Br. J. Pharmacol. (1990), **101**, 297–300

Effects of galanin, its analogues and fragments on rat isolated fundus strips

Stavros Katsoulis, [1]Wolfgang E. Schmidt, Harald Schwörer & Werner Creutzfeldt

Division of Gastroenterology and Endocrinology, Department of Medicine, University of Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen, F.R.G.

1 Rat and porcine galanin (rGal and pGal) produced dose-dependent contraction of rat fundus strips in a concentration range of 6 nM–100 nM.

2 The stimulatory effect of rGal on rat fundus strips was not modified in the presence of somatostatin (250 nM), naloxone (1 μM), guanethidine (10 μM), a mixture of propranolol (3 μM) and phentolamine (3 μM), tetrodotoxin (1 μM), indomethacin (10 μM), atropine (1 μM), a mixture of methysergide (2.5 μM) and ketanserin (2.5 μM), a mixture of mepyramine (10 μM) and cimetidine (10 μM), and saralasin (10 μM) or when strips were desensitized to substance P and neurotensin.

3 These results suggest the localization of specific Gal receptors on the surface of smooth muscle cells of rat fundus.

4 The galanin analogues [D-Trp^2]-rGal, [NLe^4]-rGal, [D-Ala^7]-rGal, [D-Trp^2-NLe^4-D-Ala^7]-rGal and fragments [Cys^{23}]-Gal (1–23), Gal (1–18) were fully active. In contrast, rGal (3–29) was completely inactive and showed no antagonistic properties to the contractile effect of intact galanin.

5 The order of potency of the galanin peptides, analogues and fragments to contract rat fundus strips was: pGal > rGal > [NLe^4]-rGal > [Cys^{23}]-Gal (1-23) > Gal (1-18) > [D-Ala^7]-rGal > [Trp^2]-rGal > [D-Trp^2-NLe^4-D-Ala^7]-rGal.

6 The data originating from our structure-activity study suggest that the C-terminal portion of Gal contributes mainly to the affinity of Gal receptors whereas the N-terminal portion of Gal is responsible for the full activation of Gal receptors in this tissue. In particular the amino acids in position 1 and 2 of Gal (Gly-Trp) appear to be essential for binding and intrinsic activity.

Introduction

Porcine galanin (pGal) is a 29 amino acid peptide isolated from porcine intestine by Tatemoto *et al.* (1983). Galanin occurs in the brain, spinal cord, urogenital tract and gastrointestinal tract of several mammalian species including man (Rokaeus *et al.*, 1984; Ekblad *et al.*, 1985a; Melander *et al.*, 1985; Bishop *et al.*, 1986; Furness *et al.*, 1987; Rokaeus, 1987). *In vitro* studies have shown that pGal evokes a contractile action on rat fundus, jejunum, ileum, colon and urinary bladder (Tatemoto *et al.*, 1983; Ekblad *et al.*, 1985b), human small intestine (Maggi *et al.*, 1989), human appendix (Ekblad *et al.*, 1989) and mouse colon (Fontaine & Lebrun, 1989). By contrast pGal relaxes canine ileum (Fox *et al.*, 1986) and has a neuromodulatory action on rat deferens (Ohhashi & Jacobowitz, 1985), guinea-pig taenia coli (Ekblad *et al.*, 1985b), guinea-pig small intestine (Palmer *et al.*, 1986; Yau *et al.*, 1986; Tamura *et al.*, 1987; 1988; Kuwahara *et al.*, 1989), and human urinary bladder (Maggi *et al.*, 1987).

Recently, some studies have been presented concerning the structure-activity relationship of galanin by use of galanin fragments and substitutions in rat isolated islets (Gregersen *et al.*, 1989), isolated perfused dog pancreas (Hermansen *et al.*, 1989) and in the pancreatic β-cell line Rin m 5F (Amiranoff *et al.*, 1989). So far there is little information about structure-activity relationships on smooth muscle tissues. Ekblad and his colleagues (1985b) reported that the N-terminal galanin fragment 1–10 was able to contract rat jejunum but was inactive, in contrast to intact galanin, in neuromodulation of guinea-pig taenia coli and rabbit iris sphincter.

The aim of this study was to compare the effects of pGal and the recently identified rat galanin (rGal) which differs at 3 positions in the C-terminal part (residue 23, 26 and 29, Table 1) (Vrontakis *et al.*, 1987; Kaplan *et al.*, 1988). Moreover, we analysed the mode of action of galanin on rat isolated fundus strips and used galanin analogues and fragments (Table 1) to

[1] Author for correspondence.

Table 1 Primary structure of porcine and rat galanin and various analogues and fragments

	1	5	10	15	20	25	29
Galanin (porcine):	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Arg-Ser-Phe-His-Asp-Lys-Tyr-Gly-Leu-Ala-NH_2						
Galanin (rat):	.....				Ser	His	Thr-NH_2
[D-Trp^2]-galanin (rat):	... DTrp				Ser	His	Thr-NH_2
[NLe^4]-galanin (rat):	..... NLe				Ser	His	Thr-NH_2
[D-Ala^7]-galanin (rat):	..... DAla				Ser	His	Thr-NH_2
[D-Trp^2-NLe^4-D-Ala^7]-galanin (rat):	... DTrp.. NLe..... DAla				Ser	His	Thr-NH_2
[Cys^{23}]-galanin (1-23):	.....				Cys-NH_2		
Galanin (1-18):	.....						
Galanin (3-29) (rat):	.....				Ser	His	Thr-NH_2

Dots indicate conserved amino acid residues, compared with the porcine galanin sequence.

identify the molecular domains responsible for binding and activation of the receptor in this tissue. Data derived from structure-activity studies should be very useful for constructing specific galanin antagonists which are not yet available.

Methods

Animals and tissue preparations

Albino Wistar rats of either sex (weighing 150–300 g) were killed by a blow on the head; the abdomen was opened via an incision in the midline. The stomach was taken out and the fundus was separated from the whole organ. Longitudinal muscle strips were prepared from the fundic region as previously described (Katsoulis & Conlon, 1989). The strips were kept at a resting tension of 2.0 g in oxygenated (95% O_2 and 5% CO_2) Krebs solution maintained at 37°C. The composition of the Krebs solution was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.18, $NaHCO_3$ 25, KH_2PO_4 1.18 and glucose 5.55. Responses were measured isometrically with Grass FT03 force displacement transducers connected to a Grass multichannel polygraph (model 79D). Tissue was allowed to equilibrate for 30–60 min and the superfusion buffer was changed every 15–20 min.

Concentration-response curves

Experiments were begun when reproducible contractile responses to carbachol (10 μM) were obtained. Then, conventional dose-response curves were constructed. No more than two concentration-response studies (one for rat galanin and one for a galanin analogue or galanin fragment, applied alternately) were performed on each strip. Peptides were injected in increasing concentrations by non-cumulative additions, until maximum muscular responses occurred. The maximal effect was defined as the contractile response that could not be further increased by higher concentrations of peptide. The tissue was washed as soon as the peak contraction had developed. The contact time of the peptides with muscle strips was 1–3 min. Agonist applications were performed at 20–30 min intervals to avoid tachyphylaxis. Results are expressed as a percentage of the maximum response induced by each peptide. Potencies (ED_{50}) were calculated by interpolation from the appropriate dose-response curve. Effectiveness refers to the maxium response produced by intact rat galanin compared with the maximum effect produced by galanin analogues or galanin fragments.

Characterization of the contracting effect of galanin on rat fundus strips

In order to establish whether the stimulatory action of galanin is due to specific receptor-activation located on the smooth muscle cell or mediated by the release of endogenous substances, galanin and control agonists were tested in the absence and presence of various antagonists. The concentrations used for the antagonists were sufficiently high to block the corresponding agonists (see Table 3). The rat stomach strips were incubated with the inhibitors for 15–20 min before addition of test agents. The contractile effect of galanin was also investigated on preparations desensitized with substance P and neurotensin for which no potent antagonists were available. Tachyphylaxis to substance P was obtained by the method described by Holzer-Petsche *et al.* (1989). To induce tachyphylaxis to neurotensin five test concentrations of peptide (10 nM) were applied at 6 min intervals without washout; 1 min after the administration of the last test concentration, galanin was added to the bath. Test doses of [D-Trp^2-NLe^4-D-Ala^7]-rGal and rGal (3-29) were injected 5 min before application of rGal to examine their antagonistic properties to the effect of rGal.

Statistical analysis

All data are expressed as means ± s.e.mean. Student's t test for paired data was used for statistical evaluation of differences. A P value of less than 0.05 was taken to indicate a significant difference.

Drugs

The following drugs were used: atropine sulphate, cimetidine maleate, carbachol, indomethacin, histamine hydrochloride, 5-hydroxytryptamine creatinine sulphate, tetrodotoxin, naloxone hydrochloride, (±)-propranolol and guanethidine (all from Sigma Chemie). Galanin (porcine), angiotensin II, neurotensin, substance P and somatostatin (Peninsula Laboratories Europe). Saralasin (gift from Rhöm Pharma, F.R.G.), ketanserin tartrate (gift from Janssen, Belgium), methysergide hydrogenmaleate (gift from Sandoz, Switzerland), phentolamine methansulphate (gift from Ciba Geigy, Switzerland) and mepyramine maleate (gift from May and Baker, U.K.). Rat galanin and its analogues and fragments were synthesized by Fmoc solid phase peptide synthesis on a LKB Biolynx peptide synthesizer and purified to homogeneity by high performance liquid chromatography.

Results

Effects of galanin, its analogues and fragments on rat fundus strips

Both rGal and pGal elicited concentration-dependent contractions of rat fundus strips. Reproducible effects were observed at concentrations as low as 6 nM. Maximum contraction was obtained with pGal and rGal at a concentration of 100 nM; larger concentrations failed to produce a greater response. The potencies and effectiveness of rGal and pGal were not significantly different (Table 2) and the slopes of the dose-response curves were nearly identical (Figure 1). The maximum contraction induced by rGal (100 nM) and pGal (100 nM) was 51.5% and 50.5% respectively of that achieved with carbachol (30 μM) in this tissue. The dose-response curves produced by the galanin fragments and analogues are shown in Figure 1. Effectiveness, ED_{50} values and relative potencies are presented in Table 2. The concentration-response curves to [NLe^4]-rGal, [Cys^{23}]-Gal (1-23) and Gal (1-18) were displaced slightly to the right of that to rGal and pGal but remained parallel and exhibited the same maximum. Only minor losses (13 to 55%) of potency were observed with these compounds when compared to native galanin. On the other hand [D-Ala^7]-rGal, [D-Trp^2]-rGal and [D-Trp^2-NLe^4-D-

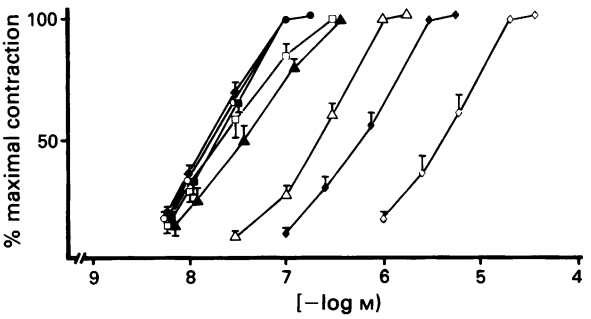

Figure 1 Dose-response curves for the contractile effect of porcine galanin (●), rat galanin (○), (NLe^4)-(rat) galanin (■), (Cys^{23})-galanin (1-23) (□), galanin (1-18) (▲), (D-Ala^7)-(rat) galanin (△), (D-Trp^2)-(rat) galanin (◆) and (D-Trp^2-NLe^4-D-Ala^7)-(rat) galanin (◇) on the rat fundus *in vitro*. Results are expressed as the percentage of the maximum effect induced by each peptide. Each point represents the mean and vertical bars show s.e.mean. The number of individual determinations is given in Table 2.

Table 2 A comparison of the effectiveness, potencies and relative potencies of galanin, its analogues and fragments on the contraction of the rat fundus

	Effectiveness (%)	ED_{50} (nM)	*Relative potency*	*Number of experiments*
Galanin (porcine)	99 ± 2.5	16.7 ± 1.0	100	5
Galanin (rat)	100.0	18.2 ± 0.8	92	65
[NLe^4]-galanin (rat)	101 ± 7.0	19.2 ± 1.3	87	8
[Cys^{23}]-galanin (1-23)	98 ± 7.0	27.6 ± 5.3*	61	12
Galanin (1-18)	97 ± 4.0	36.9 ± 5.7**	45	8
[D-Ala^7]-galanin (rat)	97 ± 3.5	180.0 ± 11.0***	9	8
[D-Trp^2]-galanin (rat)	103 ± 5.0	514.0 ± 47.0***	3	8
[D-Trp^2-NLe^4-D-Ala^7]-galanin (rat)	96 ± 5.0	3810.0 ± 500.0***	0.4	8
Galanin (3-29) (rat)	No effect	—	—	8

Data are expressed as means ± s.e.mean. Effectiveness refers to the maximum response produced by the test peptides compared with the maximum response produced by rat galanin. Potencies (ED_{50}) were calculated by interpolation from the appropriate dose-response curves. Relative potency refers to the ED_{50} of each peptide compared to the ED_{50} of porcine galanin.
* $P < 0.5$; ** $P < 0.01$; *** $P < 0.001$ compared to the ED_{50} of rat galanin.

Ala^7]-rGal showed the same maximum to intact Gal and pGal but their dose-response curves were shifted strongly to the right. [D-Ala^7]-rGal, [D-Trp^2]-rGal and [D-Trp^2-NLe^4-D-Ala^7]-rGal were 11, 33 and 250 times less potent compared to Gal in rat fundus strips (Figure 1, Table 2). rGal (3-29) was completely inactive as an agonist in these preparations even in concentrations up to 10 μM (Table 2).

Effects of various agents on the action of rGal in rat fundus strips

The contractions induced by rGal were not significantly modified in the presence of atropine, a mixture of methysergide and ketanserin, a mixture of mepyramine and cimetidine, and saralasin while those produced by their respective agonists (carbachol, 5-HT, histamine, angiotensin II) were abolished. Somatostatin, naloxone, guanethidine, a mixture of propranolol and phentolamine, TTX and indomethacin also failed to influence the contractile effect of rGal. Preparations desensitized to neurotensin or substance P remained fully responsive to rGal. None of the rGal derivatives rGal (3-29) and [D-Trp^2-NLe^4-D-Ala^7]-rGal behaved as an antagonist of rGal in rat fundus strips. These results are summarized in Table 3.

Discussion

A preliminary account has been published of the contractile effect of pGal in rat stomach strips (Tatemoto *et al.*, 1983). So far a pharmacological characterization of this effect has not been performed. The present study suggests that Gal contracts rat fundus by a direct interaction with specific galanin receptors located on the smooth muscle cells. This is supported by the observation that the action of Gal was unmodified by TTX, atropine, a mixture of methysergide and ketanserin, a mixture of mepyramine and cimetidine, saralasin, a mixture of propranolol and phentolamine, guanethidine, naloxone, somatostatin and indomethacin. Rat fundus strips desensitized with neurotensin or substance P were found to maintain their sensitivity to galanin. The existence of specific galanin receptors makes the rat fundus strip an appropriate bioassay preparation for investigating structure-function relationships of galanin and its myotropic activity.

Table 3 Myotropic effects of rat galanin (rGal) and other agonists on rat isolated stomach strips, as obtained in the absence and presence of specific antagonists, autacoids and peptides

Antagonist (M)	*Agonist*	*Dose* (M)	*Contraction (%) In the absence of the antagonist*	*Contraction (%) In the presence of the antagonist*
Rat galanin (3-29) (10^{-5})	rGal	3×10^{-8}	100	111 ± 9
[D-Trp^2-NLe^4-D-Ala^7]-rat galanin (10^{-6})	rGal	3×10^{-8}	100	98 ± 9
Somatostatin (2.5×10^{-7})	rGal	10^{-7}	100	97 ± 3.5
Naloxone (10^{-6})	rGal	10^{-7}	100	106 ± 8
Guanethidine (10^{-5})	rGal	10^{-7}	100	101 ± 5
Propranolol (3×10^{-6}) + phentolamine (3×10^{-6})	rGal	10^{-7}	100	103 ± 7
TTX (10^{-6})	rGal	10^{-7}	100	93 ± 3
Indomethacin (10^{-5})	rGal	10^{-7}	100	96 ± 7
Atropine (10^{-6})	Carbachol	10^{-5}	100	0*
	rGal	10^{-7}	100	104 ± 2
Methysergide (2.5×10^{-6}) + ketanserin (2.5×10^{-6})	5-Hydroxytryptamine	10^{-5}	100	0*
	rGal	10^{-7}	100	95 ± 8
Mepyramine (10^{-5}) + cimetidine (10^{-5})	Histamine	10^{-4}	100	0*
	rGal	10^{-7}	100	97 ± 5
Saralasin (10^{-5})	Angiotensin II	10^{-6}	100	0*
	rGal	10^{-7}	100	107 ± 4
Substance P (tachyphylaxis)	Substance P	5×10^{-7}	100	43 ± 4.5*
	rGal	10^{-7}	100	100 ± 10
Neurotensin (tachyphylaxis)	Neurotensin	10^{-8}	100	0*
	rGal	10^{-7}	100	105 ± 9

The results are expressed as means ± s.e.mean of 8 experiments. The incubation time of the antagonists with the tissues was 15–20 min except rGal (3-29) and [D-Trp^2-NLe^4-D-Ala^7]-rGal which were injected 5 min before repeating application of agonists. The desensitizing procedures performed with substance P and neurotensin are described in methods. M is molar concentration. P values < 0.05 were considered significant (see asterisk).

Both rGal and pGal were equipotent and equally effective in producing contractions of rat fundus strips. Using galanin fragments we have found that deletion of the C-terminal portion of the molecule led only to a minor loss of the potency but the effectiveness was fully conserved. In contrast, substitutions in the N-terminal part of the molecule led to a major loss of potency. Nevertheless, although considerably less potent than Gal, the N-terminal modified galanin peptides were able to elicit a comparable maximum response to Gal. The present findings support earlier conclusions that the C-terminal residue of the galanin molecule contributes mainly to the affinity of Gal for its receptor whereas the N-terminal portion is responsible for binding and ability to convey biological effects (Amiranoff *et al.*, 1989; Gregersen *et al.*, 1989). Particularly the two first amino acids (Gly-Trp) at the N-terminus appear to be crucial for binding and/or receptor activation. Whether these residues interact directly with the receptor binding site or are essential for the conformation of the ligand, cannot be decided on the basis of this study. This conclusion is consistent with the absence of effect of rGal (3-29) on rat fundus strips. Moreover, binding studies with Gal and galanin fragments and analogues performed on RIN m 5F cells in our laboratory have revealed that rGal (3-29) does not bind to the galanin receptor (W.E. Schmidt, B. Gallwitz, R. Schwarzhoff, personal communication). In this light, the lack of antagonistic properties of rGal (3-29) to the contractile action of Gal is not unexpected.

Earlier reports on discrepancies in pGal action on rat smooth muscle compared to other species have been attributed to the supposedly different amino acid sequence of pGal and rGal (Rokaeus, 1987). However, the present work suggests that reported differences in responses to galanin may be species and tissue-dependent and not a consequence of the difference in primary structures of the galanins. The fact that intact Gal, unlike N-terminal galanin fragments, reduced the cholinergic contractile response to electrical stimulation on guinea-pig taenia coli and rabbit iris sphincter (Ekblad *et al.*, 1985b) and inhibited somatostatin secretion of the endocrine pancreas of the dog (Hermansen *et al.*, 1988) raises the possibility of the existence of different galanin receptor subtypes. Further studies to support this hypothesis are in progress at present.

In conclusion, the C-terminal region contributes mainly to the affinity of Gal for its smooth muscle receptors while the N-terminal region plays a critical role for its biological activity, particularly the first two amino acids (Gly-Trp), which seem crucial for receptor activation.

The authors thank Dr B. Gallwitz for critical reading of the manuscript and Mrs M. Wilke for typing it.

References

AMIRANOFF, B., LORINET, A.-M., YANAIHARA, N. & LABURTHE, M. (1989). Structural requirement for galanin action in the pancreatic β-cell line Rin m 5F. *Eur. J. Pharmacol.*, **163**, 205–207.

BAUER, F.E., ADRIAN, T.E., CHRISTOFIDES, N.D., FERRI, G.-L., YANAIHARA, N., POLAK, J.M. & BLOOM, S.R. (1986). Distribution and molecular heterogeneity of galanin in human, pig, guinea pig, and rat gastrointestinal tracts. *Gastroenterology*, **91**, 877–883.

BISHOP, A.E., POLAK, J.M., BAUER, F.E., CHRISTOFIDES, N.D., CARLEI, F. & BLOOM, S.R. (1986). Occurrence and distribution of a newly discovered peptide, galanin, in the mammalian enteric nervous system. *Gut*, **27**, 849–857.

EKBLAD, E., ROKAEUS, A., HÅKANSON, R. & SUNDLER, F. (1985a). Galanin nerve fibers in the rat gut: distribution, origin and projections. *Neuroscience*, **16**, 355–363.

EKBLAD, E., HÅKANSON, R., SUNDLER, F. & WAHLSTEDT, C. (1985b). Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations. *Br. J. Pharmacol.*, **86**, 241–246.

EKBLAD, E., ARNBJÖRNSSON, E., EKMAN, R., HÅKANSON, R. & SUNDLER, F. (1989). Neuropeptides in the human appendix. Distribution and motor effects. *Dig. Dis. Sci.*, **34**, 1217–1230.

FONTAINE, J. & LEBRUN, P. (1989). Galanin: Ca^{2+}-dependent contractile effects on the isolated distal mouse colon. *Eur. J. Pharmacol.*, **164**, 583–586.

FOX, J.E.T., McDONALD, T.J., KOSTOLANSKA, F. & TATEMOTO, K. (1986). Galanin: an inhibitory neural peptide in the canine small intestine. *Life Sci.*, **39**, 103–110.

FURNESS, J.B., COSTA, M., ROKAEUS, A., McDONALD, T.J. & BROOKS, B. (1987). Galanin-immunoreactive neurons in the guinea pig small intestine: their projections and relationships to other enteric neurons. *Cell Tiss. Res.*, **250**, 607–615.

GREGERSEN, S., AHREN, B. & HERMANSEN, K. (1989). Galanin fragments and substitutions; effects on glucose stimulated insulin secretion from isolated rat islets. *Diabetologia*, **32**, 493A.

HERMANSEN, K., AHREN, B. & YANAIHARA, N. (1989). On the nature of galanin action on the endocrine pancreas. *Diabetologia*, **32**, 49A.

HOLZER-PETSCHE, U., SEITS, H. & LEMBECK, F. (1989). Effect of capsaicin on gastric corpus smooth muscle of the rat *in vitro*. *Eur. J. Pharmacol.*, **162**, 29–36.

KAPLAN, L.M., SPINDEL, E.R., ISSELBACHER, K.J. & CHIN, W.W. (1988). Tissue-specific expression of the rat galanin gene. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 1065–1069.

KATSOULIS, S. & CONLON, J.M. (1989). Calcitonin gene-related peptides relax guinea pig and rat gastric smooth muscle. *Eur. J. Pharmacol.*, **161**, 129–134.

KUWAHARA, A., OZAKI, T. & YANAIHARA, N. (1989). Galanin suppresses neurally evoked contractions of circular muscle in the guinea-pig ileum. *Eur. J. Pharmacol.*, **164**, 175–178.

MAGGI, C.A., SANTICIOLI, P., PATACCHINI, R., TURINI, D., BARBANTI, G., BENEFORTI, P., GIULIANI, S. & MELI, A. (1987). Galanin: a potent modulator of excitatory neurotransmission in the human urinary bladder. *Eur. J. Pharmacol.*, **143**, 135–137.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., TURINI, D., BARBANTI, G., BENEFORTI, P., MISURI, D. & MELI, A. (1989). Human isolated small intestine: motor responses of the longitudinal muscle to field stimulation and exogenous neuropeptides. *Naunyn Schmiedebergs Arch. Pharmacol.*, **339**, 415–423.

MELANDER, T., HÖKFELT, T., ROKAEUS, A., FAHRENKRUG, J., TATEMOTO, K. & MUTT, V. (1985). Distribution of galanin-like immunoreactivity in the gastro-intestinal tract of several mammalian species. *Cell Tiss. Res.*, **239**, 253–270.

OHHASHI, T. & JACOBOWITZ, D.M. (1985). Galanin potentiates electrical stimulation and exogenous norepinephrine-induced contractions in the rat deferens. *Regul. Pept.*, **12**, 163–171.

PALMER, J.M., SCHEMANN, M., TAMURA, K. & WOOD, J.D. (1986). Galanin mimics slow synaptic inhibition in myenteric neurons. *Eur. J. Pharmacol.*, **124**, 379–380.

ROKAEUS, A. (1987). Galanin: a newly isolated biologically active neuropeptide. *Trends Neurosci.*, **10**, 158–164.

ROKAEUS, A., MELANDER, T., HÖKFELT, T., LUNDBERG, J.M., TATEMOTO, K., CARLQUIST, M. & MUTT, V. (1984). A galanin-like peptide in the central nervous system and intestine of the rat. *Neurosci. Lett.*, **47**, 161–166.

TAMURA, K., PALMER, J.M., WINKELMANN, C.K. & WOOD, J.D. (1988). Mechanism of action of galanin on myenteric neurons. *J. Neurophysiol.*, **60**, 966–979.

TAMURA, K., PALMER, J.M. & WOOD, J.D. (1987). Galanin suppresses nicotinic synaptic transmission in the myenteric plexus of guinea pig small intestine. *Eur. J. Pharmacol.*, **136**, 445–446.

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

VRONTAKIS, M.E., PEDEN, L.M., DUCKWORTH, M.L. & FRIESEN, H.G. (1987). Isolation and characterization of a complementary DNA (Galanin) clone from estrogen-induced pituitary tumor messenger RNA. *J. Biol. Chem.*, **262**, 16755–16758.

YAU, W.M., DORSETT, J.A. & YOUTHER, M.L. (1986). Evidence for galanin as an inhibitory neuropeptide of myenteric cholinergic neurons in the guinea pig small intestine. *Neurosci. Lett.*, **72**, 305–308.

(Received March 15, 1990
Revised May 24, 1990
Accepted June 11, 1990)