Br. J. Pharmacol. (1988), **94**, 1241–1249

Contribution of galanin to non-cholinergic, non-adrenergic transmission in rat ileum

[1]Ikunobu Muramatsu & *Noboru Yanaihara

Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan and *Department of Bioorganic Chemistry, Shizuoka College of Pharmacy, Shizuoka 422, Japan

1 We examined the possibility that the neuropeptide, galanin, may act as a transmitter in longitudinal muscle isolated from the rat ileum.

2 Galanin at nanomolar concentrations produced a phasic contraction with a concomitant increase in rhythmic activity. At concentrations in excess of 3×10^{-8} M, the contraction was followed by a rapid desensitization; hence, with the cumulative re-addition of galanin, there was no response. This desensitization was probably selective for galanin because there was no attenuation of the contractile responses to substance P, neurokinin A and B, bradykinin or carbachol.

3 The phasic contraction induced by galanin was not inhibited by atropine, guanethidine, hexamethonium, naloxone, tetrodotoxin or [D-Pro^2, D-Trp^{7,9}]-substance P.

4 Electrical stimulation of intramural nerves at low frequencies (1–5 Hz) led to an augmentation of spontaneous rhythmic contractions, which were completely or partially inhibited by atropine. However, guanethidine, hexamethonium, naloxone, [D-Pro^2, D-Trp^{7,9}]-substance P and desensitization to galanin were without effect on the response to such electrical stimulation.

5 In contrast, transmural electrical stimulation at higher frequencies in the presence of atropine and guanethidine produced biphasic contractile responses with transient and slow components. The slow component was selectively attenuated by galanin desensitization.

6 The slow component induced by high frequency stimulation was markedly attenuated by repeated electrical stimulation at short intervals (2.5 min between 30 s trains). Following repeated stimulation, the contractile response to galanin was also attenuated. Thus, a cross-desensitization between the mediator of the slow component and galanin had to be considered. In contrast, responses to tachykinins and the transient component induced by electrical stimulation were without effect.

7 Somatostatin, vasoactive intestinal polypeptide and α,β-methylene ATP were without effect on the tone of the muscle. Calcitonin gene-related peptide, neurotensin, gastrin-releasing peptide, neuropeptide Y and capsaicin produced either a transient arrest of the spontaneous rhythmic activity or a transient relaxation.

8 These results suggest that the slow component of the non-cholinergic non-adrenergic contraction, as induced by intramural nerve stimulation is apparently due to the endogenous release of galanin, presumably released from galanin-containing nerves in the rat ileum.

Introduction

Intestinal smooth muscle activity is affected by various non-cholinergic, non-adrenergic nerves, in addition to the classical cholinergic and adrenergic nerves, and stimulation of the intramural nerves can cause atropine- and guanethidine-resistant contractions or relaxations (Gershon, 1981; Burnstock, 1986). A number of neuropeptides have been considered to be transmitter candidates of non-cholinergic non-adrenergic neuro-effector transmission (Schultzberg *et al.*, 1980; Costa & Furness, 1982; Burnstock, 1986). Galanin was originally isolated from the intestinal tract of pigs (Tatemoto *et al.*, 1983) and galanin-like immunoreactivity has been detected in neural structures throughout the gastrointestinal tract of rats (Rökaeus *et al.*, 1984; Ekblad *et al.*, 1985b) and other mammalian species (Melander *et al.*, 1985; Rökaeus, 1987). Galanin exerts a direct contractile or relaxant effect on intes-

[1] Author for correspondence.

tinal smooth muscles of rat (Tatemoto *et al.*, 1983; Ekblad *et al.*, 1985a) and dog (Fox *et al.*, 1986), inhibits the release of acetylcholine and substance P from myenteric neurones (Ekblad *et al.*, 1985a; Yau *et al.*, 1986) and hyperpolarizes the myenteric neurones (Palmer *et al.*, 1986). Thus, galanin may be a neurotransmitter or neuromodulator in the gut (Rökaeus, 1987).

The present study describes the effects of galanin on intestinal smooth muscle and provides pharmacological evidence for the possible involvement of galanin in non-cholinergic, non-adrenergic transmission in the rat ileum.

Methods

Male Wistar rats (270–350 g) were killed by a blow on the head, exsanguinated from the common carotid arteries and the ileum removed. The strips were prepared by cutting along the longitudinal muscle layer under a dissection microscope; thus, the circular muscle and myenteric plexus remained on the longitudinal muscle strips. The width and length of the preparations were approximately 3 mm and 10 mm, respectively. Each preparation was mounted vertically in a plastic organ bath containing 3 ml of Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, $NaHPO_4$ 1.2 and glucose 11.5. The bathing medium was maintained at 37°C and aerated before and during each experiment with a mixture of 95% O_2 and 5% CO_2. Under these conditions, the pH (7.4) remained constant. A resting tension of 0.3 g was applied and maintained during the experiments; tension changes of longitudinal muscle were recorded isometrically with force-displacement transducers. Preparations were equilibrated for 90 min before starting the experiments. Drugs were added directly to the bath, and concentrations were calculated accordingly.

Electrical transmural stimulation was via a pair of platinum-wire electrodes (15 mm long, 0.6 mm diameter, 3 mm distance between the electrodes) from an electronic stimulator (Nihon Kohden, SEN-3201) and a current booster (Muramatsu, 1987). Stimuli parameters were of 0.3 ms duration and supramaximal current (50 mA) and were delivered in trains of 10 s duration, unless otherwise mentioned. Stimulus frequency was varied in the range from 1 Hz to 30 Hz.

Galanin was prepared by solid-phase technique (Yanagisawa *et al.*, 1986). Purity of the product was assessed by routine analytical criteria. The synthetic peptide was eluted as a single-peak at a retention time of 16.3 min in high performance liquid chromatography (h.p.l.c.) on a TSK GEL OEL ODS-120T column (Toyo Soda Co., Tokyo) (0.46 × 25 cm) in 0.01 N HCl/CH_3CN (80/20–60/40, v/v) over 30 min at a flow rate of 1.0 ml min^{-1}. The peptide was dissolved immediately before use in 0.01% acetic acid solution and added to the organ bath. The final concentration of acetic acid in the bath never exceeded 0.0005% (this concentration of acetic acid alone caused no significant effect). Other drugs were purchased: substance P, neurokinin A, [D-Pro^2, D-Trp^{7,9}]-substance P (SP), porcine vasoactive intestinal polypeptide, neurotensin, and gastrin-releasing peptide (Peptide Institute, Osaka, Japan), calcitonin gene-related peptide, neuropeptide Y and neurokinin B (Peninsula Laboratories, CA, U.S.A.), atropine sulphate and capsaicin (Merck Sharp & Dohme, PA, U.S.A), α,β-methylene ATP and carbachol (Sigma Chemical, MO, U.S.A.), guanethidine sulphate (Tokyo-Kasei, Tokyo, Japan), hexamethonium bromide (Nakarai, Kyoto, Japan), naloxone HCl (Endo Laboratories, New York, U.S.A.) and tetrodotoxin (Sankyo, Tokyo, Japan).

Statistical analysis was performed by Student's *t* test for paired data and all results are expressed as mean ± s.e.mean.

Results

Response to galanin

Figure 1 shows representative responses to various concentrations of galanin; each concentration of galanin was administered non-cumulatively at intervals of 1 h. Galanin caused a phasic contraction with concomitant augmentation of rhythmic activity. The contraction began within 5–10 s, gradually increased to a maximum at about 1 min and then reverted toward the baseline tension within several minutes. The minimum effective concentration was approximately 3×10^{-9} M and the maximum response was produced at 10^{-7} M, the contractile amplitude being about 80% of the response to 10^{-5} M carbachol. Close inspection revealed that duration of the phasic contraction induced at 10^{-7} M was shorter than that at lower concentrations. The contractile response to galanin was not affected by atropine (10^{-6} M), guanethidine (3×10^{-6} M), hexamethonium (10^{-5} M), tetrodotoxin (5×10^{-7} M), naloxone (10^{-5} M) or [D-Pro^2, D-Trp^{7,9}]-substance P (10^{-5} M) (5 experiments for each drug, Figure 2).

The contractile response to galanin was reproducible, provided the preparation was not exposed to galanin for more than 10 min and there were intervals of 40 min or longer between exposures. However, when galanin was cumulatively administered without exchanging the bath solution, the second response to galanin was markedly attenuated. Figure 3 (closed symbols) shows the time

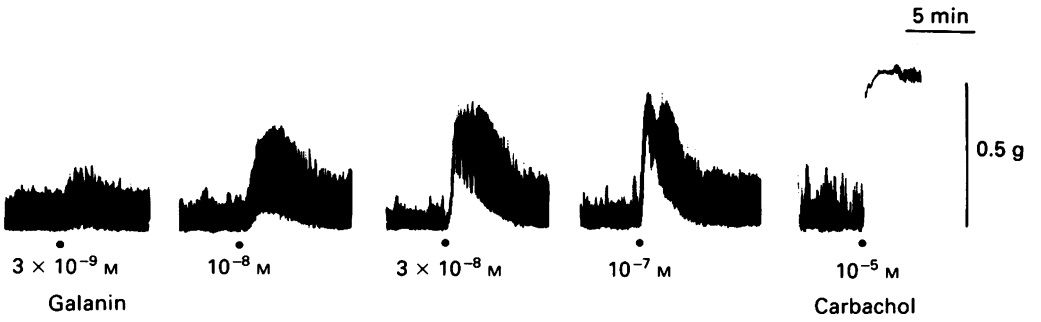

Figure 1 Representative tracings of the responses to various concentrations of galanin applied to a longitudinal muscle preparation isolated from rat ileum. Each response was recorded at intervals of 1 h, during which the bath medium was exchanged with the peptide-free solution at intervals of 20 min (three times on each occasion).

course of the reproducibility of the response to 3×10^{-8} M galanin during treatment with three different concentrations of galanin. Inhibition of the test response was complete within 10 min after the addition of 10^{-7} or 5×10^{-7} M galanin and then gradually diminished with an increase in the treatment period. This maximum inhibition could not be attributed to the disappearance or degradation of galanin in the bath solution, since the transfer of the galanin-containing medium from one preparation, which had been incubated with galanin for 5 or 10 min, to another preparation, caused a significant contraction (Figure 3, open circles). These data indicated the occurrence of a comparatively rapid desensitization phenomenon in galanin response.

Whether or not desensitization was selective to the galanin response was examined against the responses to other agents. The experiments were carried out in the presence of 10^{-6} M atropine and 3×10^{-6} M guanethidine in order to eliminate possible involvement in the test responses of cholinergic and adrenergic components. As shown in Figure 4, contractile responses to substance P (10^{-7} M), neurokinin A (10^{-7} M) and neurokinin B (10^{-7} M) were not affected by galanin desensitization (Table 1). Bradykinin (10^{-7} M) and carbachol (10^{-6} and 10^{-5} M, in the absence of atropine) also produced the same amplitude of contraction either before or after galanin desensitization (4 experiments for each drug, data not shown).

Table 1. Effects of various treatments on the contractile responses to galanin, tachykinins and transmural electrical stimulation (ES) on longitudinal muscle preparations of rat ileum

Test stimulus		% *response*[a] *after treatment with* *Galanin*[b]	[*D-Pro*², *D-Trp*⁷·⁹]-*SP*[b]	*Repeated ES*[c]
Galanin	3×10^{-8} M	2 ± 1[e] (6)	109 ± 4 (6)	29 ± 10[e] (6)
	10^{-7} M	4 ± 2[e] (5)	—[d]	20 ± 6[e] (5)
Substance P	10^{-7} M	94 ± 3 (5)	50 ± 6[e] (4)	108 ± 8 (4)
Neurokinin A	10^{-7} M	99 ± 3 (4)	46 ± 8[e] (4)	102 ± 4 (4)
Neurokinin B	10^{-7} M	91 ± 4 (4)	4 ± 6[e] (4)	100 ± 6 (4)
ES (20 Hz, 10 s)	transient component[f]	91 ± 3 (9)	101 ± 1 (6)	89 ± 4 (6)
	slow component[f]	37 ± 4[e] (7)	96 ± 2 (6)	19 ± 4[e] (5)

All responses were recorded in the presence of 10^{-6} M atropine and 3×10^{-6} M guanethidine.
Numbers in parentheses represent the number of experiments.
[a] % response compared with that before treatment with drug or repeated ES.
[b] Galanin (10^{-7} M) or [D-Pro², D-Trp⁷·⁹]-SP (10^{-5} M) was applied 7 min before and during application of test stimulus.
[c] 30 s trains of ES (30 Hz) were applied 5 times at intervals of 2.5 min, as shown in Figure 7, and the effects on test response were examined 2.5 min after the end of repeated ES.
[d] Not estimated.
[e] Significantly different from the time control obtained in parallel experiments with no treatment (paired t test, $P < 0.01$).
[f] The response to test ES (20 Hz, for 10 s) was divided into transient and slow components and the peak amplitude of each component was measured (see the text for further explanation).

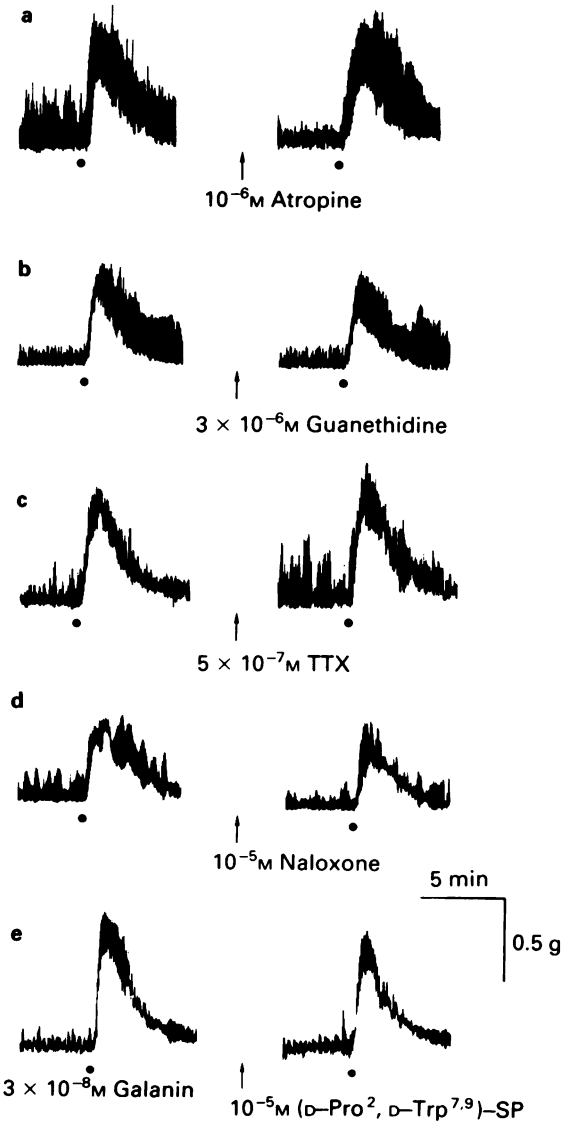

Figure 2 Effects of various drugs on responses to galanin (3×10^{-8} M) in longitudinal muscles of the rat ileum. Left: before. Right: after treatment with drug.

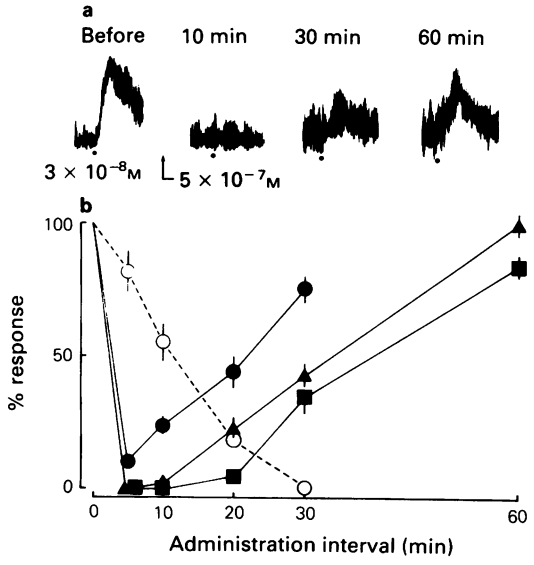

Figure 3 Reproducibility of galanin response after pretreatment with various concentrations of galanin. The upper panel shows a typical result, in which the responses to a test concentration of galanin (3×10^{-8} M) were recorded before and 10 min, 30 min and 60 min after treatment with 5×10^{-7} M galanin. In (b) closed symbols show the recovery time courses of the test response (3×10^{-8} M galanin) under treatment with three concentrations of galanin (●: 3×10^{-8} M; ▲: 10^{-7} M; ■: 5×10^{-7} M). The control response to galanin (3×10^{-8} M) before such treatment was taken as 100%, in each preparation; (○) show the relative contractions induced by galanin (3×10^{-8} M)-containing medium, which was incubated with one preparation for the period indicated on the abscissa scale and then transferred to another preparation. Four experiments for each symbol.

Among the other agents tested, capsaicin (10^{-6} M, $n = 6$), calcitonin gene-related peptide (5×10^{-7} M, $n = 5$), neuropeptide Y (10^{-7} M, $n = 4$), neurotensin (5×10^{-7} M, $n = 5$) and gastrin-releasing peptide (10^{-7} M, $n = 4$) produced a transient relaxation or suppressed the thythmic activity. Somatostatin (5×10^{-7} M, $n = 4$), vasoactive intestinal polypeptide (10^{-7} M, $n = 4$) and α,β-methylene ATP (10^{-5} M, $n = 5$) were without effect on the muscle tone. Thus, possible effects of galanin-desensitization on the responses to these drugs were not examined.

Responses to electrical transmural stimulation

Electrical transmural stimulation produced complex responses, which were variously influenced by stimulus frequencies and drugs. Figures 5 and 6 show representative results. At low frequencies (1, 3 or 5 Hz) the amplitude of rhythmic contraction was augmented during the stimulation period (10 s). At higher frequencies the muscle contracted promptly in response to electrical stimulation and reached a peak during the stimulation. This contraction was followed by a slow contractile phase. Atropine (10^{-6} M) abolished (in 10 preparations, Figure 5b) or attenu-

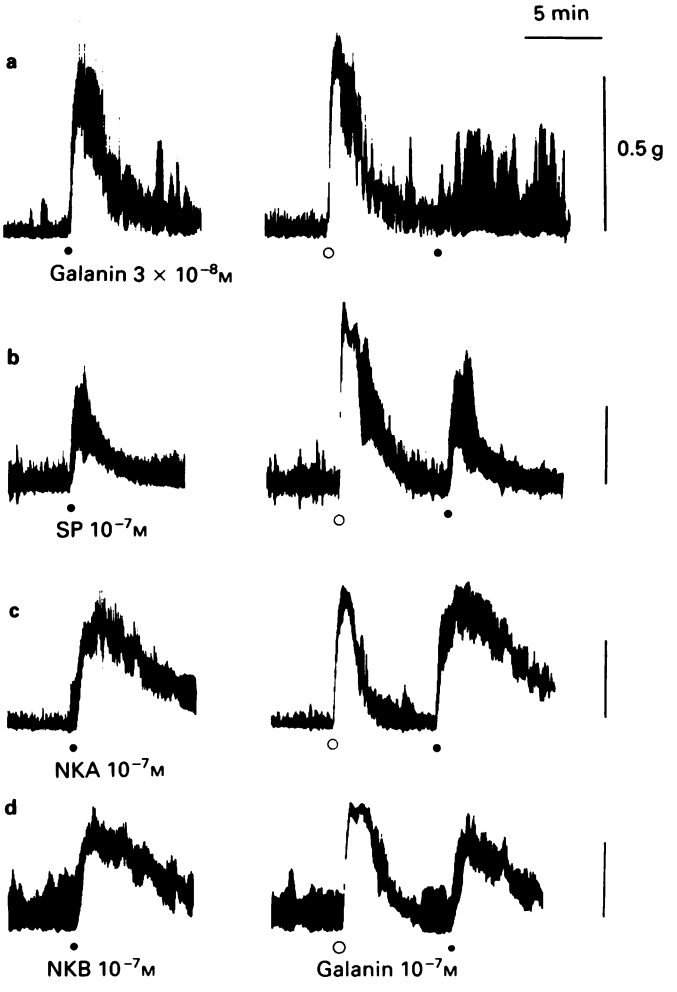

Figure 4 Effects of galanin desensitization on the responses to galanin, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). Galanin (10^{-7} M, open circle) was added 7 min before the test responses. Atropine (10^{-6} M) and guanethidine (3×10^{-6} M) were present throughout these experiments. Left side: controls.

ated (in 12 preparations, Figure 6a) the response to low frequency stimulation. The prompt contraction induced by high frequency stimulation was also inhibited (Figure 5b). Thus, in the presence of atropine and at high stimulus frequencies, two contractile phases were elicited (after initial transient arrest of spontaneous rhythmic activity): the 'transient component', which developed gradually, reached a peak immediately after the stimulation and then relaxed rapidly, and the 'slow component' which reached a peak more than 20 s after the stimulation and relaxed slowly. The slow component was frequently accompanied by an increase in rhythmic activity.

The contractile components induced in the presence of atropine and guanethidine were not affected by naloxone (10^{-5} M, $n = 4$), hexamethonium (10^{-5} M, $n = 4$) and [D-Pro^2, D-Trp^{7,9}]-substance P (10^{-5} M, $n = 6$), but were completely suppressed by tetrodotoxin (5×10^{-7} M, $n = 22$).

Galanin desensitization selectively inhibited the slow component induced by high frequency stimulation. In Figure 5c, the preparation was at first treated with a high concentration of galanin (10^{-7} M), thereafter 3×10^{-8} M galanin was cumulatively applied 3 min before each stimulation, the objective being to maintain and confirm the state of desensitization. The slow component was markedly

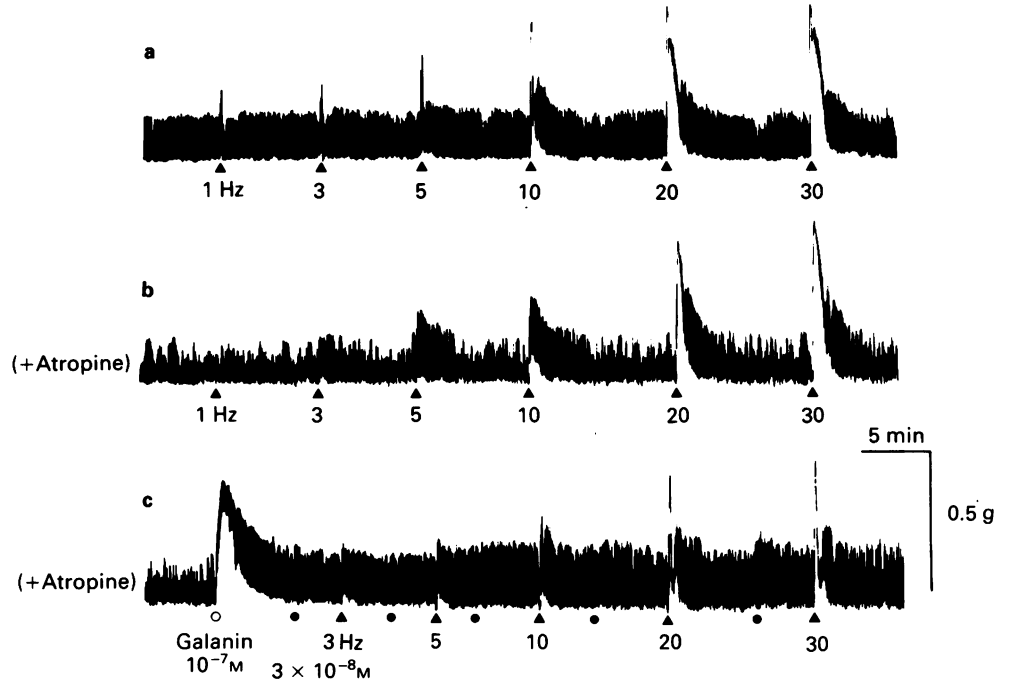

Figure 5 Representative recordings of the responses to electrical stimulation in the longitudinal muscle of rat ileum. The preparation was electrically stimulated at various frequencies for 10 s. All the records were obtained from a preparation treated with 3×10^{-6} M guanethidine. After recording the upper panel (a), the preparation was treated with 10^{-6} M atropine (b). In (c), the preparation was further treated with 10^{-7} M galanin; thereafter 3×10^{-8} M galanin was added repeatedly 3 min before each stimulation.

attenuated or abolished; thus electrical stimulation produced only a transient contraction which corresponded to the 'transient component' designated above.

The inhibition of the slow component after galanin treatment gradually diminished with the passage of time after the treatment period, as shown in Figure 6b. Complete recovery was obtained approximately 60 min after the treatment with 5×10^{-7} M galanin. Thereafter, further addition of the same concentration of galanin produced the same amplitude of contraction as the first one and again attenuated the slow component induced by electrical stimulation (20 Hz).

In contrast, galanin failed to inhibit the responses to low frequency stimulation. Figure 6a shows a representative result, in which the desensitization state was induced by repeated application of 10^{-7} M galanin. In this preparation, atropine (10^{-6} M) did not completely suppress the contractile response to electrical stimulation (5 Hz).

If the slow component were indeed caused by endogenously released galanin, then this endogenous galanin should also produce a state of desensitization. This possibility was examined with the use of strong stimulus conditions. Figure 7 shows representative results in which 30 s trains of stimuli (30 Hz) were applied 5 times at intervals of 2.5 min. In the course of repeated conditioning stimulations, the slow component was reduced more rapidly and more conspicuously than the transient component; thus the contraction became more transient in responses to later stimulation. After such conditioning stimulation, the response to exogenously applied galanin (10^{-7} M) and the slow component induced by test stimulation (20 Hz for 10 s) were markedly attenuated; this inhibition diminished with time after the conditioning stimulus (Figure 7a and c). However, the contractile responses to substance P, neurokinin A (Figure 7b) and neurokinin B were not affected by the conditioning stimulation. These results are summarized in Table 1.

Discussion

The present study clearly showed that the longitudinal muscle of the rat ileum is highly sensitive to

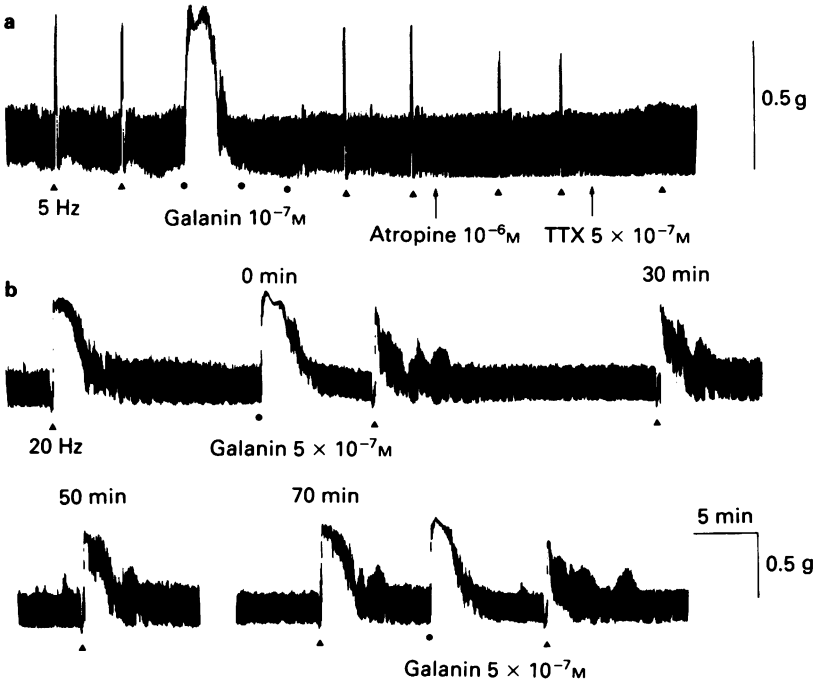

Figure 6 Effects of galanin desensitization on the responses to electrical transmural stimulation (5 or 20 Hz, for 10 s). In (a), 10^{-7} M galanin was cumulatively added three times (●). In (b) the preparation was treated with 10^{-6} M atropine and was stimulated electrically at 20 Hz. The time above the recordings represents the period after the first application of 5×10^{-7} M galanin. At the end of this experiment, the same concentration of galanin was again applied cumulatively. Guanethidine (3×10^{-6} M) was present throughout both these experiments. Records (a) and (b) were obtained from two different preparations.

galanin; at nanomolar concentrations, the peptide produced an augmentation of spontaneous rhythmic activity and a contraction. These effects of galanin were not inhibited by atropine, guanethidine, hexamethonium, tetrodotoxin, naloxone and [D-Pro^2, D-Trp^{7,9}]-substance P, suggesting that galanin acts directly on the smooth muscle of the rat ileum, probably through galanin receptors. Such a contractile action of galanin has been noted in the rat fundus, jejunum, colon and urinary bladder (Tatemoto *et al.*, 1983; Ekblad *et al.*, 1985a).

The response to galanin was transient in the rat ileum, the contraction reverting toward the baseline within several minutes and the cumulative re-addition of galanin no longer led to a response. It would appear that the phasic nature of the contraction and the rapid desensitization phenomenon reflect an intrinsic property of galanin receptors, rather than liberation by the tissue of an inhibitory substance or the disappearance from the bathing solution of a sufficient amount of galanin to cause contraction (Figure 3). In fact, no inhibitory action was evident in the desensitized state, with regard to responses to substance P, neurokinin A and B, carbachol, bradykinin and electrical transmural stimulation (at low frequency). Furthermore, the duration of phasic contraction induced by 10^{-7} M galanin was slightly shorter than that with 3×10^{-8} M galanin (Figure 1). The state of desensitization lasted for a longer period with higher concentrations of galanin.

At present, an antagonist selective to galanin is not available. Since the rat intestine is innervated by many types of peptidergic nerves, including galanin-containing ones (Schultzberg *et al.*, 1980; Melander *et al.*, 1985; Ekblad *et al.*, 1985b), the desensitization phenomenon observed in the present study can serve as an appropriate probe for analysing the galaninergic innervation, as in the case of the demonstration of transmission mediated by substance P in the guinea-pig ileum (Gintzler & Scalisi, 1982).

Electrical transmural stimulation produced a complex pattern in the longitudinal muscle of rat ileum. However, the responses were completely inhibited by tetrodotoxin, indicating that all the responses elicited were neurogenic. Under treatment with atropine and guanethidine, there appeared to

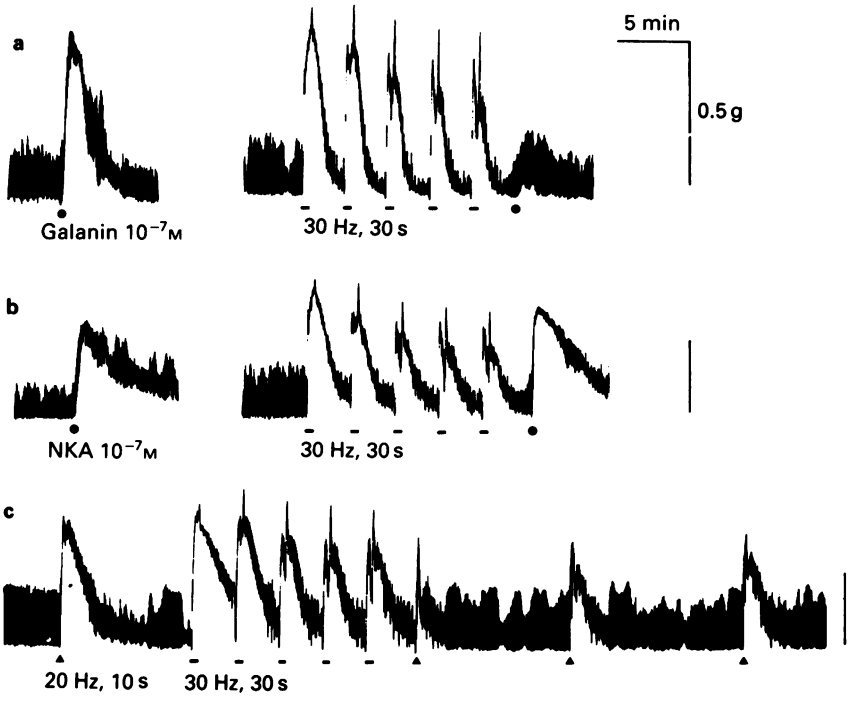

Figure 7. Response to repeated application of electrical stimulation at a high frequency (30 Hz) and its effects on responses to galanin (a), neurokinin A (b) and electrical stimulation (c: 20 Hz, for 10 s). The responses to galanin, neurokinin A and electrical stimulation (20 Hz, 10 s) were recorded before and 2.5 min after 5 trains of stimuli (30 Hz for 30 s) at intervals of 2.5 min. The preparations were treated with 10^{-6} M atropine and 3×10^{-6} M guanethidine throughout the experiments. Each of the three results was obtained from a different preparation.

be two components to the contractile response, namely, an initial transient and a subsequent, slow component. Several features of the slow component are similar to those observed with the response to galanin. For example, at moderate stimulus frequencies (such as 10 Hz) or at low concentrations of galanin (3×10^{-9} or 10^{-8} M) an augmentation of rhythmic activity was dominantly elicited, while at higher frequencies or at higher concentrations the muscle produced a phasic contraction. Secondly, both the slow component and the galanin response were resistant to atropine, guanethidine, [D-Pro^2, D-Trp^{7,9}]-substance P, hexamethonium or naloxone. Thirdly, both the responses were inhibited by galanin desensitization and the inhibition diminished with time. Furthermore, repeated application of long train stimuli at a high frequency with short intervals resulted in a gradual decline of the slow component; after such potent stimulation, not only the slow component but also the response to exogenous galanin were markedly attenuated. This provides evidence for a cross-desensitization between the mediatior of the slow component and exogenous galanin. Immunocytochemically, galanin-immunoreactive fibres were found to innervate directly the smooth muscle of the rat small intestine (Ekblad *et al.*, 1985b). The present results strongly suggest that the slow component elicited by electrical stimulation may be caused by endogenous galanin.

Diverse pharmacological activities of galanin have been noted in many tissues (Rökaeus, 1987). Prejunctionally, galanin reduces release of acetylcholine and substance P in the guinea-pig taenia coli and rabbit iris sphincter (Ekblad *et al.*, 1985a; Yau *et al.*, 1986) and suppresses nicotinic synaptic transmission in the myenteric plexus of guinea-pig ileum (Tamura *et al.*, 1987). Such prejunctional effects may be involved in the inhibition of the slow component observed in the present study. However, the cholinergic and non-cholinergic responses induced by low frequency stimulation were not inhibited by galanin in the rat ileum. Further, the slow component was evoked in the presence of hexamethonium, with no reduction in the amplitude. Thus, it appears that attenuation of the slow component after galanin treatment is mainly caused by postjunctional event(s), that is by galanin desensitization, rather than by prejunctional inhibition of the transmitter release. However, inhi-

bition of the slow component after repeated application of strong stimulation (Figure 7) may in part reflect a transient reduction in the amount of releasable transmitter during repetitive stimulation, in addition to galanin desensitization.

In addition to the slow component, electrical stimulation produced a transient contractile component in the presence of atropine and guanethidine. Lack of any inhibitory effect of galanin desensitization on the transient contraction indicates that all components of the non-cholinergic contraction cannot be accounted for only by the 'galaninergic' mechanism.

The digestive tract of mammals is rich in neuropeptides (Schultzberg *et al.*, 1980; Melander *et al.*, 1985; Ekblad *et al.*, 1985b). Among the gut neuropeptides demonstrated immunochemically and immunocytochemically, somatostatin and vasoactive intestinal polypeptide produced no change in the muscle tone of rat ileum. Calcitonin gene-related peptide, neurotensin, gastrin-releasing peptide and neuropeptide Y relaxed the muscle or suppressed the rhythmic activity. Although three known mammalian tachykinins produced a contraction, the response was inhibited by [D-Pro^2, D-Trp^{7,9}]-substance P. These results suggest that such neuropeptides are not directly involved in the galanin-resistant, non-cholinergic non-adrenergic contractions induced by electrical stimulation.

In conclusion, the present study provides pharmacological evidence that the slow component of the non-cholinergic non-adrenergic contraction elicited by transmural electrical stimulation may be caused by endogenous galanin, presumably released from galanin-containing nerves in the rat ileum.

We thank M. Ohara for reading the manuscript and N. Yamada for secretarial assistance. This work was supported in part by a grant from the Ministry of Education, Japan and by a grant from the Smoking Research Foundation in Japan.

References

BURNSTOCK, G. (1986). The changing face of autonomic neurotransmission. *Acta Physiol. Scand.*, **126**, 67–91.

COSTA, M. & FURNESS, J.B. (1982). Neuronal peptides in the intestine. *Br. Med. Bull.*, **38**, 247–252.

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

EKBLAD, E., RÖKAEUS, Å., HÅKANSON, R. & SUNDLER, F. (1985b). Galanin nerve fibers in the rat gut: distribution, origin and projections. *Neurosci.*, **16**, 353–363.

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

GERSHON, M.D. (1981). The enteric nervous system: multiplicity of neurotransmitters outside of the brain. In *Smooth Muscle: An Assessment of Current Knowledge.* ed. Bülbring, E., Brading, A.F., Joes, A.W. & Tomita, T. pp. 263–284. London: Edward Arnold.

GINTZLER, A.R. & SCALISI, J.A. (1982). Effects of opioids on noncholinergic excitatory responses of the guinea-pig isolated ileum: Inhibition of release of enteric substance P. *Br. J. Pharmacol.*, **75**, 199–205.

MELANDER, T., HÖKFELT, T., RÖKAEUS, Å., FAHRENKRUG, K., TATEMOTO, K. & MUTT, V. (1985). Distribution of galanin-like immunoreactivity in the gastrointestinal tract of several mammalian species. *Cell Tissue Res.*, **239**, 253–270.

MURAMATSU, I. (1987). The effect of reserpine on sympathetic, purinergic neurotransmission in the isolated mesenteric artery of the dog: a pharmacological study. *Br. J. Pharmacol.*, **91**, 467–474.

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.

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

RÖKAEUS, Å., 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.

SCHULTZBERG, M., HÖKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J.F., BROWN, M., ELDE, R., GOLDSTEIN, M. & SAID, S. (1980). Distribution of peptide- and catecholamine containing neurones in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalines, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine β-hydroxylase. *Neurosci.*, **5**, 689–744.

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

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

YANAGISAWA, M., YAGI, N., OTSUKA, M., YANAIHARA, C. & YANAIHARA, N. (1986). Inhibitory effects of galanin on the isolated spinal cord of the newborn rat. *Neurosci. Lett.*, **70**, 278–282.

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

(Received November 24, 1987
Revised March 26, 1988
Accepted April 15, 1988)