Drug-induced defaecation in rats: role of central 5-HT_{1A} receptors

¹Tiziano Croci, Marco Landi, ²Alberto Bianchetti & Luciano Manara

SANOFI-MIDY S.p.A. Research Center, Via G.B. Piranesi 38, 20137 Milan, Italy

1 We investigated the acute effects of 5-hydroxytryptamine (5-HT), and of the 5-HT_{1A} receptor agonists, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), buspirone and SR 57746A, on rat faecal pellet output and water content.

2 5-HT, 8-OH-DPAT, buspirone and SR 57746A, a new selective 5-HT_{1A} receptor agonist, displaced [³H]-8-OH-DPAT from specific binding sites in rat hippocampus membranes (*K_i*, nM: 1.8, 1.2, 15, 3.1 respectively) and stimulated rat defaecation dose-dependently. SR 57746A and buspirone induced 1 g dry weight of faeces at 1.3 and 6.1 mg kg⁻¹, p.o. (AD₁) respectively. 8-OH-DPAT and 5-HT stimulated defaecation after s.c. injection (AD₁, 0.07 and 7.5 mg kg⁻¹, respectively). All these agents increased faecal water content.

3 The putative 5-HT_{1A} receptor antagonist, pindolol, injected s.c. or i.c.v., significantly reduced the defaecation induced by systemically administered 8-OH-DPAT, buspirone or SR 57746A, but not 5-HT.

4 Pretreatment with *p*-chlorophenylalanine (i.p.) or 5,7-dihydroxytryptamine (i.c.v.), according to protocols designed to cause either generalized or CNS-limited 5-HT depletion respectively, also reduced the defaecation induced by buspirone or SR 57746A.

5 No specific 5-HT_{1A} binding sites could be labelled by incubating rat colon membranes with [³H]-8-OH-DPAT, and *in vitro* preparations of rat colon segments showed no response to 8-OH-DPAT or SR 57746A up to 5 µM.

6 After eight days' repeated daily treatment, complete tolerance developed to the stimulant effects of SR 57746A and buspirone on faecal water content, but not on faecal pellet output. This suggests that faecal mass excretion and water exchange through the gut wall are affected by independent mechanisms.

7 The present findings support the involvement of central 5-HT_{1A} receptors in intestinal propulsion and regulation of luminal fluid content, presumably accounting for the drug-induced defaecation in rats.

Keywords: Defaecation; 5-HT_{1A} receptor; faecal water content; buspirone; 8-OH-DPAT; SR 57746A; 5-HT

Introduction

The role of 5-hydroxytryptamine (5-HT) in the regulation of intestinal motor activity and endoluminal fluid content has long been recognized (Costa & Furness, 1979; Costa *et al.*, 1982). However, the underlying mechanisms are still largely unknown. In the gut 5-HT may affect motility either directly by stimulating smooth muscle receptors (Buchheit *et al.*, 1985) or indirectly through enteric 5-hydroxytryptaminergic interneurons, which modulate the intestinal peristaltic reflex by releasing other neurotransmitters (Costa *et al.*, 1982; Buchheit *et al.*, 1985; De Ponti *et al.*, 1991). 5-HT itself is highly concentrated in enterochromaffin cells and can be released by appropriate stimuli into the portal circulation or gut lumen (Racke & Schworer, 1991), thus influencing fluid balance and intestinal motility.

Gut motility is under the influence of the central nervous system (CNS) which may affect the intrinsic automaticity of the enteric nervous system through efferent pathways. Although different gut-located 5-HT receptor subtypes are certainly involved in the modulation of intestinal motility and secretion (Costall & Naylor, 1990), the role of CNS 5-HT in enteric functions is virtually unknown.

In a preliminary study, we found that putative 5-HT_{1A} receptor agonists, reportedly active as central antidepressant and anxiolytic drugs (Goa & Ward, 1986; Traber & Glaser, 1987; Robinson *et al.*, 1989; Jenkins *et al.*, 1990; Cervo *et al.*, 1994) stimulated faecal excretion by rats (Croci *et al.*, 1990a,b; Bianchetti *et al.*, 1990; 1991). The present work

was aimed at clarifying the importance of central 5-HT_{1A} receptors in the effects on faecal output and water content of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), buspirone and the potent, selective new 5-HT_{1A} receptor agonist SR 57746A (4-(3-trifluoromethylphenyl)-*N*-[2-(naphth-2-yl)-ethyl]-1,2,3,6-tetrahydropyridine HCl) (Bachy *et al.*, 1993).

Methods

Male CrI:CD BR (Charles River Italia) rats weighing 220 ± 20 g were used, handled according to internationally accepted principles for the care and use of laboratory animals (E.E.C. Council directive 86/609, OJ L358, 1, Dec. 12, 1987). Rats were humanely killed by cervical dislocation.

Faecal excretion

Faecal excretion was assessed according to the method described by Croci & Bianchetti (1992). On the day of the experiment, the rats were placed individually in grid-floor cages, with food and water *ad libitum*, and randomly allocated to different treatments. The food was withdrawn at 08 h 00 min and 3 h later the animals were given one of the following drugs in 2 ml kg⁻¹ of the appropriate vehicle: 5-HT or 8-OH-DPAT dissolved in saline, SR 57746A or buspirone suspended in 0.5% carboxymethylcellulose (CMC). Drug-free control rats received only the vehicles (each animal was given s.c. saline and p.o. CMC).

Immediately after treatment, gentle thumb pressure was applied to the perianal region to expel faecal pellets from the rectum. The pellets discharged during the next 90 min (s.c.

¹ Author for correspondence.

² Present address: PHARMACIA, Farmitalia Carlo Erba; Preclinical Research, Via Giovanni XXIII 23, 20014 Nerviano, Italy.

treatment) or 210 min (oral treatment) were collected and weighed immediately (wet weight) and after drying (10 h at 50°C) to constant weight (dry weight). The doses inducing 1 g (dry weight) faecal excretion (AD₁) were extrapolated from log-dose response-curves (Finney, 1964). Action on secretion and/or reabsorption of fluids was assessed from the ratio of wet to dry faecal weight. For drug-free controls this was obtained from the weights of the faeces excreted by all rats in the 2–3 h before treatment, since control rats generally did not defaecate during the 90 or 210 min observation period.

Antagonism of faecal excretion stimulated by 5-hydroxytryptamine receptor agonists was assessed after either s.c. (2 ml kg⁻¹) or i.c.v. (10 µl/rat) pindolol, according to the protocols described in the Tables.

To deplete brain 5-HT, rats were treated with i.c.v. 5,7-dihydroxytryptamine (5,7-DHT) following the procedure described by Carli *et al.* (1989), or i.p. *p*-chlorophenylalanine (*p*CPA) according to Hutson *et al.* (1987).

Rats anaesthetized with ethyl-ether were immobilized in a Kopf stereotaxic instrument. 5,7-DHT dissolved in 0.1% ascorbic acid was infused (20 s) i.c.v. in a volume of 20 µl into the right lateral ventricle. The control animals received ascorbic acid only. To protect noradrenaline-containing neurones from the action of 5,7-DHT, the rats received desmethylimipramine, 25 mg kg⁻¹, i.p. 30 min before 5,7-DHT. These animals were used for faecal excretion experiments on the third day after 5,7-DHT. *p*CPA was injected daily for three consecutive days and rats were used for faecal excretion experiments 24 h after the last injection.

To assess whether the acute effects on faecal output and water content changed after repeated treatment with 5-HT_{1A} receptor agonists, rats were treated daily at 11 h 00 min with buspirone, SR 57746A or CMC for eight consecutive days. Faecal excretion (wet and dry weight of faeces) was assessed daily as described above.

Statistical analysis was based on ANOVA plus Duncan's test (Kramer, 1956).

Binding assays

Radioligand binding assays with [³H]-8-OH-DPAT were carried out either on rat hippocampus previously frozen and stored at -20°C or on fresh rat distal colon segments (5–6 cm) taken 10 cm from the ileo-caecal junction. Membranes from rat hippocampus or colon were prepared according to Bennett & Snyder (1976) or Landi *et al.* (1992) respectively.

The membrane preparation, containing about 2 mg protein ml⁻¹, was used immediately for binding experiments. Protein was assayed according to Lowry *et al.* (1951) with bovine serum albumin as standard.

In both preparations (colon and hippocampus), the binding assay for [³H]-8-OH-DPAT (180–264 Ci mmol⁻¹, radiochemical purity >98%) was run in triplicate at 25°C for 30 min incubation. Samples contained 500 µl membrane suspension and 1 nM [³H]-8-OH-DPAT in a total volume of 1 ml. The compounds tested for competition with the binding of the radiolabelled ligand were dissolved in distilled water or ethanol; the ethanol concentration in the assay (10 µl ml⁻¹) did not affect binding.

Incubation of samples was started by the addition of membranes and stopped by rapid vacuum filtration through Whatman GF/B filters, followed by two consecutive 5-ml cold Tris-HCl (50 mM, pH 7.4) washes on a Brandel Cell Harvester. The [³H]-8-OH-DPAT bound was measured by liquid scintillation spectrometry in a Beckman LS 6000IC beta counter. Non-specific binding was determined in the presence of 10 µM 5-HT. In the hippocampus, specific binding was calculated as the difference between total and non-specific binding, and accounted for 85–90% of the total.

Data were plotted and analysed according to published procedures (Scatchard, 1949). Saturation curves were fitted to a one-site model, using a non-linear regression computer

programme, Accufit Saturation (Beckman, London Software). The inhibitory constant (K_i) was calculated according to Cheng & Prusoff (1973) while the IC₅₀ was obtained by converting the binding data to logits and plotting them against the log of the inhibitor concentration to obtain linear regression (Finney, 1964).

Isolated distal colon preparation

Rat distal colon segments (about 5 cm, 2 cm from the rectum) were carefully dissected and mounted in a 20 ml organ bath containing warm (32°C) aerated (100% O₂) Tyrode solution of the following composition, mM: NaCl 136.9, KCl 2.68, MgCl₂ 1.05, CaCl₂ 1.82, NaH₂PO₄ 0.417, NaHCO₃ 11.9, glucose 5.5. The tissue was stretched with a weight of 0.5 g and tonic contractions elicited by 5-HT were recorded isotonicity and scored as the percentage of maximal effect. After 60 min equilibration, 5-HT or the 5-HT_{1A} receptor agonists were added, the concentrations being increased stepwise. Only one cumulative curve was obtained for each preparation (contact time for each concentration 3 to 5 min). The concentration of 5-HT (EC₅₀) raising tonus by 50% over the basal value was extrapolated from the regression lines obtained by plotting log-concentration against response.

Chemicals

The following drugs were purchased from commercial sources: Sigma-Aldrich Corp., St-Louis, Missouri, U.S.A., buspirone HCl, (±)-pindolol, *p*CPA, 5,7-DHT, carbachol, 5-HT creatinine sulphate, pargyline; RBI, Natick, Ma, U.S.A., 8-OH-DPAT HBr, ketanserin; Amersham, Buckinghamshire, England, [³H]-8-OH-DPAT. Mesulergine was kindly provided by Sandoz Pharma, Switzerland and desmethylimipramine HCl by Ciba-Geigy, Varese, Italy.

SR 57746A was synthesized in the Chemistry Section of the SANOFI-MIDY S.p.A. Research Centre, Milan, Italy.

Results

Binding of drugs acting at 5-HT receptors

In hippocampal membranes [³H]-8-OH-DPAT bound saturably with an affinity constant (K_d) of 0.9 ± 0.2 nM and a B_{max} of 185 ± 22 fmol mg⁻¹ protein (mean $\pm$ s.e. mean of three experiments), as revealed by saturation analysis with eight concentrations of [³H]-8-OH-DPAT ranging from 0.125 to 16 nM. Binding was rapid, reversible and stable for at least 60 min. [³H]-8-OH-DPAT specific binding to hippocampal membranes was competitively inhibited by the 5-HT receptor agonists, 5-HT, 8-OH-DPAT, SR 57746A and buspirone; the K_i (nM, 95% confidence limits) were 1.8 (1.7–1.9), 1.2 (1–1.5), 3.1 (2.9–3.4) and 15 (13–16), in that order. The putative 5-HT_{1A} receptor antagonist, pindolol also competed for [³H]-8-OH-DPAT binding sites with high affinity, K_i 36 (31–41) nM. The slope of the Hill plot was never significantly different from unity.

No specific binding could be detected in rat distal colon membranes incubated with [³H]-8-OH-DPAT (1 nM).

Stimulation of faecal excretion by 5-HT_{1A} receptor agonists

Table 1 shows the dose-related effects of 5-HT_{1A} receptor agonists and 5-HT on rat faecal excretion. After s.c. injection, 8-OH-DPAT was about 100 times more potent than 5-HT in inducing defaecation (AD₁ 0.07 and 7.5 mg kg⁻¹ respectively) and increasing the faecal water content, as indicated by the higher than control ratios of wet to dry weights. However, 8-OH-DPAT (1 mg kg⁻¹) and 5-HT (12 mg kg⁻¹) were completely inactive when given orally (faecal dry weights, $g \pm$ s.e. mean, 0.08 ± 0.03 and 0.02 ± 0.02

Table 1 Effects of 5-HT, 8-OH-DPAT, buspirone and SR 57746A on rat defaecation

		Dose (mg kg ⁻¹)	Faecal dry weight (g)	AD ₁ ^a (mg kg ⁻¹)	Wet/dry weight of faeces
Control		–	0.01 ± 0.01		1.98 ± 0.05 ^b
5-HT	s.c.	4	0.80 ± 0.04**		3.43 ± 0.13**
		12	1.17 ± 0.05**	7.5	3.16 ± 0.11**
		36	1.36 ± 0.05**	(5.9–9.8)	3.60 ± 0.13**
8-OH-DPAT	s.c.	0.0125	0.46 ± 0.16*		2.44 ± 0.15**
		0.05	0.76 ± 0.15**	0.07	2.37 ± 0.11**
		0.2	1.59 ± 0.10**	(0.04–0.12)	2.63 ± 0.12**
Buspirone	p.o.	1	0.18 ± 0.08		NE ^c
		4	0.75 ± 0.09**	6.1	2.41 ± 0.17*
		16	1.54 ± 0.12**	(4.7–7.7)	2.69 ± 0.11**
SR 57746A	p.o.	0.25	0.41 ± 0.09*		NE ^c
		1	0.64 ± 0.12**	1.3	2.30 ± 0.12*
		4	1.56 ± 0.15**	(0.5–3.1)	2.73 ± 0.05**

^aAD₁: dose inducing excretion of 1 g (dry weight) of faeces during 90 min (s.c.) or 210 min (p.o.) after drugs.

^bFaeces collected 2–3 h before treatment.

^cNot evaluable.

Data are mean ± s.e.mean from 8 rats. In parentheses 95% confidence limits. Control (drug-free) rats received only vehicles (saline and CMC). See Methods for details. **P* < 0.05, ***P* < 0.01 vs control (Duncan's test).

respectively). SR 57746A and buspirone were both effective orally, the former being about five times more potent (Table 1). 8-OH-DPAT (1 µg/rat, i.c.v.) caused the excretion of 0.8 ± 0.1 g of faeces during the 30-min collection time. All agents significantly increased the wet/dry weight ratio of faeces.

Prevention of 5-HT_{1A} receptor agonist-induced faecal excretion by pindolol

As shown in Table 2, pindolol (1 or 5 mg kg⁻¹, s.c.) significantly prevented defaecation and the increase of the wet/dry weight ratio of faeces induced by 8-OH-DPAT (0.2 mg kg⁻¹, s.c.), SR 57746A (4 mg kg⁻¹, p.o.), or buspirone (12 mg kg⁻¹, p.o.), but not by 5-HT (12 mg kg⁻¹, s.c.). In rats chronically implanted with cannulae, given pindolol i.c.v. (1 or 2.5 µg/rat), faecal excretion (dry weight) induced by SR 57746A or 8-OH-DPAT, but not by 5-HT, was likewise prevented (Table 3). Unlike its effect when given s.c., the i.c.v. administration of pindolol (a) slightly but significantly increased faecal output and (b) did not prevent the increase in faecal water content induced by the 5-HT_{1A} receptor agonists (Table 3).

Effect of 5,7-DHT and pCPA on drug-induced faecal excretion

As shown in Tables 4 and 5, in rats pretreated with pCPA (160 mg kg⁻¹ per day i.p. for three days) or 5,7-DHT (150 µg/rat i.c.v.), SR 57746A and buspirone were less effective in promoting the excretion of faeces (dry weight), although the wet/dry weight ratio was still as high as in non-pretreated animals. However, pretreatment with either pCPA or 5,7-DHT by themselves significantly raised the wet:dry weight ratio (Tables 4, 5). Rats given pCPA or 5,7-DHT also had lower body weight than untreated controls (about 25 g). In the same experiments, in additional groups of rats pretreated with pCPA or 5,7-DHT the scores for which are not presented in the Tables, the cholinomimetic carbachol (0.2 mg kg⁻¹, s.c.) was as effective in promoting defaecation as in non-pretreated animals (dry weight of faeces, $\bar{x} \pm \text{s.e.mean}$: carbachol, 1.14 ± 0.16; pCPA + carbachol, 0.89 ± 0.18; 5,7-DHT + carbachol, 1.0 ± 0.2). Carbachol also increased the wet/dry weight ratio of faeces but this was not affected by pCPA pretreatment.

Table 2 Antagonism of 5-HT, 8-OH-DPAT, buspirone and SR 57746A-induced rat defaecation by s.c. pindolol

	Pindolol dose (mg kg ⁻¹)	Faecal dry weight (g)	Wet/dry weight of faeces
Control	–	0.03 ± 0.03	1.88 ± 0.07*
–	1	0.06 ± 0.05	NE ^b
–	5	0.02 ± 0.02	NE ^b
5-HT	–	1.31 ± 0.15**	3.05 ± 0.06**
	1	1.05 ± 0.10**	2.92 ± 0.10**
8-OH-DPAT	–	1.60 ± 0.11**	2.65 ± 0.09**
	1	0.34 ± 0.13* ^{○○}	2.01 ± 0.10 ^{○○}
	5	0.16 ± 0.06 ^{○○}	NE ^b
Buspirone	–	1.73 ± 0.09**	2.79 ± 0.07**
	1	1.23 ± 0.09** ^{○○}	2.66 ± 0.07**
	5	0.20 ± 0.08* ^{○○}	2.06 ± 0.06 ^{○○}
SR 57746A	–	1.75 ± 0.06**	2.60 ± 0.08**
	1	1.24 ± 0.08***	2.60 ± 0.07**
	5	0.17 ± 0.07 ^{○○}	1.85 ± 0.09 ^{○○}

^aFaeces collected 2–3 h before treatment. ^bNot evaluable. Data are mean ± s.e.mean from 8 rats. 5-HT (12 mg kg⁻¹, s.c.) and 8-OH-DPAT (0.2 mg kg⁻¹, s.c.) were given 30 min after pindolol and buspirone (12 mg kg⁻¹, p.o.) and SR 57746A (4 mg kg⁻¹, p.o.) immediately after. Control (drug-free) rats received only vehicles (saline and CMC). See Methods for details.

P* < 0.05, *P* < 0.01 vs control; ^{○○}*P* < 0.01 vs 8-OH-DPAT, buspirone or SR 57746A alone (Duncan's test).

Effect of repeated treatment with 5-HT_{1A} receptor agonists on faecal excretion

Throughout repeated oral treatment (8 days) with either buspirone (4 or 16 mg kg⁻¹) or SR 57746A (1 or 4 mg kg⁻¹), stimulation of faeces excretion was dose-related with no real difference between the results on any given day in terms of faecal dry weight (Figure 1) and faecal pellet number (data not shown). During the first four days of treatment with either compound, some rats (10 to 30%) presented loose stools, especially at the higher doses. This was no longer apparent as treatment continued. The ratio of wet to dry weight of faeces after the first dose was always considerably higher than in drug-free controls, but tolerance developed

within a few days (days 5 to 6 for buspirone and 2 to 5 for SR 57746A depending on the dose) and the wet/dry weight of faeces returned to drug-free ratios. The body weight increase of rats on the 8th day was not significantly different from controls in the buspirone or SR 57746A groups (Δ g $\pm$ s.e.mean: buspirone 4 and 16 mg kg⁻¹, 40 $\pm$ 3 and 39 $\pm$ 2; SR 57746A 1 and 4 mg kg⁻¹, 40 $\pm$ 2 and 38 $\pm$ 3; controls, 41 $\pm$ 3).

Table 3 Antagonism of 5-HT, 8-OH-DPAT, buspirone and SR 57746A-induced rat defaecation by i.c.v. pindolol

	Pindolol dose (μ g/rat)	Faecal dry weight (g)	Wet/dry weight of faeces
Control	–	0.02 $\pm$ 0.02	1.86 $\pm$ 0.10 ^a
–	1	0.20 $\pm$ 0.07*	NE ^b
–	2.5	0.41 $\pm$ 0.12**	2.90 $\pm$ 0.06
5-HT	–	1.48 $\pm$ 0.13**	2.85 $\pm$ 0.08**
	1	1.24 $\pm$ 0.10**	2.92 $\pm$ 0.10**
	–	1.83 $\pm$ 0.06**	2.75 $\pm$ 0.08**
8-OH-DPAT	1	0.42 $\pm$ 0.03** ^{○○}	2.91 $\pm$ 0.10**
	2.5	0.42 $\pm$ 0.09** ^{○○}	3.00 $\pm$ 0.13**
	–	1.82 $\pm$ 0.15**	2.76 $\pm$ 0.07**
SR 57746A	1	0.70 $\pm$ 0.14** ^{○○}	2.78 $\pm$ 0.09**
	2.5	1.11 $\pm$ 0.10** ^{○○}	2.85 $\pm$ 0.11**

^aFaeces collected 2–3 h before treatment. ^bNot evaluable. Average faecal excretion (g $\pm$ s.e.mean) from at least 12 rats. Pindolol in a volume of 10 μ l/rat was given i.c.v. concurrently with 5-HT (12 mg kg⁻¹, s.c.) and 8-OH-DPAT (0.2 mg kg⁻¹, s.c.) or 45 min after SR 57746A (4 mg kg⁻¹, p.o.). Control (drug-free) rats received only vehicles (saline and CMC). See Methods for details.

* $P < 0.05$, ** $P < 0.01$ vs control; [○] $P < 0.05$, ^{○○} $P < 0.01$ vs SR 57746A or 8-OH-DPAT alone (Duncan's test).

Effect of 5-HT receptor agonists on isolated distal colon

The isolated colon preparation presented a low degree of spontaneous contractions. 5-HT induced a concentration-dependent increase of the basal tonus: EC₅₀ = 130 (100–160) nM, that was partially prevented by the 5-HT₂ receptor antagonist, ketanserin and the 5-HT_{1C} receptor antagonist, mesulergine, but not by pindolol. 8-OH-DPAT and SR 57746A up to 5 μ M did not induce any contraction, or affect the tonus of the colon.

Discussion

Defaecation by rats is environmentally influenced and drugs can either increase or reduce it depending on experimental conditions (Sanberg *et al.*, 1989; Croci *et al.*, 1994). Our model is suitable for the evaluation of defaecation induced acutely by different agents, since control animals do not defaecate throughout the standard observation period (Croci & Bianchetti, 1992).

We investigated the effects of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, buspirone and SR 57746A, either *in vitro* on 5-HT_{1A} binding and rat isolated colon contractility or *in vivo* on rat faecal pellet output and water content. SR 57746A bound to [³H]-8-OH-DPAT-labelled rat hippocampal membranes at nanomolar concentrations and induced faecal excretion as did 8-OH-DPAT and buspirone, suggesting that 5-HT_{1A} receptors are involved in their intestinal effects. Moreover 8-OH-DPAT and buspirone promoted defaecation at doses within the range of those reportedly effective in several behavioural and other *in vivo* functional tests (Kennet *et al.*, 1987; Cerro & Samanin, 1987; Moser *et al.*, 1990; Martin *et al.*, 1991). Activation of 5-HT_{1A} receptors is further supported as a common mechanism for increasing faecal pellet output and water content, since both were prevented by pindolol at doses previously found to antagonize 5-HT_{1A}-mediated responses (Hjorth & Carlsson, 1986; Sharp *et al.*,

Table 4 Effect of pCPA on faecal excretion stimulated by buspirone or SR 57746A

	Dose (mg kg ⁻¹ , p.o.)	pCPA	Faecal dry weight (g)	Wet/dry weight of faeces
Control	–	–	0 $\pm$ 0	1.87 $\pm$ 0.03 ^a
	–	+	0.04 $\pm$ 0.04	2.43 $\pm$ 0.12***
Buspirone	12	–	1.84 $\pm$ 0.08** ^{□□}	3.26 $\pm$ 0.11** [□]
	12	+	0.75 $\pm$ 0.19** ^{□□○○}	2.69 $\pm$ 0.11**
SR 57746A	4	–	2.36 $\pm$ 0.12** ^{□□}	3.03 $\pm$ 0.12** [□]
	4	+	0.40 $\pm$ 0.13* ^{○○}	2.53 $\pm$ 0.24*

^aFaeces collected 2–3 h before treatment.

Data are mean $\pm$ s.e.mean from at least 8 rats. pCPA (160 mg kg⁻¹ per day) was injected i.p. daily for three consecutive days. Drugs were administered 24 h after the last pCPA injection. Control (drug-free) rats received only vehicles (saline and CMC). See Methods for details. * $P < 0.05$, ** $P < 0.01$ vs control; [□] $P < 0.05$, ^{□□} $P < 0.01$ vs pCPA alone; [○] $P < 0.05$, ^{○○} $P < 0.01$ vs SR 57746A or buspirone alone (Duncan's test)

Table 5 Effect of i.c.v. 5,7-DHT on faecal excretion stimulated by buspirone or SR 57746A

	Dose (mg kg ⁻¹ , p.o.)	5,7-DHT	Faecal dry weight (g)	Wet/dry weight of faeces
Control	–	–	0.02 $\pm$ 0.02	2.00 $\pm$ 0.09 ^a
	–	+	0.04 $\pm$ 0.03	2.74 $\pm$ 0.10***
Buspirone	12	–	2.01 $\pm$ 0.17** ^{□□}	3.04 $\pm$ 0.11**
	12	+	1.30 $\pm$ 0.09** ^{□□○○}	3.29 $\pm$ 0.18**
SR 57746A	4	–	2.22 $\pm$ 0.10** ^{□□}	2.93 $\pm$ 0.10**
	4	+	1.21 $\pm$ 0.08** ^{□□○○}	2.67 $\pm$ 0.12**

^aFaeces collected 2–3 h before buspirone or SR 57746A.

Data are mean $\pm$ s.e.mean from at least 8 rats. Rats were injected concurrently with i.c.v. 5,7-DHT (150 μ g/rat) and i.p. DMI (25 mg kg⁻¹) three days before drugs. Control (drug-free) rats received only vehicles (saline and CMC). See Methods for details. Faeces were collected for 210 min after buspirone or SR 57746A.

** $P < 0.01$ vs control; ^{□□} $P < 0.01$ vs 5,7-DHT alone; ^{○○} $P < 0.01$ vs SR 57746A or buspirone alone (Duncan's test).

Figure 1 Effect of repeated daily oral doses of (a) buspirone ($\square$, 4 mg kg⁻¹; Δ , 16 mg kg⁻¹) and (b) SR 57746A ($\square$, 1 mg kg⁻¹; Δ , 4 mg kg⁻¹) on rat faecal pellet output (dry weight of faeces; $\circ$, control) and water content (wet/dry weight of faeces). Data are mean $\pm$ s.e.mean from 10 rats in each experimental group. The mean $\pm$ s.e.mean of wet/dry weight of faeces in control rats (dotted line) was 1.96 ± 0.06 and 1.92 ± 0.05 for buspirone and SR 57746A respectively. * $P < 0.01$ vs control (Duncan's test).

1993). It is worth noting that 5-HT acts peripherally on different 5-HT receptor subtypes (Croci et al., 1990b) and indeed, in spite of its high affinity for 5-HT_{1A} binding sites, it promotes faecal excretion at s.c. doses 100 times higher than 8-OH-DPAT.

The overall evidence produced by the present study certainly upholds the view that in rats, 5-HT_{1A} receptor agonists promote defaecation by stimulating receptors in the CNS rather than peripherally in intestinal smooth muscle and/or myenteric plexus. Thus the representative 5-HT_{1A} receptor agonist, 8-OH-DPAT, injected i.c.v., induced defaecation. We found no intestinal 5-HT_{1A} sites, since there was no specific [³H]-8-OH-DPAT binding in rat distal colon membranes and neither 8-OH-DPAT nor SR 57746A had any effect on the tonus of the isolated distal colon. This, however, was dose-dependently contracted by 5-HT, an effect partially prevented by the 5-HT_{1C} and 5-HT₂ receptor antagonists mesulergine and ketanserin, but not by the putative 5-HT_{1A} receptor antagonists, pindolol.

Pindolol i.c.v. significantly reduced the defaecation promoting effects of SR 57746A and 8-OH-DPAT, but not of 5-HT. This is unlikely to be a result of pindolol diffusing into the periphery because: (a) in an additional experiment (results not shown), the same dose of pindolol given i.v. was unable to prevent faecal excretion induced by 5-HT_{1A} receptor agonists; (b) pindolol, i.c.v. or s.c., did not prevent the presumed peripheral action of 5-HT on defaecation. In addition, it is worth pointing out that the incomplete prevention of the defaecation promoting action of 5-HT_{1A} receptor agonists by i.c.v. pindolol is inherent to its dose-dependent intrinsic agonist activity under these conditions, which is consistent with its otherwise mixed agonist-antagonist properties at central 5-HT_{1A} receptors (Hjorth & Carlsson, 1986).

In rat brain, 5-HT_{1A} receptors are located both pre- and postsynaptically where they subserve different functions

(Tricklebank et al., 1984; Goodwin et al., 1985; 1986; Dourish et al., 1986; Gilbert et al., 1988). In the present study, the fact that pretreatment with i.c.v. 5,7-DHT or i.p. pCPA (both known to deplete 5-HT stores in the CNS) prevented buspirone- or SR 57746A-induced faecal excretion in rats suggests that presynaptic 5-HT_{1A} receptors on central 5-hydroxytryptaminergic neurones are involved in the intestinal effects of these agonists. The fact that only the increase in dry weight of faeces, not in water content, was prevented to a greater extent in rats pretreated with 5,7-DHT or pCPA is accounted for by their ability to increase faecal water content on their own. Carbachol-induced faecal excretion was not prevented by pretreating rats with 5,7-DHT or pCPA, indicating the 5-HT specificity of their inhibition of drug-induced defaecation.

Buspirone or SR 57746A-induced faecal excretion was prevented more by pCPA than by 5,7-DHT. Although we did not measure the actual CNS and intestinal 5-HT depletion by these agents, with the protocols adopted 5,7-DHT (i.c.v.) should have depleted rat brain 5-hydroxytryptaminergic neurones by about 70% (Cervo & Samanin, 1987) while pCPA (i.p.) should deplete brain 5-HT by about 90% and 5-HT in the colon by about 50% (Weber, 1970). Thus greater central and/or peripheral 5-HT depletion could explain the more substantial prevention by pCPA of 5-HT_{1A} receptor agonist-induced defaecation in our experiments. In addition SR 57746A was somewhat more susceptible than buspirone to both the 5-HT depleting agents. Since buspirone in rats is actively biotransformed into the α_2 -adrenoceptor antagonist, 1-(2-pyrimidinyl) piperazine (Caccia et al., 1986), which is also a potent inducer of rat defaecation, through a different, non-5-hydroxytryptaminergic mechanism (Croci & Bianchetti, 1992), this could partly explain the difference from SR 57746A.

Repeated daily doses of the 5-HT_{1A} receptor agonists led to progressive loss of their overall effectiveness on faecal excretion. After eight days of treatment, complete tolerance developed to the effects of SR 57746A or buspirone on faecal water content, but not on faecal dry weight. These results suggest that the drug mechanisms stimulating faecal pellet output and affecting water exchange through the gut wall are independent. Tolerance to the faecal water content increasing effect seemed to arise sooner with SR 57746A than with buspirone. Unlike SR 57746A, which appears to be a full 5-HT_{1A} receptor agonist (Bachy et al., 1993), buspirone has partial agonist properties (Millan et al., 1991), possibly accounting for the slower tolerance.

In conclusion the present findings support a role of 5-HT_{1A}-5-hydroxytryptaminergic mechanisms in the CNS in drug-induced defaecation in rats. Our *in vitro* radioligand binding or functional assays, provided no evidence of 5-HT receptor subtypes accounting for such mechanisms in the rat distal colon, although 5-HT_{1A} receptors are present in the guinea-pig ileum, as shown by electrophysiologically (Galligan et al., 1988) and mechanically monitored (Fozard & Kilbinger, 1985) responses to appropriate agonists and antagonists in isolated preparations. Yet acceleration of postprandial colonic transit by 5-HT_{1A} receptor agonists in an *in vivo* rat model, has been recently attributed to a local rather than central receptor subtype-specific 5-HT mechanism (Gué et al., 1994). We assume that the experimental condition we adopted involves the stimulation of central 5-HT_{1A} receptors presumably activating efferent cholinergic pathways (Croci et al., 1990b), leading to propulsive contraction of gut smooth muscle (Croci et al., 1994).

The relevance of these findings for other species is unknown but, should they apply to man, 5-HT_{1A} receptor agonists might have clinical potential for treating hypomotility disorders of the lower intestinal tract, such as idiopathic constipation.

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