The Topographical Basis and Frequency-Dependence in the Effect of Different Compounds on Neurogenic Contractions of the Guinea-Pig Ileum

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Abstract. The site of action of compounds affecting either Na^+/K^+ or Ca^{2+} conductances in nerve terminals was 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 a strip into three segments Neurogenic stimulation of the oral segment, set up nerve action potentials in the neurons projecting axons up to the aboral segment These axons, turning into varicose nerve terminals, conducted action potentials aborally across the middle segment (10 mm) Finally, the nerve terminals, extending into the aboral segment, might be also invaded and trigger aboral twitches Compounds were added, either to the oral segment, to affect the genesis and spread of action potentials in the proximal parts of cholinergic neurons (cell bodies) or they were added to the middle segment to affect propagation of action potentials in axon preterminals and the proximal parts of varicose nerve terminals, or they were added to the aboral segment to affect the propagation in the endings of varicose nerve terminals As a result, the amplitude of aboral twitches reflected drug effects at each site quantitatively Interference with Na^+/K^+ conductance by an elevation of K^+ concentration or by cisapride or neuropeptide Y at non-aboral segments modulated the amplitude of aboral twitches evoked by low-frequency stimulation but did not affect post-tetanic potentiation of twitches, on the other hand, changes in Ca^{2+} concentration of compounds affecting excitatory amino acids receptor system or omega-Conotoxin, when applied to the aboral segment, affected post-tetanic potentiation Thus the effects of cisapride and neuropeptide Y (NPY) during low-frequency stimulation could be located to axon preterminals or the proximal parts of the terminals and their polarization On the other hand, a new role for glutamatergic system in post-tetanic potentiation was bound to the endings of varicose nerve terminals with Ca-sensitive component

in conduction.

Key words: Guinea-pig ileum — Myenteric plexus — Transmission-topography — Low- and high-frequency stimulation — Cisapride — Neuropeptide Y — Glutamatergic system — Omega-Conotoxin

Introduction

Electrical neurogenic stimulation of the guinea-pig ileum excites postganglionic cholinergic fibres of the myenteric plexus at the axon hillock; action potentials set up there propagate in nerve fibres to invade varicose terminals and release acetylcholine from axon varicosities evoking contractions of the longitudinal muscle (Paton et al. 1971). In most studies biologically active agents, drugs and other treatments were applied to the bath containing the preparation of the guinea-pig ileum in order to affect the activity of cholinergic neurons indiscriminately of the possible site of their action. Recently attempts were made to resolve topographically some of these sites of action (Kadlec et al. 1991a,b).The aim of the present study was therefore to discriminate topographically between different targets in this preparation for agents like omega-Conotoxin or N-methyl-D-aspartic acid (NMDA). The method of neurogenic stimulation of myenteric plexus-longitudinal muscle strip

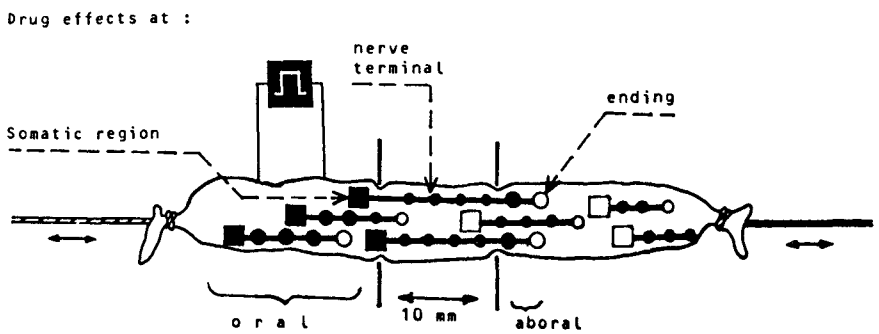

Figure 1. Wiring diagram of cholinergic neurons of the strip preparation in the triple bath. Stimulation was applied to the oral segment and the excited neurones are represented by filled square bodies. Proximal varicosities (filled circles) and distal varicosities (open circles) are distinguished. Oral contractions depend primarily on transmitter release from the proximal varicosities. Judged from the effects on aboral twitches, drugs applied to the oral segment affect the somatic region of cholinergic motoneurons; drugs applied to the middle segment affect conduction of action potentials in nerve terminals; finally, the application of drugs to the aboral segment could affect conduction in the endings of the terminals as well as transmitter release from the distal varicosities and smooth muscle responses.

from the guinea-pig ileum in a triple bath was used (Kadlec et al. 1987). A strip was divided by two rubber membranes into three segments. Nerve action potentials set up by stimulation 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 agents and drugs to the oral segment affects neuronal cell bodies and axon initial segments where excitation might be triggered. The application of drugs to the middle segment containing the proximal portion of varicose terminals gives the possibility to selectively affect the propagation of nerve action potentials in them. In both cases these non-synaptic effects changing the excitability of cholinergic neurons could be monitored by twitch amplitude of the aboral segment (Kadlec et al. 1985; 1987). Finally, drugs applied to the aboral segment containing the very endings of the varicose terminals could affect the propagation of nerve action potentials in them (Kadlec et al. 1990a). To enhance the resolution of the topographical aspect of the method used treatments like changes in Ca^{2+} concentration in the bathing fluid or twitches following a train of high-frequency stimulation were also employed.

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 strip, 30 mm long, were prepared as described by Paton et al. (1971) and were mounted into a groove (4 mm wide, 3 mm deep and 33 mm long) in a conventional sucrose gap apparatus (Burnstock and Straub 1958; Kadlec et al. 1974) (Fig. 1). Two rubber membranes divided the groove into 3 compartments with separate supplies (0.5 ml/min each) of the bathing medium filling the groove up to the brim. The bathing medium was Krebs solution (37 °C) of the following composition (mmol/l): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, and glucose 11.5; the solution was gassed with 95 % O_2 and 5% CO_2. A strip was pulled through narrow openings in the membranes so that the oral and aboral parts (10 mm each) were in the peripheral compartments with the middle compartment being 10 mm in width. The strip was firmly anchored by the membrane so that contractions of one peripheral segment caused no mechanical artifacts in the contralateral segment (Kadlec et al. 1985; 1987). At the end of experiments methylene blue was added to the solution supplying the middle compartment in order to check whether there was any leakage to the peripheral compartment through the rubber membranes. After the equilibration period, rectangular pulses of 0.2 ms duration (10–30 V) were applied at a frequency of 0.1 or 0.04 Hz to the oral segment by means of a pair of platinum wire electrodes. Submaximal isometric twitch response were recorded separately from each peripheral segment. Twitches of the aboral segment evoked by the oral stimulation are, however, expected to be mediated by the propagation of action potentials across the middle segment and by their invasion of the aboral segment (Fig. 1). All the responses were neurogenic as the addition of tetrodotoxin (1 μmol/l) to the oral, middle or aboral segments separately, always abolished twitches of the aboral segment; furthermore, these responses were not affected by the addition of hexamethonium or (+)-tubocurarine or

papaverine or cromakalim to the middle segment (Kadlec et al. 1985; 1987; 1990a) or to the oral segment (present results).

In some experiments the frequency of stimulation was increased from 0.04 to 30 Hz and 25 s tetanic stimulations were delivered at 15–30 min intervals. The post-tetanic changes in twitch amplitude were quantified from 150 s periods preceding or following tetanus; the sum of six twitch amplitudes was calculated ($\sum preT$ and $\sum postT$, respectively) and the percentage change induced by tetanus was estimated as $[(\sum postT - \sum preT)/\sum preT] \times 100$. Positive values of this index mean post-tetanic potentiation (PTP) (Kadlec et al. 1982). Drugs in their final concentrations were present in the fluid superfusing the respective compartment; similarly, the changes in ionic composition also varied. In 100 s periods preceding the addition of a drug, the average of 4 twitch amplitudes was calculated and considered as 100%. In the presence of a drug the average of 4 consecutive twitch amplitudes showing the largest effect was calculated and the change from the respective control value was expressed in per cent. Apart from affecting twitch amplitude, the basal tone might have been changed in some preparations. Therefore concentrations of agents and drugs used were selected so that the tone of the aboral segment remained usually unaffected; still, experiments in which the tone of the aboral segment was raised above the twitch amplitude were not included in the analysis. Aboral contractions were also evoked by bolus additions of acetylcholine (5.5 to 22 pmol in 10 μl) into the solution superfusing the aboral segment immediately before reaching it.

The agents used were: acetylcholine chloride, L-glutamic acid, DL-2-amino-5-phosphonovaleric acid, glycine and N-methyl-D-aspartic acid (NMDA) and tetrodotoxin (Sigma, St. Louis, USA); Bay K 8644 [Methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4(2-trifluoromethylphenyl)-pyridin-5-carboxylate] (Bayer, Wuppertal, Germany); cisapride (Janssen, Belgium); cromakalim (Beecham Pharmaceuticals, England); neuropeptide Y porcine and omega-Conotoxin GVIA (Bachem, Switzerland).

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

Results

Twitches of both oral and aboral segments were evoked by neurogenic stimulation applied to the oral segment. Agents and drugs were added to the oral and middle segments, either combined or separately; or to the aboral segment (Fig. 1). First, the effect of a drug or treatment on the twitch amplitude was determined. Prior to testing the effect on posttetanic potentiation, the amplitude of twitches was reset to that seen before drug treatment by modulating stimulation voltage; thus the conditions preceding tetanic stimulation were comparable.

Changes in Ca^{2+} and K^+ concentration

The elevation of Ca^{2+} concentration from 2.5 to 5 mmol/l either at the oral segments or at the middle segment or both changed neither twitches nor PTP of twitches nor the contractions of the aboral segment evoked by the addition of

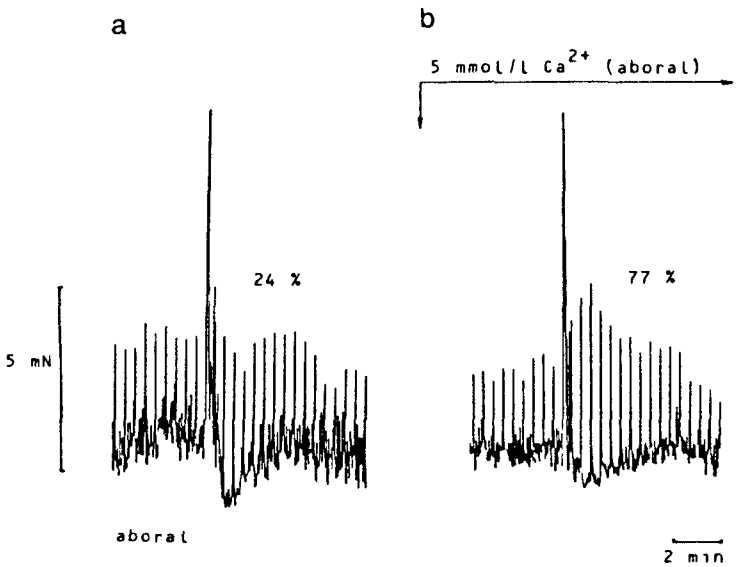

Figure 2. The effect of an elevation of Ca^{2+} concentration (to 5 mmol/l) of the solution superfusing the aboral compartment on twitches of the aboral segment Left panel (*a*) control post-tetanic potentiation (24%), right panel (*b*) experimental post-tetanic potentiation (77%) in the same preparation

Table 1. Changes in responses of the aboral segment of a preparation in the triple bath due to variation in Ca^{2+} concentrations of the solution perfusing the aboral compartment, or K^+ in the solution perfusing the oral plus middle compartments Electrical stimulation at the oral site evoked either twitches (0 04 Hz) or tetanic responses (30 Hz, 25s) Twitch amplitude at 5 9 mmol/l K^+ and 2 5 mmol/l Ca^{2+} (1 2±0 1 mN, $n = 47$) was considered as 100% and percentages of changes (Δ) are given Similarly, changes in PTP as compared with the value obtained under control conditions (14 ± 11%, $n = 18$) are shown Means ± S E M and the number of experiments (in parentheses) are given The significance of differences between the respective groups were tested by Student's *t*-test for paired data ($^*p < 0\ 05$, $^{**}p < 0\ 005$)

	Twitches (Δ%)		PTP (Δ%)	
$[Ca^{2+}]$ mmol/l	$[K^+]$ mmol/l			
	5 9	12	5 9	12
0 6	$-36 \pm 6^{**}$ (22)		$-51 \pm 8^{**}$ (10)	
2 5	0	$111 \pm 17^{**}$ (11)	0	-4 ± 19 (8)
5 0	$17 \pm 6^{*}$ (22)		$25 \pm 5^{**}$ (10)	

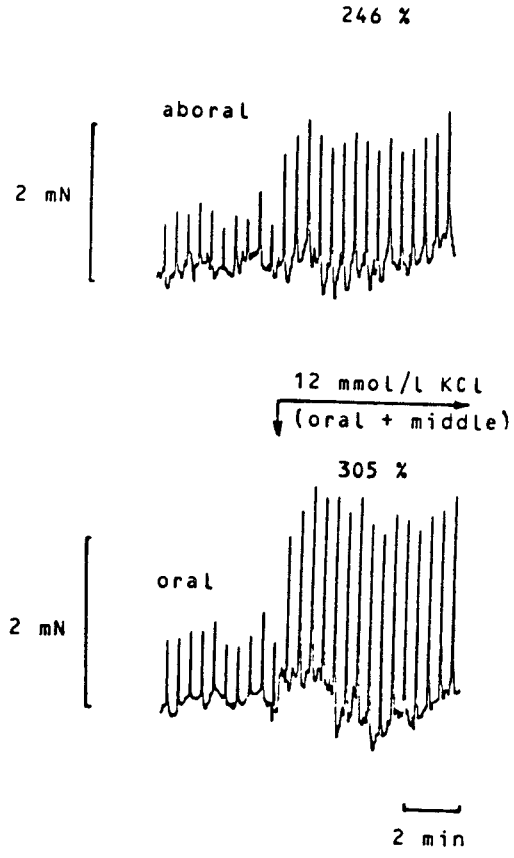

Figure 3. The effect of an elevation of K^+ concentration (to 12 mmol/l) in the solution superfusing both, the oral and middle compartments, on the twitches of the oral segment (lower panel) and aboral segment (upper panel). Percentages indicate twitch height following the change of solutions (in % of preceding control period).

acetylcholine to the aboral segment. The lowering of Ca^{2+} to 0.6 mmol/l at the oral segment tended to decrease PTP but other parameters were not affected.

The elevation and lowering of Ca^{2+} concentration at the aboral segment augmented and depressed twitch height, respectively (Table 1) and the changes of PTP in the same sense were even more expressed (Fig. 2); there were no changes in contractions evoked by acetylcholine in 34 experiments.

An elevation of K^+ concentration from 5.9 to 12 mmol/l at the oral and middle segments impressively augmented the amplitude of aboral twitches (Fig. 3). However no changes in PTP were observed (Table 1); neither were affected contractions of the aboral segment evoked directly by acetylcholine ($92 \pm 5\%$; $n = 32$). An elevation of K^+ concentration at the aboral segment doubled the amplitude of twitches ($208 \pm 20\%$; $n = 4$; $p < 0.02$) but PTP was but insignificantly reduced.

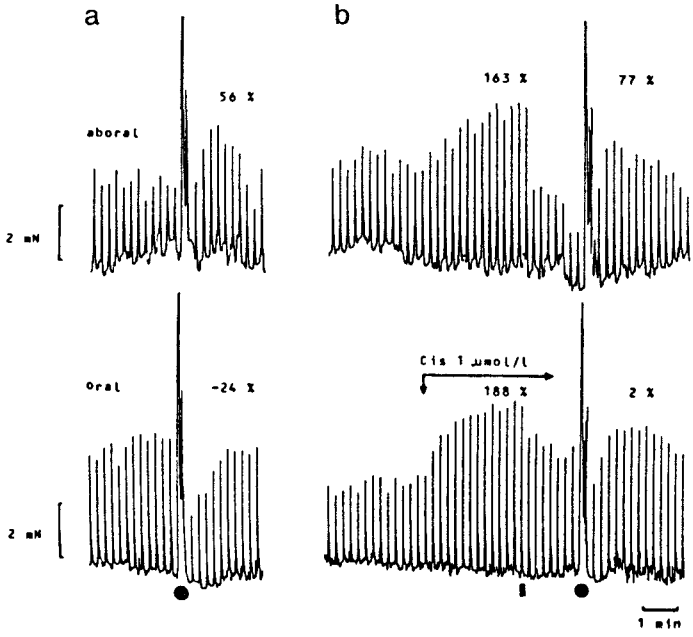

Figure 4. The effect of cisapride (1 μmol/l) added into the solution perfusing the oral compartment on twitches of the oral segment (lower panels) and aboral segment (upper panels). Tetanus (filled circle) and post-tetanic potentiation (−24% and 56%, respectively) under control conditions (*a*; left panels) or in the presence of cisapride (*b*; 2%; 77%; right panels); all the same experiment. After the addition of cisapride, the twitch amplitude rose (188% and 163%) and the stimulation voltage dropped from 18 V to 16 V (filled rectangle).

Drugs changing twitch amplitude but not PTP

The application of cisapride (0.1–10 μmol/l) or neuropeptide Y (0.1–10 μmol/l) at any segment did not affect PTP significantly. Cisapride, apart from augmenting twitch amplitude of the segment to which it was added, enhanced aboral twitches upon its addition to the oral as well as middle segments (Fig. 4; Table 2). The latter effect of cisapride applied to the oral segment was not accompanied by changes in contractions evoked directly by acetylcholine applied to the aboral segment (93±8% of the control value; $n = 21$; n.s.). Furthermore, depression of oral twitches to less than 20% of the control value by local application of cromakalim (1 μmol/l) with no effect on aboral twitches did not itself prevent the augmentation of aboral twitches by simultaneous application of cisapride to the oral segment in 22 experiments (not shown).

Neuropeptide Y, when added to the oral compartment, depressed twitch am-

Table 2. The effects of cisapride (1 μmol/l) and neuropeptide Y (NPY) (1 μmol/l) on twitch amplitude of the oral and aboral segments of strip preparations in the triple bath The drugs were applied separately to the respective segment Twitch amplitude before drug addition was considered as 100% Means ± S E M and the number of experiments (in parentheses) are given The significance of differences between the respective groups was tested by Student's *t*-test for paired data ($^{*}p < 0\ 05$, $^{**}p < 0\ 005$)

Drug	Applied to segment	Twitches (%) Oral	Aboral
Cisapride	Oral	156 ± 12** (20)	148 ± 10** (20)
	Middle	104 ± 4 (20)	125 ± 6** (20)
	Aboral	102 ± 3 (20)	135 ± 6** (20)
NPY	Oral	44 ± 12* (4)	89 ± 10 (4)
	Middle	109 ± 5 (8)	87 ± 5* (8)
	Aboral	104 ± 2 (8)	94 ± 6 (8)

plitude of the segment Neuropeptide Y depressed aboral twitches when present with the middle segment, when present with the oral or aboral segments, the depression of aboral twitches was but insignificant (Table 2) In the presence of cisapride at the oral segment with oral twitches augmented (and considered as 100% here) the addition of neuropeptide Y to the oral segment caused a depression of the oral twitches, again (67 ± 10%, $n = 8$), the depression of the aboral twitches (70 ± 11%, $n = 8$) was significant ($p < 0\ 05$) under these circumstances

Drug effects on PTP

At the end of 20 min superfusion with Bay K 8644 (5 50 nmol/l) at the aboral segment, contractions evoked by acetylcholine as well as twitches but not PTP were augmented in a concentration-dependent manner With 10 nmol/l Bay K 8644, acetylcholine contractions amounted to 141 ± 9% ($n = 16$, $p < 0\ 001$) of the pretreatment control and twitches to 136 ± 5% ($n = 21$, $p < 0\ 001$), however PTP (38 ± 6%, $n = 39$) was not different from the control value (37 ± 5%, $n = 61$) Following washout of Bay K 8644 the responses to acetylcholine and twitches gradually declined but PTP tended to increase so that 30 min later it amounted to 92 ± 14%, ($n = 49$, $p < 0\ 001$)

The presence of NMDA (100 μmol/l) for 30 min at the aboral segment affected neither contractions evoked by acetylcholine nor aboral twitches Control PTP (25 ± 7%, $n = 20$) was not augmented significantly by NMDA (36 ± 6%), 30 min after its washout PTP tended to augment further (46 ± 15%) but these effects were not significant either The same time pattern was also used in a control run of 21 experiments where PTP was measured at 30 min intervals The first PTP was

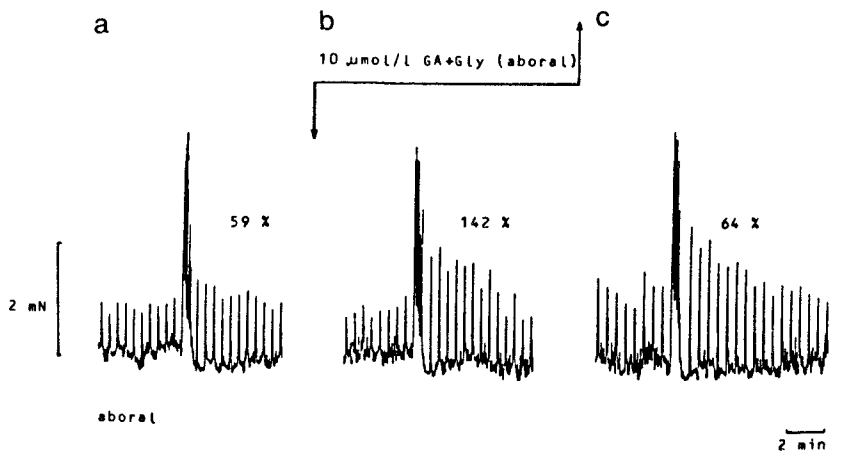

Figure 5. The effect of glutamic acid plus glycine (both 10 μmol/l) added to the aboral compartment on twitches of the aboral segment. Left panel (*a*): control post-tetanic potentiation (59%); middle (*b*) and right (*c*) panels: the responses after 30 min perfusion with the compounds (142%) and 30 min after their washout (64%).

$17 \pm 4\%$ ($n = 16$), the 2nd $26 \pm 9\%$ ($n = 16$), the 3rd was $27 \pm 8\%$ ($n = 16$), not differing significantly from each other.

Glutamic acid and glycine (both 10 μmol/l) were added to the aboral segment affecting neither contractions evoked by bolus addition of acetylcholine ($102 \pm 11\%$; $n = 12$) nor aboral twitches ($102 \pm 3\%$; $n = 17$). PTP was augmented 30 min after the beginning of superfusion with glutamic acid plus glycine, and such increase outlasted their presence by another 15 min (Fig. 5). The effect of glutamic acid plus glycine was completely prevented by the simultaneous application of 2-amino-5-phosphonovaleric acid; actually, the latter drug either in the presence of glutamic acid plus glycine or alone caused an insignificant decrease in PTP (Table 3). The elevation of Ca^{2+} concentration to 5 mmol/l in the presence of glutamic acid plus glycine did not further enhance PTP significantly.

Omega-Conotoxin (1 and 10 nmol/l) did not affect the contractions evoked by acetylcholine ($101 \pm 8\%$; $n = 9$). After 30 min presence, only the larger concentration depressed the amplitude of twitches ($64 \pm 10\%$; $n = 9; p < 0.01$); no significant recovery of the amplitude was observed within 30 min of the drug washout. PTP was decreased from the control level of $40 \pm 9\%$; ($n = 14$) to $29 \pm 5\%$ ($n = 14$; n.s.) by 1 nmol/l, and from $43 \pm 10\%$ ($n = 14$) to $0 \pm 4\%$ ($n = 14$; $p < 0.002$) by 10 nmol/l (Fig. 6); full recovery of PTP was achieved within 15 and 30 min after washout of 1 and 10 nmol/l of omega-Conotoxin, respectively.

Table 3. Post-tetanic potentiation (%) of twitches of the aboral segment under control conditions and in the presence of glutamic acid (GA) plus glycine (Gly, both 10 μmol/l) and/or 2-amino-5-phosphonovaleric acid (A5V) Post-tetanic potentiation was evoked eve ry 15 min and the values obtained are either given separately (1st column) or two adjacent values from the same group were combined as they did not differ from each other Means ± S E M and the number of experiments (in parentheses) are given The significance of differences between the average of the two control values and the respective experimental value was tested by Student's *t*-test for paired data ($^*p < 0\ 05$)

Condition	GA + Gly	GA + Gly + A5V	A5V
Control	30 ± 14 (8) 33 ± 14 (8)	30 ± 9 (12)	19 ± 15 (8)
Drug treatment	35 ± 15 (8) 60 ± 19* (8)	21 ± 7 (12)	13 ± 7 (8)
After drug washout	63 ± 18*(8) 30 ± 15 (8)	29 ± 10 (12)	16 ± 4 (8)

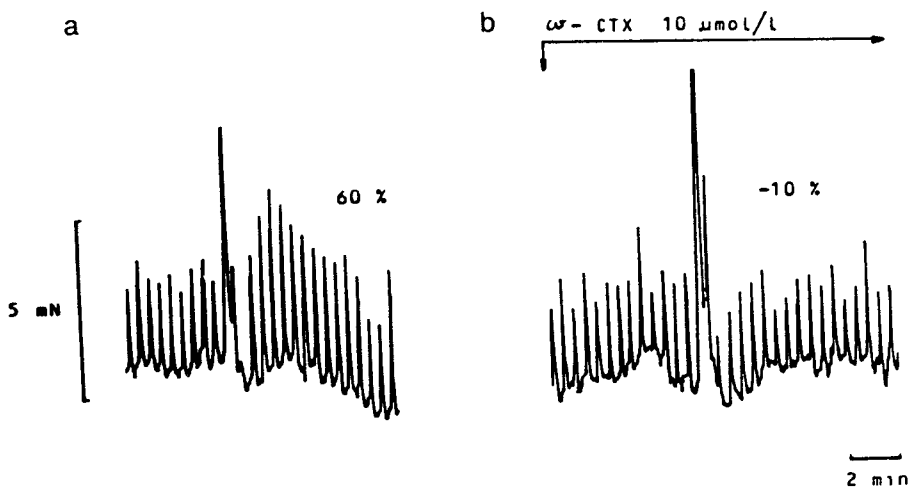

Figure 6. The effect of omega-Conotoxin 10 nmol/l added to the aboral compartment on twitches of the aboral segment Left panel (*a*) control post-tetanic potentiation (60%) right panel (*b*) response after 15 min perfusion with omega-Conotoxin (−10%)

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). These twitches were also resistant to hexamethonium or *d*-tubocurarine applied to the middle segment (Kadlec et al. 1985; 1987). The possibility that more cholinergic neurons were recruited by other interneurons which utilized peptides as their neurotransmitters did not seem to be of primary importance either (North 1982; Ševčík et al. 1990; Kadlec et al. 1990b). The possibility that the aboral segment could be activated by the passage of propagating muscle action potentials generated in the middle segment and affected by the application of a stimulant there was ruled out in previous experiments with papaverine (Kadlec et al. 1987) or with the potassium channel opener cromakalim (Schwörer and Kilbinger 1989; Kadlec et al. 1991b) as well as in the present experiments with cromakalim. Electrical stimulation triggered action potentials in cholinergic neurons of the oral segment of myenteric plexus-longitudinal muscle strips; action potentials propagated in axons projecting aborally across the middle segment invaded the aboral segment and set up twitches there (Fig. 1). The addition of agents to the oral segment might affect the regions of the neuronal soma, axon hillock and the initial segment of preterminal axon. The application of drugs to the oral segment caused also changes in twitch amplitude of the oral segment; these changes served as a control for the overall effect of a drug or treatment on strip preparations. The addition to the middle segment might affect the proximal portion of varicose nerve terminals. Twitch amplitudes of the oral segment served as a control either of a leakage of a drug from the middle to the peripheral compartments or of an asymmetric polarity of a drug effect which was prominent in the aboral segment in line with the projection of cholinergic motor terminals (Fig. 1). The application of drugs to the middle compartment did not cause significant changes in oral twitch amplitude throughout this study. The effects of all agents applied to the oral and middle segments on aboral twitches were presynaptic as also evident from the fact that contractions evoked by exogenous acetylcholine applied to the aboral segment were not affected. As a result of a drug application to the oral and middle segment a variable fraction of cholinergic nerve terminals projecting into the aboral segment was recruited to participate in neuro-effector transmission. The twitch amplitude of aboral segment was thus taken to reflect the effect located at these presynaptic targets (Kadlec et al. 1991b).

The addition of agents to the aboral segment was always accompanied by testing the effect of acetylcholine in order to differentiate possible postsynaptic action. Attention was paid to treatments not affecting contractions evoked directly by acetylcholine and thus the effect on the endings of nerve terminals was, at least

partly separated. The very endings of nerve terminals contained in the aboral segment were shown to be transiently recruited to participate in transmission during PTP and this was connected with a Ca-sensitive component in conduction of nerve action potential within them (Cunnane and Stjärne 1984; Kadlec et al. 1990a). On the other hand, drugs interfering with Na^+/K^+ spikes modulated the amplitude of twitches evoked by low frequency stimulation and did not affect PTP unless twitches were completely abolished (Kadlec et al. 1990a,b; 1991a,b).

Truly, the elevation of K^+ concentration at non-aboral segments augmented single twitches but not PTP whereas changes in Ca^{2+} concentration at the aboral segment alone affected also PTP. In turn, the potency of drugs to affect PTP when applied aborally or to affect the amplitude of single twitches when applied non-aborally could assist in judging on their mechanism of action. In this sense cisapride and neuropeptide Y were exemplified as drugs affecting selectively single twitches and not PTP and being most potent when applied non-aborally. Similar actions were described previously with cholinergic drugs affecting nerve terminal excitability; cholinomimetics causing delicate depolarization of terminals based on changes in potassium conductance could promote variable invasion of them by nerve action potentials and to recruit them into the secretory process (Smith 1988; Kadlec et al. 1991a). This is in agreement with general motor-stimulating properties of cisapride on isolated gastrointestinal preparation of the guinea-pig described previously (Schuurkes et al. 1985). The mechanism of action of neuropeptide Y was weighed from the same viewpoint, and Stjärne et al. (1986) suggested that instead of depressing stimulus-secretion coupling it might reduce the invasion of varicosities by action potentials (Potter 1988). The fact that neuropeptide Y was effective only at non-aboral segments and did not affect PTP stressed the involvement of polarization change based on potassium rather than calcium conductances (Smith 1988; Kadlec et al. 1990a; 1991a; Giuliani et al. 1989).

Bay K 8644 augmented PTP but its effect was delayed and nonselective as smooth muscle contractions evoked directly by acetylcholine as well as twitches evoked by low-frequency stimulation were also enhanced. On the other hand, compounds affecting excitatory amino acid (EAA) receptor system, NMDA and glutamic acid plus glycine and their antagonist 2-amino-5-phosphonovaleric acid as well as omega-Conotoxin were selective in that only PTP was affected but acetylcholine contractions were not; single twitches were decreased in amplitude only after 30 min presence of the larger concentration of omega-Conotoxin. PTP was significantly augmented after 15 min delay in the presence of glutamic acid plus glycine; the increase was completely prevented by 2-amino-5-phosphono-valeric acid. Omega-Conotoxin suppressed PTP in a concentration-dependent manner. This suggested that functional Ca^{2+} channels of N-type in the very endings of nerve terminals (Glossmann and Striessnig 1988; Stanley and Atrakchi 1990) were indispensable for PTP; these channels could be selectively blocked by omega-Conotoxin

which has also been reported to block glutamatergic transmission (Lundy and Frew 1988; Dutar et al. 1989). This is in contrast with dihydropyridine-sensitive *L*-type channels modified by Bay K 8644 and nifedipine (Rijnhout et al. 1990; Wessler et al. 1990) which seemed to be less directly involved in PTP modulation (Kadlec et al. 1990a).

It was very interesting to observe the effect of a low concentration of glutamic acid potentiated by glycine (Bonhaus et al. 1987) on presynaptic cholinergic PTP (Kadlec et al. 1979; 1982) as described above. This contrasted with a much larger concentration of glutamate used by Moroni et al. (1986) to evoke cholinergic contractions of the guinea-pig ileum, which was possible only after a 3-hour-exposure to magnesium-free solution. In both cases, however, the antagonism of glutamic acid effects by NMDA receptor antagonist, namely 2-amino-5-phosphonovaleric acid, suggested that the same receptor for excitatory amino acids of NMDA class was involved. The pivotal role for NMDA receptors in long-term potentiation at some synapses has been gradually established (Dolphin 1985; Collingridge and Bliss 1987). Further, long-term potentiation and PTP, despite their different time courses (Racine and Kairiss 1987), were linked by a common denominator, namely an elevation of presynaptic calcium levels in terminal varicosities (Delaney et al. 1989). Now, it seems that PTP at muscarinic synapses of the guinea-pig ileum could be also intimately bound with the glutamatergic system.

The triple bath arrangement with the stimulation of the oral segment, detection of aboral twitches and addition of drugs to segments oral, middle and aboral, combined with the effect of either low- or high-frequency stimulation proved useful to distinguish the specific sites of action and distinct effects of different drugs within the cholinergic motor neuron of the guinea-pig ileum myenteric plexus as opposed to their overall effect.

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