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Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations

E. Ekblad[1], R. Håkanson, F. Sundler & C. Wahlestedt

Departments of Histology and Pharmacology, University of Lund, Lund, Sweden

1 The effects of galanin, a newly isolated neuropeptide, and of a galanin fragment (galanin 1–10) were studied on various smooth muscle preparations *in vitro*. Direct motor effects as well as effects on electrically induced (neuronally mediated) responses (neuromodulatory effects) were observed.

2 Both gatanin and galanin 1–10 evoked a strong contractile response in rat jejunal longitudinal muscle. This effect was a direct one on the smooth muscle.

3 Addition of galanin to guinea-pig taenia coli inhibited the contractile responses to electrical stimulation, mediated by endogenous substance P and acetylcholine. In the rabbit iris sphincter, galanin reduced the acetylcholine-mediated but not the substance P-mediated contraction evoked by electrical stimulation. The neuromodulatory effects seem to be presynaptic and require the whole or possibly only the C-terminal part of the galanin molecule, since galanin 1–10 was ineffective.

4 Rabbit femoral artery and vein, gastroepiploic and basilar arteries and guinea-pig trachea and main bronchi did not respond to either galanin or galanin 1–10.

Introduction

Recently, Tatemoto *et al.* (1983) isolated and characterized a 29 amino acid peptide from porcine upper small intestine. The peptide was found to have N-terminal glycine and C- terminal alanin amide and was named galanin. Immunocytochemistry revealed galanin immunoreactive nerve fibres in the mucosa, smooth muscle and intramural ganglia and around blood vessels of the gastro-intestinal tract (Rökaeus *et al.*, 1984; Ekblad *et al.*, 1985). A contractile effect of porcine galanin on strips of rat stomach, ileum, colon and urinary bladder was also demonstrated (Rökaeus *et al.*, 1984). It is not yet known if the motor effects of galanin are exerted on the smooth muscle directly or mediated by neuronal elements in the muscle.

The present study examines the effects of synthetic porcine galanin and galanin 1–10 on smooth muscle activity *in vitro* in a variety of preparations: guinea-pig taenia coli, trachea and main bronchi, rat small intestine, rabbit blood vessels and iris sphincter muscle.

[1] Author for correspondence at Department of Histology, Biskopsgatan 5, S-223 62 Lund, Sweden.

Methods

Sprague-Dawley rats (150–180 g), guinea-pigs (300–400 g) and pigmented rabbits (1.5–3 kg) of either sex were used. They were killed by a blow on the neck and exsanguinated.

Intestinal smooth muscle preparations

Taenia coli strip preparations (Burnstock *et al.*, 1966) from 15 guinea-pigs and jejunal strip preparations from 15 rats, consisting of the outer longitudinal smooth muscle layer with adherent myenteric ganglia were dissected out and placed in a modified Krebs solution (for composition see below) at 4°C. The strip preparations were mounted vertically on a Perspex holder in a 7 ml organ bath with the thermostat set at 37°C. One end was attached to a rigid support and the free end to a lever connected via a spring to a Grass FT 04 force displacement transducer for isotonic registration of mechanical activity (Ishida & Krakowa, 1974; Leander *et al.*, 1981b). The load on the muscle was set at 0.2 g. Platinum electrodes were placed around the muscle strip with a constant electrode distance of 5 mm. The electrodes were connected to a Grass S4C

stimulator for field stimulation with square wave pulses (10–15 V over the electrodes, 1 ms duration, 1–10 Hz frequency, pulse train lasting for 3 s). The polarity of the electrodes was reversed after each stimulation in order to avoid polarization. The mechanical activity was recorded continuously throughout the experiment on a Grass model 7 Polygraph. The preparations were allowed to equilibrate in the bath for 1 h before experimentation started.

Blood vessel preparations

The left gastro-epiploic artery, femoral artery and vein and basilar artery from six rabbits were dissected out and placed in a modified Krebs solution (for composition see below). Vascular segments, 2–3 mm long, were mounted on two L-shaped metal prongs in a tissue bath at 37°C (Högestätt *et al.*, 1983). Isometric tension was measured with Grass FT03 force-displacement transducers and recorded on a Grass Polygraph. The preparations were given a tension of 4 mN (arteries) or 2 mN (veins). Platinum electrodes were placed in the bath (electrode distance 20 mm). They were connected to a Grass S4 stimulator for field stimulation with square wave pulses (frequency 5–10 Hz, 5–15 V over the electrodes, duration 0.3 ms, pulse train lasting for 3 s). The specimens were allowed to equilibrate for 1 h before experiments were begun.

Iris sphincter muscle

The eyes were removed from six rabbits and opened by an incision 2–3 mm posteriorly to the limbus. The iris was excised from the ciliary margin, opened, freed from the dilator muscle (leaving the approx. 2 mm wide sphincter muscle) and cut in half. The two halves were mounted vertically on separate Perspex holders in 7 ml tissue baths maintained at 37°C. The medium was a modified Krebs solution (see below). The mechanical activity was recorded isometrically with a Grass FT03 force displacement transducer and Grass model 7 polygraph. Before starting the experiments the sphincter muscle was allowed to equilibrate for about 90 min under a constant tension of 1.5 mN maintained throughout the experiments. Electrical field stimulation with square wave pulses (14–17 V over the electrodes, 0.3 ms duration) was applied by means of a pair of platinum ring electrodes connected to a S4C stimulator. The preparations were stimulated with trains of pulses lasting for 10 s, the pulse frequency being 20 Hz.

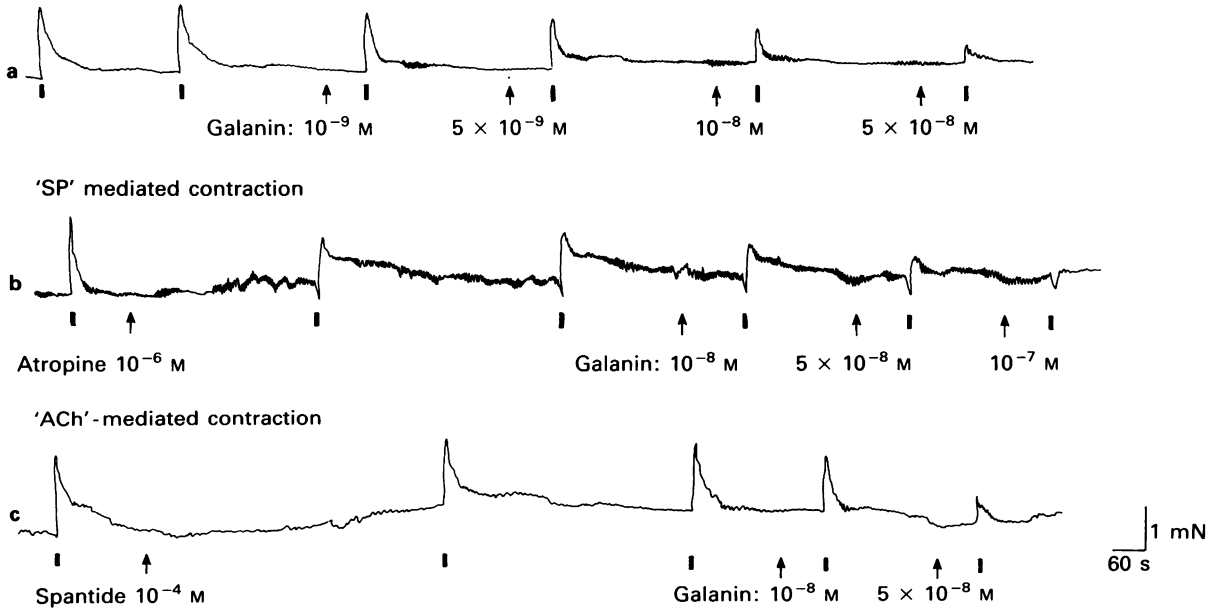

Figure 1 Typical tracings of the electrically induced contractions of guinea-pig taenia coli. Stimulation (10–15 V, 1 ms duration, 2 Hz in (a) and (c), 5 Hz in (b), was maintained for 3 s (indicated by black rectangles). (a) Electrical stimulation evoked a contractile response that was greatly reduced by galanin. The contractile response was also reduced in the presence of atropine (leaving the Spantide-sensitive contraction, probably mediated by substance P (SP) or related peptides) (b) or Spantide (leaving the atropine-sensitive contraction, probably mediated by acetylcholine (ACh)) (c). Galanin (10^{-9}–10^{-7} M) had no contractile effect by itself.

Airway preparations

The trachea and main bronchi were removed from five guinea-pigs. Ring preparations 3–4 mm long were obtained from the main bronchi and the trachea. The preparations were mounted on Perspex holders to allow the measurement of isometric tension. The medium was a modified Krebs solution (see below). An initial tension of 3 mN (bronchi) or 6 mN (trachea) was applied; the preparations were then allowed to equilibrate for at least 60 min. The preparations were stimulated electrically by means of two platinum electrodes placed on each side (electrode distance 20 mm) and a Grass S4C stimulator. Stimulation parameters were: 0.5 ms, 10-15 V over the electrodes, 10 Hz, pulse trains lasting for 10 s.

Experimental

The bathing fluid had the following composition (mM): NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. The solution was bubbled with a gas mixture of 5% CO_2 in O_2 giving pH of 7.2–7.3. The response to galanin and galanin 1–10 was tested by cumulative application of the peptide. The contractions are given in mN or in percentage of acetylcholine-induced (10^{-5} M) contractions (gut preparations only).

Drugs

Synthetic porcine galanin and substance P (SP) were obtained from Peninsula, Belmont, CA, U.S.A. Synthetic galanin 1–10 and Spantide, (D- Arg^1, D-$Trp^{7,9}$, Leu^{11})-SP, which is a potent SP antagonist (Folkers *et al.*, 1984), were generously provided by Ferring Pharmaceuticals, Malmö, Sweden (courtesy of Dr J. Trojnar). The purity of galanin and galanin 1–10 was better than 98% as indicated by high performance liquid chromatography and amino acid analysis. Atropine was purchased from ACO; Stockholm, Sweden, tetrodotoxin and acetylcholine from Sigma, St Louis, MO, U.S.A., carbachol from Merck, Darmstadt, FRG, and guanethidine from Ciba-Geigy, Basel, Switzerland.

Results

Guinea-pig taenia coli

Electrical stimulation produced frequency-dependent contraction. After blockade of muscarinic receptors with atropine (10^{-6} M), the response to stimulation at 5 Hz was biphasic: a relaxation followed by a contraction. Both responses could be blocked by tetrodotoxin (TTX) (10^{-6} M) (cf. Leander *et al.*, 1981a). The atropine-resistant contraction could be blocked by the adddition of Spantide, an SP antagonist (cf. Leander *et al.*, 1981b; Folkers *et al.*, 1984). After blockade of SP receptors the preparation responded to electrical stimulation at 2 Hz and above with a contraction that could be abolished by atropine (not shown). Galanin (10^{-9} – 5×10^{-7} M) was without effect on the basal tension of the taenia coli preparation but dose-dependently reduced the electrically induced contractile response ($n = 8$) (Figure.1a and 2a). In the presence of atropine (10^{-6} M), galanin reduced the electrically induced, Spantide-sensitive contraction ($n = 8$) Figure 1b and 2b), leaving the relaxation unaffected (Figure

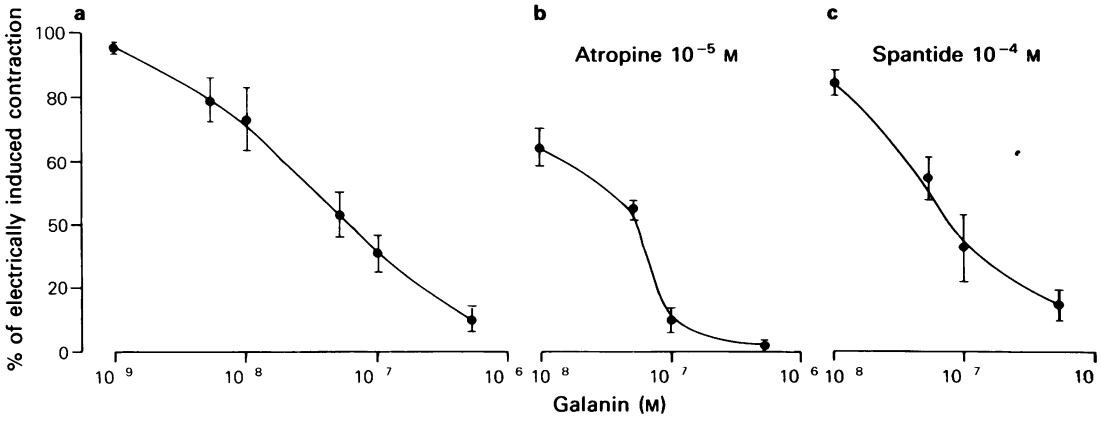

Figure 2 Concentration-response curves showing the inhibitory effect of galanin on the electrically induced contractions of guinea-pig taenia coli (a–c). By the addition of atropine (b) or Spantide (c) it could be shown that galanin is equally effective in inhibiting both the 'SP'-mediated (Spantide-sensitive) and the 'ACh'-mediated (atropine-sensitive) contractile responses. Vertical bars give s.e.mean ($n = 8$).

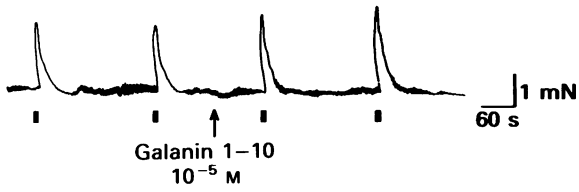

Figure 3 Typical tracing of the electrically induced contractions of the guinea-pig taenia coli. Stimulation (2 Hz, 10–15 V, 1 ms duration) for 3 s (black rectangles) evoked a contractile response that was unaffected by galanin 1–10.

1b). In the presence of Spantide (10^{-4} M), galanin reduced the electrically evoked atropine-sensitive contraction ($n = 8$) (Figure 1c and 2c). Galanin (5×10^{-7} M) did not affect the contractile response to exogenous SP (10^{-9} M) or to acetylcholine (10^{-5} M) ($n = 6$) (not shown). Galanin 1–10 (10^{-7} – 10^{-4} M) had no effect on the basal tension or the electrically induced responses ($n = 12$) (Figure 3).

Rat jejunum

Electrical stimulation evoked a frequency dependent contraction that was abolished by TTX (10^{-6} M) or atropine (10^{-6} M) (not shown). Addition of galanin (10^{-9} – 5×10^{-7} M) or galanin 1–10 (10^{-7} – 10^{-4} M) induced dose-dependent contractions that were unaffected by TTX (10^{-6} M), atropine (10^{-6} M) or Spantide (10^{-4} M) ($n = 7$–15) (Figure 4 and 5). The effectiveness and potency of galanin as a contractile agent prohibited studies on its possible neuromodulatory effects. The possibility that galanin and galanin 1–10 interacted with the same receptor population was tested in the following manner: near-maximal concentrations of galanin (2.5×10^{-8} M) and galanin 1–10 (5×10^{-6} M) were added to the bath separately and in combination. The contractions evoked by the two peptides were of similar magnitude. The contraction evoked by the two peptides in combination was not stronger than that induced by either peptide alone (not shown) ($n = 12$). Carbachol at 10^{-4} M evoked a much stronger contraction. The results suggest that galanin and galanin 1–10 interact with the same receptor population.

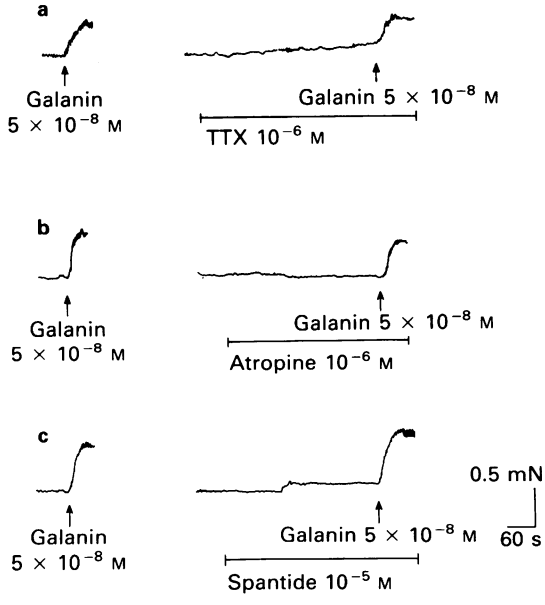

Figure 4 Typical tracings of the galanin-induced contractions in the longitudinal smooth muscle of rat jejunum. The contractile response was unaffected by tetrodotoxin (TTX) (a), atropine (b) or Spantide (c).

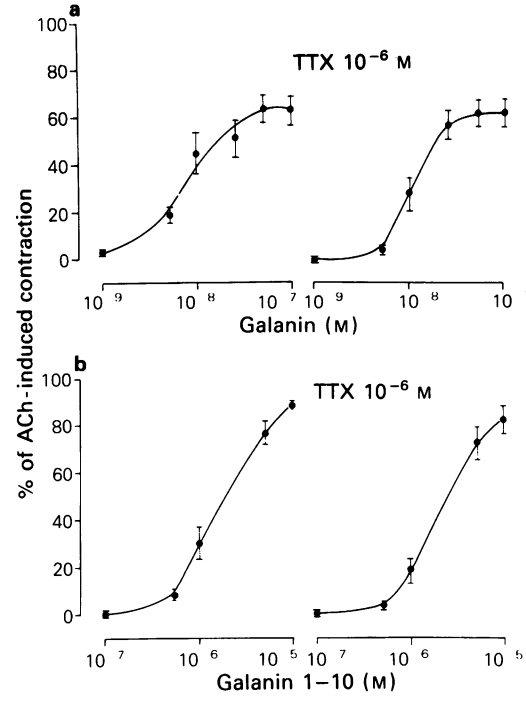

Figure 5 Concentration-response curves showing the contractile effect of galanin (a) and galanin 1–10 (b) on longitudinal smooth muscle of rat jejunum. Addition of tetrodotoxin (TTX) (right) was without effect on the response to galanin and galanin 1–10. Results after addition of atropine and Spantide were identical (not shown). Hence, the effect of galanin and galanin 1–10 is probably a direct one on the smooth muscle. The contractile responses are expressed as a percentage of the contraction induced by acetylcholine (ACh, 10^{-5} M). Each value is the mean of 7–15 experiments. Vertical bars give s.e.mean.

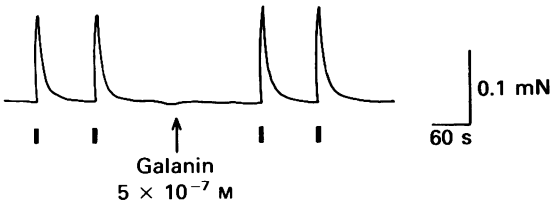

Figure 6 Typical tracing of the electrically induced constriction of the isolated gastro-epiploic artery of the rabbit. Stimulation (5 Hz, 5–15 V over the electrodes 0,3 ms) for 3 s (black rectangles) evoked a contractile response that was unaffected by the addition of galanin.

Rabbit blood vessels

Isolated vascular segments responded to electrical stimulation with a contraction that could be blocked with guanethidine (10^{-6} M) (not shown). Addition of galanin ($10^{-9} - 5 \times 10^{-7}$ M) did not induce contraction (tested on all the vessels listed in Methods), neither did it affect the electrically evoked contractions (tested on the femoral and gastro-epiploic arteries) ($n = 7$) (Figure 6).

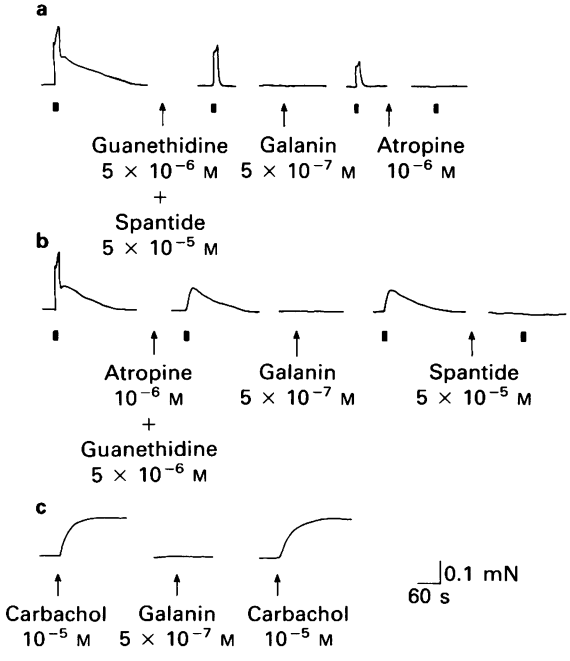

Figure 7 Responses of the isolated iris sphincter muscle of the rabbit to electrical stimulation (20 Hz, 0,25 ms; pulse trains lasting 10 s) and to exogenously applied agents. Galanin (5×10^{-7} M) alone did not induce any change in the muscle tone (a, b and c). The atropine-sensitive components in the response to electrical stimulation were attenuated by galanin (a), while the Spantide-sensitive component was unaffected (b). The response to carbachol (10^{-5} M) was sustained in the presence of galanin (c).

Rabbit iris sphincter

The isolated iris sphincter muscle did not respond to galanin *per se* ($10^{-9} - 10^{-6}$ M). In the presence of Spantide (5×10^{-5} M) and guanethidine (5×10^{-6} M), electrical stimulation evoked a biphasic twitch that could be completely blocked by atropine (10^{-6} M) (cf. Wahlestedt *et al.*, 1985). This twitch was dose-dependently reduced by galanin ($n = 8$) (Figure 7a) but not by galanin 1–10 ($10^{-7} - 10^{-4}$ M) ($n = 6$) (not shown). The effect of galanin could be reversed by extensive washing. After addition of galanin (10^{-7} M) the amplitude of the twitch was $62 \pm 4\%$ (mean $\pm$ s.e.mean, $n = 8$) of the original value; after washing, the amplitude was $82 \pm 4\%$. Control preparations not receiving galanin were run in parallel and showed no time-dependent decline of the twitch amplitude. Electrical stimulation evoked not only a cholinergic twitch but also a slow atropine- and guanethidine-resistant contraction that could be abolished by Spantide. This slow contraction was not affected by galanin ($n = 8$) (Figure 7b). Carbachol gave the same contraction regardless of whether or not galanin (5×10^{-7} M) was present ($n = 8$) (Figure 7c).

Guinea-pig airways

Galanin ($10^{-9} - 10^{-6}$ M) did not contract resting preparations of the isolated trachea or main bronchus ($n = 6$) (Figure 8), neither did it relax preparations contracted by carbachol (5×10^{-7} M). Further, galanin seemed ineffective in modulating the response to electrical stimulation in these specimens ($n = 6$) (Figure 8).

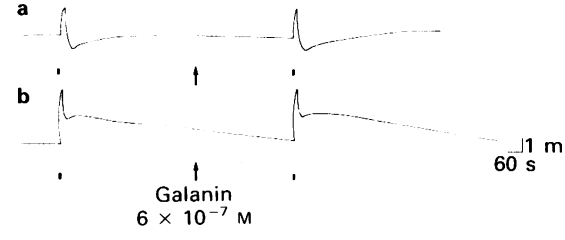

Figure 8 (a) Responses of guinea-pig trachea to electrical stimulation (10 Hz, 0,5 ms; pulse trains lasting 10 s) in the absence or presence of galanin (5×10^{-7} M). Galanin was ineffective in changing the muscle tone or in altering the response to electrical stimulation. (b) Responses of the guinea-pig bronchus to electrical stimulation (10 Hz, 0,5 ms; pulse trains lasting 10 s) in the absence or presence of galanin (5×10^{-7} M). Galanin was ineffective in changing the muscle tone or in altering the response to electrical stimulation.

Discussion

The results of the present study show that synthetic porcine galanin affects the various smooth muscle preparations differentially. Neither galanin nor galanin 1–10 had any motor effect on the guinea-pig taenia coli. Unlike galanin 1–10, galanin inhibited both the cholinergically mediated and the SP-mediated contractile responses to electrical stimulation. Since galanin did not affect the responses evoked by exogenously applied acetylcholine and SP, the effect of galanin on the guinea-pig taenia coli may be presynaptic, exerted by a suppression of the release of acetylcholine and SP. This is in contrast to the rat jejunum in which both galanin and galanin 1–10 evoked a strong contractile effect, unaffected by atropine, Spantide and TTX; hence, galanin and galanin 1–10 probably act directly on rat jejunal smooth muscle. Also, results from experiments with addition of galanin and galanin 1–10 separately and together suggested that the two peptides interact with the same receptor population on the small intestine. Galanin and galanin 1–10 had no direct effect on the rabbit iris sphincter. Unlike galanin 1–10, galanin reduced the cholinergically but not the SP-mediated contractile response to electrical stimulation. The effect on the iris seems to be presynaptic, since the contraction evoked by carbachol was unaffected. It may be noted that the SP nerves of the iris, that failed to respond to galanin, are sensory (Tervo *et al.*, 1982), while the SP nerves of the taenia coli, that responded to galanin are thought to have a motor function (Leander *et al.*, 1981a). Galanin had no effect on either the vascular tension or the electrically evoked, adrenergic vasoconstriction as studied in rabbit blood vessels. Guinea-pig airway preparations (trachea and main bronchi) also seemed unresponsive to galanin.

Like galanin, galanin 1–10 had a direct contractile effect on the rat jejunum but, unlike galanin, displayed no neuromodulatory effects on guinea-pig taenia coli or rabbit iris sphincter.

Taken together the results suggest that only certain types of smooth muscle preparations respond to galanin and that only certain populations of autonomic nerve fibres are affected by the peptide. The results further suggest that the smooth muscle receptor population responding to both galanin and galanin 1–10, recognizes the N-terminal portion of the peptide whereas the receptor population mediating the neuromodulatory effects of galanin requires the whole molecule or possibly only the C-terminal portion.

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