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Role of tachykinins in castor oil diarrhoea in rats

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1 We set out to ascertain the role of tachykinins, neurokinin A and substance P, in castor oil-induced diarrhoea in rats as disclosed by the inhibitory effect of the non-peptide NK_1- and NK_2-receptor antagonists, SR 140333 and SR 48968, respectively.

2 SR 48968 (0.02 to 20 μg kg^{-1}, s.c. or p.o.), and the opioid receptor agonist loperamide (1–10 mg kg^{-1}, p.o.), dose-dependently prevented castor oil effects: % inhibition vs castor oil, diarrhoea 0 to 100, increase in faecal mass 7 to 90 and water content 16 to 90. SR 140333 (0.02 to 20 μg kg^{-1}, s.c.) and the platelet activating factor antagonist SR 27417 (5 to 500 μg kg^{-1}, p.o.) did not prevent the increase in faecal water content, but reduced faecal mass (35 to 66%) and diarrhoea (0 to 57%).

3 The **R**-enantiomers of tachykinin NK_1 and NK_2 receptor antagonists, SR 140603 and SR 48605 (both at 2 or 20 μg kg^{-1}, s.c.) had no effect other than reducing faecal mass at the highest dose tested.

4 SR 48968 (20 μg kg^{-1}, p.o.) but not loperamide (10 mg kg^{-1}, p.o.) given 24 h before castor oil, still slightly but significantly reduced by 30% the increase of faecal mass output; both treatments significantly reduced (30 to 70%) the effect of castor oil on faecal water content, although the incidence of diarrhoea was only slightly less than in controls.

5 In castor oil-treated rats, naloxone (2 mg kg^{-1}, s.c.) completely blocked the antidiarrhoeal action of loperamide (10 mg kg^{-1}, p.o.) but not of SR 48968 (20 μg kg^{-1}, p.o.); a similar result was obtained on faecal mass and water content.

6 Castor oil strongly increased the occurrence of manometrically recorded propulsive giant contractions (500 to 1000% over control values) of transverse and distal colon, this effect being significantly prevented (80 to 100%) by SR 48968 and loperamide and partially by SR 140333 (35% distal colon, 70% transverse colon).

7 In castor oil free rats, loperamide but not SR 48968 or SR 140333 significantly reduced by 50% the gastrointestinal transit of a charcoal test meal, as well as 24 h faecal mass output. Consistently, loperamide, unlike the tachykinin receptor antagonists, had a dramatic effect on manometric recordings of intestinal motility, reducing all kinds of colonic contractions.

8 Our findings suggest that castor oil diarrhoea in rats entails activation of NK_1 and NK_2 receptors by endogenous tachykinins, whose antagonists may have a potential as antidiarrhoeal agents free from the constipating action of opioids.

Keywords: Castor oil diarrhoea; tachykinins; NK_1-receptor; NK_2-receptor; SR 140333; SR 48968; loperamide; naloxone; gut motility

Introduction

Endogenous tachykinins, neurokinin A (NKA) and substance P (SP), have a potent spasmogenic effect on the mammalian gut through activation of NK_2 and NK_1 receptors (Croci *et al*., 1995; Holzer-Petsche, 1995). Tachykinin NK_2-receptors and, to a lesser extent, NK_1-receptors are thought to play a key role in contractions and peristalsis in isolated preparations from the colon of rats and humans (Giuliani *et al*., 1991; Holzer-Petsche, 1995). *In vivo* too, the activation of NK_2 rather than NK_1 or NK_3 receptors increases faecal excretion and water content in rats (Croci *et al*., 1994b); these effects are mediated through tachykinin receptor activation, either directly by selective agonists, or indirectly by endogenous peptides (i.e. NKA and/or SP).

In anaesthetized animals, tachykinins are involved in rat colonic hypersecretion induced by interleukin 1β (Eutamene *et al*., 1995) and in the rabbit ileal secretory and inflammatory response to *Clostridium difficile* toxin A (Pothoulakis *et al*., 1994). These studies have suggested the activation of neuronal SP-dependent pathways and mast cells degranulation by SP as the targets of the action of the toxin. Finally, enhanced colonic generation of platelet-activating factor (PAF) *in vitro*, following intestinal inflammation, may raise the tissue levels of vasointestinal-peptide (VIP) and SP, probably accounting for altered gut motility during inflammatory conditions (Deshpande *et al*., 1994).

In view of the above and of the availability of potent and selective receptor antagonists (Croci *et al*., 1995), the present study was designed to investigate the involvement of tachykinin NK_1 and NK_2 receptors in castor oil-induced diarrhoea in rats. A preliminary account of this work was presented at the 8th Symposium on Gastrointestinal Motility 5–8 June 1996, Copenhagen, Denmark (Croci *et al*., 1996).

Methods

Animals

Male Crl:CD BR rats (Charles River Italia, Italy) weighing 220±20 g, were used according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, OJ L358; 1; Dec. 12, 1987). Animals were

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housed for at least seven days before the experiment was carried out. They were kept under controlled environmental conditions (22±1°C, 55±15% relative humidity, 12 h light, 06 h 30 min–18 h 30 min) and were given a pellet diet (4RF21, Mucedola, Italy) and water *ad libitum*.

Faecal excretion and gastrointestinal transit

Faecal excretion was assessed according to the method of Croci *et al.* (1994b) in rats housed individually in grid-floor cages; at 08 h 00 min, the food was withdrawn and 3 h later rats were given castor oil (1 ml 100 g^{-1} body weight) by gavage. Immediately after the oil, gentle thumb pressure was applied to the perianal region to expel any faecal pellet from the rectum. The pellets discharged during the next 210 min were collected and weighed immediately (wet weight) and after drying for 18 h at 50°C to constant weight (dry weight). Any action on intestinal secretion or reabsorption of fluids was scored as the ratio of wet to dry faecal weights. The basal ratio was assessed from the faeces excreted in the 2 h before treatment, since control rats generally did not defecate during the observation period. The incidence of diarrhoea was assessed as the number of animals presenting unformed stools by a team member unaware of the treatment code. To measure 24 h defaecation, castor oil-free rats were placed in metabolic cages and allowed to have free access to food and water throughout the observation time.

Gastrointestinal transit (GIT) was also measured in castor-oil free, 24 h fasted rats, as the percentage of small intestine traversed in 15 min by a charcoal meal (2 ml/rat of a distilled water suspension containing 10% gum arabic, 10% vegetable charcoal and 20% starch).

Manometric recordings

Rats to be used for manometric recordings of large intestine motility from chronically implanted catheters were anaesthetized with sodium pentobarbitone 40 mg kg^{-1}, i.p., and laparatomized, under an infrared lamp to keep their body temperature at 37±0.5°C throughout the surgical procedure. Open-tipped polyvinylchloride catheters (1 mm i.d., 2 mm o.d., Portex Ltd., Hythe, Kent, U.K.) were inserted into the colon, 6 (transverse), or 12 cm (distal colon) from the ileocaecal junction. The catheters, held in place by purse-string sutures at the point of exit from the colonic wall (1 cm proximally to the recording point), were brought out together through the abdominal wall musculature and tunneled subcutaneously to exit at the back of the neck, where they were sutured to the skin and protected by a glass tube. Before surgery and at the end of each recording session, catheters were filled with distilled water and closed by heating at the proximal ends. Rats were allowed to recover from surgery for five to six days, then intraluminal intestinal pressure was recorded as previously described (Croci *et al.*, 1994a) by use of a hydraulic capillary infusion system. A low-compliance perfusion apparatus (Sensor Medics, Milan, Italy), consisting of a tank of bidistilled water, was subjected to a nitrogen pressure of 418 mmHg from a gas cylinder connected in series through a regulator. The capillary infusion system was adapted to the small size of the rat, to minimize the flow of water delivered to the colon. Stainless steel capillary tubing 2.5 m long (1 m, 0.2 mm i.d., plus 1.5 m, 0.1 mm i.d.) ensured high resistance to water flow from the reservoir to a pressure transducer (P23XL, Gould Inc., Cleveland, Ohio). Transducers were connected to the

Table 1 Effects of loperamide, PAF and tachykinin receptor antagonists on castor oil-induced diarrhoea in rats

		Castor oil	*Incidence[a] of diarrhoea*	*Dry weight of faeces* (g)	*Wet/dry weight of faeces*
Vehicles		–	0/50	0.16±0.05	1.85±0.06[b]
		+	50/50	2.67±0.12**	3.20±0.12**
Loperamide, mg kg^{-1}, p.o.					
	1	+	7/7	1.64±0.15**,°°	2.86±0.13**
	10	+	0/7	0.40±0.06°°	1.99±0.12°°
SR 140333, μg kg^{-1}, s.c.					
(**S**-isomer)	0.02	+	7/7	1.80±0.20**,°	3.60±0.25**
	0.2	+	3/7	1.59±0.22**,°°	3.37±0.41**
	2	+	3/7	1.29±0.06**,°°	2.93±0.17**
	20	+	4/7	1.00±0.10**,°°	2.66±0.17**
SR 140603, μg kg^{-1}, s.c.					
(**R**-isomer)	2	+	7/7	2.12±0.19**	3.47±0.18**
	20	+	5/6	1.52±0.36**,°	3.06±0.26**
SR 48968, μg kg^{-1}, s.c.					
(**S**-isomer)	0.02	+	7/7	2.50±0.24**	2.98±0.10**
	0.2	+	4/7	1.81±0.24**,°	2.87±0.25**
	2	+	2/7	0.96±0.14**,°°	2.61±0.15**,°°
	20	+	0/7	0.58±0.05°°	2.39±0.16*,°°
	p.o.				
	0.02	+	7/7	2.28±0.17**	2.87±0.16**
	0.2	+	5/7	1.14±0.08**,°°	2.59±0.16**,°°
	2	+	1/7	0.56±0.07°°	2.26±0.16°°
	20	+	1/8	0.61±0.06*,°°	2.00±0.10°°
SR 48965, μg kg^{-1}, s.c.					
(**R**-isomer)	2	+	6/6	1.83±0.18**,°	3.50±0.19**
	20	+	5/6	0.95±0.12**,°°	2.95±0.17**
SR 27417, μg kg^{-1}, p.o.					
	5	+	6/6	1.77±0.15**,°	2.80±0.17**
	50	+	5/7	1.07±0.20**,°°	2.77±0.34**
	500	+	3/6	1.02±0.12**,°°	2.62±0.22**

Data are mean±s.e.mean of faeces collected in the 210 min after castor oil (1 ml 100 g^{-1} body weight, p.o.). The test compounds were given 30 min (s.c.) or 60 min (p.o.) before castor oil. At the doses employed, the drugs had no intrinsic stimulating effect on faecal output (dry weight of faeces (g), mean±s.e.mean by the highest dose tested: loperamide 0±0, SR 140333 0.2±0.1, SR 140603 0.1±0.1, SR 48968 0.15±0.05, SR 48965 0.1±0.1, SR 27417 0.18±0.07).

[a]Number of rats presenting unformed stools; [b]From faeces collected in the 2 h period before treatment $*P<0.05$, $**P<0.01$ compared with vehicle alone; $°P<0.05$,$°°P<0.01$ compared with castor oil (Duncan's test).

smaller end of the capillary tubing at one outlet and to an intraluminal catheter (inserted into a connecting 40 cm polyethylene tube, 1.98 mm i.d., 2.5 mm o.d.) at the other. Pressure signals from the transducers were recorded by a polygraph (TA4000, Gould). Recordings in freely moving animals housed in individual wire-bottom cages always started at 10 h 00 min, after 30 min adaptation from the beginning of the perfusion. After 60 min basal recordings, rats were treated with the test compounds or their solvent and 30 min later were given castor oil.

Treatment, experimental design and data analysis

Treatments were assigned from random tables. Castor oil (1 ml 100 g^{-1} body weight) was given by gavage. The test compounds, dissolved in bidistilled water or suspended in Na-carboxymethyl cellulose (loperamide), were given in 2 ml kg^{-1} vehicle.

Computer-based recording and analysis of pressure waves was done by a programme called Mot An 1 (Gate House, Nørresundby, Denmark) (Andersen *et al*., 1991). Results are expressed as mean ± s.e.mean. The means were compared by completely randomized one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons (Kramer, 1956) by use of RS/1 software. A probability less than 0.05 was considered statistically significant.

Chemicals

SR 140333, (**S**)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2,2,2]octane chloride, SR 48968, (**S**)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide hydrochloride, and their (**R**)-enantiomers, SR 140603 and SR 48965 were synthetized at Sanofi Recherche, Montpellier, France; SR 27417, N-(2-dimethylaminoethyl)-N-(3-pyridinylmethyl)[4-(2,4,6-triisopropylphenyl)thiazol-2-yl]-amine fumarate at Sanofi Recherche, Toulouse, France. The following chemicals were purchased from the commercial sources as indicated: Sigma-Aldrich Corp. (St Louis, Mo, U.S.A.): loperamide, naloxone and castor oil; Prodotti Gianni (Milan, Italy): low-viscosity Na-carboxymethyl-cellulose of alimentary grade (E466); E. Merck (Darmstadt, Germany): vegetable charcoal, gum arabic and extra-pure starch.

Results

Faecal excretion, diarrhoea and gastrointestinal transit

The effects of the tachykinin NK_1- and NK_2-receptor antagonists, SR 140333 and SR 48968, on faecal excretion and diarrhoea induced by castor oil were compared with those of their **R**-enantiomers (i.e. SR 140603 and SR 48965), the opioid agonist loperamide and the platelet activating factor (PAF) antagonist, SR 27417 (see Table 1 and Figure 1). Unlike its **R**-enantiomer, SR 48968 (0.02 to 20 $\mu g\ kg^{-1}$, p.o. or s.c.), potently and dose-dependently prevented diarrhoea (% inhibition vs castor oil alone, 0 to 100) and by 7 to 93% the increase in faecal mass and water content induced by castor oil (Table 1). Loperamide was likewise fully effective (25 to 90%) in preventing the actions of castor oil, but at substantially higher doses (1 and 10 mg kg^{-1}, p.o.).

SR 140333 (0.02 to 20 $\mu g\ kg^{-1}$, s.c.), although potent, only partially reduced (0 to 66%) the increase in faecal mass and diarrhoea and did not reduce faecal water content (Table 1). The **R**-enantiomer of SR 140333, SR 140603, likewise SR 48965, had no effect other than reducing faecal mass by 46% at the top dose tested.

The PAF antagonist SR 27417 (5 to 500 $\mu g\ kg^{-1}$, p.o.) also partially antagonized the effects of castor oil in reducing diarrhoea (0 to 50%) and pellet output (35 to 66%), without affecting the increase in faecal water content.

SR 48968 (20 $\mu g\ kg^{-1}$, p.o.) but not loperamide (10 mg kg^{-1}, p.o.) given 24 h before castor oil, still slightly but significantly reduced by 30% the increase of faecal mass output (Figure 1a); both treatments significantly reduced (30 to 70%) the effect of castor oil on faecal water content although only the reduction by SR 48968 was substantial (Figure 1b). However, the incidence of diarrhoea was only slightly less than in controls when loperamide or SR 48968 was given 24 h before castor oil: control 10/10; SR 48968, 8/10; loperamide 8/10.

In castor oil-free rats, loperamide but not SR 48968 or SR 140333 significantly ($P<0.01$ vs control) reduced by 53% the GIT of a charcoal test meal (Figure 2), as well as 24 h faecal

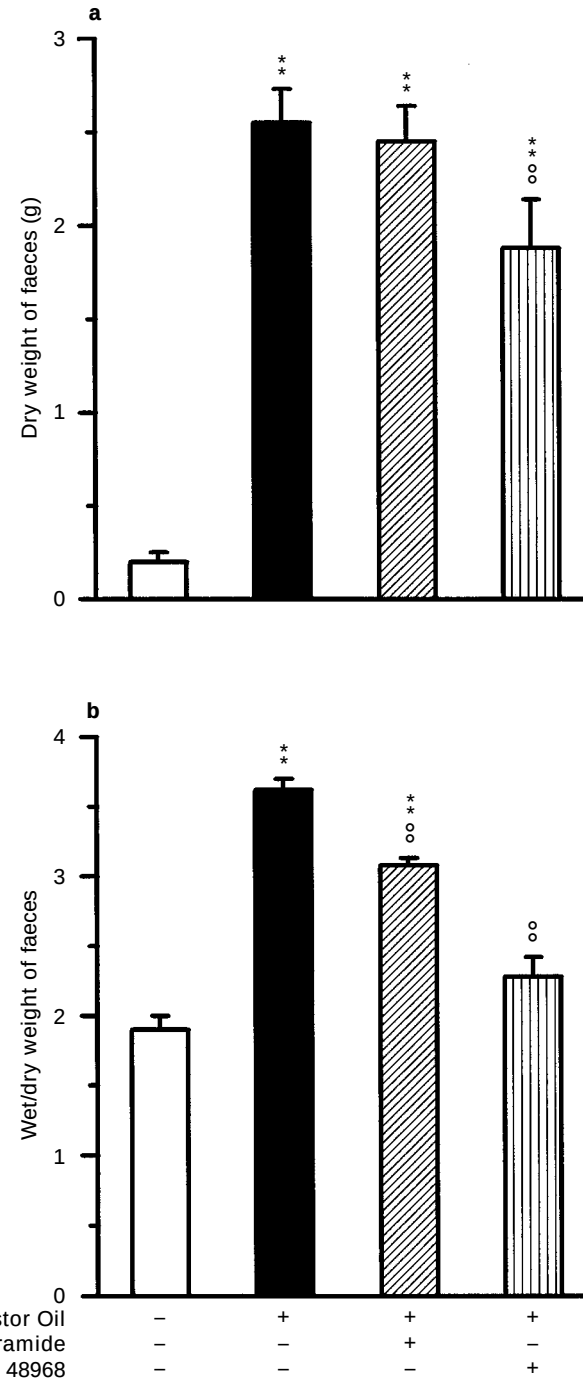

Figure 1 Effect of 24 h-pretreatment with the tachykinin NK_2-receptor antagonist SR 48968 (20 $\mu g\ kg^{-1}$, p.o.) or loperamide (10 mg kg^{-1}, p.o.) on the increase in faecal mass output (a) and faecal water content (b) induced by castor oil (1 ml 100 g^{-1} body weight, p.o.). Data are mean ± s.e.mean of 10 rats. $^{**}P<0.01$ vs vehicle, $^{\circ\circ}P<0.01$ vs vehicle + castor oil (Duncan's test).

mass output: pellets dry weight, g, $n=8$: vehicle 6.8 ± 0.4; loperamide, 10 mg kg^{-1}, p.o., 3.8 ± 0.3, SR 48968, 20 μg kg^{-1}, s.c., 6.0 ± 0.3 and SR 140333, 20 μg kg^{-1}, s.c., 7.0 ± 0.5.

In castor oil treated rats, naloxone (2 mg kg^{-1}, s.c.) completely blocked the antidiarrhoeal action of loperamide (10 mg kg^{-1}, p.o.) but not of SR 48968 (20 μg kg^{-1}, p.o.) (Table 2). A similar result was obtained on faecal mass and water content (Table 2).

Colonic pressure waves

As shown in Figure 3, castor oil significantly increased by 500 to 1000% the number of giant contractions (drug-free rats: 1.6 transverse, 1.3 distal colon contractions in 210 min), i.e. the number of intestinal pressure waves reflecting motor activity (Croci *et al*., 1994a); this effect was 80% prevented by SR 48968 (20 μg kg^{-1}, s.c.) in both colonic segments. SR 140333 (20 μg kg^{-1}, s.c.), caused a substantial reduction (70%) of giant contractions occurring in the transverse colon but not in the distal colon where its inhibitory action was scant (about 35%, $P<0.05$) (Figure 3). Loperamide (10 mg kg^{-1}, p.o.) also completely prevented the increased occurrence of giant propulsive contractions induced by castor oil in both colonic segments, but, unlike the tachykinin antagonists, SR 48968 and SR 140333, it reduced significantly (80 to 90%) their frequency also in castor oil free rats (Figure 3). Moreover, loperamide significantly ($P<0.01$) reduced the frequency of total contractions (i.e. giant ones plus the normally more frequent short duration lower amplitude pressure waves) in both transverse and distal colon (% inhibition of control vehicle-treated rats, mean $\pm$ s.e.mean, $n=6$, 30 ± 5 and 60 ± 10).

Neither castor oil nor the tachykinin antagonists modified the frequency of total contractions in the transverse or distal colon (contractions min^{-1}, mean $\pm$ s.e.mean, 210 min recording time, 5 to 8 rats: transverse colon, vehicle 0.50 ± 0.05, castor oil 0.68 ± 0.1, SR 48968 0.55 ± 0.1, SR 140333 0.63 ± 0.20; distal colon, vehicle 0.40 ± 0.10, castor oil 0.43 ± 0.15, SR 48968 0.35 ± 0.10, SR 140333 0.47 ± 0.10).

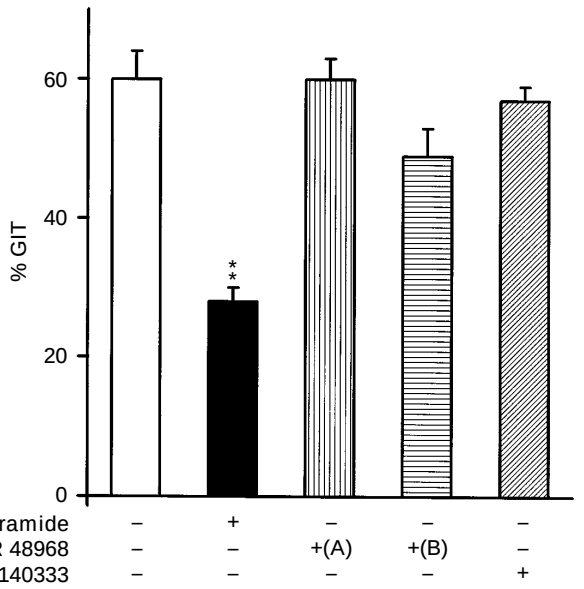

Figure 2 Effect of loperamide (10 mg kg^{-1}, p.o.) or the tachykinin NK_2 and NK_1 receptor antagonists, SR 48968 (A 20 and B 250 μg kg^{-1}, s.c.) and SR 140333 (20 μg kg^{-1}, s.c.) on gastrointestinal transit (GIT) of a charcoal test meal. The test compounds were given 30 min (s.c.) or 60 min (p.o.) before the charcoal meal. Data are mean $\pm$ s.e.mean of 7 rats. $^{**}P<0.01$ vs vehicle (Duncan's test).

Discussion

The intestinal effects of castor oil have been associated with mucosal injury and the release of several endogenous mediators, such as prostaglandins (Pons *et al*., 1994), PAF (Pinto *et al*., 1992) and nitric oxide (NO) (Mascolo *et al*., 1994), but not of tachykinins. We have investigated the role of endogenous tachykinins in castor oil diarrhoea as disclosed by their specific receptor antagonists, SR 140333 and SR 48968. In rat, guinea-pig and human intestinal and non-intestinal *in vitro* functional preparations, these compounds have been shown to be selective and potent tachykinin NK_1 and NK_2 receptor antagonists, respectively, with affinity in the subnanomolar range (Advenier *et al*., 1992; Maggi *et al*., 1993; Croci *et al*., 1995). The apparent affinities of SR 48968 (pA_2) and SR 140333 (pK_B) specifically for rat tachykinin NK_2 and NK_1 receptors were shown to be 9.5 (Croci *et al*., 1995) and 9.0 (Meini & Maggi, 1994), respectively.

In the present study, SR 48968, and to a lesser extent its **R**-enantiomer (SR 48965), potently and dose-dependently prevented diarrhoea and the increase of faecal mass output and water content after the administration of castor oil to rats. Oral or subcutaneous treatment with SR 48698 was virtually equi-effective, indicating good bioavailability or local action. Comparable effects with loperamide required a substantially higher dose (about 500 times). SR 48968 had a remarkably long duration of action, lasting up to 24 h.

We had previously shown (Croci *et al*., 1994b) that SR 48968 dose-dependently inhibited faecal excretion induced by the selective tachykinin NK_2 receptor agonist [βAla^8]NKA(4–10) with potency (ID_{50}, about 0.3 μg kg^{-1}, equal after oral or subcutaneous treatment) and a remarkably long duration of action. SR 48968 also reduced faecal excretion stimulated by the α_2-adrenoceptor antagonist, idazoxan, or by Salmonella endotoxin, presumably through the release of endogenous ta-

Table 2 Effects of naloxone on loperamide and SR 48968 inhibition of castor oil-induced diarrhoea in rats

	Naloxone	*Castor oil*	*Incidence*[a] *of diarrhoea*	*Dry weight of faeces* (g)	*Wet/dry weight of faeces*
Vehicles	–	–	0/6	0.20 ± 0.06	1.96 ± 0.10[b]
Vehicles	–	+	6/6	2.53 ± 0.12**	3.70 ± 0.10**
Vehicles	+	–	0/6	$0.25\pm0.06^{\circ\circ}$	$1.98\pm0.07^{\circ\circ}$
Vehicles	+	+	6/6	2.52 ± 0.19**	3.65 ± 0.12**
Loperamide	–	+	0/6	$0.42\pm0.08^{\circ\circ}$	$2.17\pm0.15^{\circ\circ}$
Loperamide	+	+	6/6	$2.78\pm0.27^{**,\bullet\bullet}$	$3.77\pm0.14^{**,\bullet\bullet}$
SR 48968	–	+	1/6	$0.77\pm0.11^{**,\circ\circ}$	$2.43\pm0.21^{*,\circ\circ}$
SR 48968	+	+	1/6	$0.98\pm0.16^{**,\circ\circ}$	$2.57\pm0.11^{*,\circ\circ}$

Data are mean $\pm$ s.e.mean of faeces collected in the 210 min after castor oil (1 ml 100 g^{-1} body weight, p.o.). Naloxone (2 mg kg^{-1}, s.c.) immediately after castor oil; loperamide (10 mg kg^{-1}, p.o.) and SR 48968 (20 μg kg^{-1}, p.o.) were given 60 min before castor oil.
[a]Number of rats presenting unformed stools; [b]From faeces collected in the 2 h period before treatment. $^{*}P<0.05$, $^{**}P<0.01$ compared with vehicle alone or vehicle plus naloxone. $^{\circ\circ}P<0.01$ compared with castor oil alone or castor oil plus naloxone. $^{\bullet\bullet}P<0.01$ compared with loperamide plus castor oil (Duncan's test).

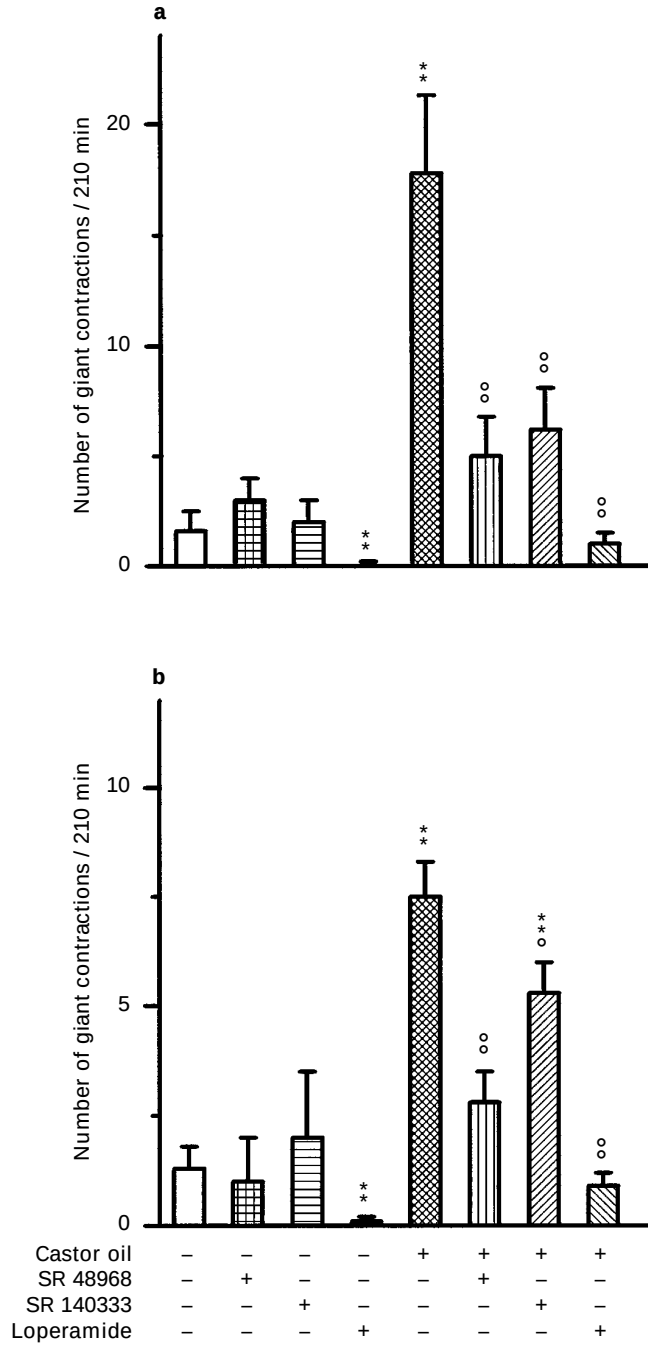

Figure 3 Effect of loperamide (10 mg kg^{-1}, p.o.), SR 140333 (20 μg kg^{-1}, s.c.) and SR 48968 (20 μg kg^{-1}, s.c.) on the increase in giant colonic contractions induced by castor oil (1 ml 100 g^{-1} body weight, p.o.) during 210 min in (a) transverse colon and (b) distal colon. The test compounds were given 30 min (s.c.) or 60 min (p.o.) before castor oil. Data are mean ± s.e.mean of 4 to 8 rats. $^{**}P<0.01$ vs vehicle, $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$ vs vehicle + castor oil (Duncan's test).

chykinins, whereas it did not prevent the similar stimulating action of the other agents such as the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin, 5-hydroxytryptamine, carbachol and PAF (Croci *et al*., 1994b).

These earlier observations with SR 48968 are fairly consistent with those obtained in the present study, supporting NK_2-receptor blockade as the mechanism by which the compound prevents castor oil diarrhoea. The tachykinin NK_1 antagonist SR 140333, but not its **R**-enantiomer (SR 140603), also potently, but only partially, reduced the effects of castor oil, thus suggesting that NK_2 rather than NK_1 receptors play a major role in this model of diarrhoea in which endogenously released tachykinins, i.e. NKA and/or SP, presumably account for the enhanced gut propulsive and secretory activity. In addition, our findings, whose specificity was further strengthened by the lower activity of the two **R**-enantiomers of SR 140333 and SR 48968, showed an *in vivo* stereoselectivity of these antagonists substantially lower than that observed *in vitro* (Croci *et al*., 1995).

Manometric recordings of colonic motility with SR 48968 and SR 140333 are also consistent with their ability to prevent castor oil diarrhoea. In animals not given castor oil, SR 48968 and SR 140333 did not affect colonic contractions or defaecation. Under these conditions, drug-free control animals defaecate only occasionally and giant migrating contractions seldom occur (Croci *et al*., 1994a,b; Croci & Manara, 1994). However, in the present study, the frequency of these propulsive contractions significantly increased in the transverse and distal colon after castor oil treatment; this effect was fully prevented by SR 48968 and partially by SR 140333, which only prevented the giant contractions in the transverse colon to a substantial extent. Since the giant migrating contractions in the distal colon are the ones that best reflect faecal excretion in rats (Croci *et al*., 1994a), the scant ability of SR 140333 to prevent their increase by castor oil might explain why the compound had less antidiarrhoeal action than SR 48968. However, a different site of action along the gut and/or a more important role of NK_2-receptors in intestinal fluid balance might also explain why SR 48968 was definitely more effective under the test conditions. Indeed, a different localization of tachykinin NK_1 and NK_2 receptors in the rat gastrointestinal tract by immunocytochemical techniques was found (Sternini *et al*., 1995; Grady *et al*., 1996). Castor oil-induced diarrhoea in rats can also be reduced by the inhibitor of NO-synthetase N^G-nitro-L-arginine methyl ester (Mascolo *et al*., 1994; Capasso *et al*., 1994) and, as shown in the present study, by the PAF antagonist SR 27417, that proved a powerful, selective and lasting inhibitor of PAF-induced gastrointestinal lesions in rats (Giudice *et al*., 1996). Thus activation of tachykinin receptors by castor oil might release PAF and NO, both contributing to impaired fluid balance and mucosal damage in the gut (Pinto *et al*., 1989; 1992; Mascolo *et al*., 1994; Capasso *et al*., 1994).

SR 140333 was recently shown to be effective in a rat model of experimental colitis and in preventing the concurrent alterations of *in vitro* intestinal contractility (Di Sebastiano *et al*., 1996). SR 48968 has additionally been shown to interact *in vitro* with μ-opioid receptors, at quite high concentrations (μmolar range) (Boyle *et al*., 1993; Martin *et al*., 1993). However these latter *in vitro* findings do not seem to account for the *in vivo* results of the present study. Unlike loperamide whose constipating properties apparently cannot be dissociated from its antidiarrhoeal efficacy, SR 48968 did not slow gastrointestinal transit or produce constipation, even at the maximally effective antidiarrhoeal doses. In addition, naloxone completely blocked the antidiarrhoeal action of loperamide but had no influence on that of SR 48968. It is also worth noting that loperamide, unlike the tachykinin antagonists, strongly inhibited – most probably through opiate receptors activation – all kinds of colonic contractions, even in the absence of castor oil.

A different role of tachykinin and opiate receptors in modulating gut motility is also suggested by the fact that loperamide and the tachykinin receptor NK_1 antagonist RP67580, but not SR 48968 prevented rat defecation induced by acute restraint stress (Barone *et al*., 1990; Ikeda *et al*., 1995). The overall outcome of our study supports the view that tachykinin antagonists might offer a new therapeutic approach to the treatment of diarrhoea in inflammatory and possibly also non-inflammatory gut diseases.

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