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The role of tachykinin NK_1 and NK_2 receptors in atropine-resistant colonic propulsion in anaesthetized guinea-pigs

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1 The role of endogenous tachykinins on guinea-pig colonic propulsion was investigated by using potent and selective tachykinin NK_1 and NK_2 receptor antagonists. Colonic propulsion and contractions were determined by means of a balloon-catheter device, inserted into the rectum of guanethidine (68 μmol kg^{-1}, s.c., 18 and 2 h before)-pretreated, urethane-anaesthetized guinea-pigs. Propulsion of the device (dynamic model) was determined by measuring the length of the catheter expelled during 60 min filling of the balloon (flow rate 5 μl min^{-1}).

2 In control conditions the tachykinin NK_1 receptor antagonist SR 140333 (1 μmol kg^{-1}, i.v.) did not affect either colonic propulsion or the amplitude of contractions. The tachykinin NK_2 receptor antagonists MEN 10627 and MEN 11420 (1 μmol kg^{-1}, i.v.) increased colonic propulsion at 10 min (+120% and 150%, respectively) but at 60 min the effect was significant only for MEN 10627 (+84%). SR 48968 (1 μmol kg^{-1}, i.v.) did not significantly enhance the colonic propulsion. None of these tachykinin NK_2 receptor antagonists modified the amplitude of colonic contractions. In contrast, both atropine (6 μmol kg^{-1}, i.v., plus infusion of 1.8 μmol h^{-1}) and hexamethonium (55 μmol kg^{-1}, i.v., plus infusion of 17 μmol h^{-1}) abolished propulsion (81% and 87% inhibition, respectively) and decreased the amplitude of contractions (68% inhibition for either treatment).

3 In atropine-treated animals (6 μmol kg^{-1}, i.v., plus infusion of 1.8 μmol h^{-1}), apamin (30 nmol kg^{-1}, i.v.) restored colonic propulsion (+416%) and increased the amplitude of contractions (+367% as compared to atropine alone). Hexamethonium (55 μmol kg^{-1}, i.v., plus infusion of 17 μmol h^{-1}) abolished the apamin-induced, atropine-resistant colonic propulsion (97% inhibition) and reduced the amplitude of the atropine-resistant contractions (52% inhibition).

4 The apamin-induced, atropine-resistant colonic propulsion was inhibited by SR 140333 (−69% at 1 μmol kg^{-1}), SR 48968 (−78% at 1 μmol kg^{-1}), MEN 11420 (−59% at 1 μmol kg^{-1}) and MEN 10627 (−50% at 1 μmol kg^{-1}), although the latter effect was not statistically significant. The combined administration of SR 140,333 and MEN 10,627 (1 μmol kg^{-1} for each antagonist) almost completely abolished colonic propulsion (90% inhibition). The amplitude of colonic contractions was also reduced by SR 140333 (−42%), SR 48968 (−29%), MEN 11420 (−45%) but not by MEN 10627 (−16%). The combined administration of SR 140333 and MEN 10,627 reduced the amplitude of contractions by 47%. SR 140603 (1 μmol kg^{-1}, i.v.), the less potent enantiomer of SR 140333, was inactive.

5 In control animals, apamin (30 nmol kg^{-1}, i.v.) enhanced colonic propulsion (+84%) and increased the amplitude of contractions (+68%), as compared to the vehicle. Hexamethonium (55 μmol kg^{-1}, i.v. plus infusion of 17 μmol h^{-1}) inhibited propulsion (86% inhibition) and decreased the amplitude of contractions (49% inhibition). SR 140333, SR 48968, MEN 11420, MEN 10627, or the coadministration of SR 140333 and MEN 10627 had no effect.

6 In a separate series of experiments, the mean amplitude of colonic contractions was also recorded under isovolumetric conditions through the balloon-catheter device kept in place at 75 mm from the anal sphincter (static model). In control conditions, neither SR 140333 nor MEN 11420 modified the amplitude of contractions. In atropine-pretreated guinea-pigs, SR 140333 and MEN 11420 (0.1–1 μmol kg^{-1}) dose-dependently decreased the amplitude of contractions. In apamin- and atropine-pretreated animals, only the highest (1 μmol kg^{-1}) dose of SR 140333 or MEN 11420 significantly decreased the amplitude of contractions. The inhibitory potency of atropine (0.3–1 μmol kg^{-1}) was similar in apamin-pretreated animals and in controls.

7 It was concluded that, in anaesthetized guinea-pigs, endogenous tachykinins, acting through both NK_1 and NK_2 receptors, act as non-cholinergic excitatory neurotransmitters in promoting an apamin-evoked reflex propulsive activity of the distal colon.

Keywords: Apamin; atropine; colon; hexamethonium; MEN 10627; MEN 11420, SR 48968, peristalsis; SR 140333; tachykinin antagonists

Introduction

Substance P (SP) and neurokinin A (NKA) are peptides of the tachykinin family which are abundantly expressed in the mammalian enteric nervous system. Although SP and NKA display some selectivity for tachykinin NK_1 and NK_2 receptors, respectively, the effects of these two peptides involve extensive cross-talk between different (NK_1, NK_2 and NK_3/NK_4) tachykinin receptors (Maggi, 1995; Maggi & Schwartz, 1997). Functional and anatomical studies indicate that tachykinin receptors are involved in both neuroneuronal and neuromuscular transmission and/or modulation in the enteric nervous system (Holzer & Holzer-Petsche, 1997a,b).

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The motor effects of tachykinins have been widely investigated in the gut: in general, both SP and NKA directly contract the enteric smooth muscle, although the tachykinin receptors involved in this effect exhibit marked species-related variations and regional differences (Holzer-Petsche, 1995; Shuttleworth & Keef, 1995; Furness *et al*., 1995).

Functional and autoradiographic studies indicate that tachykinin NK_1 and NK_2 receptors are both present in the circular muscle of the guinea-pig colon (Burcher *et al*., 1986; Maggi *et al*., 1994b), whereas only NK_1 receptors seem to be present in the longitudinal muscle (Briejer *et al*., 1993).

There is evidence that SP and NKA are evenly co-released by enteric neurones in guinea-pig colon following application of graded depolarizing stimuli (Lippi *et al*., 1998) and that tachykinin NK_1 and NK_2 receptors are both junctionally activated to produce neuromuscular excitation to the circular muscle of the guinea-pig colon. In particular, the electrically-evoked, atropine-resistant excitatory junction potential in the circular muscle, in response to depolarizing stimuli of short duration, is apparently mediated by NK_1 receptors only (Zagorodnyuk *et al*., 1993): a junctional activation of NK_2 receptors is highlighted by increasing the duration of applied stimuli (Maggi *et al*., 1994b; Santicioli *et al*., 1997). Moreover, when the temporal sequence of activation of different receptors in the circular muscle of the guinea-pig colon is analysed, it appears that acetylcholine activates muscarinic receptors to produce a full contractile response at a time when tachykininergic transmission is not yet activated (Maggi *et al*., 1997).

In view of this specialized pattern of excitatory neuromuscular transmission, it would be of interest to establish how these differences translate into more physiological, reflexly-activated and even propulsive motor events. We have previously shown that, under isovolumetric recording conditions, distension determines a local reflex contractile activity in colon of anaesthetized guinea-pigs, which has both an atropine-sensitive and an atropine-resistant component, the latter definitely involving both NK_1 and NK_2 receptors (Giuliani *et al*., 1993; Santicioli *et al*., 1997).

In this study we have developed a new model which enables the investigation of the above problems at a further level of complexity, i.e. by measuring propulsive activity of the guinea-pig colon through the expulsion of a balloon-catheter device placed at some distance from the anal sphincter in anaesthetized guinea-pigs. This model, hereafter referred to as the 'dynamic' model, enables the study of both atropine-sensitive and atropine-resistant propulsive activity, the latter being unmasked by concomitant pretreatment with apamin, a blocker of small conductance calcium-activated potassium channels that inhibits part of the inhibitory junction potential evoked by inhibitory neuroeffector transmitters (Zagorodnyuk *et al*., 1996). Since apamin pretreatment seemingly affects the doses of tachykinin antagonists needed to inhibit the atropine-resistant propulsion, a separate series of experiments was also performed by recording the local reflex response to distension under isovolumetric conditions (cf. Giuliani *et al*., 1993; Santicioli *et al*., 1997), which is hereafter referred to as the 'static' model.

In both models we have examined the effect of selective tachykinin NK_1 (SR 140333) and NK_2 receptor antagonists (SR 48968, MEN 10627 and MEN 11420) in guanethidine-pretreated, urethane-anaesthetized guinea-pigs.

Methods

Male albino guinea-pigs weighing 350–400 g (Rodentia, Italy) received guanethidine (136 μmol kg^{-1}, s.c. in two equal divided doses 18 h and 2 h before experiments); concomitant with the first dose of guanethidine, the food was withdrawn. On the day of the experiment, the animals were anaesthetized with urethane (1.5 g kg^{-1}, s.c.), trachetomized, and the left jugular vein was cannulated for drug injections.

Dynamic model

A latex balloon (Hugo Sachs, size 10, capacity 0.5 ml) was tied on the top of a silicon catheter (length 80 mm, outer diameter 3 mm). A polyethylene catheter (PE 90) was inserted into the silicon catheter connected to a pressure transducer in order to record intracolonic pressure. The transducer was connected to a peristaltic pump to provide filling of the latex balloon. The guinea-pigs were placed on steel platforms and their temperature was kept constant between 36 and 36.5°C by means of infra-red heater lamps controlled by a thermistor probe placed into the mouth. The balloon-catheter device was lubricated with liquid silicon, inserted through the rectum for 75 mm and secured to the platform to avoid propulsion before the beginning of experiments. After 30 min equilibration, the block to the catheter was removed and water was infused into the catheter-balloon device at a flow rate of 5 μl min^{-1}. Test drugs were administered 25 min before the beginning of the experiment.

The length of the catheter expelled from the rectum (propulsion, mm) and the maximal amplitude of colonic contractions (MAC, mmHg) were measured every 10 min for 60 min. Cumulative responses on these parameters were calculated at the end of the experiment (60 min from the start of the infusion) by measuring the length of the device expelled from the rectum and the amplitude of the highest contraction that occurred during the 60 min of infusion. In those cases where the balloon-catheter was totally expelled, the amplitude of the contraction immediately preceding balloon expulsion was not considered for the calculation of MAC, to exclude the possible involvement of sphincter muscle activity.

Static model

In a separate series of experiments, the amplitude of colonic contractions was measured by means of the same device described above, which was inserted for 75 mm through the rectum but fixed in this position into the colon to avoid propulsion. The amplitude of distension-induced colonic contractions was recorded under isovolumetric conditions (0.2 ml) for 60 min before (stabilization) and 90 min after drug administration. Treatments (atropine, SR 140333 or MEN 11420) or vehicle were cumulatively administered in three doses (0.1, 0.3 or 1 μmol kg^{-1}) at 30 min intervals. Pretreatments (atropine and/or apamin) were given at the beginning of the stabilization period.

Statistics

All values represent means$\pm$s.e.mean. Drugs were administered to animals chosen by means of a random schedule. Results were analysed by repeated measures (when applicable) or completely random factorial (2 way) analysis of variance (ANOVA) followed by Fisher LSD (least significant difference) test for multiple comparisons. P values <0.05 were regarded as significant.

Drugs

Guanethidine sulphate was purchased from Sigma (St. Louis, Mo, U.S.A.) and dissolved in saline (34 μmol kg^{-1} 3 ml^{-1}).

Atropine hydrochloride (Serva, Heidelberg, Germany) was dissolved in saline (6 μmol kg^{-1} ml^{-1} as bolus, plus infusion of 1.8 μmol 300 μl^{-1} h^{-1}, i.v.). Hexamethonium bromide (Sigma, St. Louis, Mo, U.S.A.) was dissolved in saline (55 μmol kg^{-1} ml^{-1} as bolus, plus infusion of 17 μmol 300 μl h^{-1} h^{-1}, i.v.). Apamin (L.C. Laboratories, Laufenfingen, Switzerland) was dissolved in saline (30 nmol kg^{-1} 100 μl^{-1}, i.v. as bolus). SR 140333 (Emonds-Alt *et al*., 1993), ((**S**)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl) -piperidin-3-yl]ethyl}-4 -phenyl-1-azoniabicyclo[2.2.2.] octane chloride), SR 140603, its (**R**)-enantiomer and SR 48968 (Emonds-Alt *et al*., 1992), ((**S**)-N-methyl-N[4-acetylamino-4-phenylpiperidino- 2 - (3, 4 - dichlorophenyl) butyl] benzamide) were a kind gift of Dr X. Emonds-Alt (Sanofi, Montpellier, France). MEN 10,627 (Maggi *et al*., 1994a), (c[Met-Asp-Trp-Phe-Drp-Leu]c(2β-5β)) and MEN 11420 (Nepadutant) (Catalioto *et al*., 1998), (c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β-5β)}) were synthesized in the Chemistry Research Department of Menarini Pharmaceuticals (Florence, Italy). SR 140333, SR 140603 and MEN 10627 (1 μmol kg^{-1} 100 μl^{-1}, i.v.) were dissolved in dimethylsulphoxide (DMSO) which was also used as vehicle. MEN 11420 and SR 48968 (1 μmol kg^{-1} 100 μl^{-1}, i.v.) were dissolved in distilled water that was also administered in a separate control group.

Results

Dynamic model

Effect of atropine, hexamethonium or apamin During the 60 min observation period the average propulsion of the balloon-catheter device in untreated animals was 32$\pm$9 mm (n=10), that is about 40% of the maximal possible effect. Either atropine (6 μmol kg^{-1}, i.v. plus infusion of 1.8 μmol h^{-1}) or hexamethonium (55 μmol kg^{-1}, i.v. plus infusion of 17 μmol h^{-1}) totally abolished propulsion (Figure 1a) and markedly decreased the amplitude of colonic contractions (Figure 1b). Apamin markedly potentiated the colonic propulsive activity in both control and atropine-treated animals (Figure 1a): in controls, the average propulsion of the balloon-catheter device (59$\pm$8 mm, n=10) approached 80% of the maximal possible effect; indeed the device was expelled in 6 out of 10 animals tested. In atropine-treated animals, apamin restored propulsion at a level (31$\pm$8 mm, n=10, about 40% of the maximum possible effect) comparable to that observed in controls. Notably, apamin was unable to restore colonic propulsion in hexamethonium-pretreated animals, indicating the reflex origin of the apamin-evoked propulsive activity (Figure 1a). It is also interesting to note that apamin increased the maximal amplitude of colonic contractions (MAC) in both vehicle-, atropine- and hexamethonium-pretreated animals: although the MAC evoked by apamin in hexamethonium-treated animals (24$\pm$4 mmHg, n=10) was comparable to that observed in vehicle-treated guinea-pigs (28$\pm$4 mmHg, n=10), no propulsive activity was detected in the former group. This dissociation clearly indicates that the propulsive activity measured with this model involves mechanisms more complex than the simple contractile action of a particular colonic segment.

Effect of tachykinin NK$_1$ and/or NK$_2$ receptor antagonists In control animals the tachykinin NK$_1$ receptor antagonist SR 140333 (1 μmol kg^{-1}, i.v.) did not change colonic propulsion or the amplitude of colonic contractions (Table 1A). Among

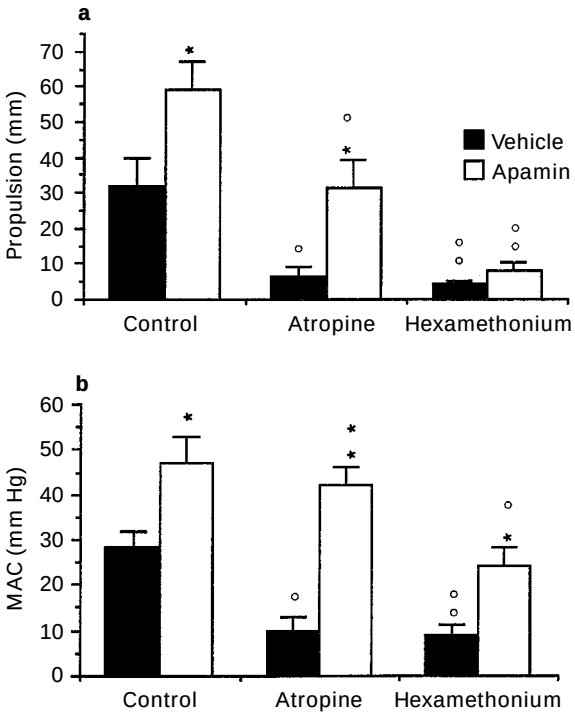

Figure 1 Cumulative responses in colonic propulsion (a) and maximal amplitude of colonic contractions (MAC, b) in vehicle- and in apamin (30 nmol kg^{-1})-treated animals after pretreatment with atropine (6 μmol kg^{-1} plus infusion of 1.8 μmol h^{-1}) or hexamethonium (55 μmol kg^{-1} plus infusion of 17 μmol h^{-1}). Each column and vertical line represents mean$\pm$s.e.mean of 10 animals. Fisher's LSD test: *P<0.05 vs vehicle, ^{o}P<0.05 and ^{oo}P<0.01 vs control.

the tachykinin NK$_2$ receptor antagonists, MEN 10627 and MEN 11420 (both at 1 μmol kg^{-1}) enhanced colonic propulsion at 10 min after the start of the balloon filling; at 60 min, only the effect of MEN 10627 was statistically significant. The non-peptide tachykinin NK$_2$ receptor antagonist SR 48968 (1 μmol kg^{-1}) or the combined administration of SR 140333 (1 μmol kg^{-1}) and MEN 10627 (1 μmol kg^{-1}) also increased colonic propulsion but this effect was not statistically significant (Table 1A). Tachykinin NK$_1$ or NK$_2$ receptor antagonists, either alone or in combination, had no effect on the amplitude of colonic contractions (Table 1B).

Tachykinin NK$_1$ or NK$_2$ receptor antagonists, given alone or in combination at a dose of 1 μmol kg^{-1}, did not alter either colonic propulsion or the amplitude of colonic contractions in apamin-pretreated guinea-pigs (Table 2A and B).

In apamin- and atropine-pretreated guinea-pigs, SR 140333 (1 μmol kg^{-1}, i.v.), SR 48968 (1 μmol kg^{-1}, i.v.) and MEN 11420 (1 μmol kg^{-1}, i.v.) decreased colonic propulsion and reduced the amplitude of contractions. MEN 10,627 (1 μmol kg^{-1}, i.v.) also decreased both parameters, although these effects were not statistically significant. A typical tracing of the effect of the combined administration of SR 140333 and MEN 10627 (both at 1 μmol kg^{-1}) in apamin- and atropine-pretreated animals is shown in Figure 2. The combined administration of SR 140333 and MEN 10627 further reduced colonic propulsion, but not the amplitude of colonic contractions as compared to the effect of SR 140333 alone. SR 140603 (1 μmol kg^{-1}. i.v.). the less active enantiomer of SR 140333, did not

Table 1 Effect of tachykinin NK_1 or NK_2 receptor antagonists on colonic propulsion (A) and colonic contractions (B) in control guinea-pigs

A			*Colonic propulsion* (mm)			
	Dose		*Time* (min)			
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(17)	18±4	100	31±6	100
MEN 10627	1	(10)	40±7*	222	57±8**	184
MEN 11420	0.3	(8)	21±4	116	32±7	103
MEN 11420	1	(10)	45±8**	250	48±8	155
SR 48968	0.3	(8)	27±6	150	33±6	106
SR 48968	1	(10)	32±9	178	44±9	142
SR 140333	1	(9)	22±4	122	37±8	119
MEN 10627 plus	1					
SR 140333	1	(9)	32±7	178	47±9	152
B			*Maximal amplitude of colonic contractions* (mmHg)			
	Dose				*Time* (min)	
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(17)	16±3	100	23±3	100
MEN 10627	1	(10)	22±3	138	31±3	135
MEN 11420	0.3	(8)	8±2	50	15±2	65
MEN 11420	1	(10)	22±4	138	26±3	113
SR 48968	0.3	(8)	18±4	113	26±3	113
SR 48968	1	(10)	19±3	119	29±3	126
SR 140333	1	(9)	18±5	113	33±4	143
MEN 10627 plus	1					
SR 140333	1	(9)	17±5	106	23±5	100

Values represent means±s.e.mean from *n* experiments. Fisher's LSD test: $*P<0.05$ and $**P<0.01$ vs vehicle.

Table 2 Effect of tachykinin NK_1 or NK_2 receptor antagonists on colonic propulsion (A) and colonic contractions (B) in apamin (30 nmol kg^{-1})-pretreated guinea-pigs

A			*Colonic propulsion* (mm)			
	Dose		*Time* (min)			
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(18)	36±5	100	47±6	100
MEN 10627	1	(9)	45±9	125	60±8	128
MEN 11420	1	(9)	31±9	86	34±8	72
SR 48968	1	(9)	33±6	92	36±6	77
SR 140333	1	(9)	46±10	128	54±10	115
MEN 10627 plus	1					
SR 140333	1	(9)	55±10	153	58±9	123
B			*Maximal amplitude of colonic contractions* (mmHg)			
	Dose				*Time* (min)	
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(18)	38±5	100	48±4	100
MEN 10627	1	(9)	37±5	97	49±5	102
MEN 11420	1	(9)	31±9	86	43±3	90
SR 48968	1	(9)	29±4	76	34±3	72
SR 140333	1	(9)	43±8	113	48±6	100
MEN 10627 plus	1					
SR 140333	1	(9)	36±5	95	40±4	83

Values represent means±s.e.mean from *n* experiments.

significantly reduce the propulsion or the amplitude of contractions (Table 3A and B). The apamin-evoked, atropine-resistant propulsion was abolished by hexamethonium, which also reduced the amplitude of contractions (Table 3A and B).

The administration of different vehicles (dimethylsulphoxide, 100 μl kg^{-1} twice, $n=9-10$, or distilled water, 100 μl kg^{-1} once, $n=6-16$), given in order to match the various experimental conditions, never yielded different results, therefore these data have been pooled together in Tables 1–3.

Static model

The balloon-catheter device filled with a constant volume of 200 μl and fixed in the distal colon at 75 mm from the anal sphincter, evoked rhythmic colonic contractions the mean amplitude of which was quite regular over the experimental period (90 min) (data not shown). Administration of dimethylsulphoxide ($n=4$, 100 μl kg^{-1}, i.v., 3 times) or distilled water ($n=4$, 100 μl kg^{-1}, i.v., 3 times) did not change the amplitude of colonic contractions. Atropine (6 μmol kg^{-1}, i.v., plus infusion of 1.8 μmol h^{-1}) reduced by about 35% the

mean amplitude of colonic contractions as compared to control; apamin (30 nmol kg^{-1}, i.v.) induced a similar enhancement of this parameter (35–40%) in atropine-pretreated or in control animals (Table 4).

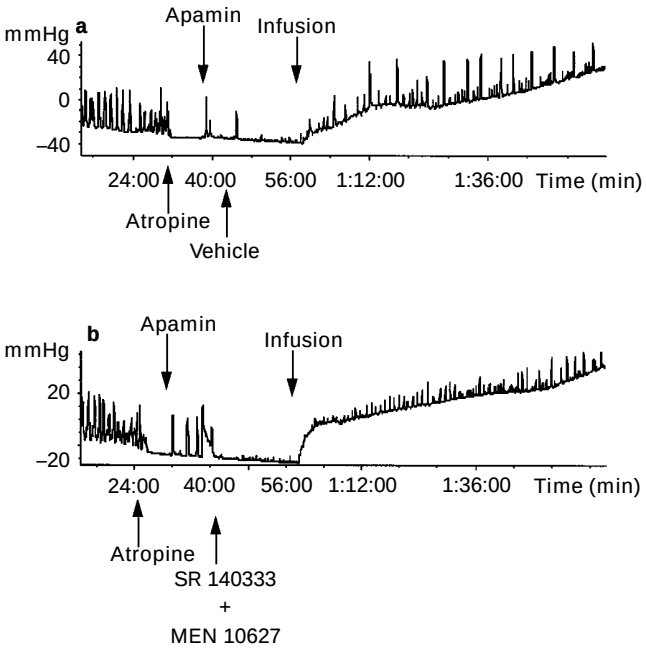

Figure 2 Typical tracings showing intracolonic pressure in atropine (6 μmol kg^{-1} plus infusion of 1.8 μmol h^{-1})- and apamin (30 nmol kg^{-1})-pretreated guinea-pigs after (a) administration of vehicle or (b) SR 140333 (1 μmol kg^{-1}) plus MEN 10627 (1 μmol kg^{-1}).

The effect of atropine was then investigated in more detail by studying the effect of different doses in vehicle- or apamin-pretreated animals. Atropine (0.1–1 μmol kg^{-1}, i.v.) induced a dose-dependent reduction in the amplitude of colonic contractions in both groups; the least effective dose was 0.3 μmol kg^{-1} and the intensity of the inhibitory effect was comparable in the two groups (Figure 3a). Neither SR 140333 nor MEN 11420 (0.1–1 μmol kg^{-1}, i.v.) changed the amplitude of colonic contractions in vehicle-treated guinea-pigs. In atropine-pretreated animals (6 μmol kg^{-1}, i.v., plus infusion of 1.8 μmol h^{-1}), SR 140333 or MEN 11420 dose-dependently reduced the amplitude of colonic contractions; the minimal effective dose was 0.1 μmol kg^{-1} for both drugs. SR 140333 and MEN 11420 also reduced the amplitude of colonic contractions in atropine- and apamine-pretreated guinea-pigs but, in this case the minimal effective dose was 1 μmol kg^{-1} for both drugs, i.e., the inhibitory effect of SR 140333 and MEN 11420 in atropine-pretreated animals was significantly reduced by apamin pretreatment (Figure 3b and c).

Table 4 Effect of atropine (6 μmol kg^{-1}, i.v., plus infusion of 1.8 μmol h^{-1}), apamin (30 nmol kg^{-1}, i.v.) or atropine and apamin on the mean amplitude of colonic contractions in the static model

	Mean amplitude of colonic contractions (mmHg)	
Pretreatments	*Vehicle*	*Atropine*
Control	6.8±0.3	4.6±0.2**
Apamin	9.5±0.4$^{\circ\circ}$	6.3±0.3**$^{\circ\circ}$

Values represent means±s.e.mean from 16 experiments. Fisher's LSD test: **$P<0.01$ vs vehicle and $^{\circ\circ}P<0.01$ vs control.

Table 3 Effect of tachykinin NK$_1$ or NK$_2$ receptor antagonists on colonic propulsion (A) and colonic contractions (B) in atropine (6 μmol kg^{-1} plus infusion of 1.8 μmol h^{-1})- and apamin (30 nmol kg^{-1})-pretreated guinea-pigs

A			*Colonic propulsion* (mm)			
	Dose		*Time* (min)			
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(26)	19±4	100	32±5$^{\$}$	100
MEN 10627	1	(10)	15±7	79	16±7	50
MEN 11420	0.3	(8)	19±7	100	29±9	91
MEN 11420	1	(14)	11±3	58	13±4**	41
SR 48968	0.3	(8)	12±4	63	18±3	56
SR 48968	1	(10)	6±3	32	7±4**	22
SR 140333	1	(9)	7±2	37	10±2**	31
SR 140603	1	(9)	15±5	79	23±5	72
MEN 10627 plus	1					
SR 140333	1	(9)	1±1$^{\circ\circ}$	3	3±1$^{\circ\circ}$	9
Hexameth.	55	(8)	1±1**	3	1±1**	3
B			*Maximal amplitude of colonic contractions* (mmHg)			
	Dose		*Time* (min)			
Treatment	(μmol kg^{-1})	n	*10*	*%*	*60*	*%*
Vehicle		(26)	26±3	100	38±3$^{\$\$}$	100
MEN 10627	1	(10)	17±4	65	32±6	84
MEN 11420	0.3	(8)	19±6	73	29±6	76
MEN 11420	1	(14)	14±3*	54	21±3**	55
SR 48968	0.3	(8)	22±5	85	33±3	87
SR 48968	1	(10)	12±4**	46	27±4*	71
SR 140333	1	(9)	13±3	50	22±2**	58
SR 140603	1	(9)	22±5	85	34±3	89
MEN 10627 plus	1					
SR 140333	1	(9)	9±2	35	20±4	53
Hexameth.	55	(8)	9±2**	35	20±3**	53

Values represent means±s.e.mean from *n* experiments. Fisher's LSD test: *$P<0.05$ and **$P<0.01$ vs vehicle; $^{\circ\circ}P<0.01$ vs SR 140333; $^{\$}P<0.05$ and $^{\$\$}P<0.01$ vs Time 10.

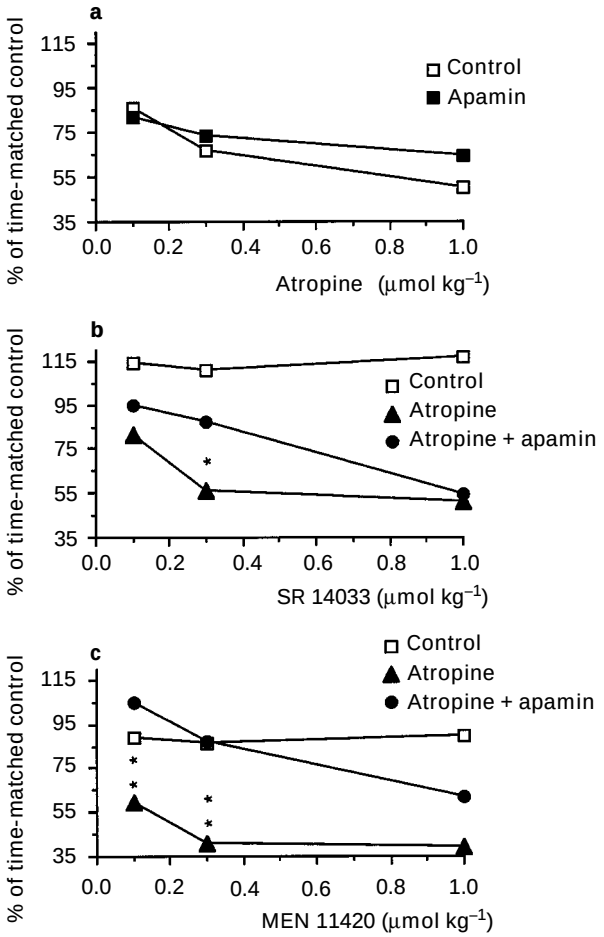

Figure 3 Cumulative dose-response curves for the inhibitory effect on the mean amplitude of colonic contractions in the static model of atropine in apamin (30 nmol kg^{-1})-pretreated or in control animals (a) SR 140333 and MEN 11420 in controls, atropine (6 μmol kg^{-1} plus infusion of 1.8 μmol h^{-1})- or apamin- and atropine-pretreated animals (b) and (c), respectively. Each point represents the mean from 6–8 experiments, s.e.mean have been omitted for the sake of clarity. Fisher's LSD test: $*P<0.05$ and $**P<0.01$ vs atropine + apamin.

Discussion

In the present study we have developed a model for studying atropine-sensitive vs atropine-resistant colonic propulsion in anaesthetized guinea-pigs. The ability of hexamethonium to abolish the expulsion/progression of the balloon-catheter device warrants the involvement of coordinated reflexes in the propulsive activity measured in our experimental conditions. It is interesting to note that some contractile activity was recorded after hexamethonium administration: the nature (myogenic or neurogenic) of which cannot be decided at present. The intestinal propulsion of *digesta* (peristalsis) is driven by coordinated excitatory and inhibitory reflexes which can be activated by distension of the intestinal wall and/or mucosal stroking (Furness & Costa 1987). Since the coordination between these reflexes is essential for maintaining peristalsis, propulsive activity can be potentially inhibited either by blocking the excitatory transmission or through blockade of the inhibitory reflex.

The *in vivo* conditions of the present study restrict the possibility of drawing firm conclusions about the sites of actions of atropine and tachykinin receptor antagonists in inhibiting colonic propulsion. In particular, sites of actions outside the colonic wall cannot be excluded *a priori*. Moreover, both muscarinic and tachykinin NK_1 and NK_2 receptors are expressed on enteric neurones (Portbury *et al*., 1996a,b) and a possible effect of these drugs on neuro-neuronal enteric transmission is conceivable (see below). Despite these limitations, we shall discuss the effects of tachykinin receptor antagonists on colonic propulsion on the assumption that blockade of excitatory neuromuscular transmission is the main site of action of these drugs. This assumption is supported by the following observations. First, nicotinic receptors activate postganglionic excitatory motor neurones containing acetylcholine (and tachykinins) that acts on muscarinic receptors located on the smooth muscle (Furness *et al*., 1995). According to this concept, we have shown that in control animals the inhibitory effect of hexamethonium and atropine are qualitatively and quantitatively similar (see Figure 1). Furthermore, either nicotinic or muscarinic antagonists have only a minimal inhibitory effect on the descending inhibitory reflex (Johnson *et al*., 1996). Second, the tachykinin NK_1 receptor antagonist SR 140,333, which could potentially block nitric oxide synthase-containing inhibitory interneurones (Portbury *et al*., 1996a; Holzer, 1997), inhibits colonic propulsion only in atropine- (and apamin-) pretreated animals. This result is consistent with the observation that a significant tachykininergic contribution to smooth muscle contraction leading to intestinal propulsion is evident only when muscarinic receptors are blocked (Barthó & Holzer, 1985; Giuliani *et al*., 1993; Holzer & Maggi, 1994). Moreover, in the guinea-pig isolated small intestine, tachykinin NK_1 receptor agonists inhibit (and NK_1 antagonists facilitate) the atropine-sensitive peristalsis (Holzer *et al*., 1995; Holzer, 1997): therefore, if a similar mechanism operates in the colon, SR 140,333 should have enhanced peristalsis rather than have produced inhibition. Therefore, although an effect of SR 140333 on NK_1 receptor-bearing enteric neurones cannot be excluded, we interpret its inhibitory effect in distinct experimental groups of this study as evidence for participation of smooth muscle (or interstitial cells) NK_1 receptors in colonic motility. Third, the inhibition of propulsion by atropine, hexamethonium or SR 140,333 was always paralleled by a reduction in the amplitude of colonic contractions. Although the contractions we recorded are not necessarily coupled to propulsion, this result shows that acetylcholine and tachykinin antagonists inhibit excitatory mechanisms.

The administration of apamin, a blocker of small conductance, calcium-activated potassium channels, enabled us to observe an atropine-resistant but hexamethonium-sensitive propulsion. Apamin blocks part of the inhibitory neuromuscular transmission to the circular muscle generating the fast component of the inhibitory junction potential, i.e. the ATP-mediated component (Maggi & Giuliani, 1993; Zagorodnyuk & Maggi, 1994; Zagorodnyuk *et al*., 1996). It appears unlikely that this effect can explain the ability of apamin to restore colonic propulsion in atropine-treated animals, since, as suggested above, a reduction of the inhibitory reflex should have impaired the efficiency of propulsion. An effect of apamin on excitability of muscle cells is a possible mechanism, since apamin increased the amplitude of colonic contractions even in hexamethonium-treated animals. However, since hexamethonium totally blocked the apamin-induced propulsive activity, we postulate that apamin increased the efficiency of neuronal pathways sustaining the hexamethonium-sensitive, atropine-resistant (as well as atropine-sensitive) propulsion. Whether this effect is produced on neuronal structures intrinsic to the colonic wall or may involve the extrinsic innervation of the intestine cannot be determined at present.

SR 140,333, but not its less active enantiomer SR 140,603, reduced colonic propulsion and the amplitude of the atropine-resistant, apamin-evoked contractions. Likewise, tachykinin NK_2 receptor antagonists, namely MEN 11420 (Nepadutant), SR 48968 and, to a lesser extent MEN 10627, inhibited both propulsion and contractions in atropine- and apamin-pretreated guinea-pigs. These results demonstrate the involvement of endogenous tachykinins, acting through both NK_1 and NK_2 receptors, as the main non-cholinergic excitatory neuroeffectors in determining colonic propulsion in anaesthetized guinea-pigs. Accordingly, both NK_1 and NK_2 receptor antagonists reduced the hexamethonium-sensitive, atropine-resistant phasic pressure waves evoked by distension in the guinea-pig proximal (Giuliani *et al*., 1993; Maggi *et al*., 1994b) or distal colon (see Figure 3). Moreover, the inhibitory effects produced by the combined administration of both NK_1 and NK_2 receptor antagonists are qualitatively and quantitatively similar to those induced by hexamethonium (see Table 3). However, it should be noted that the doses of NK_1 and NK_2 receptor antagonists required for inhibiting the atropine-resistant colonic propulsion and contractions in the presence of apamin are higher than those necessary for producing a similar degree of inhibition in the absence of the toxin (see Figure 3). Whether apamin enhances the release of tachykinins from nerve terminals, or affects the sensitivity of smooth muscle cells to tachykinins to render colonic contractions less sensitive to tachykinin NK_1 and NK_2 receptor antagonists, cannot be decided on the basis of the present experiments. It is worth noting that the inhibitory effect of atropine was unchanged by apamin pretreatment: therefore the effect of apamin either exerted at the prejunctional or postjunctional level (or even at both sites) seems somewhat selective for tachykinin-mediated as opposed to acetylcholine-mediated neuromuscular transmission.

Unlike SR 140333, the tachykinin NK_2 receptor antagonists MEN 11420 and MEN 10627 significantly facilitated, and SR 48968 tended to facilitate colonic propulsion in control animals in the absence of any effect on the amplitude of contractions. Owing to the high selectivity of MEN 11420 and MEN 10627 as ligands for NK_2 receptors (Maggi *et al*., 1997; Santicioli *et al*., 1997; Catalioto *et al*., 1998), we speculate that this facilitatory effect involves a pharmacological blocking effect on an inhibitory mechanism activated by NK_2 receptors, possibly through the activation of inhibitory interneurones (Zagorodnyuk & Maggi, 1995; Portbury *et al*., 1996b). The failure of SR 48968 to reproduce this effect of MEN 11420 and MEN 10627 may involve either distribution/kinetic factors or some non-specific pharmacological effect of this non-peptide ligand (Martin *et al*., 1993; Wang *et al*., 1994; Lombet & Spedding, 1994).

This aspect is worthy of further investigation, not only for unravelling the physiological role of neuronal NK_2 receptors, but also because the apparent order of potency for producing this excitatory effect (MEN 10627 > MEN 11420 > SR 48968) is somewhat different from that observed for the inhibitory effect (SR 48968 $\geqslant$ MEN11420 > MEN 10627) on colonic propulsion.

In conclusion, the present study demonstrates the involvement of endogenous tachykinins acting through both NK_1 and NK_2 receptors, as neuroeffector transmitters for the induction of apamin-induced, atropine-resistant peristalsis.

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