Non-Junctional Modulation of Neurogenic Twitches of the Guinea-Pig Ileum by Some Peptides and Other Compounds in the Triple Bath

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Abstract. The effects of some neuropeptide transmitter candidates and of some other neurotoxins or drugs on conduction of neural excitation were studied in myenteric plexus-longitudinal muscle strips from the guinea-pig ileum. A preparation in a special triple bath was drawn through two rubber membranes dividing the strip into three segments. Neurogenic stimulation of the oral segment set up nerve action potentials propagating aborally across the middle segment so that the aboral segment might also be invaded. Drugs were added to the middle segment to affect neuronal propagation (non-junctional effects) which was monitored by twitch amplitude of the aboral segment. The application of bradykinin and cromakalim did not affect aboral twitches although strong contractile and relaxatory effects were observed when the drugs were applied directly to the aboral segment; no neurogenic effects thus manifested. Capsaicin and neurotensin, when applied both to the middle and aboral segments, elevated the tone of the preparations accompanied with a decrease in twitch amplitude; these effects may have been due to neurogenic stimulation and release of other motor neurotransmitters. The application of VIP, apamin and dendrotoxin to the middle as well as to the aboral segments augmented aboral twitches, which might be at least partly due to facilitation of nerve action potential propagation in nerve terminals of cholinergic motor fibres.

Key words: Guinea-pig ileum — Neurogenic twitches — Neuropeptides — Non-junctional modulation

Introduction

Electrical stimulation of preparations of the guinea-pig ileum excites postganglionic cholinergic fibres of the myenteric plexus; action potentials generated in the nerve fibres propagate to varicose terminals which release acetylcholine from the axon varicosities and thus evoke contractions of the longitudinal muscle (Paton et al. 1971). Action potentials may either fail to propagate as far as the terminal endings (Stjärne 1978; Morita and North 1981; Kadlec et al. 1984); or, the failure of all the varicosities to participate in transmission may also be due to the intermittent nature of depolarization-secretion coupling (Cunnane and Stjärne 1982; Brock and Cunnane 1988). These hypotheses prompted us to investigate the possibility whether the conduction of action potentials within the varicose terminal could be modulated; previously, drugs known to affect calcium 'utilization' (Kadlec et al. 1987) or substance *P* (Ševčík et al. 1990) were found to be effective in this respect; now we investigated the actions of some putative neuropeptide transmitters. In our previous works the method of neurogenic stimulation of myenteric plexus-longitudinal muscle strip from the guinea-pig ileum in a triple bath was described (Kadlec et al. 1985). According to this method, the strip is pulled through two rubber membranes dividing it into three segments, supplied separately by perfusing solutions. The site of stimulation at the oral segment is separated by a 10 mm wide middle segment from the aboral segment from which the contractions are recorded. Thus, nerve action potentials generated in the oral segment propagate through the interconnecting middle segment and may even partially invade varicose nerve terminals extending up to the aboral segment to trigger twitches there (Fig. 1). The addition of drugs to the middle segment containing the varicose terminals allows to selectively affect the propagation of nerve action potentials in them and to monitor the non-junctional effects by twitch amplitude of the aboral segment. The effects of some peptide neurotransmitter candidates (VIP, neurotensin and bradykinin) and of some compounds used as pharmacological tools known to affect potassium channels (animal and plant toxins apamin, dendrotoxin and capsaicin, as well as cromakalim) were tested in our experimental arrangement; the effect of substance *P* was reported elsewhere (Ševčík et al. 1990).

Materials and Methods

The experiments were carried out on pieces of the central part of ileum isolated from male short-hair guinea-pigs weighing 200—400 g. Myenteric plexus-longitudinal muscle strips, 40 mm long, were prepared as described by Paton et al. (1971) and were mounted into a groove (4 mm wide, 3 mm deep and 40 mm long) in a conventional sucrose-gap apparatus (Burnstock and Straub 1958; Kadlec et

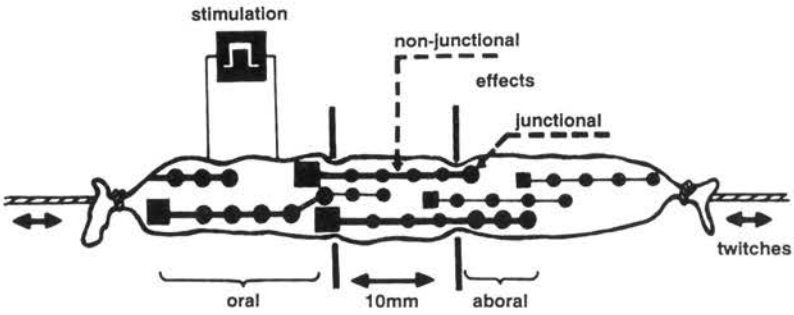

Fig. 1. Wiring diagram of cholinergic neurons of the strip preparation in the triple bath. Stimulation was applied to the oral segment, the excited neurons are represented by full squares and the thick and enlarged lines and circles represent axons and varicosities participating in conduction of action potentials and transmitter secretion responsible for contractions monitored. Judged from effects on aboral twitches, drugs applied to the middle segment affected conduction of action potentials toward the aboral segment (non-junctional effects); on the other hand, the application of drugs to the aboral compartment primarily affected transmitter release and smooth muscle contractions (junctional effects).

al. 1974; 1985). Two rubber membranes divided the groove into 3 compartments with separate supplies (1 ml/min each) of the bathing medium filling the groove up to the brim. The bathing medium was Krebs solution of the following composition (mmol/l): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1.2, and glucose 11.5, gassed with a stream of 95 % O_2 and 5 % CO_2. The strip was pulled through narrow openings in the membranes so that the oral and aboral segments (15 mm each) were in the peripheral compartments; the middle compartment was 10 mm wide. At the end of some experiments methylene blue was added to the solution supplying the middle compartment in order to check whether there was leakage to the peripheral compartments through the rubber membranes.

After the equilibration period, rectangular pulses of 0.2 ms duration (16—20 V) were applied at a frequency of 0.04 Hz to the oral segment by means of a pair of platinum wire electrodes. Submaximal isometric twitch responses were recorded separately from each peripheral segment. These were twitches of the oral segment evoked by local stimulation; moreover, such stimulation of the oral segment generated nerve impulses propagating across the middle segment and reaching to the aboral segment to trigger twitches there. The responses were neurogenic as the addition of tetrodotoxin (1 μmol/l) to the oral, middle or aboral segments separately always abolished the contractions of the aboral segment (Kadlec et al. 1985).

In five min periods preceding the addition of a drug, the average of 12 twitch amplitudes was calculated and taken for 100 %. In the presence of a drug the average of 6 consecutive twitch amplitudes showing the largest effect was calculated and expressed in percent of the respective control. Apart from affecting twitch amplitude, basal tone was changed in some preparations. However, only the amplitude of twitches was considered in the evaluation of drug effects. Contractions were also evoked by bolus addition of acetylcholine (1.1 pmol in 6.5 μl) added into the solution superfusing the aboral segment immediately before reaching it.

The agents used were: acetylcholine chloride, apamin, bradykinin, capsaicin, neurotensin, tetrodotoxin and vasoactive intestinal peptide (Sigma); cromakalim (Beecham); and dendrotoxin (DpI or toxin I from black mamba *Dendroaspis polylepsis*, a gift from Prof. A. L. Harvey).

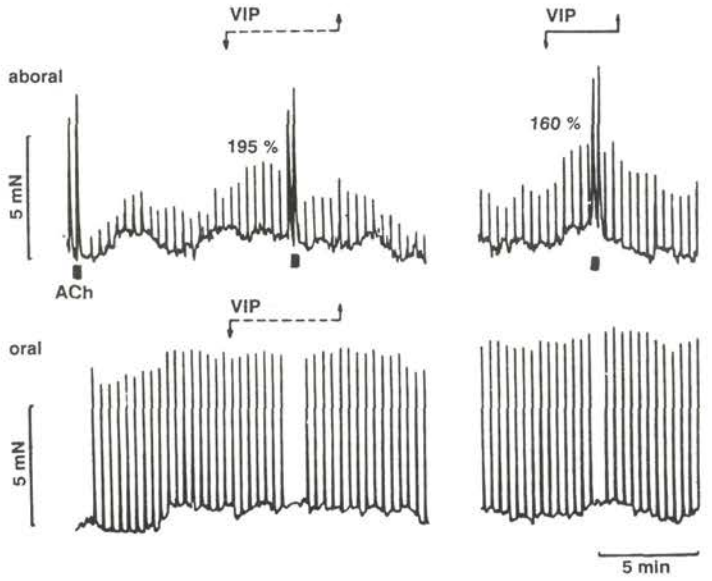

Fig. 2. The effect of VIP (100 nmol/l) on contractions of a strip preparation in the triple bath. Neurogenic stimulation (0.04 Hz) applied to the oral segment evoked twitches of both oral (*lower row*) and aboral (*upper row*) segments. VIP was added either to the middle segment (*left panels*; broken lines); or to the aboral segment (*right panels*; full line); percent changes in twitch amplitude are shown above each panel. Double addition of acetylcholine (Ach; 1.1. pmol bolus) to the aboral segment is indicated by full rectangles.

The results were expressed as means ± S. E. M., the numbers of experiments are shown in parentheses. The significance of differences was assessed with Student's two-tailed *t*-test for paired and unpaired data as indicated.

Results

Twitches of both segments were always evoked by neurogenic stimulation applied to the oral segment. Compounds were first added directly to the contracting aboral segment to study their junctional effects. Further, compounds were added to the middle segment to study their non-junctional effects.

Vasoactive intestinal peptide (VIP) dose-dependently augmented the amplitude of aboral segment twitches when applied both to the middle and aboral segments (Fig. 2). Dendrotoxin and apamin evoked similar effects (Table 1).

Capsaicin when applied both to the middle and aboral segments transiently elevated the tone of the aboral segment and the twitches were depressed. Neurotensin applied to the middle compartment caused a similar effect; its

Table 1. The effects of different compounds on twitches of the aboral segment of the strip preparation in the triple bath. Stimulation evoking twitches of the aboral segment was applied to the oral segment. Acetylcholine was given as a bolus into the solution superfusing the aboral segment. The compounds were superfusing either the middle or the aboral segment as indicated. The values are percentages of the control amplitude obtained before the addition of a drug in each group. Means ± SEM, figures in the parentheses indicate number of experiments, $^{*}p < 0.05$, $^{**}p < 0.005$.

Added to segment:	Middle		Aboral	
Compounds (nmol/l)	Twitches	Acetylcholine	Twitches	Acetylcholine
VIP 10	126 ± 9* (10)	98 ± 6 (9)	133 ± 6** (8)	101 ± 7 (8)
100	179 ± 14** (4)	106 ± 8 (6)	154 ± 13** (3)	95 ± 8 (3)
Dendrotoxin 10	157 ± 13** (14)	90	168 ± 17** (20)	113 ± 6 (11)
Apamin 100	135 ± 9* (6)	105 ± 7 (10)	159 ± 7** (6)	112 ± 7 (10)
Capsaicine 1000	63 ± 6** (7)	95 ± 6 (15)	64 ± 5** (8)	101 ± 4 (14)
Neurotensin 10	67 ± 9* (6)	105 ± 9 (10)	1st 53 ± 7** (6)	63 ± 4** (10)
			2nd 113 ± 9 (6)	
Bradykinin 100	84 ± 8 (5)	100 ± 6 (9)	26 ± 2** (5)	21 ± 7** (10)
Cromakalim 1000	96 ± 3 (8)	89 ± 6 (16)	29 ± 7** (9)	12 ± 4** (16)

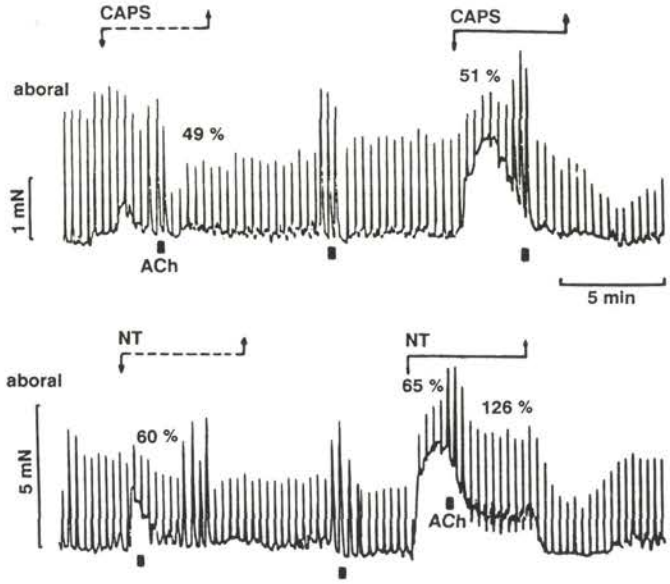

Fig. 3. Non-junctional effects of capsaicin (CAPS; 1 μmol/l; *upper panel*) and neurotensin (NT; 10 nmol/l; *lower panel*) on twitches of the aboral segment. Drugs were added either to the middle segment (broken lines) or to the aboral segment (full lines); percent changes in twitch amplitude are shown above each panel. Repeated addition of acetylcholine (Ach; 1.1 pmol, bolus) to the aboral segment is indicated by full rectangles.

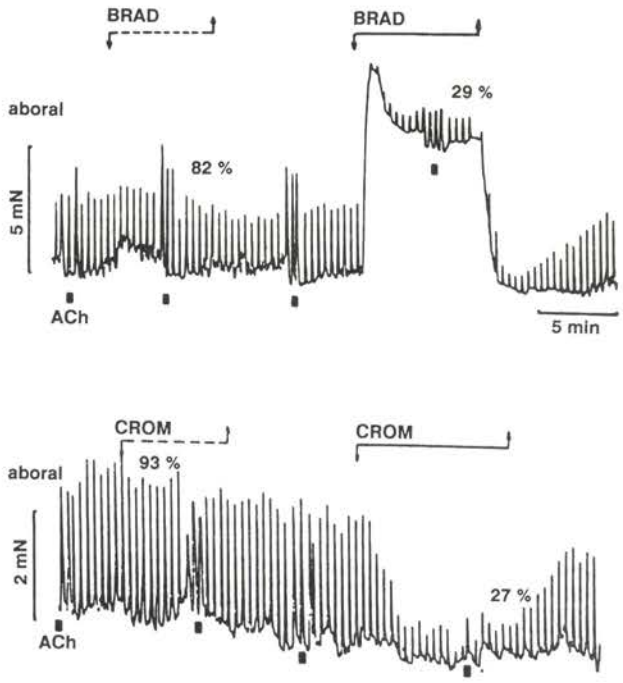

Fig. 4. Non-junctional effects of bradykinin (BRAD; 100 nmol/l; *upper panel*) and cromakalim (CROM; 1 μmol/l; *lower panel*) on twitches of the aboral segment. Drugs were added either to the middle segment (broken lines) or to the aboral segment (full lines); percent changes in twitch amplitude are shown above each panel. Triple addition of acetylcholine (Ach; 1.1. pmol bolus) to the aboral segment is indicated by full rectangles.

application to the aboral compartment also transiently elevated the tone with reduced twitch amplitude in the 1st phase; after 3 min the tone partially subsided and twitch amplitude was no longer reduced in this 2nd phase of the drug action (Fig. 3).

Bradykinin and cromakalim did not affect aboral twitches when applied to the middle segment; when applied to the aboral segment bradykinin contracted it so that twitches were reduced, whereas cromakalim suppressed the amplitude of twitches (Fig. 4).

The contractions evoked by the bolus addition of acetylcholine to the aboral segment were not significantly affected by any compound applied to the middle segment (Table 1). When the compounds were applied directly to the contracting aboral segment, these contractions were unaffected by VIP, dendrotoxin, apamin and capsaicin. Neurotensin and bradykinin elevated the tone

of the aboral segment and reduced the amplitude of contractions evoked by acetylcholine; this depression was proportional to the elevation of tone (Figs. 3 and 4). Cromakalim largely suppressed or even abolished the contractions evoked by acetylcholine (Fig. 4).

Discussion

The contractions of myenteric plexus-longitudinal muscle strips evoked by electrical stimulation in these and previous experiments were neurogenic and cholinergic as they were completely abolished by tetrodotoxin and atropine (Kadlec et al. 1985). Electrical stimulation triggered action potentials in cholinergic neurones of the oral segment of myenteric plexus-longitudinal muscle strips; action potentials propagated in axons projecting aborally across the middle segment, reached the aboral segment and induced twitches there. The addition of drugs to the middle segment thus could affect propagation of action potentials in nerve terminals (Fig. 1); as a result, a variable fraction of only some terminals of the aboral segment was reached by action potentials and this non-junctional effect was monitored by twitch amplitude of the aboral segment (Kadlec et al. 1987; 1990).

The possibility that the aboral segment could be activated by the passage of the propagating muscle action potential generated in the middle or oral segments was excluded in the experiments with papaverine (Kadlec et al. 1987). D-Tubocurarine or hexamethonium were also added to the middle compartment with no effect on the aboral segment contractions evoked by oral segment stimulation; the participation of a chain of cholinergic interneurones in the aboral segment contraction was thus unlikely (Kadlec et al. 1985). However, the possibility remained open that more cholinergic neurones were recruited by other interneurones which utilize e. g. peptides as their neurotransmitters (North 1982). Therefore, some neurotransmitter candidates as well as some compounds used as pharmacological tools to modify the effect of neurotransmitters were tested to detect their non-junctional effects.

The application of VIP to the guinea-pig ileum excites myenteric cholinergic neurones (Williams and North 1979; Yau et al. 1986a). In the present experiments VIP augmented only neurogenic responses both when applied to the middle or aboral segment. Apamin and dendrotoxin that could block certain types of calcium-dependent potassium channels (Habermann 1984; Harvey and Anderson 1985) evoked effects similar to VIP as well as to substance *P* (Ševčík et al. 1990). Substance *P* was reported to stimulate prejunctional cholinergic terminals in the myenteric neurones by an enhanced Ca^{2+} conductance and suppressed K^+ conductance in the neuronal membrane (Kadlec et al. 1984; Yau

et al. 1985; Hanani et al. 1988). Thus, substance *P*, and perhaps also VIP, apamin and dendrotoxin, might facilitate propagation of nerve action potential into the distal regions of these terminals (Ševčík et al. 1990). The effect of substance *P* was much larger when the drug was added to the aboral segment; the effect of VIP applied to the middle segment was however not smaller than the effect of the aboral application. This could suggest that VIP was more selectively affecting propagation than the subsequent processes of transmitter secretion and postjunctional action.

Capsaicin, neurotensin and bradykinin contracted aboral segment when applied to it. The contraction was prominent with bradykinin so that twitches and acetylcholine-evoked contractions were reduced in amplitude; on the other hand, bradykinin had no prominent effect when applied to the middle compartment, suggesting that propagation of action potentials in terminals was not affected although a neuromodulatory role for bradykinin has also been proposed (Bauer and Kuriyama 1982; Yau et al. 1986b).

Neurotensin and capsaicin were shown to induce a release of acetylcholine and substance *P*, respectively, thus contracting the myenteric plexus-longitudinal muscle preparation (Nakamoto et al. 1987; Bartho et al. 1982). The reduced twitch amplitude during the first phase of the neurotensin action could thus be explained by its contractile effect similar to that of bradykinin. CGRP-like peptide might also be released by capsaicin which could contribute to the decrease in amplitude of aboral twitches (Maggi et al. 1988; Holzer et al. 1989).

The potassium channel opener cromakalim which is known to reduce contractility of the intestinal smooth muscle (Buchheit and Bertholet 1988; Schwörer and Kilbinger 1989) did not affect aboral contractions when applied to the middle segment but suppressed them when applied aborally; apparently, the propagation of nerve action potentials in the nerve terminals of cholinergic fibres has not been affected.

From all the compounds tested which could be considered as endogenous neuropeptides, only VIP similarly to substance *P* (Ševčík et al. 1990), seemed to facilitate propagation of nerve action potentials in cholinergic nerve terminals when present in the solution superfusing the myenteric plexus-longitudinal muscle preparation. However, it is not yet clear to what extent such a model neuropeptide can be released and operate either during neurogenic stimulation, evoking single cholinergic twitches in our triple-bath studies, or physiologically (Burnstock 1981; Taylor and Bywater 1989).

Acknowledgements. We wish to thank Mrs. Claire West (Beecham Pharmaceuticals) for the supply of cromakalim, Professor Alan L. Harvey (Strathclyde) for his providing us with dendrotoxin, Professor Konrad Löffelholz for the supply of neurotensin, and Mrs. L. Němcová for excellent technical assistance.

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Final version accepted March 9, 1990