British Journal of Pharmacology (1995) 116, 2076-2080

New high affinity peptide antagonists to the spinal galanin receptor

Xiao-Jun Xu, [1]Zsuzsanna Wiesenfeld-Hallin, *Ülo Langel, *Katarina Bedecs & *Tamás Bartfai

Department of Laboratory Medical Science and Technology, Section of Clinical Neurophysiology, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden and *Department of Neurochemistry and Neurotoxicology, Stockholm University, Stockholm, Sweden

1 The role of endogenous galanin in somatosensory processing has been studied with galanin receptor antagonists. The new galanin receptor ligands C7, M32, M38 and M40 bind with high affinity (K_d in nanomolar range) to spinal cord galanin receptors and possess oxidative stability as compared to earlier generations of peptide ligands. These peptides have been examined in the spinal flexor reflex model where exogenous galanin exhibited biphasic excitatory and inhibitory effects.

2 Intrathecal administration of C7 [galanin(1-13)-spantide] and M32 [galanin (1-13)-neuropeptide Y(25-36) amide] blocked facilitation of the nociceptive flexor reflex induced by 30 pmol intrathecal galanin in decerebrate, spinalized rats in a dose-dependent manner, thus behaving as antagonists of the galanin receptor. In contrast, M38 [galanin(1-13)-(Ala-Leu)$_3$-Ala amide] and M40 [galanin(1-13)-Pro-Pro-(Ala-Leu)$_2$-Ala amide], exhibited only weak antagonism at high doses in this model. Moreover, lower doses of M40 potentiated galanin-induced reflex facilitation. C7 was neurotoxic at high doses in the rat spinal cord.

3 M32 and C7 were potent antagonists of galanin receptors in rat spinal cord, in correlation with their *in vitro* binding characteristics. In contrast, M38 and M40, despite their high *in vitro* affinity, exhibited only very weak antagonism. Moreover, M40 may also behave as a partial agonist.

4 Previous studies have shown that the galanin receptor may be heterogeneous. The discrepancy between *in vitro* binding and *in vivo* antagonistic potency of M38 and M40 may also suggest the presence of different galanin receptor subtypes within the rat spinal cord. However, other explanations for the discrepancy, such as differences in metabolic stability, diffusion rates and penetration to the site of action are also possible.

Keywords: Flexor reflex; galanin; galanin receptor; antagonist; pain; spinal cord

Introduction

Since its discovery in 1983 (Tatemoto *et al.*, 1983), galanin has received much attention as a potent neuropeptide with widespread distribution in the endocrine, peripheral and central nervous systems (Ch'ng *et al.*, 1985; Melander *et al.*, 1986; Rossowski *et al.*, 1990; Bartfai *et al.*, 1992; 1993a). Numerous properties and functions of galanin have been identified by use of exogenously applied synthetic peptides (Bartfai *et al.*, 1992; 1993a). In the somatosensory system, galanin occurs in a relatively small population of dorsal root ganglion cells, primarily with small somata (Ch'ng *et al.*, 1985; Skofitsch & Jacobowitz, 1985). Previous studies have indicated that galanin has a complex, biphasic effect upon nociceptive transmission at spinal level (Wiesenfeld-Hallin *et al.*, 1992a).

The synthesis of the first generation of galanin receptor antagonists was based on the finding that the biological activity of the full length peptide, galanin(1-29), resides in the N-terminus (Fisone *et al.*, 1989; Crawley *et al.*, 1990; Xu *et al.*, 1990) and that its N-terminal, as well as C-terminal, parts from two α-helices separated by Pro[13] (Rigler *et al.*, 1991). These compounds were chimeric, bioreceptor recognizing peptides with galanin(1-13) as the N-terminal fragment and the carboxy-terminus of some other bioactive peptides whose activity was known to reside in the C-terminus (Bartfai *et al.*, 1991; 1992; 1993b; Langel *et al.*, 1992). Two of these chimeric peptides, M15 [galanin(1-13)-substance P(5-11) amide] and M35 [galanin(1-13)-bradykinin(2-9) amide], have been extensively tested in the spinal flexor reflex model (Bartfai *et al.*, 1991; Wiesenfeld-Hallin *et al.*, 1992b) and both peptides dose-dependently antagonized intrathecal (i.t.) galanin-induced facilitation of the flexor reflex. Moreover, M15 and M35 were also able to reverse galanin-induced antagonism of reflex hyperexcitability following conditioning stimulation (CS) of unmyelinated afferents. M35, by blocking the effect of endogenous galanin, potentiated C-fibre CS-induced flexor reflex facilitation (Wiesenfeld-Hallin *et al.*, 1992b). Both M15 and M35 have been shown to be high-affinity antagonists (K_D=0.1 nM) at the spinal galanin receptor (Bartfai *et al.*, 1991; Wiesenfeld-Hallin *et al.*, 1992b), with Hill coefficients of unity, indicating a single class of recognition sites.

To evaluate further the structural requirements for high-affinity binding and functional agonism/antagonism we have studied the displacement of [^{125}I]-galanin from spinal galanin receptors and the effects on the spinal flexor reflex of four additional chimeric peptides/galanin analogues. The peptides studied here were: M32, consisting of galanin(1-13)-NPY(25-36) amide; C7, galanin(1-13)-spantide; M40, galanin(1-13)-Pro-Pro-(Ala-Leu)$_2$-Ala amide and M38, galanin(1-13)-(Ala-Leu)$_3$-Ala amide. The peptides with non-sense carboxy terminals, M38 and M40, have been designed in order to avoid possible bi-receptor recognition and activation, as well as to stabilize the interaction of the ligand with the galanin receptor.

Methods

Protocol of the electrophysiological study

Twenty female Sprague-Dawley rats (200-250 g, ALAB, Sweden) were used. The animals were initially briefly anaesthetized with methohexital (Brietal, Lilly, Indianapolis, USA, 70 mg kg^{-1}, i.p.), ventilated and decerebrated by aspiration of

[1] Author for correspondence.

the forebrain and midbrain. The spinal cord was exposed by a laminectomy at mid-thoracic level and sectioned at Th8-9. An i.t. catheter (PE 10) was implanted caudally to the transection with its tip on the lumbar spinal cord (L4–5). The flexor reflex was elicited by supramaximal electric shocks to the sural nerve or its innervation area in the left foot (0.5 ms, 10 mA, 1 min^{-1}) that activated A- and C-afferents. In some experiments, a train of CS (0.9 Hz, 20 stimuli) of the same strength as the test stimulus was administered to facilitate briefly the magnitude of the reflex (Wall & Woolf, 1984).

The flexor reflex was recorded as EMG activity via stainless steel needle electrodes inserted into the ipsilateral posterior biceps femoris/semitendinosus muscles. The number of action potentials elicited during the reflex was integrated over 2 s and recorded on a chart recorder. During the experiments the heart rate and rectal temperature of the rat were monitored.

Synthetic peptides

The structure of the chimeric peptides used in the present study is summarized in Table 1. The peptides were assembled in a stepwise manner on a solid support using an Applied Biosystems Model 431A Peptide Synthesizer and the standard DCC/HOBt Solvent-Activation strategy on a 0.1 mmol scale (small scale). *tert*-Boc-amino acids were coupled to MBHA (Bachem Feinchemikalien AG, Switzerland) resin as hydroxybenzotriazole (HOBt) esters.

Deprotection, cleavage and purification of the peptides has been described earlier (Langel *et al.*, 1992). Purity of the individual peptides was checked by analytical Nucleosil 120-3 C_{18} reversed-phase h.p.l.c. column (0.4 cm × 10.0 cm) and determined to be ≥98%. Molecular weights of the peptides were determined with Plasma Desorption Mass Spectrometer (PDMS) Model Bioion 20, Applied Biosystems, the calculated values were obtained in each case.

Binding studies

Membrane preparation from rat spinal cord and receptor binding analysis was carried out by filtration technique as described earlier (Land *et al.*, 1991), using 5 mM HEPES-buffered (pH 7.4) Krebs-Ringer solution containing 1 mg ml^{-1} bacitracin and 0.5 mg ml^{-1} bovine serum albumin (BSA). The mixtures were incubated for 30 min at 37°C with 0.1–0.2 nM porcine [^{125}I]-galanin (NEN, specific activity 2200 Ci $mmol^{-1}$) as a tracer at increasing concentrations (0.001–1000 nM) of the peptides to be tested. Specific binding was defined as that displaceable by 1 mM galanin. The K_d values of the displacing ligands were calculated from the computer-generated IC_{50} values using the correction of Cheng & Prusoff (1973). Fitting of the experimental data was carried out be means of a non-linear least squares method using the programme Kaleidograph on a Macintosh SE/30.

Results

Data presented in Table 1 and Figure 1 demonstrate that all four peptides/galanin analogues tested, C7, M32, M38 and M40, are high-affinity ligands at the spinal cord galanin receptors characterized with K_d values ranging from 0.01–6.8 nM. The displacement of [^{125}I]-galanin by some of the ligands, C7, M32 and M38, was characterized with Hill coefficients of 0.3–0.5, being significantly below unity (*vs* $n_H = 1$ for galanin and M40), indicating that the galanin recognition sites in spinal cord can be heterogeneous.

In order to evaluate the functional nature (antagonists *vs* agonists) of these galanin receptor ligands, we have determined the effect of these ligands on the galanin induced (30 pmol, i.t.) facilitation of the flexor reflex. It has been demonstrated earlier (Wiesenfeld-Hallin *et al.*, 1989) that i.t. galanin exerts a complex dose-dependent effect on the spinal flexor reflex; facilitatory at low doses (30–300 pmol) and inhibitory at high doses (30 nmol).

I.t. galanin (30 pmol) briefly facilitated the flexor reflex (Figure 2), with an average peak reflex facilitation of 115.2±15.4% above baseline, lasting 5.7±0.5 min ($n = 19$). Both M32 and C7 applied i.t. dose-dependently blocked reflex facilitation induced by i.t. galanin (Figure 3) with ED_{50} of 71.5 pmol for M32 and 23.2 pmol for C7. M38 only weakly antagonized galanin-induced reflex facilitation, with a ≈100 fold lower inhibitory potential ($ID_{50} = 2.1$ nmol) than M32 and C7 (Figure 3). In contrast, M40 potentiated galanin-induced

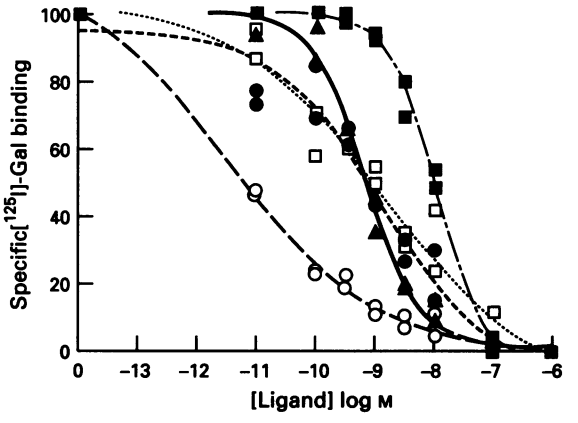

Figure 1 The inhibition of specific [^{125}I]-galanin (0.1–0.2 nM) binding to membranes from lumbar dorsal spinal cord by galanin (▲), M32 (○), M38 (□), M40 (■) and C7 (●), at 37°C for 30 min, with increasing concentrations of displacing ligand under equilibrium conditions. Non-specific binding was defined as that displaceable by 1 μM galanin. Two independent experiments were performed and each value was dertermined in duplicates.

Table 1 Displacement of 0.1–0.2 nM [^{125}I]-galanin from rat spinal cord membranes by galanin receptor ligands

Peptide	*Sequence*	K_d, (nM)	n_H
Rat galanin	GWTLNSAGYLLGP-HAIDNHRSFSDKHGLT amide	0.53±0.2	0.97±0.03
M32 Galanin(1-13)-NPY(26-36)	GWTLNSAGYLLGP-RHYINLITRQRY amide	0.010±0.001	0.33±0.06
M38 Galanin(1-13)-ALALALA	GWTLNSAGYLLGP-ALALALA amide	0.70±0.07	0.41±0.12
M40 Galanin(1-13)-PPALALA	GWTLNSAGYLLGP PPALALA amide	6.8±2.2	0.93±0.13
C7 Galanin(1-13)-Spantide	GWTLNSAGYLLGP-(D-R)PKPQQ(D-W)F(D-W)LL amide	1.16±0.12	0.46±0.04

The data are the means ± s.e.mean from two independent experiments and each value was determined in duplicate. Specific binding was defined as that displaceable by 1 μM unlabelled galanin, representing 80–90% of total binding.

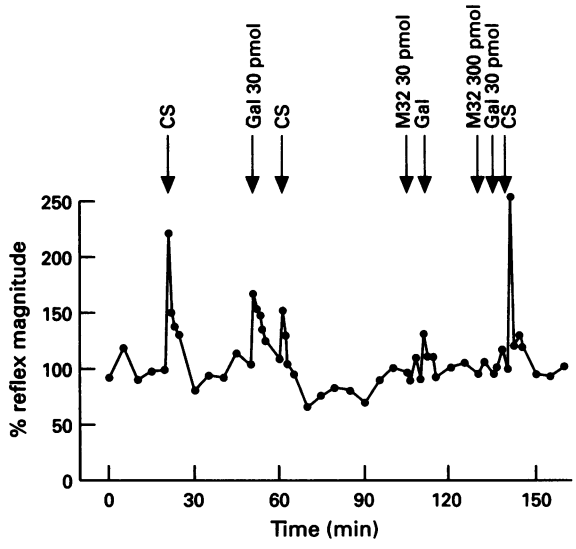

Figure 2 Illustration of the effect of conditioning stimulation (CS) of unmyelinated afferents, GAL and M32 in rat spinal cord. The baseline flexor reflex was defined as 100%. Note that i.t. GAL by itself facilitated of the flexor reflex, but it also antagonized the reflex facilitation by the C-fibre CS. Both actions of GAL were blocked by M32.

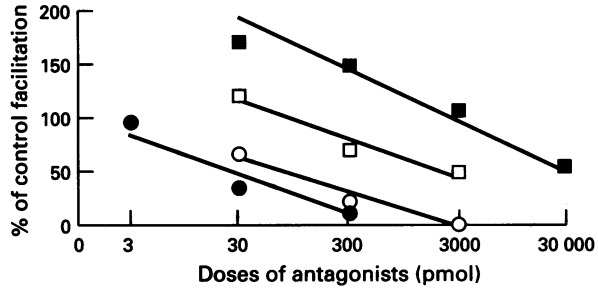

Figure 3 Summary of the effects of C7 (●), M32 (○), M38 (□) and M40 (■) on the facilitation of the flexor reflex induced by 30 pmol i.t. GAL. Data from 3–4 experiments are presented for each dose and expressed as mean ± s.e.m. The regressions for all compounds were calculated. C7; $y = -35.8x + 98.8$ ($P < 0.01$), M32; $y = -32.2x + 109.7$ ($P < 0.001$), M38; $y = -35.8x + 169.2$ ($P < 0.05$) and M40; $y = -48.2x + 269.5$ ($P < 0.01$). The ED_{50} values with 95% confidence limits are 23.2 pmol (6.7–42.2 pmol) for C7; 71.5 pmol (22.1–178.4 pmol) for M32; 2.1 nmol (178.4 pmol–∞) for M38 and 39.8 nmol (7.9 nmol–∞) for M40.

facilitation at low doses, but attenuated it at very high doses, thus behaving as a mixed agonist-antagonist at the spinal galanin receptor(s).

Besides the galanin-induced complex facilitatory-inhibitory effect on the flexor reflex, i.t. galanin dose-dependently (30 pmol–30 nmol) depressed the reflex facilitation induced by the C-fibre CS (Wiesenfeld-Hallin *et al.*, 1989). I.t. galanin (30 pmol) applied prior to the CS caused a brief reflex facilitation and significantly inhibited the subsequent CS-induced facilitation (Figure 2). This galanin receptor-mediated depression was also potently reversed by M32 and C7, confirming their antagonistic properties (Figure 2). Furthermore, C7 at a high dose (3 nmol) totally blocked the flexor reflex, such that the reflex response to strong, even tissue damaging, stimuli could not be evoked for up to 60 min after drug administration, indicating the presence of neurotoxicity. All the chimeric ligands caused brief facilitation of the flexor reflex upon i.t. injection. However, this facilitation could be correlated with neither affinity nor antagonism.

Discussion

In earlier studies we have shown the presence of a G-protein coupled single class of galanin receptors in the spinal cord, with a K_d for galanin of 0.6 nM present in a concentration of ≈ 50 fmol mg^{-1} protein (Bedecs *et al.*, 1992). In the present study we have evaluated the binding properties and functional effects of a series of galanin chimeras which in their C-terminal contain either other bioactive peptides (C7 and M32) or nonsense sequences (M38 and M40). All the chimeric peptides displaced [^{125}I]-galanin from rat spinal cord membranes with high affinities, in the range of K_d from 0.01 to 6.8 nM (Table 1).

M32, M38 and C7 had K_d values of 0.01 nM, 0.7 nM and 1.16 nM with Hill coefficients of 0.33, 0.41 and 0.46, respectively, showing a clear correlation between high-affinity recognition and low Hill coefficients, indicating heterogeneity of recognition sites in the spinal cord. M32 actually showed the highest affinity toward a galanin receptor yet reported in any system. However, in the electrophysiological studies, only M32 and C7 potently antagonized the galanin-induced facilitation of the flexor reflex, whereas M38 and M40 only weakly or partly antagonized the effect of galanin. The antagonistic potencies of M32 and C7 are comparable with those of M15 and M35, two previously tested chimeric galanin receptor antagonists (Bartfai *et al.*, 1991; Wiesenfeld-Hallin *et al.*, 1992b), although significantly differing from these in binding properties since M15 and M35 both displaced [^{125}I]-galanin in spinal cord membranes with Hill coefficients of unity.

M32 and C7 also potently reversed the galanin-mediated depression on C-fibre CS-induced reflex facilitation, their effective doses being comparable with M15 and M35. This suggests that spinal cord galanin receptors mediating the excitatory and inhibitory effects on the flexor reflex are similar in their structural requirement for functional agonism and antagonism and cannot be distinguished by the structurally divergent chimeric antagonists used so far.

An interesting finding is that both C7 [galanin(1–13)-spantide] and M15 [galanin(1–13)-substance P(5–11 amide] (Bartfai *et al.*, 1991) behaved as galanin receptor antagonists in the spinal cord, thus excluding any substance P receptor interaction, since the galanin receptor antagonism is similar if the C-terminal part of the galanin chimera contains a substance P agonist [substance P(5–11)] or an antagonist (spantide). However, C7, which in its C-terminal contains the entire spantide molecule, was shown to be neurotoxic at high doses. Previous studies in this and other laboratories have shown that i.t. neuropeptides and neuropeptide antagonists could be toxic at high concentrations, in particular the substance P receptor antagonist, spantide (Post & Paulsson, 1985; Wiesenfeld-Hallin & Duranti, 1987). Thus, the neurotoxicity of C7 is most probably due to spantide on its C-terminal.

In contrast to the efficacy of M32 and C7, the other two chimeras, M38 and M40, exhibited low or no antagonism, respectively. M38, though having an affinity of 0.7 nM at spinal cord galanin receptors, could block the galanin-induced facilitation only slightly even at quite a high dose (30 nmol), thus behaving as a weak antagonist. The potentiation of galanin-induced facilitation by M40 at low doses and the fact that a thousand fold excess of M40 was required to produce moderate antagonism of galanin-mediated reflex facilitation indicate that M40 is probably a partial agonist with a weak antagonistic property in this system.

The fact that only the chimeras containing bioactive peptides in their C-terminal, M32 [NPY (25–36)] and C7 (spantide), behaved as antagonists favours a bi-receptor interaction-mediated galanin receptor antagonism (Langel *et al.*, 1992). This is excluded for C7 (see above). However, in the case of M32, this is not clear as the Hill coefficient in the [^{125}I-galanin displacement experiment was significantly below unity, indicating putative multiple recognition sites of M32. Autoradiographic and functional studies have shown the presence of NPY receptors on DRG cells and in the dorsal horn of the spinal cord (Kar & Quirion, 1992), where NPY was shown to

reduce depolarization-induced SP-release through an inhibition of Ca^{2+} currents (Walker *et al.*, 1988). I.t. NPY has antinociceptive effects and depresses the spinal flexor reflex (Hua *et al.*, 1991; Xu *et al.*, 1994). These studies have suggested a possible presynaptic localization of NPY binding sites and NPY-mediated effects, whereas the antagonism of M32 on exogenously applied galanin-induced reflex facilitation is postsynaptic on second order spinal neurones. Thus, involvement of a NPY receptor activation as part of the M32-mediated galanin receptor antagonism is unlikely.

Based on the differential pharmacological effects of these chimeric peptides, especially that of M40, the presence of galanin receptor subtypes has been suggested (Bartfai *et al.*, 1993b; Wynick *et al.*, 1993). Hypothalamic and hippocampal galanin receptors represent a putative central galanin receptor subtype (GL_1-receptor) which is blocked by M40 (Bartfai *et al.*, 1993b; Crawley *et al.*, 1993), whereas the pancreatic galanin receptor represents a peripheral subtype (GL_2-receptor) which recognizes M40, but as a weak agonist. The galanin receptors in the spinal cord (GL_3) may occupy an intermediate position between these two putative subtypes. However, it should be noted that the discrepancy between *in vitro* and *in vivo* results with these chimeric peptides may also be explained in terms of different metabolic stability, diffusion rates and penetration to the site of action. Recent cloning of galanin receptors from the human Bowes melanoma cell line (Habert-Ortoli *et al.*, 1994) opens the possibility of finally revealing in terms of their amino acid sequences the galanin receptor subtypes whose classification so far is based on pharmacological studies.

This study was supported by the Swedish MRC (07913, O4X-2887), the Bank of Sweden Tercentenary Foundation, Marcus och Amalia Wallenbergs Minnesfond, Astra Pain Control AB, The Swedish Society of Medicine, Konung Gustav V:s och Drottning Victorias Stiftelse, Ivar Bendixons Foundation, Pharmacia's Scientific Foundation and Marianne och Marcus Wallenbergs Stiftelse.

References

BARTFAI, T., BEDECS, K., LAND, T., LANGEL, Ü., BERTORELLI, R., GIROTTI, P., CONSOLO, S., XU, X.-J., WIESENFELD-HALLIN, Z., NILSSON, S., PIERIBONE, V.A. & HÖKFELT, T. (1991). M-15: high-affinity chimeric peptide that blocks the neuronal actions of galanin in the hippocampus, locus coeruleus, and spinal cord. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 10961–10965.

BARTFAI, T., FISONE, G. & LANGEL, Ü. (1992). Galanin and galanin antagonists; molecular and biochemical perspectives. *Trends Pharmacol. Sci.*, **13**, 312–316.

BARTFAI, T., HÖKFELT, T. & LANGEL, Ü. (1993a). Galanin – a neuroendocrine peptide. *Crit. Rev. Neurobiol.*, **7**, 229–274.

BARTFAI, T., LANGEL, Ü., BEDECS, K., ANDELL, S., LAND, T., GREGERSEN, S., AHRÉN, B., GIROTTI, P., CONSOLO, S., CORVIN, R., CRAWLEY, J., XU, X.-J., WIESENFELD-HALLIN, Z. & HÖKFELT, T. (1993b). Galanin-receptor ligand M40 peptide distinguish between putative galanin-receptor subtypes. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 11287–11291.

BEDECS, K., LANGEL, Ü., BARTFAI, T. & WIESENFELD-HALLIN, Z. (1992). Galanin receptors and their second messengers in the lumbar dorsal spinal cord, the effect of GTP and sciatic nerve transection. *Acta Physiol. Scand.*, **144**, 213–220.

CHENG, Y.-C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.

CH'NG, J.L.C., CHRISTOFIDES, N.D., ANAND, P., GIBSON, S.J., ALLEN, Y.S., SU, H.C., TATEMOTO, K., MORRISON, J.F.B., POLAK, J.M. & BLOOM, S.R. (1985). Distribution of galanin immunoreactivity in the central nervous system and the response of galanin-containing neuronal pathways to injury. *Neuroscience*, **16**, 343–354.

CRAWLEY, J.N., AUSTIN, M.C., FISKE, S.M., MARTIN, B., CONSOLO, S., BERTHOLD, M., LANGEL, Ü., FISONE, G. & BARTFAI, T. (1990). Activity of centrally administered galanin fragments on stimulation of feeding behavior and on galanin receptor binding in the rat hypothalamus. *J. Neurosci.*, **10**, 3695–3700.

CRAWLEY, J., ROBINSON, J.K., LANGEL, Ü. & BARTFAI, T. (1993). Galanin receptor antagonists M40 and C7 block galanin-induced feeding. *Brain Res.*, **600**, 268–272.

FISONE, G., BERTHOLD, M., BEDECS, K., UNDÉN, A., BARTFAI, T., BERTORELLI, R., CONSOLO, S., CRAWLEY, J., MARTIN, B., NILSSON, S. & HÖKFELT, T. (1989). N-terminal galanin (1–16) fragment is an agonist at the hippocampal galanin receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 9588–9591.

HABERT-ORTOLI, E., AMIRANOFF, B., LOQUET, I., LABURTHE, M. & MAYAUX, J.-F. (1994). Molecular cloning of a functional galanin receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 9780–9783.

HUA, X.Y., BOUBLIK, J.H., SPICER, M.A., RIVER, J.E., BROWN, M.R. & YAKSH, T.L. (1991). The antinociceptive effects of spinally administered neuropeptide Y in the rat: systematic studies on structure-activity relationship. *J. Pharmacol. Exp. Ther.*, **258**, 243–248.

KAR, S. & QUIRION, R. (1992). Quantitative autoradiographic localization of [^{125}I]neuropeptide Y receptor binding sites in rat spinal cord and the effects of neonatal capsaicin, dorsal rhizotomy and peripheral axotomy. *Brain Res.*, **574**, 333–337.

LAND, T., LANGEL, Ü., FISONE, G., BEDECS, K. & BARTFAI, T. (1991). Assay for galanin receptor. In *Methods in Neuroscience*. Vol. 5, *Neuropeptide Technology: Gene Expression and Neuropeptide Receptors*. ed. Conn, P.M. pp. 225–234. San Diego: Academic Press.

LANGEL, Ü., LAND, T. & BARTFAI, T. (1992). Design of chimeric peptide ligands to galanin receptor and substance P receptors. *Int. J. Pept. Protein Res.*, **39**, 516–522.

MELANDER, T., HÖKFELT, T. & RÖKAEUS, Å. (1986). Distribution of galanin-like immunoreactivity in the rat central nervous system. *J. Comp. Neurol.*, **248**, 475–517.

POST, C. & PAULSSON, I. (1985). Antinociceptive and neurotoxic actions of substance P analogues in the rat's spinal cord after intrathecal administration. *Neurosci. Lett.*, **57**, 159–164.

RIGLER, R., WENNERBERG, A., COOKE, R.M., ELOFSSON, A., NILSSON, L., VOGEL, H., HOLLEY, L.H., CARLQUIST, M., LANGEL, Ü., BARTFAI, T. & CAMPBELL, I.D. (1991). On the solution structure of galanin. In *Galanin: a New Multifunctional Peptide in the Neuro-endocrine System*. ed. Hökfelt, T., Bartfai, T., Jacobowitz, D. & Ottoson, D. pp. 17–25. London: McMillan Press.

ROSSOWSKI, W.J., ROSSOWSKI, T.M., ZACHARIA, S., ERTAN, A. & COY, D.H. (1990). Galanin binding sites in rat gastric and jejunal smooth muscle membrane preparations. *Peptides*, **11**, 333–338.

SKOFITSCH, G. & JACOBOWITZ, D. (1985). Galanin-like immunoreactivity in capsaicin sensitive sensory neurons and ganglia. *Brain Res. Bull.*, **15**, 191–195.

TATEMOTO, K., RÖKAEUS, Å., JÖRNVALL, H., McDONALD, T.J. & MUTT, V. (1983). Galanin- a novel biologically active peptide from porcine intestine. *FEBS Lett.*, **164**, 124–128.

WALKER, M.W., EWALD, D.A., PERNEY, T.M. & MILLER, R.G. (1988). Neuropeptide Y modulates neurotransmitter release and Ca2+ currents in rat sensory neurons. *J. Neurosci.*, **8**, 2438–2446.

WALL, P.D. & WOOLF, C.J. (1984). Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. *J. Physiol.*, **356**, 443–458.

WIESENFELD-HALLIN, Z., BARTFAI, T. & HÖKFELT, T. (1992a). Galanin in sensory neurons in the spinal cord. *Frontiers Neuroendocrinol.*, **13**, 319–343.

WIESENFELD-HALLIN, Z. & DURANTI, R. (1987). D-Arg^{1}, D-Trp^{7,9}, Leu^{11}-substance P (spantide) does not antagonize substance P-induced hyperexcitability of the nociceptive flexion withdrawal reflex in the rat. *Acta Physiol. Scand.*, **129**, 55–59.

WISENFELD-HALLIN, Z., VILLAR, M.J. & HÖKFELT, T. (1989). The effect of intrathecal galanin and C-fibre stimulation on the flexor reflex in the rat. *Brain Res.*, **486**, 205–213.

WIESENFELD-HALLIN, Z., XU, X.-J., LANGEL, Ü., BEDECS, K., HÖKFELI, T. & BARTFAI, T. (1992b). Galanin-mediated control of pain: enhanced role after nerve injury. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 3334–3337.

WYNICK, D., SMITH, D.M., GHATEI, M., AKINSANYA, K., BHOGAL, R., PURKISS, P., BYFIELD, P., YANAIHARA, N. & BLOOM, S.R. (1993). Characterization of a high-affinity galanin receptor in the rat anterior pituitary-absence of biological effect and reduced membrane binding of the antagonist M15 differentiate it from the brain/gut receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 4231–4235.

XU, X.-J., WIESENFELD-HALLIN, Z., FISONE, G., BARTFAI, T. & HÖKFELT, T. (1990). The N-terminal, but not C-terminal, galanin fragment effects the flexor reflex in rats. *Eur. J. Pharmacol.*, **182**, 137–141.

(Received March 20, 1995
Revised June 19, 1995
Accepted June 22, 1995)