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Non-synaptic Cholinergic Modulation of Neurogenic Twitches of the Guinea-pig Ileum

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Abstract—The effect of cholinergic and anticholinergic compounds on conduction of neuronal excitation has been 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 (10 mm) so that the aboral segment might be also invaded, eventually. Drugs were added to the middle segment to affect neuronal propagation (non-synaptic effects) which was monitored by twitch height of the aboral segment. The application of acetylcholine to the middle segment augmented aboral twitches. The effects of nicotine, pilocarpine and oxotremorine were selectively blocked by (+)-tubocurarine, pirenzepine and atropine, respectively. The effect of acetylcholine was suppressed by pirenzepine and atropine and mimicked by doubling of KCl concentration. The effect of acetylcholine may be thus explained by the facilitated propagation of nerve action potentials in partially depolarized cholinergic terminals via stimulation of muscarinic receptors. The adenylate cyclase system is not directly involved in the mechanism of muscarinic facilitation of neuronal propagation in the terminals; however, it may participate in the modulation of a final common effector mechanism.

The release of acetylcholine from myenteric neurons of the guinea-pig ileum and its modulation by cholinomimetics and other drugs has been widely studied (Szerb 1980; Kilbinger 1984; Vizi 1984). Cholinomimetics were shown to affect the cholinergic neuron at different sites and via different receptors. Stimulation of nicotinic as well as non-nicotinic receptors of neuronal cell bodies caused tetrodotoxin-sensitive release of acetylcholine from the terminals (Vizi et al 1973; Hanani et al 1988). Excitatory muscarinic receptors located at cell bodies of enteric ganglia activated by for example, pilocarpine, and selectively blocked by pirenzepine (M_1-receptors) and inhibitory presynaptic muscarinic receptors at nerve terminals (M_2) were also described (Kilbinger & Nafziger 1985; North et al 1985); the M_2-muscarinic receptor-type is further present postsynaptically at smooth muscle cells and its stimulation produces smooth muscle contractions (Kilbinger & Wessler 1980). Apart from the two localizations of cholinergic regulatory sites at ganglionic and presynaptic extremities of enteric cholinergic neurons, an axonal or non-synaptic release of acetylcholine from cholinergic neurons was noticed; such a release was, however, not modulated by for example, noradrenaline or ouabain in a manner observed with presynaptic release sites (Vizi et al 1983; Vizi 1984).

In our previous work a 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, 1987). The strip was pulled through two rubber membranes dividing it into three segments supplied separately by perfusing solutions. The site of stimulation at the oral segment was separated by a 10 mm wide middle segment from the aboral segment in which the contractions were recorded. Thus, the nerve action potentials set up 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 cholinergic drugs to the middle compartment containing varicose terminals gives the possibility of selectively affecting the propagation of nerve action potentials in them and the ability to monitor the non-synaptic effects by twitch amplitude of the aboral segment. Some of the results have been previously communicated (Kadlec et al 1990a).

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Materials and Methods

The experiments were carried out on pieces of the central part of ileum isolated from male short-hair guinea-pigs, 200–400 g. Myenteric plexus-longitudinal muscle strips, 40 mm long, were prepared as described by Paton et al (1971) and

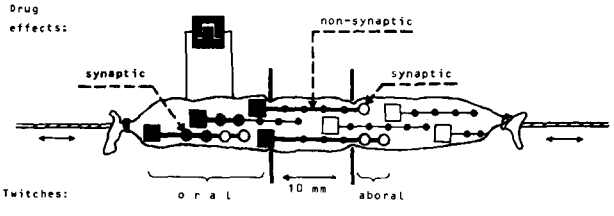

FIG. 1. Wiring diagram of cholinergic neurons of the strip preparation in the triple bath. Stimulation was applied to the oral segment, the excited neurons are depicted by filled square bodies and the regions participating in conduction of action potentials and transmitter secretion responsible for contractions monitored have their axons and varicosities thickened and enlarged, respectively. Proximal varicosities (full circles) and distal varicosities (open circles) are differentiated. Judged from the effects on aboral twitches, drugs applied to the middle segment could affect primarily conduction of action potentials toward the aboral segment (non-synaptic effects); on the other hand, the application of drugs to the peripheral compartment could primarily affect transmitter release and smooth muscle membrane (synaptic effects).

were mounted into a groove (4 mm wide, 3 mm deep and 40 mm long) in a conventional sucrose-gap apparatus (Burnstock & Straub 1958; Kadlec et al 1974). Two rubber membranes divided the groove into three compartments with separate supplies (1 mL min^{-1} each) of the bathing medium filling the groove up to the brim. The bathing medium was Krebs solution of the following composition (mmol L^{-1}): 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 and gassed with a constant stream of 95% O_2–5% CO_2. The strip was pulled through narrow openings in the membranes so that the oral and aboral parts (15 mm each) were in the peripheral compartments with the middle compartment of 10 mm width. At the end of the experiments methylene blue was added to the solution supplying the middle compartment to check whether there was any leakage to the peripheral compartments through the rubber membranes. After the equilibration period, rectangular pulses of 0·2 ms duration (16–30 V) at a frequency of 0·1 Hz were applied to the oral segment by means of a pair of platinum wire electrodes. Submaximal isometric twitch responses were recorded separately from each peripheral segment. Twitches of the aboral segment also evoked by the oral stimulation should, however, 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^{-1}) to the oral, middle or aboral compartments separately always abolished twitches of the aboral segment; furthermore, these responses were not affected by the addition of hexamethonium, (+)-tubocurarine or papaverine to the middle segment (Kadlec et al 1985, 1987).

Contractions were also evoked by bolus additions of acetylcholine (1·1 pmol in 10 μL) added to the solution superfusing the aboral segment immediately before reaching it. Contractions of one peripheral segment caused no mechanical artifacts in the contralateral segment (Kadlec et al 1985, 1987).

In one min periods preceding the addition of a drug, the average of 6 twitch amplitudes was calculated and considered as 100%; when the contractions were more irregular, the control period of twitch evaluation was extended to 2 min to increase the reliability of the calculation. In the presence of a drug the average of four consecutive twitch amplitudes showing the largest effect was calculated and expressed in per cent of the respective control. Apart from affecting twitch amplitude, the basal tone was changed in some preparations. However, only the amplitude of twitches was considered in the evaluation of drug effects; should the basal tone elevation surpass the twitch height before the addition of drugs, the data were discarded.

The agents used were: acetylcholine chloride, substance P and tetrodotoxin (Sigma, St. Louis, USA); aminophylline chloride, atropine sulphate, isoprenaline chloride, nicotine bitartrate, noradrenaline bitartarate, pilocarpine chloride and yohimbine chloride (Spofa, Prague, Czechoslovakia); Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (Bayer, Wuppertal, Germany); clonidine hydrochloride and pirenzepine dihydrochloride monohydrate (Boehringer Ingelheim, Germany); (+)-tubocurarine hydrochloride (Wellcome, UK); forskolin (Calbiochem-Behring, San Diego, USA); oxotremorine sesquifumarate (Aldrich, Milwaukee, USA); propranolol hydrochloride (ICI, Macclesfield, UK); regitine methane sulphonate (Ciba, Basel, Switzerland).

The results were expressed as means ± s.e.m., with the number of experiments 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. Drugs were first added directly to the contracting oral and aboral segments to study their synaptic effects. Further, drugs were added to the middle segment to study their non-synaptic effects.

Synaptic effects

The addition of atropine or pirenzepine to either the oral or aboral segments dose-dependently reduced the neurogenic twitches of each segment, respectively. There was no significant difference in the effect of both drugs on either oral or aboral responses (Fig. 2). EC50 for atropine and pirenzepine was 0·028 ± 0·002 μmol L^{-1} (n = 4) and 0·33 ± 0·047 μmol L^{-1} (n = 5), respectively. The addition of acetylcholine (0·01–10 μmol L^{-1}), oxotremorine (0·01–25 μmol L^{-1}), pilocarpine (1–10 μmol L^{-1}) and nicotine (1–10 μmol L^{-1}) caused contractions of both segments so that twitches were reduced in amplitude or abolished (not shown).

The contractions evoked by the bolus addition of acetylcholine to the aboral segment were not significantly affected by the above cholinomimetics applied to the middle segment (Fig. 3).

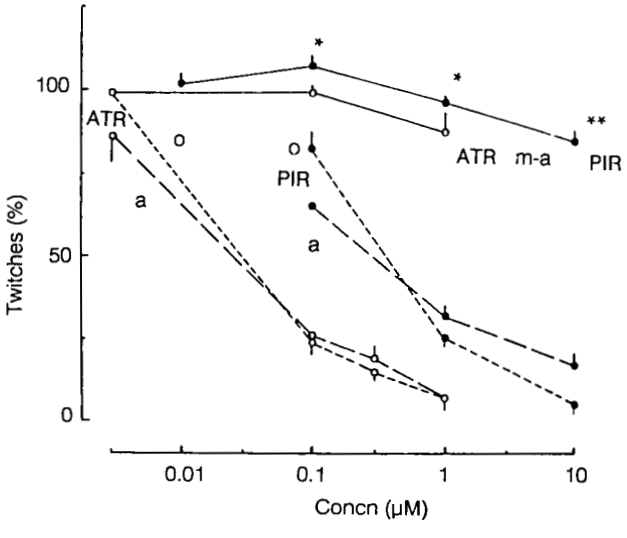

FIG. 2. The dose-response curves for atropine (ATR) or pirenzepine (PIR) on twitches of strip preparations in the triple bath. Each point represents mostly five determinations. Neurogenic stimulation was applied to the oral segment and twitches either of the oral segment (o) or aboral segment (a) were monitored. Drugs were added either directly to the contracting oral and aboral segments (dashed lines); or, drugs were added to the middle segment and their effects on twitches of the aboral segment were displayed (m-a; full lines). In the latter curves the significant changes against the initial twitch amplitude (100%) are indicated: * $P < 0.05$; ** $P < 0.005$.

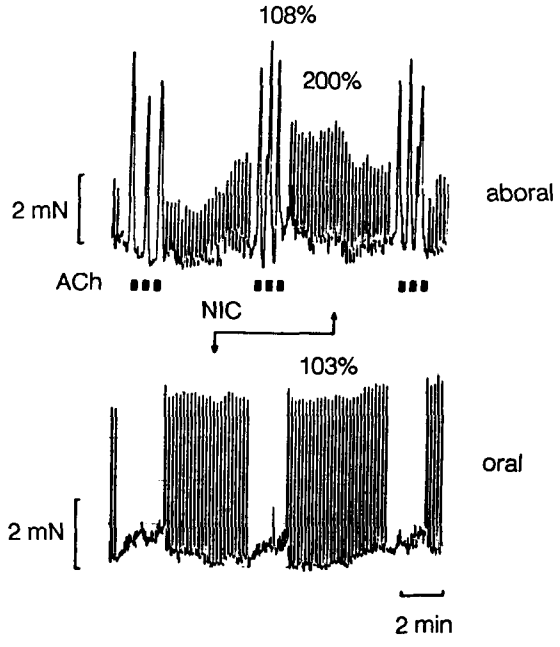

FIG. 3. Non-synaptic effects of nicotine (NIC; 1 μmol L^{-1}) on neurogenic twitches evoked by electrical stimulation of the oral segment (lower row) and on contractions evoked directly by a bolus addition of acetylcholine (ACh; 1·1 pmol; rectangle) to the aboral segment (upper row). Nicotine was added to the middle segment and percent change in twitch amplitude as well as in amplitude of responses to acetylcholine evoked by nicotine is given above each panel.

Non-synaptic effects

The addition of the largest concentration of atropine from the range used (0·003–1 μmol L^{-1}) or of pirenzepine (1 and 10 μmol L^{-1}) to the middle segment decreased twitches of the aboral segment but only to a limited extent (Fig. 2). The addition of 0·1 μmol L^{-1} pirenzepine induced an increase in twitch amplitude; lowering calcium concentration to 0·6 mmol L^{-1} and/or applying trains of 3–6 impulses at 30 Hz (stimulation voltage was halved; cf. Wessler et al 1987) neither augmented this enhancing effect of pirenzepine nor did it change the effect of atropine (0·1 μmol L^{-1}).

Acetylcholine (0·01–1 μmol L^{-1}), oxotremorine (0·01–25 μmol L^{-1}), pilocarpine (1–10 μmol L^{-1}) and nicotine (1–10 μmol L^{-1}) were added to the middle segment for 2 min. In the higher concentrations from the range indicated, cholinomimetics caused a contraction or an elevation of the basal tone of the aboral segment so that twitches were reduced in amplitude or abolished. The lower concentrations of these drugs caused an increase in the amplitude of aboral twitches and only those results were further evaluated (Fig. 4; Table 1); twitches of the oral segment were usually not affected. The enhancement of twitch amplitude by oxotremorine was abolished only by atropine (0·03 μmol L^{-1}); the effect of nicotine was significantly reduced only by (+)-tubocurarine (25 μmol L^{-1}); and the effect of pilocarpine was decreased by both pirenzepine (1 μmol L^{-1}) and atropine. The effect of acetylcholine was not affected by (+)-tubocurarine but was suppressed by atropine and pirenzepine (Table 1).

Table 1. Non-synaptic effects of cholinergic drugs added to the middle segment on the amplitude of aboral twitches. The effect of agonists on the amplitude was expressed in percentages of the respective values (* $P < 0.05$; ** $P < 0.005$) obtained before the addition of an agonist either in the control situation or after pretreatment with an antagonist indicated. The significance of differences in the effect of agonists either in the control situation or in the presence of an antagonist is also indicated (*P*).

	Twitches		
Agonist (μmol L^{-1})	Control	*P* <	Pretreatment by antagonist (μmol L^{-1})
Acetylcholine (0·1)	125 ± 5** (37)	0·05	Atropine (0·03) 98 ± 7 (8)
		0·02	Pirenzepine 1 94 ± 7 (7)
Oxotremorine (0·2)	128 ± 12* (8)	0·01	Atropine (0·03) 87 ± 6 (13)
Pilocarpine (1)	133 ± 9* (9)	0·02	Pirenzepine (1) 100 ± 7 (7)
		0·01	Atropine (0·03) 93 ± 5 (6)
Nicotine 1	166 ± 16* (6)	0·05	(+)-Tubocurarine (25) 120 ± 9 (5)

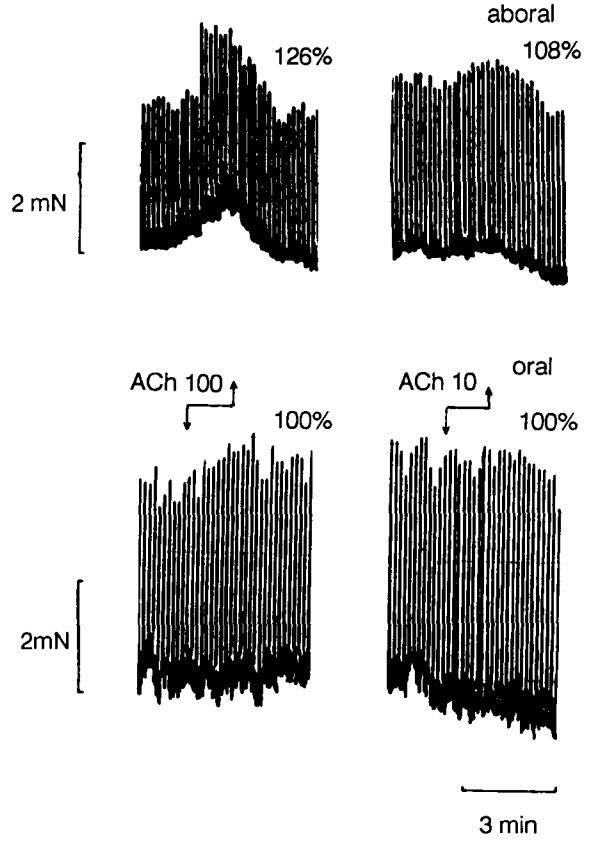

FIG. 4. Non-synaptic effects of acetylcholine (ACh; 10 and 100 nmol L^{-1}) on twitches of the oral (lower row) and aboral (upper row) segments. Acetylcholine was added to the middle segment and percent change in twitch amplitude evoked by acetylcholine is given above each panel.

Mechanism of action

The site of action of cholinomimetics was studied by comparison with a drug known to be selective in this respect; further procedures affecting the function of a second messenger system that could mediate or at least parallel the observed effects of cholinomimetics were utilized.

Ouabain. Following the addition of ouabain (10 μmol L^{-1}) for 10 min to the middle segment, the amplitude of aboral twitches was augmented to 135±8% (n=17), culminated in 2–4 min and tended to return to the pretreatment level in spite of the presence of ouabain. Oral twitches were not affected by ouabain.

Calcium. The stimulatory effect of acetylcholine (0·01 μmol L^{-1}) on the amplitude of twitches (126±7%; n=19) has not been influenced by elevation of calcium concentration in the Krebs solution to 5 mmol L^{-1} (124±13%; n=8) or by its reduction to 0·6 mmol L^{-1} (122±8%; 8); neither were the stimulatory effects of oxotremorine, pilocarpine or nicotine affected.

Drugs affecting adenylate cyclase system. The addition of forskolin (0·5 and 1 μmol L^{-1}) for 2 min to the middle segment gradually augmented the amplitude of aboral twitches (119±10%; n=9; n.s.; and 139±11%; n=15; $P<0·005$, respectively); in contrast to cholinomimetics the onset and offset were slow (Fig. 5). The time from the addition of forskolin till the return of twitch amplitude to half of the maximum elevation above the pretreatment level (offset half-time) was 3·3±1·4 min (n=3) and 3·4±0·5 min (n=15), respectively. Aminophylline (100 μmol L^{-1}) alone augmented twitch amplitude insignificantly (111±8%; n=6). The offset half-time after the washout of 0·5 μmol L^{-1} forskolin was prolonged in the presence of aminophylline to 11·5±3·1 min (n=4; $P<0·05$, one-way analysis of variance). Twitch enlargement by acetylcholine or substance P in the presence of aminophylline was not changed significantly compared with its absence; neither was the time course of these effects. The effect of a calcium agonist Bay K 8644 (0·05 μmol L^{-1}) present for 4 min (129±8%; n=17; $P<0·05$) was still slower and less regular than the effect of forskolin. The time to reach the maximum effect was therefore evaluated, taking 6·1±0·7 min (n=14) in the absence and 10·0±0·7 min ($P<0·05$) in the presence of aminophylline.

Clonidine suppressed aboral twitches after its application for 2 min to the middle segment; the effect of clonidine outlasted its presence in the middle segment by another 5–15 min. The effect of noradrenaline was less potent than that of clonidine and was limited to the time of its presence in the bath; the decrease in twitch amplitude evoked by isoprenaline was not significant. The inhibitory effect of clonidine (0·1 μmol L^{-1}), noradrenaline (1 μmol L^{-1}) and isoprenaline (10 μmol L^{-1}) on aboral twitches were prevented in the presence of yohimbine (10 μmol L^{-1}), regitine (10 μmol L^{-1}) and propranolol (10 μmol L^{-1}), respectively (not shown). The effects of forskolin and Bay K 8644 were largely reduced in the presence of clonidine whereas the effects of acetylcholine and substance P were not affected by this α_2-agonist.

Elevation of potassium concentration. The concentration of potassium was elevated for 1 min to 9–24 mmol L^{-1} in the solution perfusing the middle segment. The application of solutions containing 9 and 12 mmol L^{-1} KCl to the middle segment always augmented the amplitude of aboral twitches reaching 124±4% (n=4) and 153±10% (n=20), respectively; 18 mmol L^{-1} KCl tended either to further increase or to decrease the amplitude of aboral twitches (Fig. 6) and 24 mmol L^{-1} KCl usually caused a contraction with suppression of aboral twitches.

Although aboral twitches were augmented by perfusion of the middle segment with 12 mmol L^{-1} KCl (174±18%; n=10; $P<0·005$), the contractions evoked by the bolus

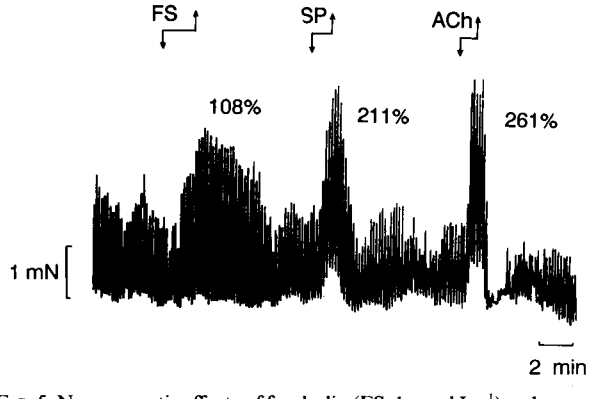

FIG. 5. Non-synaptic effects of forskolin (FS; 1 μmol L^{-1}), substance P (SP; 1 nmol L^{-1}) and acetylcholine (ACh; 100 nmol L^{-1}) on twitches of the aboral segment. Drugs were added to the middle segment and percent change in twitch amplitude is given above each record. Note that the effect of acetylcholine has rapid onset and offset of action in contrast with the effect of forskolin.

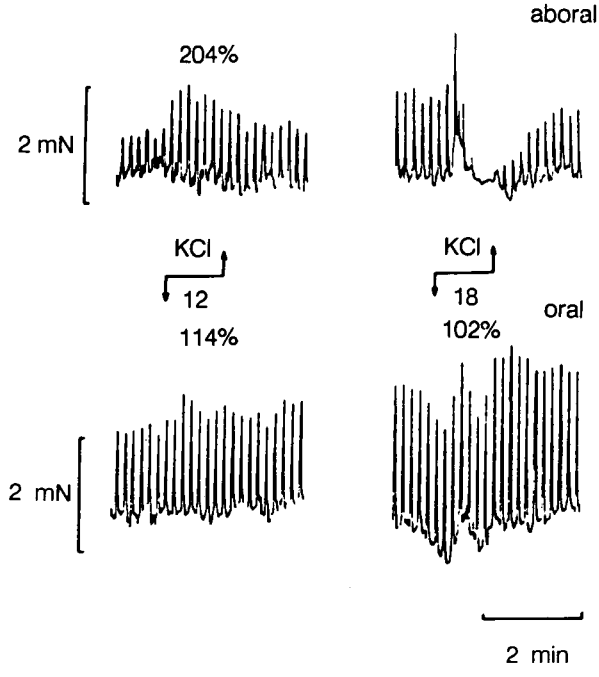

FIG. 6. Non-synaptic effects evoked by the elevation of KCl concentration from 5·9 to 12 and 18 mmol L^{-1} on twitches of the aboral segment. Solution with the elevated KCl concentration was added to the middle segment and percent change in twitch amplitude is given above each panel where it was possible.

addition of acetylcholine applied directly to the aboral segment were not significantly affected ($94 \pm 5\%$; $n = 10$).

Discussion

The effects of cholinomimetic drugs on postsynaptic M_2-receptors causing contractions or on presynaptic M_2-receptors causing autoinhibition of transmitter release have been extensively studied (Kilbinger 1984). The main aim of our study, however, was to distinguish the non-synaptic effects of cholinomimetic drugs which might be explained by their interference with propagation of action potentials in cholinergic nerve terminals; such study was enabled by using the triple bath arrangement (Kadlec et al 1985, 1987).

Synaptic effects

First, postsynaptic effects of atropine and pirenzepine in the triple bath were confirmed. Both drugs inhibited neurogenic twitches of the myenteric plexus-longitudinal muscle strip when applied to the contracting segments. The concentration of pirenzepine required to cause the same degree of inhibition were by one to two log units greater than those of atropine. The postsynaptic site of action and the difference in antagonistic potencies of pirenzepine and atropine corresponded well to their action at M_2-muscarinic receptors of the smooth muscle (North et al 1985; Freedman et al 1988; Jeck et al 1988; Kilbinger & Stein 1988). The inhibitory effects of both drugs did not differ appreciably when applied either to the oral or aboral segments suggesting that their main effect was postsynaptic as it did not indicate a different presynaptic arrangement in the respective segments (Kadlec et al 1987, 1990b).

Non-synaptic effects

Electrical stimulation triggered action potentials in cholinerneurons 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 (Kadlec et al 1985). The addition of drugs to the middle segment thus could affect propagation of action potentials and this was monitored by twitch amplitude of the aboral segment. These twitches were tetrodotoxin-sensitive but resistant to hexamethonium or (+)-tubocurarine applied to the middle segment (Kadlec et al 1985, 1987). The possibility that the aboral segment could be activated by the passage of the propagating muscle action potential generated in the middle segment was previously excluded in the experiments with papaverine (Kadlec et al 1987); in the present experiments, similarly, the contractions evoked directly by acetylcholine added to the aboral segment were unchanged in spite of drug additions to the middle segment and ensuing changes in the amplitude of aboral neurogenic twitches. Another possibility that more cholinergic neurons were recruited by other interneurons which utilized peptides as their neurotransmitters did not seem to be of primary importance (North 1982; Ševčík et al 1990; Kadlec et al 1990c).

The addition of agonists to the middle segment, i.e. nicotine stimulating N-receptors, pilocarpine and oxotremorine with some selectivity for M_1- and M_2-receptors, respectively, and acetylcholine stimulating all types of cholinoceptors, evoked an increase in the amplitude of aboral twitches. The increases in twitch amplitude evoked by nicotine, pilocarpine and oxotremorine were blocked by (+)-tubocurarine, pirenzepine and atropine, respectively, suggesting possible involvement of all types of cholinoceptors (Freedman et al 1988; Jeck et al 1988; Kilbinger & Stein 1988). The increase in twitch amplitude evoked by acetylcholine was, however, suppressed by pirenzepine and atropine but not affected by (+)-tubocurarine. Thus, acetylcholine as a natural neurotransmitter may facilitate the propagation of nerve action potentials in cholinergic terminals of the guinea-pig ileum myenteric plexus via stimulation of muscarinic receptors.

The addition of both pirenzepine (1–10 μmol L^{-1}) and atropine (1 μmol L^{-1}) to the middle segment reduced the amplitude of aboral twitches. As no reduction of twitches of the oral segment was observed, the leakage of drugs from the middle to the peripheral compartments was unlikely. The inhibitory effect of atropine and pirenzepine added to the middle segment on twitches of the aboral segment might mean that some positive cholinergic influence present physiologically was removed, i.e. muscarinic facilitation of propagation of nerve action potentials in the terminals (cf. Nachmansohn 1971).

On the other hand, a small increase in twitch amplitude in the presence of 0·1 μmol L^{-1} pirenzepine was also observed. The increase was not augmented under conditions considered optimal for the manifestation of the removal of muscarinic autoinhibition by pirenzepine (Wessler et al 1987); no such increase was observed in the presence of atropine under any conditions. Thus, presynaptic autoinhibition of transmitter secretion was probably not a major factor involved in the described effects of pirenzepine and atropine.

Mechanism of muscarinic facilitation

Ouabain inhibition of sodium-potassium activated ATPase was shown to increase the release of acetylcholine of cytoplasmic origin from axon terminals of the guinea-pig ileum myenteric plexus (Vizi 1977; Vizi et al 1986). The release of acetylcholine from preterminal axons was, however, neither increased by ouabain nor decreased by noradrenaline or morphine (Vizi et al 1983). In the present experiments ouabain distinctly, although transiently, augmented aboral twitches which might be due to the release of acetylcholine from terminals passing the middle segment. This is in accord with our previous results showing that the addition of opiate ligands and noradrenaline to the middle segment also affected invasion by nerve action potentials of the aboral segment only when varicose cholinergic terminals were present in the middle segment; such conditions were fulfilled with the 10 mm wide middle segment (Kadlec et al 1985). Thus in our experimental arrangement the addition of drugs to the middle segment probably affected varicose cholinergic terminals and not preterminal axons (Kadlec et al 1987, 1990b).

The fact that all the cholinomimetics used could augment the amplitude of aboral twitches led us to look for a common denominator in their action. Changes in calcium concentration were ineffective; they were shown to affect propagation of nerve action potentials and twitches during

post-tetanic potentiation or high-frequency train stimulation (Kadlec et al 1983, 1990b). The changes in twitch amplitude evoked by cholinomimetic drugs during low-frequency stimulation in the present experiments were, however, mimicked by the doubling of KCl concentration in the solution perfusing the middle segment. There is reasonable evidence that an elevated extracellular potassium concentration may concentration-dependently first increase and then decrease nerve terminal excitability and effect variable nerve terminal invasion involving failure and recruitment (for review see Smith 1988). Thus the effect of delicate depolarization of cholinergic nerve terminals by potassium could be a common mechanism for all the cholinergic drugs tested.

Activation of adenylate cyclase and subsequent elevation of intraneuronal cyclic adenosine 3′, 5′-phosphate (cAMP) mimicked slow synaptic excitation in some myenteric neurons (Nemeth et al 1986; Palmer et al 1986) and enhanced the secretion of acetylcholine in the guinea-pig ileum myenteric plexus (Alberts & Ögren 1988). In the present experiments the adenylate cyclase activator, forskolin (Seamon & Daly 1986) like acetylcholine augmented aboral twitches when added to the middle segment suggesting an effect on propagation of action potentials in cholinergic nerve terminals. The slow time course of the onset and offset of such effects of forskolin and its prolongation by the cyclic nucleotide phosphodiesterase inhibitor aminophylline (Chasin & Harris 1976) is consistent with an involvement of this second messenger system (Dunwiddie & Hoffer 1982) in the proposed mechanism of action. In turn, stimulation of inhibitory receptors coupled with adenylate cyclase by clonidine (Čepelík & Hynie 1990) substantially reduced the effect of forskolin. The calcium entry promoter Bay K 8644 (Hagiwara & Byerly 1981) was affected by aminophylline and clonidine in a similar way to forskolin whereas substance P and acetylcholine were not influenced. These data suggest that adenylate cyclase was not directly involved in the muscarinic effect although a final common effector mechanism within the membrane may exist for muscarinic agonists, adenylate cyclase activators and a calcium entry promoter (Palmer et al 1987; Scott & Dolphin 1988).

It was proposed that the combined effect of autoinhibition and intermittence both of transmitter release as well as of invasion of the distal regions of nerve terminals might serve to protect the innervated organ against undue activation (Stjärne 1978; Alberts et al 1981; Kadlec et al 1984). The fact that the released transmitter could also promote invasion of the distal regions of a nerve terminal may serve the same goal by a redistribution of the available secretory sites. During repetitive stimulation the proximal varicosities are protected against undue activation by secreting intermittently (Brock & Cunnane 1988) or by autoinhibition (Starke et al 1989) but the transmitter released from them may recruit the distal varicosities (Kadlec et al 1990b) so that the latter gradually take over the secretory burden.

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