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SR 48968 selectively prevents faecal excretion following activation of tachykinin NK_2 receptors in rats*

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Abstract—We tested the ability of SR 48968, (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl)benzamide, a non-peptide antagonist highly selective for tachykinin NK_2 receptors, to prevent defecation induced in rats by several agents. The tachykinin agonists substance P, [MePhe^7]neurokinin B and [β-Ala^8]neurokinin A (4–10) all promoted defecation and increased faecal water content, the last compound being over ten times more potent than the other two (intraperitoneal dose inducing the excretion of 1 g faeces dry weight = 6·7 μg kg^{-1}). SR 48968 given either orally (p.o.) or subcutaneously (s.c.) was similarly potent in dose-dependently inhibiting faecal output stimulated by the selective NK_2-agonist [β-Ala^8]neurokinin A (4–10) (doses causing 50% inhibition 0·4 μg kg^{-1}, p.o. and 0·3 μg kg^{-1}, s.c.). This inhibition was long-lasting (more than 18 h after 1 μg kg^{-1} SR 48968 either s.c. or p.o.). At the higher doses tested, SR 48968 also significantly prevented the increase in faecal water content produced by [β-Ala^8]neurokinin A (4–10). In rats treated with SR 48968, stimulation of faecal output by the α_2-adrenergic antagonist idazoxan and by salmonella endotoxin (LPS), but not by the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin, 5-HT, carbachol or platelet-activating factor, was partially prevented. The present results suggest that activation of intestinal NK_2 receptors, either directly by the selective agonist [β-Ala^8]neurokinin A (4–10) or indirectly through the release of endogenous neurokinin A (by idazoxan or LPS), promotes defecation, presumably as a consequence of increased gut motility or secretion, or both. SR 48968 should therefore be useful for studying the role of neurokinin A-dependent mechanisms in health and disease, including those of the gastrointestinal system, and possibly for developing new therapeutic agents.

Neurokinin A (NKA) is structurally related to substance P and neurokinin B, but its pharmacological action is mainly mediated through a distinct tachykinin receptor subtype called NK_2 (Nakanishi 1991). Although it has been suggested that NK_2 receptors are involved in a variety of physiological and pathological processes, supporting evidence has been largely contingent upon the discovery of potent, selective and biologically stable NK_2 antagonists. In this respect, Emonds-Alt et al (1992) recently described the first non-peptide NK_2-selective receptor antagonist SR 48968, (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl)benzamide.

NKA is thought to play an important role in the gastrointestinal system, where it may modulate intestinal propulsion and fluid secretion. In-vitro, NKA induced gastrointestinal smooth muscle contractions in different animal species (Regoli et al 1990; Croci et al 1992; Parsons et al 1992) and in man (Giuliani et al 1991; Maggi et al 1992).

The aim of the present study was to evaluate the possible role of NK_2 receptors in stimulated faecal excretion in conscious rats. We assessed the ability of SR 48968 to counteract the defecation-promoting effects of several agents presumably acting through different mechanisms.

Materials and methods

Faecal excretion was assessed according to the method described by Croci & Bianchetti (1992). Male Crl:CD BR rats (Charles River Italia), 220–250 g, were kept individually in grid-floor cages, with food and water freely available. At 0800 h food was withdrawn and 3 h later the rats were given the test compounds subcutaneously (s.c.) or intraperitoneally (i.p.). If not otherwise indicated, SR 48968 was given 30 min (s.c.) or 60 min (p.o.) before the test compounds. Drugs were dissolved in 0·9% NaCl (saline) or in distilled water and administered in a volume of 2 mL kg^{-1}.

Treatments were assigned from random tables, each group consisting of seven animals. Immediately after the test compound or its vehicle had been given, gentle thumb pressure was applied to the perianal region to expel any faecal pellets from the rectum. The pellets discharged during the next 90 min were collected and weighed immediately (wet weight) and after drying (10 h at 50°C) to constant weight (dry weight).

The drug doses that induced 1 g (dry weight) faecal excretion (AD_1) or those of SR 48968 causing 50% inhibition (ID50) of [β-Ala^8]neurokinin A (4–10) ([β-Ala^8]NKA(4–10)) effects were both extrapolated from log-dose response-lines (Finney 1964). Any action on secretion or reabsorption of fluids was presumably evidenced from the ratio of wet to dry faecal weights. The normal ratio was calculated from the faeces excreted in the 2 h before treatment, since control rats generally did not defecate during the observation period (90 min). In addition, to assess the effect of SR 48968 on 24-h defecation, rats were placed in metabolic cages (two per cage, eight per group) where they were allowed free access to food and water throughout the observation time.

Data are presented as mean ± s.e. and were analysed statistically by Dunnett's test (Dunnett 1955).

The following drugs were purchased from commercial sources as indicated: Novabiochem (Laufelfingen, Switzerland): substance P, [β-Ala^8]NKA(4–10), [MePhe^7]NKB; Sigma-Aldrich Co. (St Louis, MO, USA): 5-hydroxytryptamine, (5-HT) creatine sulphate, idazoxan, platelet-activating factor (PAF), carbachol, *Salmonella enteritidis*-LPS; RBI (Natick, MA, USA): 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) HBr. SR 48968 was synthesized in the chemistry section of the Sanofi Recherche Centre in Montpellier, France, by X. Emonds-Alt and colleagues.

Results

The tachykinin agonists, substance P, [β-Ala^8]NKA(4–10), and [MePhe^7]NKB, all promoted defecation (Fig. 1). Their potencies were compared on the basis of the doses inducing the excretion of 1 g faeces dry weight (AD_1) by rats that normally do not defecate during the 90-min observation period. The selective NK_2-receptor agonist [β-Ala^8]NKA(4–10) was the most potent with an AD_1 value of 6·7 (4·7–9·5) μg kg^{-1} i.p. (95% confidence limits in parentheses), followed by substance P (NK_1 agonist, $AD_1 = 70$ μg kg^{-1}) and the selective NK_3 agonist [MePhe^7]NKB ($AD_1 > 100$ μg kg^{-1}). Faecal water content was also significantly increased ($P < 0.01$) by the tachykinin agonists, as indicated by the higher than control ratios of wet to dry weight (mean ± s.e.): control, 1·88 ± 0·05; [β-Ala^8]NKA(4–10) at 1 μg kg^{-1},

* A preliminary partial account of this study was presented at the XXVI National Congress of the Italian Society of Pharmacology, Naples, Italy, September 29–October 3, 1992 (Croci et al 1992).

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Table 1. Effect of SR 48968 on rat faecal excretion stimulated by [β-Ala^8]NKA(4–10).

SR 48968 (μg kg^{-1})	[β-Ala^8]NKA (μg kg^{-1}, i.p.)	Dry weight[a] of faeces (g)	ID50[b] (μg kg^{-1})	Wet/dry weight[a] of faeces
—	—	0±0		1·86±0·05[c]
—	14	1·30±0·06**		3·10±0·07**
s.c.				
0·05	14	0·84±0·04**°°		2·94±0·15**
0·5	14	0·70±0·05**°°	0·4 (0·2–0·7)	2·94±0·15**
5	14	0·36±0·05**°°		2·69±0·36*
50	14	0·23±0·17°°		d
p.o.				
0·05	14	0·91±0·05**°°		2·99±0·08**
0·5	14	0·53±0·04**°°	0·3 (0·2–0·5)	2·59±0·20*
5	14	0·34±0·05**°°		2·20±0·18°°
50	14	0·07±0·05°°		d

[a] Data are mean±s.e. of faeces collected from seven rats 90 min after [β-Ala^8]NKA(4–10). [b] Dose of SR 48968, extrapolated from log-dose response-line, that reduces the faeces dry weight by 50%. The dose of 50 μg kg^{-1} was not included in the ID50 calculation (95% confidence interval). [c] Obtained from faeces collected in the 2 h before treatment. [d] Not evaluable. *$P<0·05$, **$P<0·01$ compared with saline alone; °°$P<0·01$ compared with saline+[β-Ala^8]NKA(4–10) (Dunnett's test).

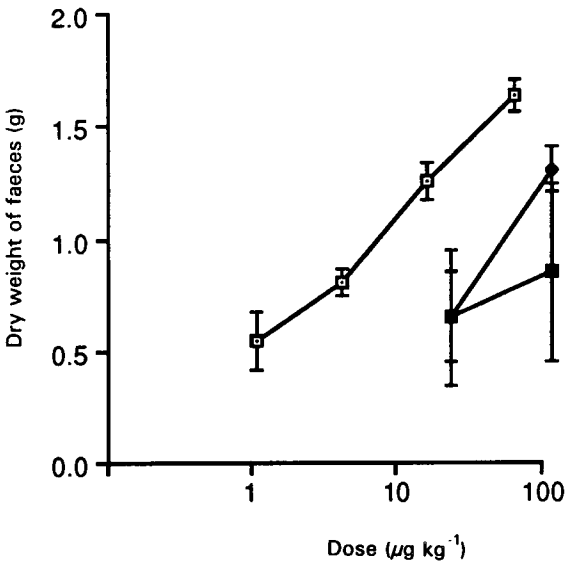

FIG. 1. Effect of tachykinin agonists [β-Ala^8]NKA(4–10) (□), substance P (●) or [MePhe^7]NKB (■) on faecal excretion by rats. Each point is the mean±s.e.; faeces collected from seven rats during 90 min after intraperitoneal treatment.

2·60±0·18; at 50 μg kg^{-1}, 3·21±0·06; substance P at 100 μg kg^{-1}, 3·2±0·17; [MePhe^7]NKB at 100 μg kg^{-1}, 2·9±0·45.

Table 1 shows the results of the experiments designed to ascertain the potency of SR 48968 in preventing faecal excretion stimulated by [β-Ala^8]NKA(4–10). SR 48968, administered either s.c. or p.o., dose-dependently prevented the effects of a submaximal dose (14 μg kg^{-1}, i.p.) of [β-Ala^8]NKA(4–10); the calculated dose of SR 48968 causing 50% inhibition of stimulated faecal excretion (ID50) was 0·4 μg kg^{-1}, s.c. and 0·3 μg kg^{-1}, p.o. However, a relatively high dose of SR 48968 (50 μg kg^{-1}) was needed for maximal inhibition (82–95%) of faecal excretion. SR 48968 at 5 or 50 μg kg^{-1} also prevented the increase of faecal water content.

SR 48968 (0·05, 0·5, 50, 250 μg kg^{-1}, s.c. or p.o.) had no intrinsic effect on faecal excretion (data not shown). In addition rats given SR 48968 1 or 10 μg kg^{-1} s.c. during the following 24 h defecated normally (number of faecal pellets, mean±s.e.: control, 85±7; SR 48968, 78±6 and 83±10, as listed) thus indicating that the compound has no constipating action.

Table 2. Effect of SR 48968 on rat faecal excretion stimulated by idazoxan and salmonella endotoxin (LPS).

SR 48968 (μg kg^{-1}, s.c.)		Dry weight[a] of faeces (g)	Wet/dry weight[a] of faeces
—	Saline	0·04±0·04	1·97±0·07[b]
—	Idazoxan	1·20±0·08**	3·27±0·20**
1		0·93±0·12**°°	3·31±0·20**
10		0·66±0·07**°°	3·34±0·22**
250		0·53±0·06**°°	2·59±0·34*°
—	LPS	1·40±0·10**	3·66±0·23**
10		1·04±0·12**°	3·65±0·29**
50		0·46±0·04**°°	3·20±0·33**
250		0·46±0·10**°°	3·17±0·26**

[a] Data are mean±s.e. of faeces collected from seven rats 90 min after idazoxan (500 μg kg^{-1}, s.c.) or LPS (500 μg kg^{-1}, i.p.). [b] Obtained from faeces collected in the 2 h before treatment. **$P<0·01$ compared with saline alone; °$P<0·05$, °°$P<0·01$ compared with idazoxan or LPS (Dunnett's test).

SR 48968 1 μg kg^{-1}, either s.c. or p.o., still prevented the stimulation of faecal excretion by [β-Ala^8]NKA(4–10) at 14 μg kg^{-1} i.p., given 18 h later; dry weight of faeces (g, mean±s.e.): control, 0·02±0·02; [β-Ala^8]NKA(4–10), 1·57±0·12; SR 48968, s.c. 0·38±0·09, p.o. 0·50±0·08, ($P<0·01$). Further experiments showed that faecal excretion stimulated by idazoxan or LPS was only partially prevented by SR 48968, no more than 60 to 70% at the top doses tested (Table 2).

Finally, SR 48968 up to 250 μg kg^{-1} did not affect faecal excretion stimulated by several other agents including carbachol, 5-HT, 8-OH-DPAT and PAF, but slightly reduced (by 32%) the defecation-promoting effect of substance P (Table 3).

Discussion

Defecation by rats is environmentally influenced and drugs can either increase or reduce it, depending on experimental conditions (Samberg et al 1989). Our model is suitable for the evaluation of defecation acutely induced by different agents,

Table 3. Effect of SR 48968 on rat faecal excretion stimulated by several agents.

SR 48968[a] ($\mu g\ kg^{-1}$)		Dry weight[b] of faeces (g)	Wet/dry weight[b] of faeces
—	Saline	0±0	1·82±0·06[c]
—	Substance P	1·26±0·09**	3·10±0·13**
250	(0·1 mg kg^{-1}, i.p.)	0·86±0·07**°	3·36±0·11**
—	5-HT	1·14±0·13**	3·34±0·13**
250	(10 mg kg^{-1}, s.c.)	0·85±0·11**	3·23±0·16**
—	8-OH-DPAT	0·99±0·13**	3·07±0·15**
250	(0·1 mg kg^{-1}, s.c.)	1·02±0·12**	3·17±0·22**
—	Carbachol	1·29±0·19**	3·44±0·19**
250	(0·2 mg kg^{-1}, s.c.)	1·14±0·07**	3·53±0·14**
—	PAF	0·99±0·15**	3·33±0·23**
250	(0·025 mg kg^{-1}, i.p.)	0·81±0·09**	3·21±0·21**

[a] SR 48968 was injected subcutaneously 30 min before stimulating agents. [b] Data are mean ± s.e. of faeces collected from seven rats 90 min after the faecal excretion stimulating agents. [c] Obtained from faeces collected in the 2 h before treatment. **$P<0.01$ compared with saline alone, °$P<0.05$ compared with substance P alone (Dunnett's test).

since control animals consistently do not defecate throughout the standard observation period (Croci & Bianchetti 1992).

In the present study, $\mu g\ kg^{-1}$ amounts of the selective NK_2 agonist [β-Ala^8]NKA(4–10) potently stimulated faecal excretion and increased faecal water content. The other two tachykinin agonists, substance P and [MePhe^7]NKB, respectively selective for NK_1 and NK_3 receptors, were 10 to 20 times less effective on a weight basis. This suggests that NK_2 rather than NK_1 or NK_3 receptors may play an important role in modulating rat colonic propulsion or secretion.

The selective non-peptide antagonist of NK_2 receptors SR 48968 dose-dependently prevented the defecation-stimulating effects of [β-Ala^8]NKA(4–10), with impressive potency. A similar potency was obtained after oral treatment, suggesting good bioavailability. In addition, SR 48968 showed a remarkably long duration of action.

Although further studies are needed to clarify the persistent action, it is worth noting that SR 48968 does not bind covalently to the NK_2 receptor (Emonds-Alt et al 1992). SR 48968 by itself neither stimulated defecation nor produced constipation; it reduced the stimulation of faecal excretion by idazoxan and salmonella endotoxin (LPS) but not by PAF, carbachol, 5-HT, or 8-OH-DPAT; stimulation by substance P was only slightly, but significantly, reduced. Substance P, at the higher doses promoting faecal excretion, probably activates NK_2 receptors, which may account for the antagonism by SR 48968.

Altogether these results with faecal excretion-promoting agents other than [β-Ala^8]NKA(4–10) support the NK_2-selectivity of SR 48968 antagonism and suggest that LPS and idazoxan might promote defecation through the release of endogenous NKA, although non-specific effects cannot be ruled out at the moment.

In conclusion, our findings suggest the involvement of NK_2 receptors in colonic propulsion and secretion that presumably accounts for the acutely-induced defecation observed in rats. By monitoring this endpoint, we further confirmed the in-vivo potency and NK_2-selectivity of SR 48968, previously assessed on the grounds of its antagonism of tachykinin-induced bronchoconstriction in guinea-pigs (Emonds-Alt et al 1992) and rat urinary bladder contraction (Maggi et al 1993). We also found that the SR 48968 was long-acting, and this is worth investigating further. Finally, SR 48968 displayed good oral bioavailability. This new compound should therefore prove useful in studying the role of NKA-dependent mechanisms in health and disease, including those of the gastrointestinal tract, and possibly for developing new therapeutic agents.

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