SPECIAL REPORT

Promotion by SR 48692 of gastric emptying and defaecation in rats suggesting a role of endogenous neurotensin

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We investigated the influence of the nonpeptide neurotensin receptor antagonist, SR 48692, administered orally, on gastric emptying and on acute defaecation. SR 48692 dose-dependently ($ED_{50} \sim 0.7 \mu\text{g kg}^{-1}$) increased gastric emptying of a food suspension, but it had no effect on that of a non-caloric meal. SR 48692 also dose-dependently promoted defaecation and increased faecal water content. We suggest that antagonism of endogenous neurotensin accounts for the gastric emptying and defaecation promoting action of SR 48692.

Keywords: Neurotensin; SR 48692; rat gut; gastric emptying; defaecation

Introduction Although it has been suggested that neurotensin is involved in a variety of physiological processes, evidence has been largely contingent on the availability of potent, selective and biologically stable receptor antagonists. Recently we described the biochemical and pharmacological properties of the first non-peptide neurotensin receptor antagonist, SR 48692, 2-[[1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carbonyl]amino]-adamantane-2-carboxylic acid (Gully *et al.*, 1993; Vita *et al.*, 1993) that was also shown to inhibit neurotensin binding *in vitro* to specific sites in guinea-pig intestinal tissues (Labbé-Jullié *et al.*, 1994).

The present study was designed to investigate the effects of SR 48692 on rat gastric emptying and acute defaecation, with a view to clarifying the presumed underlying role of endogenous neurotensin.

Methods Crl:CD BR, male rats (220–250 g) handled according to internationally accepted principles for care of laboratory animals, were used. The rats were kept under standard conditions and had free access to water and food (4RF21, Mucedola, Italia).

For assessment of gastric emptying rats were deprived of food 24 h before the test and were placed in cages with a wire mesh bottom with water freely available. A 3 ml bolus of a standard meal at 25°C was given by gavage. The non-caloric meal contained 2 ml of BaSO₄ (113% w/v, Prontobarrio Esófago, Bracco S.p.A., Italia) plus 1 ml 0.5% carboxymethylcellulose (CMC) and 0.05% phenol-red. The caloric meal was a 0.5% CMC suspension of standard chow (2 g of 4RF21 in 3 ml of CMC). Both meals had the same viscosity of about 1900 mPa s⁻¹ at 25°C.

Rats were killed by cervical dislocation immediately ($t=0$), 15 and 30 min after the non-caloric meal, or 0, 30 and 60 min after the caloric meal. The pylorus and the oesophageal junction were clamped before excision of the stomach.

After the caloric meal, the gastric content was assessed by weighing the stomachs before and after removing their contents and then cutting, rinsing and blotting on dry tissues. After the non-caloric meal, the stomachs were minced and vigorously stirred for 15 min in 20 ml 0.1 N NaOH. After centrifugation (3,000 r.p.m. for 15 min), 1 ml of supernatant was diluted with 9 ml of 0.1 N NaOH and the samples were read for absorbance at 560 nm.

At each time, the remaining content of the stomach after drug treatment was compared to that of control drug-free rats, killed at $t=0$. The percentage gastric emptying was calculated for each rat: % GE = $1 - (\text{content of treated rats} / \text{mean content of control rats}) \times 100$.

The dose of SR 48692 producing 50% of its assumed maximal effect, obtained at 0.1 mg kg^{-1} , (ED_{50}) was calculated graphically from the log-dose response-curve.

Faecal excretion was assessed according to the method previously described (Croci & Bianchetti, 1992).

Drugs were given either s.c. or p.o. (gavage) in 2 ml kg⁻¹ vehicles, i.e. CMC (SR 48692) or saline (ICS 205,930); control drug-free rats received only the vehicle.

SR 48692 was synthesized at SANOFI Recherche (Montpellier, France). ICS 205,930 HCl (endo-8-methyl-8-azabicyclo[3.2.1]oct-3-ol-indol-3-yl carboxylate.HCl) was purchased from RBI (Natick, MA, U.S.A.).

Results Gastric emptying was faster in control drug-free rats receiving a non-caloric standard meal (% GE $\pm$ s.e.mean, 52 ± 5 and 73 ± 3 respectively, at 15 and 30 min, $n=12$, $P<0.05$) than in those fed the caloric meal (at 30 and 60 min respectively 13 ± 2 and 50 ± 2 , $n=7$, $P<0.01$).

SR 48692 had no significant effect on gastric emptying of the non-caloric meal (% GE $\pm$ s.e.mean, $n=10-15$, at 15 and 30 min: SR 48692 0.1 mg kg^{-1} 49 ± 5 and 64 ± 3 ; 1 mg kg^{-1} 42 ± 6 and 68 ± 4) but dose-dependently increased that of the caloric meal (ED_{50} 0.7 and $0.8 \mu\text{g kg}^{-1}$ at 30 and 60 min) (Figure 1).

The stimulation of gastric emptying by 1 mg kg^{-1} SR 48692 was also tested in a separate experiment: after 30 min it was 38% more than control. This was slightly less than that achieved by 0.1 mg kg^{-1} SR 48692 (55%), indicating that the neurotensin receptor antagonist had already attained its maximal effect at 0.1 mg kg^{-1} .

The 5-HT₃ receptor antagonist, ICS 205,930 (ICS) (0.1 mg kg^{-1} , s.c. injected 30 min before the meal) significantly increased ($P<0.01$) gastric emptying in rats fed either of the standard meals (% GE $\pm$ s.e.mean, $n=7$, at 30 and 60 min after the caloric meal, control 5 ± 5 and 34 ± 3 ; ICS 38 ± 2 and 73 ± 5 , $P<0.01$; 15 min after the non-caloric meal, control 53 ± 5 ; ICS 79 ± 3 , $P<0.01$).

SR 48692 promoted defaecation dose-dependently ($AD_1 = 1.9 \mu\text{g kg}^{-1}$ p.o.) and the faecal water content was increased concurrently as indicated by the higher ratios of wet to dry weight (Table 1).

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Figure 1 Stimulation of gastric emptying by SR 48692 in rats fed a caloric meal. SR 48692 was given 60 min before the meal, and the gastric content was assessed after 30 (solid line) and 60 min (dotted line). The results are mean $\pm$ s.e.mean of seven rats. Values are significantly different from their respective controls ($P < 0.01$, Duncan's test) with the exception of that at $0.1 \mu\text{g kg}^{-1}$ SR 48692 (60 min).

Discussion The present study shows that the highly selective, non-peptide, neurotensin receptor antagonist, SR 48692, potently affects rat gut functions such as gastric emptying, faecal output and faecal water content. SR 48692, that is orally effective, has no intrinsic agonist activity, either *in vitro* or *in vivo*, on neurotensin receptors (Gully *et al.*, 1993). It potently and dose-dependently accelerated gastric emptying in rats fed a caloric meal, but not in those receiving a non-caloric meal. The two meals were otherwise identical as to viscosity and volume.

Although drug-free control rats receiving the non-caloric rather than the caloric meal presented substantially faster gastric emptying, the positive control provided by ICS 205,930 confirmed that the test system adequately discloses the ability of any suitable agent to shorten further the time required for stomach emptying. Our results thus suggest that neurotensin released by food, but not by gastric distension, is responsible for the lower rate of gastric emptying in drug-free control animals, and strongly support the involvement of this hormonal factor in the action of SR 48692 on the stomach.

Since SR 48692 stimulated faecal output and increased faecal water content at remarkably low doses, comparable to those effective on gastric emptying, the same neurotensin-mediated mechanism is likely to be involved. This supports an additional role already proposed for neurotensin, in influencing peristalsis as well as fluid secretion and absorption in the intestine (Hellström *et al.*, 1982; Ferris, 1989).

Table 1 Effect of SR 48692 on rat faecal excretion

SR 48692 dose ($\mu\text{g kg}^{-1}$, p.o.)	Faecal dry weight (g)	AD_{10} ^a ($\mu\text{g kg}^{-1}$, p.o.)	Wet/dry weight of faeces
—	0.10 ± 0.07		1.88 ± 0.04^b
0.1	$0.63 \pm 0.04^{**}$		$2.87 \pm 0.09^{**}$
1	$0.90 \pm 0.07^{**}$	1.9	$3.10 \pm 0.32^{**}$
10	$1.19 \pm 0.08^{**}$	(1–3.6)	$3.11 \pm 0.11^{**}$
100	$1.54 \pm 0.13^{**}$		$3.30 \pm 0.11^{**}$

^a AD_{10} = dose-inducing excretion of 1 g (dry weight) of faeces during 210 min after administration of SR 48692

^bFaeces collected 2–3 h before treatment

Data are means $\pm$ s.e.mean of eight rats with 95% confidence limits in parentheses.

^{**} $P < 0.01$ vs control drug-free rats (Duncan's test)

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