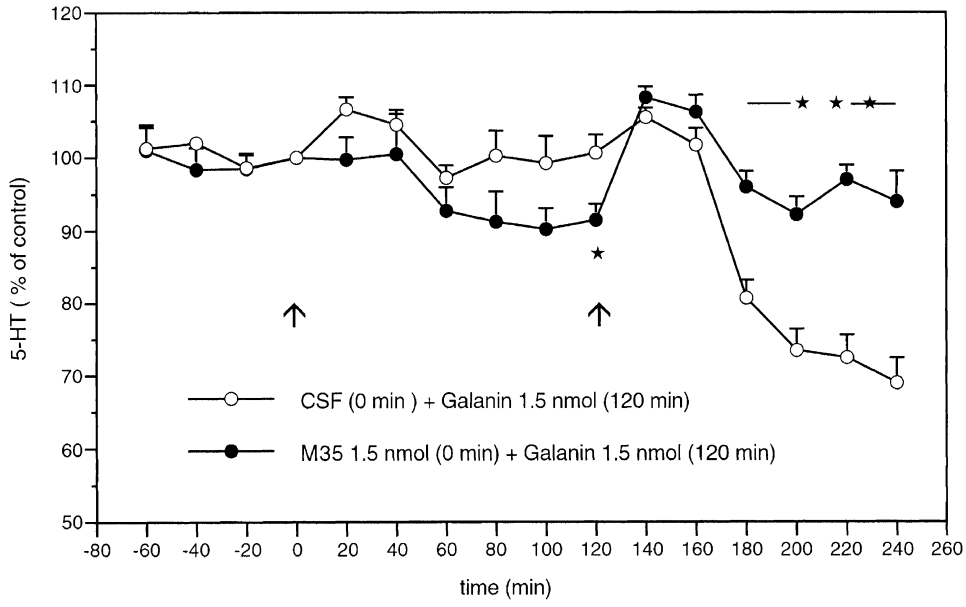

Figure 6. Pre-treatment with galanin antagonist M35 blocked the galanin-induced reduction of basal 5-HT in the ventral hippocampus. At 0 min (↑) a chimeric peptide M35 or aCSF were injected i.c.v, followed by injection of porcine galanin (1.5 nmol) 120 min later (second ↑). M35 itself caused a slight reduction (about 10%, ⋆ $p < .05$ at 120 min) of hippocampal 5-HT levels. However, M35 even 4 h after its administration blocked the reduction of extracellular 5-HT in the rat hippocampus caused by galanin injection. (Data expressed as means ± SEM, n = 4 rats, ⋆⋆⋆ $p < .005$, compared with the aCSF/galanin group).

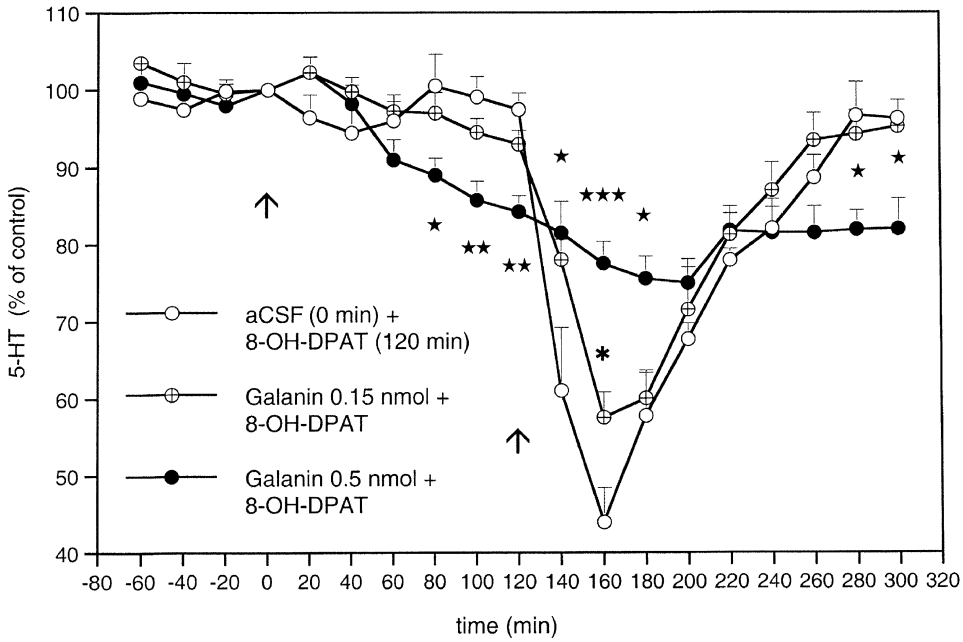

Figure 7. Attenuation, by galanin, of the 8-OH-DPAT-induced reduction of basal 5-HT in the ventral hippocampus of the awake rat. Galanin or aCSF were given i.c.v. at time 0 min (↑) and 8-OH-DPAT (0.3 mg/kg s.c.) was injected 120 min later (second ↑). The 8-OH-DPAT injection into the aCSF pretreated rats (n = 4) caused a rapid, but reversible reduction of hippocampal 5-HT levels within 40 min, i.e. at 160 min. Galanin, at a dose of 0.15 nmol, significantly attenuated the effect of 8-OH-DPAT only at its peak effect at 160 min (* $p < .05$ versus aCSF/8-OH-DPAT group, n = 4). Galanin, at the dose of 0.5 nmol, caused a moderate but significant ($\star$ $p < .05$; $\star\star$ $p < .01$, n = 4) reduction of basal 5-HT levels at 80–120 min, as compared with the aCSF injected group. However, systemic 8-OH-DPAT administration in the 0.5 nmol galanin pretreated group failed to reduce hippocampal 5-HT to the levels observed in the aCSF/8-OH-DPAT treated group ($\star$ $p < .05$; $\star\star\star$ $p < .001$). The 5-HT levels remained significantly reduced ($\star$ $p < .05$) at 82% of the control, aCSF/8-OH-DPAT group even 280–300 min after galanin injection.

DISCUSSION

The present studies have shown that galanin is a potent and long-lasting inhibitor of mesencephalic 5-HT neurotransmission in vivo. Galanin given i.c.v. caused a dose-related and long lasting (> 5 h) inhibition of 5-HT release in the ventral hippocampus (Figure 1). The observations that galanin administered directly into the ventral hippocampus (Figure 2, inset) did not affect 5-HT release strongly supports the view that the actions of galanin on 5-HT release is not exerted at the 5-HT nerve terminal level. Moreover, the injection of galanin into the vicinity of the dorsal raphe (Figure 2) decreased basal 5-HT release in the hippocampus.

After galanin infusion, the galanin peptide was visualized by immunohistochemistry in the DRN, as shown in Figure 3. In the control sections (Figure 3, panel A), there were generally low levels of GAL-IR, which is consistent with the low synthesis rate of galanin in normal rats (Hökfelt et al. 1998). However, a strong GAL-IR staining was observed in the entire DRN, 20 min after the injection of 1.5 nmol galanin (Figure 3, panel B), providing neuroanatomical support for the view that the raphe nucleus is the site of action of galanin on hippocampal 5-HT release, as shown in Figure 2. The infused galanin could act directly via activation of galanin receptors on 5-HT neurons, which was demonstrated at the ultrastructural level for galanin-positive axon terminals forming synapses with the 5-HT-immunoreactive dendrites in the DRN (Xu et al. 1998). However, some DRN neurons are surrounded by galanin-positive nerve endings, which do not express 5-HT (Xu and Hökfelt 1997; Xu et al. 1998), suggesting also a possibility for indirect interactions.

Three subtypes of galanin receptors have been cloned, GalR1 (Habert-Ortoli et al. 1994; Burgevin et al. 1995; Parker et al. 1995) GalR2 (Howard et al. 1997; Smith et al. 1997; Wang et al. 1997a) and GalR3 (Wang et al. 1997b; Smith et al. 1998), which all belong to the superfamily of G-protein-linked receptors. The autoradiographic receptor binding studies have indicated moderate galanin R1 and R2 receptor binding in the DRN (Branchek et al. 1997, 1998). However, almost no detectable expression of GalR1 receptors in the DRN was found by in situ hybridization technique (Xu et al. 1998); rather GalR1 receptors were expressed in the surrounding periacqueductal cen-

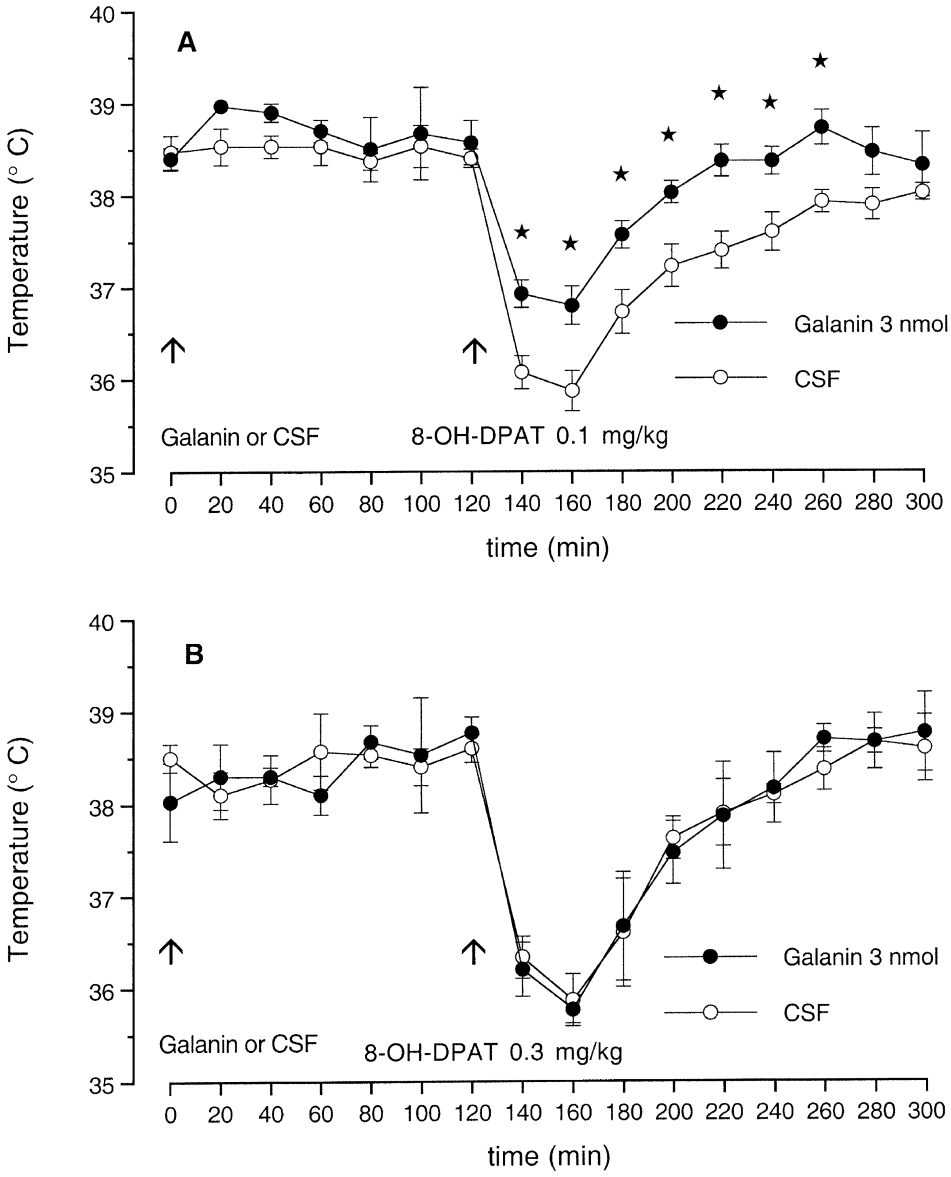

Figure 8. Galanin (3 nmol/rat i.c.v.) attenuated the 8-OH-DPAT induced hypothermia at the dose of 0.1 mg/kg s.c. (panel A) but not at 0.3 mg/kg s.c. (panel B) in awake rats. ★ $p < .05$, all data are expressed as means ± SEM, n = 4. ↑ denotes injection of the drug at 0 min and 120 min.

tral gray. Thus, it was hypothesized that GalR2 or GalR3 receptors may be responsible for galanin-mediated effects in the DRN. In this context it is important to emphasize that the modulatory effect of galanin on hippocampal 5-HT release is probably mediated by galanin receptors since the galanin receptor antagonist M35 attenuated the inhibitory effect of i.c.v. galanin on hippocampal 5-HT release (Figure 6).

M35 is a nonselective ligand for probably all three galanin receptor subtypes. Since, M35 has about the same affinity for both GalR1 (Ki = 0.3 nM) and GalR2 (Ki = 0.6 nM) receptor subtypes, it is not possible to link the blockade of the galanin effect to any specific galanin receptor subtype in the DRN. Administration of M35 itself caused a slight but significant reduction of basal extracellular 5-HT at 120 min, which is in agreement both with in vitro data in insulinoma cell lines (Kask et al. 1995) and in vivo data showing a partial agonistic mode of action of M35 (Antoniou et al. 1997). The temporal increase of the 5-HT levels at 20–40 min following galanin administration in the M35 pretreated group could be explained by an increased sensitivity to the acute stress caused during the insertion of the i.c.v. injection cannula. However, M35 even 4 h after its administration blocked the reduction of extracellular 5-HT in the rat hippocampus caused by the galanin injection.

The effects of galanin on TrpH, the rate-limiting enzyme in the biosynthesis of 5-HT (Lovenberg et al. 1967) further link the action of galanin to the DRN. The profound effect of i.c.v. galanin (3 nmol/rat) on gene expression of TrpH (Figure 4 and Figure 5), indicates that galanin also inhibits the synthesis of 5-HT. It is

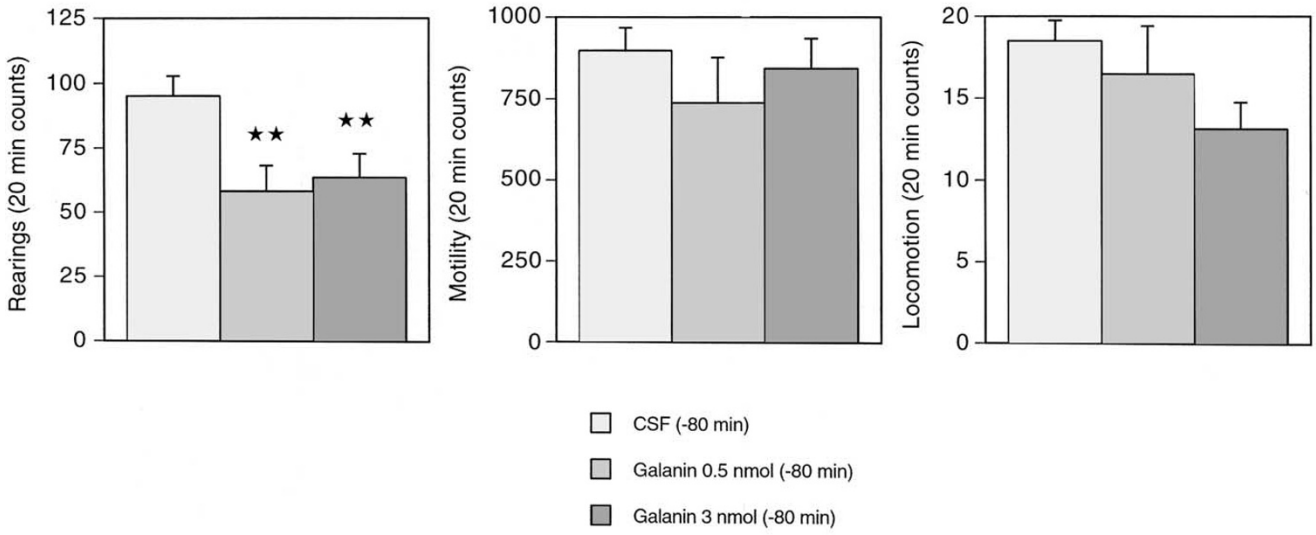

Figure 9. The effects of galanin on spontaneous locomotor activity in non-habituated rats. The total number of rearings during the initial 20 min was significantly reduced by about 40% ($F_{2,57} = 5.498$; ★★ $p < .01$) in two groups pretreated with galanin (0.5 nmol, n = 16 and 3 nmol/rat, n = 22) compared with the aCSF (n = 22) injected rats. The drugs were given bilaterally into the lateral ventricle 80 min before start of the activity recording. Locomotion and motility were not significantly different form the aCSF injected animals.

likely that the inhibition of TrpH enzyme activity by galanin is indirectly mediated by the inhibition of the raphe cell firing similarly to 5-HT_{1A} receptor agonists (Invernizzi et al. 1991).

The mechanisms which contribute to the inhibitory action of galanin on raphe neurons are not known at the present but are consistent with the electrophysiological studies in slice preparations showing that galanin can induce hyperpolarization of 5-HT sensitive neurons in the DRN (Xu et al. 1998). In those studies, the inhibitory effect of galanin was seen at high concentrations (10^{-6}–10^{-7} M), while at lower concentrations (10^{-9} M) galanin was found to enhance and prolong the 5-HT induced outward current (Xu et al. 1998). However, the present results based on in vivo microdialysis showing long-lasting effects of galanin and M35 on 5-HT release and on TrpH expression are not easily reconciled with the electrophysiological results. First, the strong inhibition found at very low doses of galanin in the present study do not correlate with the high concentrations needed in vitro. Second, the long duration of the galanin effect in vivo (see below) does not follow the time-course for galanin-induced tachyphylaxis seen in vitro with a rather fast normalization of desensitized raphe neurons within 20 min (Xu et al. 1998).

The long lasting effects of galanin i.c.v. on 5-HT neurotransmission are difficult to explain because of the fact that elimination of the infused peptide in the brain tissue appears to be rapid, in the range of 20 min (Schött et al. 1998). On the other hand, recent data based on i.c.v. administration of galanin indicate that the disappearance of infused galanin may be considerably slower, in the range of 30–60 min (Jansson et al. 2000). Galanin fragments, e.g. galanin(1–9) was shown to prolong the half-life of galanin from 140 min to 255 min during the incubation with membranes from the rat ventral hippocampus (Girotti et al. 1993). In addition, several behavioral studies have shown relatively long-lasting physiological effects of i.c.v. administered galanin. Thus, galanin was found to induce c-fos expression in the nucleus of the solitarii tract and the reticular nucleus up to six hours after intra-cisternal injection (Diaz et al. 1997). Galanin has also been reported to produce long-lasting inhibitory effects on food intake (Ervin et al. 1997). It is possible that the prolonged action of galanin can reflect its internalization in the target cells and/or a formation of galanin fragments which have prolonged action in vivo (Schött et al. 1998; Jansson et al. 2000).

The major efferents from the dorsal raphe nucleus project to the striatum and frontal cortex, whereas the hippocampal formation receives serotonergic input from both the dorsal and medial raphe nuclei (Jacobs et al. 1974; Azmitia and Segal 1978). The inhibition of basal 5-HT release in the ventral hippocampus induced by systemic 8-OH-DPAT is most likely a consequence of activation of somatodendritic 5-HT_{1A} autoreceptors in the 5-HT neurons of the raphe nuclei. Several electrophysiological and microdialysis studies have shown that iontophoretic application of 5-HT_{1A} receptor agonist such as 8-OH-DPAT, as well as systemic administration, inhibit neuronal activity in the raphe neurons, reducing 5-HT release and synthesis in forebrain termi-

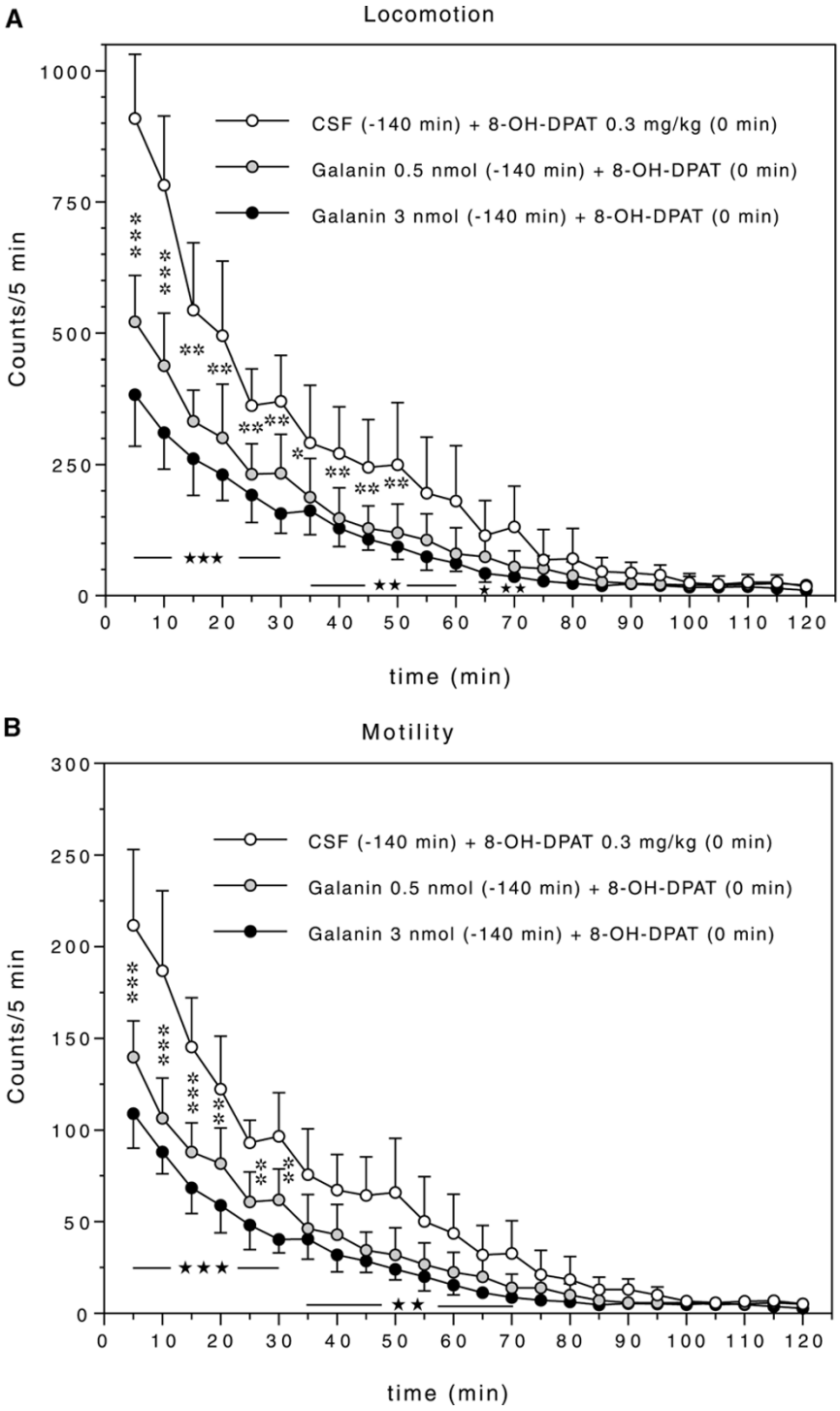

Figure 10. The effect of galanin on motor activity induced by 8-OH-DPAT (0.3 mg/kg s.c.) in habituated rats. Galanin at doses 0.5 nmol or 3 nmol, or aCSF, were given i.c.v. 140 min before 8-OH-DPAT injection (time 0 min). All data are expressed as means ± SEM, n = 8. A. Locomotion: galanin 3 nmol: ★★★ $p < .0001$, ★★ $p < .005$; ★ $p < .05$, also for 75, 80 and 85 min; galanin 0.5 nmol *** $p < .0001$, ** $p < .005$; * $p < .05$ and also for 55, 60 and 70 min. B. Motility: galanin 3 nmol: ★★★ $p < .0001$, ★★ $p < .005$; and also $p < .01$ for 75–85 min; galanin 0.5 nmol *** $p < .0001$, ** $p < .005$; and also $p < .05$ for 35–60 and 70 min intervals. C. Rearing: ★★ $p < .01$; ★ $p < .05$. Galanin/8-OH-DPAT groups were compared with the aCSF/8-OH-DPAT control group.

nal areas (Sprouse and Aghajanian 1986; Blier and de Montigny 1987; Sharp et al. 1989; Kreiss and Lucki 1994). The observation that the combination of galanin and 8-OH-DPAT did not result in an additive effect on 5-HT release is paradoxical (see Figure 7). Instead, at doses which by themselves had moderate or no effect on the basal 5-HT release, galanin blocked the inhibitory action of 8-OH-DPAT, providing the first neurochemical evidence for an important in vivo interaction between galanin and 5-HT_{1A} receptors in the dorsal raphe.

Recently, several behavioral and physiological studies have shown that galanin can counteract the effects induced by 5-HT_{1A} receptors stimulation. Thus, galanin given 10 min and 2 h before 8-OH-DPAT administration could block the deficit of passive avoidance reten-

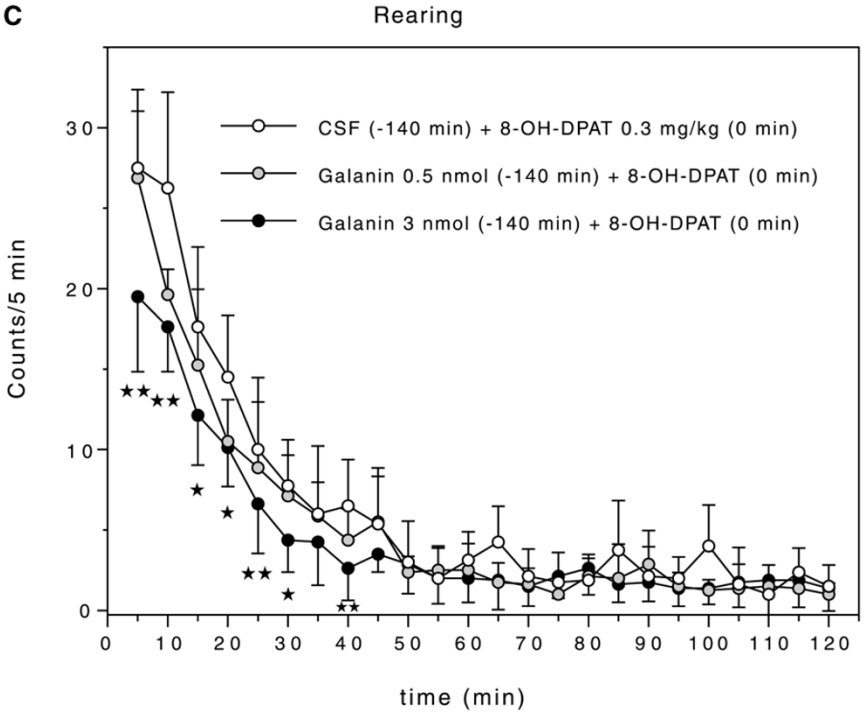

Figure 10. Continued.

tion induced by a 5-HT_{1A} receptor agonist 8-OH-DPAT in the rat (Misane et al. 1998; Razani et al. 2001). This deficit of passive avoidance is mainly related to stimulation of postsynaptic 5-HT_{1A} receptors in the limbic forebrain (Carli et al. 1993). Galanin given i.c.v. was also found to attenuate the hypothermic effect of 8-OH-DPAT in mice (Patel and Hutson 1996). This finding was confirmed in the present experiments showing that galanin, which does not alter body temperature, could exert a blocking effect of 8-OH-DPAT-induced hypothermia at the dose of 0.1 mg/kg 8-OH-DPAT s.c., as shown in Figure 8. The hypothermic effect in mice appears to be exerted at the presynaptic level (Patel and Hutson 1996), whereas in the rat, 8-OH-DPAT most likely induces the hypothermic effect via stimulation of postsynaptic receptors, probably in the hypothalamus (Millan et al. 1993).

Galanin given i.c.v. 80 min prior to the measurements showed modest effects on locomotor activity in non-habituated rats. However, galanin caused a significant reduction of number of rearings within the first 20 min in the novel environment indicating that the peptide has subtle effects on mechanisms of exploration (Figure 9). In the locomotor study with 8-OH-DPAT and galanin, the experiments were performed in habituated rats. Thus, 8-OH-DPAT has been shown to produce a more consistent hyperlocomotion (forward locomotion) in habituated compared with non-habituated rats (Evenden and Angeby-Moller 1990). 8-OH-DPAT at the 0.3 mg/kg dose was found to cause a marked increase in the three parameters of locomotor activity. However, the increase in locomotor activity induced by 8-OH-DPAT, which is mediated by activation of postsynaptic 5-HT_{1A} receptors (Tricklebank et al. 1984), was found to be significantly and dose-dependently blocked by galanin given i.c.v. (Figure 10, panels A, B, C). These findings further support the view that in vivo administration of galanin can block 5-HT_{1A} receptor mediated responses involving different functional pathways.

Taken together, the present data based on neurochemical, molecular, immunohistochemical, and behavioral analysis provide, for the first time, a direct evidence for a strong inhibitory effect of galanin on 5-HT neurotransmission in vivo. These results also emphasize the importance of the in vivo effects of galanin on central 5-HT_{1A} receptor function, both at the presynaptic and postsynaptic levels. The actions of galanin are long lasting and mediated via galanin receptors. The effects of galanin on gene expression of TrpH and 5-HT_{1A} receptor function give support for an inhibitory role of galanin on intracellular signaling in the 5-HT neurons of the DRN. In view of these findings, one can hypothesize that galanin antagonists may represent a new therapeutic principle in treatment of depression. In depressive disorders, galanin may exert a profound inhibition of dorsal raphe neuronal activity, partly mediated via galanin/5-HT_{1A} receptor interactions, which may result in a dysfunction of 5-HT neurotransmission in the cortico-limbic areas of the brain associated with depression.

REFERENCES

Antoniou K, Kehr J, Snitt K, Ögren SO (1997): Differential effects of the neuropeptide galanin on striatal acetylcholine release in anaesthetized and awake rats. Br J Pharmacol 121:1180–1186

Azmitia EC, Segal M (1978): An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641–667

Bartfai T, Fisone G, Langel Ü (1992): Galanin and galanin antagonists: molecular and biochemical perspectives. Trends Pharm Sci 13:312–317

Blier P, de Montigny C (1987): Modification of 5-HT neuron properties by sustained administration of the 5–HT1A agonist gepirone: electrophysiological studies in the rat brain. Synapse 1:470–480

Branchek TA, Gustafson EL, Walker MW, Forray C, Smith KE, Gerald C, Durkin MM (1997): Autoradiographic localization of the GALR2 receptor subtype in the rat CNS. Soc Neurosci Abstr 23:965

Branchek TA, Smith KE, Gerald C, Walker MW (2000): Galanin receptor subtypes. Trends Pharm Sci 21:109–116

Branchek T, Smith KE, Walker MW (1998): Molecular biology and pharmacology of galanin receptors. Ann N Y Acad Sci 863:94–107

Bunnemann B, Fuxe K, Metzger R, Bjelke B, Ganten D (1992): The semi-quantitative distribution and cellular localization of angiotensinogen mRNA in the rat brain. J Chem Neuroanat 5:245–262

Burgevin MC, Loquet 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

Carli M, Tatarczynska E, Cervo L, Samanin R (1993): Stimulation of hippocampal 5–HT1A receptors causes amnesia and anxiolytic-like but not antidepressant-like effects in the rat. Eur J Pharmacol 234:215–221

Crawley JN (1996): Minireview: Galanin-acetylcholine interactions: Relevance to memory and Alzheimer's disease. Life Sci 58:2186–2199

Crawley JN (1999): The role of galanin in feeding behavior. Neuropeptides 33:369–375

Dahlström A, Fuxe K (1964): Evidence for the existence of monoamine neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol Scand 62:1–55

Diaz Z, Marcos P, Cordon MP, Covenas R, Aguirre JA, Hedlund PB, Fuxe K (1997): C-fos expression induced by galanin in brainstem nuclei of the rat. Soc Neurosci Abstr 23:966

Ervin GN, Dun AP, Talbot CA, Bramer SA, Schofield A, Branchek TA, Noble SA, Heydorn WE (1997): Unique features of feeding induced by intraventricular galanin 1–29 (GAL): in the rat. Soc Neurosci Abstr 23:1074

Evenden JL, Angeby-Moller K (1990): Effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT): on locomotor activity and rearing of mice and rats. Psychopharmacology (Berl) 102:485–491

Fuxe K, Agnati LF, von Euler G, Lundgren K, Zoli M, Bjelke B, Eneroth P, Ögren SO (1990): Galanin/5-HT receptor interactions. A new integrative mechanism in the control of 5-HT neurotransmission in the CNS. In Paoletti R, Vanhoutte PM (eds), Serotonin: From Cell Biology to Pharmacology and Therapeutics. Dordrecht, Kleewer Academic Publ, pp 169–185

Fuxe K, Jansson A, Diaz-Cabiale Z, Andersson A, Tinner B, Finnman U-B, Misane I, Razani H, Wang F-H, Agnati LF, Ögren SO (1998): Galanin modulates 5-hydroxytryptamine functions. Focus on galanin and galanin fragment/5-hydroxytryptamine1A receptor interactions in the brain. Ann N Y Acad Sci 863:274–290

Fuxe K, Ögren SO, Jansson A, Cintra A, Härfstrand A, Agnati LF (1988a): Intraventricular injections of galanin reduces 5-HT metabolism in the ventral limbic cortex, the hippocampal formation and the fronto-parietal cortex of the male rat. Acta Physiol Scand 133:579–581

Fuxe K, von Euler G, Agnati LF, Ögren SO (1988b): Galanin selectively modulates 5-hydroxytryptamine 1A receptors in the rat ventral limbic cortex. Neurosci Lett 85: 163–167

Girotti P, Bertorelli R, Fisone G, Land T, Langel Ü, Consolo S, Bartfai T (1993): N-terminal galanin fragments inhibit the hippocampal release of acetylcholine in vivo. Brain Res 612:258–262

Habert-Ortoli E, Amiranoff B, Loquet I, Laburthe M, Mayaux JF (1994): Molecular cloning of a functional human galanin receptor. Proc Natl Acad Sci USA 91:9780–9783

Härfstrand A, Fuxe K, Agnati LF, Eneroth P, Zini I, Zoli M, Andersson K, von Euler G, Terenius L, Mutt V, Goldstein M (1986): Studies on neuropeptide Y-catecholamine interaction in the hypothalamus and in the forebrain of the male rat. Relationship to neuroendocrine function. Neurochem Int 8:355–376

Hedlund P, Fuxe K (1996): Galanin and 5–HT1A receptor interactions as an integrative mechanism in 5-HT neurotransmission in the brain. Ann N Y Acad Sci 780:193–212

Hökfelt T, Xu ZQ, Shi TJ, Holmberg K, Zhang X (1998): Galanin in ascending systems. Focus on coexistence with 5-hydroxytryptamine and noradrenaline. Ann N Y Acad Sci 863:252–263

Howard AD, Tan C, Shiao LL, Palyha OC, McKee KK, Weinberg DH, Feighner SD, Cascieri MA, Smith RG, Van Der Ploeg LH, Sullivan KA (1997): Molecular cloning and characterization of a new receptor for galanin. FEBS Lett 405:285–290

Invernizzi R, Carli M, Di Clemente A, Samanin R (1991): Administration of 8-hydroxy-2-(di-n-propylamino)tetralin in raphe nuclei dorsalis and medianus reduces serotonin synthesis in the rat brain: differences in potency and regional sensitivity. J Neurochem 56:243–247

Ishida J, Yoshitake T, Fujino K, Kawano K, Kehr J, Yamaguchi M (1998): Serotonin monitoring in microdialysate from rat brain by microbore-HPLC with fluorescence detection. Anal Chim Acta 365:227–232

Jacobs BL, Wise WD, Taylor KM (1974): Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. Brain Res 79:353–361

Jansson A, Tinner B, Andbjer B, Razani H, Wang F-H, Schött PA, Agnati LF, Ögren SO, Fuxe K (2000): Internalization of intracerebrally administered porcine galanin (1–29):

by a discrete nerve cell population in the hippocampus of the rat. Exp Neurol 161:153–166

Kask K, Berthold M, Bourne J, Andell S, Langel Ü, Bartfai T (1995): Binding and agonist/antagonist actions of M35, galanin(1–13)-bradykinin(2–9)amide chimeric peptide, in Rin m 5F insulinoma cells. Regul Pept 59:341–348

Kreiss DS, Lucki I (1994): Differential regulation of serotonin (5-HT) release in the striatum and hippocampus by 5–HT1A autoreceptors of the dorsal and median raphe nuclei. J Pharmacol Exp Ther 269:1268–1279

Lovenberg W, Jequier E, Sjoerdsma A (1967): Tryptophan hydroxylases: measurement in pineal gland, brain stem, and carcinoid tumor. Science 155:217–219

Mazarati A, Langel Ü, Bartfai T (2001): Galanin: An endogenous anticonvulsant? The Neuroscientist 7:506–517

Melander T, Hökfelt T, Rökaeus Å (1986): Distribution of galanin-like immunoreactivity in the rat central nervous system. J Comp Neurol 248:475–517

Millan MJ, Rivet JM, Canton H, Le Marouille-Girardon S, Gobert A (1993): Induction of hypothermia as a model of 5-hydroxytryptamine1A receptor-mediated activity in the rat: A pharmacological characterization of the actions of novel agonists and antagonists. J Pharmacol Exp Ther 264:1364–1376

Misane I, Razani H, Wang F-H, Jansson A, Fuxe K, Ögren SO (1998): Intraventricular galanin modulates a 5–HT1A receptor-mediated behavioural response in the rat. Eur J Neurosci 10:1230–1240

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

Ögren SO, Köhler C, Fuxe K, Ängeby K (1979): Behavioural effects of ergot drugs. In Fuxe K, Calne DB (eds), Dopaminergic Ergot Derivates and Motor Function. Oxford, Pergamon, pp 225–266

Ögren SO, Schött PA, Kehr J, Misane I, Razani H (1999): Galanin and learning. Brain Res 848:174–182

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. Mol Brain Res 34:179–189

Patel S, Hutson PH (1996): Effects of galanin on 8-OH-DPAT induced decrease in body temperature and brain 5-hydroxytryptamine metabolism in the mouse. Eur J Pharmacol 317:197–204

Paxinos G, Watson C (1997): The Rat Brain in Stereotaxic Coordinates. Australia, Academic Press

Pazos A, Palacios JM (1985): Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res 346:205–230

Priestley JV, Wotherspoon G, Savery D, Averill S, Rattray M (1993): A combined in situ hybridization and immunofluorescence procedure allowing visualisation of peptide mRNA and serotonin in single sections. J Neurosci Meth 48:99–110

Razani H, Diaz-Cabiale Z, Fuxe K, Ögren SO (2000): Intraventricular galanin produces a time-dependent modulation of 5–HT1A receptors in the dorsal raphe of the rat. Neuroreport 11:3943–3948

Razani H, Diaz-Cabiale Z, Misane I, Wang FH, Fuxe K, Ögren SO (2001): Prolonged effects of intraventricular galanin on a 5-hydroxytryptamine1A receptor mediated function in the rat. Neurosci Lett 299:145–149

Rökaeus Å (1987): Galanin: a newly isolated biologically active neuropeptide. Trends Neurosci 10:158–164

Schött PA, Bjelke B, Ögren SO (1998): Distribution and kinetics of galanin infused into the ventral hippocampus of the rat: relationship to spatial learning. Neuroscience 83:123–136

Sharp T, Bramwell SR, Hjorth S, Grahame-Smith DG (1989): Pharmacological characterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release in vivo as measured by microdialysis. Br J Pharmacol 98:989–997

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

Skofitsch G, Jacobowitz DM (1986): Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system. Peptides 7:609–613

Skofitsch G, Sills MA, Jacobowitz DM (1986): Autoradiographic distribution of 125I-galanin binding sites in the rat central nervous system. Peptides 7:1029–1042

Smith KE, Forray C, Walker MW, Jones KA, Tamm JA, Bard J, Branchek TA, Linemeyer DL, Gerald C (1997): Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. J Biol Chem 272: 24612–24616

Smith KE, Walker MW, Artymyshyn R, Bard J, Borowsky B, Tamm JA, Yao WJ, Vaysse PJ, Branchek TA, Gerald C, Jones KA (1998): Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. J Biol Chem 273: 23321–23326

Sprouse JS, Aghajanian GK (1986): (-)-Propranolol blocks the inhibition of serotoninergic dorsal raphe cell firing by 5–HT1A selective agonists. Eur J Pharmacol 128:295–298

Tatemoto K, Rökaeus Å, Jörnvall H, McDonald TJ, Mutt V (1983): Galanin - a novel biologically active peptide from porcine intestine. FEBS Lett 164:124–128

Tricklebank MD, Forler C, Fozard JR (1984): The involvement of subtypes of the 5–HT1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. Eur J Pharmacol 106:271–282

Wang S, Hashemi T, He C, Strader C, Bayne M (1997a): Molecular cloning and pharmacological characterization of a new galanin receptor subtype. Mol Pharmacol 52:337–343

Wang S, He C, Hashemi T, Bayne M (1997b): Cloning and expressional characterization of a novel galanin receptor. Identification of different pharmacophores within galanin for the three galanin receptor subtypes. J Biol Chem 272:31949–31952

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

Wrenn CC, Crawley JN (2001): Pharmacological evidence

supporting the role of galanin in cognition and affect. Prog Neuropsychopharmacol Biol Psychiatry 25:283–299

Xu Z-QD, Zhang X, Pieribone VA, Grillner S, Hökfelt T (1998): Galanin—5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. Neuroscience 87: 79–94

Xu ZQ, Hökfelt T (1997): Expression of galanin and nitric oxide synthase in subpopulations of serotonin neurons of the rat dorsal raphe nucleus. J Chem Neuroanat 13: 169–187

Zoli M, Zini I, Agnati LF, Guidolin D, Ferraguti F, Fuxe K (1990): Aspects of neural plasticity in the central nervous system-I. Computer-assisted image analysis methods. Neurochem Int 16:383–418