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Stimulation of faecal excretion in rats by α_2-adrenergic antagonists

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Abstract—The effects of several α-adrenoceptor antagonists on faecal output and water content in rats were investigated. Fed rats were treated either subcutaneously (s.c.) or orally with phentolamine, idazoxan, yohimbine, 1-(2-pyrimidinyl) piperazine (PmP) or prazosin. Drug potencies were compared on the basis of the dose inducing excretion of 1 g dry weight of faeces (AD1) by rats that do not normally excrete any faecal pellet during the observation time. The α_2-antagonist, idazoxan (AD1 = 0·25 mg kg^{-1}, s.c.) was approximately 2·5, 4 and 8 times more potent than PmP, phentolamine and yohimbine in promoting faecal excretion. Prazosin, an α_1-antagonist with putative affinity for the α_{2B}-receptor subtype, was the least effective (AD1 > 5 mg kg^{-1}, s.c.). The same compounds also increased the water content of faeces and had similar potencies by the oral route. Both clonidine (0·15 mg kg^{-1}, s.c.) and atropine (0·2 mg kg^{-1}, s.c.) significantly prevented the effects of all antagonists on faecal excretion. The present results are consistent with the view that rat colon is under tonic inhibitory control of prejunctional α_2-adrenergic receptors, whose blockage by specific antagonists induces faecal excretion. The α_{2A}-receptor subtype appears to be the most likely candidate for controlling faecal excretion through inhibition of acetylcholine release.

Coordination of motor activity and regulation of gastrointestinal fluid transport are important physiological functions of the enteric nervous system. In this context, the α_2-adrenoceptors were found to play a prominent role as modulators of intestinal propulsion and fluid secretion (DiJoseph et al 1984; Crema & De Ponti 1989). The α_2-agonist clonidine has been reported to be a potent antidiarrhoeal drug (Lal et al 1981; Megens & Niemegeers 1984) and an inhibitor of defaecation under normal (Doherty & Hancock 1983) and stressful conditions (Laverty & Taylor 1969). On the other hand, the α_2-antagonist yohimbine has been shown to facilitate in-vitro release of acetylcholine and to increase the peristaltic reflex (Marcoli et al 1987).

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The aim of the present study was to evaluate the functional importance of the α_2-adrenergic system in modulating faecal excretion by conscious rats, in view of the now recognized hetereogeneity of α_2-adrenergic receptors and, especially the recently postulated existence of separate α_{2A}- and α_{2B}-adrenoceptor subtypes (Bylund 1988; Young et al 1989; Gobbi et al 1990).

Materials and methods

Male Crl:CD(SD)BR rats (Charles River, Italy), 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 orally (p. o.). Drugs were dissolved in 0·9% NaCl (saline) or distilled water (except prazosin, which was dissolved in a 10% aqueous solution of polyethylene-glycol) and administered in a volume of 2 mL kg^{-1}.

Treatments were assigned from random tables, each group consisting of eight animals. 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. treatment) or 210 min (p.o. 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-lines (Finney 1964).

Any action on secretion or reabsorption of fluids was assessed from the ratio of wet to dry faecal weights. The normal ratio was calculated from the faeces excreted throughout the 2 h preceding treatment, since control rats generally did not defecate during the observation period (90–210 min).

Statistical analysis of the effects of tested compounds on faecal excretion was based on the Dunnett test (Dunnett 1955).

The following drugs were purchased from commercial sources

as indicated: Ciba-Geigy (Varese, Italy)—phentolamine methansulphonate (Regitin ampoules); Sigma-Aldrich Corp (St Louis, MO, USA)—atropine sulphate, prazosin HCl, yohimbine HCl; Boerhinger Ingelheim—clonidine (Catapresan ampoules); Reckitt & Colman (London, UK)—idazoxan. 1-(2-Pyrimidinyl) piperazine (PmP, CM 56324H) was synthesized in the chemistry section of Sanofi-Midy Research Centre, (Milan, Italy).

Results

Table 1 shows the dose-related effects of the s.c.-injected α-adrenoceptor antagonists. Idazoxan was the most potent compound to induce faecal output (AD1: 0·25 mg kg^{-1}), followed by PmP (0·66 mg kg^{-1}), phentolamine (1 mg kg^{-1}) and yohimbine (2 mg kg^{-1}). The least effective compound, prazosin, did not induce excretion of 1 g of faeces even at the highest dose tested (5 mg kg^{-1}). The water content of faeces was increased by the treatments, as indicated by the higher than normal ratios of wet to dry weight.

Idazoxan, PmP and yohimbine stimulated faecal excretion also when given orally (data not shown), with corresponding AD1 and 95% confidence limits of 0·14 (0·05–0·38), 0·56 (0·50–1·35) and 0·78 (0·42–0·75) mg kg^{-1}. Prazosin induced the excretion of 0·7 ± 0·11 g of faeces at the highest tested dose of 5 mg kg^{-1}, p.o.

Fig. 1 summarizes the results of experiments designed to ascertain the specificity of the stimulation of faecal excretion by the α-adrenergic antagonists. Atropine (0·2 mg kg^{-1}, s.c.) and the α_2-agonist clonidine (0·15 mg kg^{-1}, s.c.) completely prevented the effects of s.c. equiactive doses, extrapolated from log-dose response-lines, of idazoxan (0·5 mg kg^{-1}), PmP (2·5 mg kg^{-1}), and phentolamine (2 mg kg^{-1}) on faecal excretion. The s.c. effect of 5 mg kg^{-1} prazosin was also antagonized. Neither atropine nor clonidine had any intrinsic stimulatory effect on faecal excretion. On the other hand, in this experimental model, the virtual absence of faecal excretion in control rats throughout the observation period made it impossible to detect any potential inhibitory effects of these compounds.

Table 1. Effects of α-adrenergic antagonists on faecal excretion by rats.

Dose (mg kg^{-1}) s.c.	Dry weight of faeces (g)	AD1[a] (mg kg^{-1}) s.c.	Wet/dry weight of faeces
Control			
—	0 ± 0	—	1·78 ± 0·12[b]
Idazoxan			
0·04	0·53 ± 0·06*		2·59 ± 0·21*
0·2	0·74 ± 0·10**	0·25	2·84 ± 0·20**
1	1·56 ± 0·17**	(0·16–0·40)	3·49 ± 0·28**
PmP			
0·2	0·64 ± 0·06**		2·41 ± 0·15*
1	1·07 ± 0·16**	0·66	2·73 ± 0·09**
5	1·64 ± 0·12**	(0·46–0·96)	3·24 ± 0·29**
Phentolamine			
0·2	0·54 ± 0·14**	~1	2·52 ± 0·81*
1	0·93 ± 0·12**		2·80 ± 0·16**
Yohimbine			
0·2	0·47 ± 0·09*	~2	2·58 ± 0·12*
2	0·97 ± 0·10**		2·61 ± 0·08**
Prazosin			
1	0·41 ± 0·04**	>5	2·24 ± 0·12
5	0·74 ± 0·08**		2·59 ± 0·08**

[a] Dose causing 1 g (dry weight) faecal excretion, extrapolated from log-dose response-line. [b] Obtained from faeces collected over the 2 h preceding treatment.

Data are expressed as means ± s.e. (see methods), calculated from faeces collected 90 min after drug treatments. In parentheses 95% confidence limits.

*$P < 0·05$, **$P < 0·01$ vs control (Dunnett test).

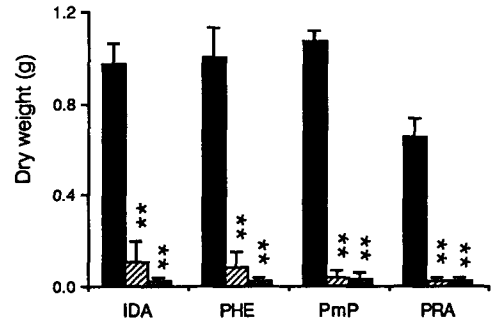

FIG. 1. Effects of clonidine and atropine on α_2-antagonist-induced faecal excretion in rats. Effects on dry weight of faeces: 0·15 mg kg^{-1}, clonidine (■), 0·2 mg kg^{-1}, atropine (▨), or saline (■) were injected, s.c., 30 min before 0·5 mg kg^{-1} idazoxan (IDA), 2 mg kg^{-1} phentolamine (PHE), 2·5 mg kg^{-1} PmP or 5 mg kg^{-1} prazosin (PRA). Each column represents the mean ± s.e. for dry weight faeces excreted during 90 min. Evaluation of wet/dry weight of faeces for clonidine- or atropine-treated rats was not possible due to minimal faecal excretion. **$P < 0·01$ vs saline (Dunett test).

Discussion

Our study shows that both subcutaneous and oral treatment with several α_2-adrenoceptor antagonists induces faecal excretion in rats. Under the influence of these compounds, rats excreted more than 1 g of faeces (dry weight) in 90 min (s.c. treatment) or 210 min (p.o. treatment). Defecation by rats is subject to environmental factors and, depending on experimental conditions, drugs can either increase or decrease defecation (Sanberg et al 1989). In our model, control animals consistently failed to defecate during an observation period suitable for the evaluation of drug-induced acute defecation. The induction of faecal excretion was accompanied by a clear-cut dose-dependent increase in faecal water content (faecal wet/dry weight significantly higher after treatment than before). These observations suggest that α-adrenergic antagonists may induce faecal excretion by either one or both of the following mechanisms: increased colonic propulsion and altered exchange of fluids from gut and lumen. The α_2-antagonist idazoxan was the most active compound, inducing excretion of 1 g dry weight of faeces at s.c. doses 2·5, 4 and 8 times lower than those of PmP, phentolamine and yohimbine, respectively. The α_1-receptor antagonist, prazosin, was less effective than idazoxan, even at doses 50 times higher. Comparable potencies were observed after p.o. and s.c. treatment, suggesting good bioavailability of the compounds. It is worth noting that the effects of idazoxan, PmP and phentolamine and that of a high dose of prazosin could be counteracted by the α_2-agonist clonidine. All these results suggest that antagonists induce faecal excretion by acting at the α_2-rather than the α_1-adrenergic receptor subtype. It has long been known that α_2-adrenoceptors play an important role in the modulation of gut motility and intestinal secretion (for review see: DiJoseph et al 1984; Daniel et al 1989). In rats, clonidine was found to inhibit castor oil-induced diarrhoea (Megens et al 1986) and gastrointestinal transit of a charcoal meal (Bianchi & Garattini 1988) and this inhibition was prevented by selective α_2-receptor antagonists. In the above studies, however, there was no evidence of any intrinsic effect af α_2-antagonists on the gut. The induction of faecal excretion by α_2-antagonists in the present

study supports the view that rat colon motility is under tonic inhibitory control by α_2-adrenergic receptors (Gillis et al 1987). Atropine completely prevented the defecation-promoting effects of α_2-antagonists, suggesting that α_2-adrenoceptors are prejunctionally located and control the release of acetylcholine from enteric neurons. This functional arrangement is supported by many in-vitro studies, such as that reporting a facilitory effect of yohimbine on both peristaltic reflex and acetylcholine release in isolated intestine of the guinea-pig (Marcoli et al 1987).

There is growing biochemical and pharmacological evidence that α_2-adrenoceptors are not homogeneous. The existence of separate subtypes, namely α_{2A} and α_{2B}, was postulated on the basis of differences in in-vitro sensitivity to specific pharmacological agents (Bylund 1988; Young et al 1989; Gobbi et al 1990). It was shown that presynaptically located α_{2A}- but not α_{2B}-adrenoceptors may modulate amine release from neurons of rat brain cortex (Gobbi et al 1990). Recently the α_{2A}- and the α_{2B}-adrenoceptor subtypes have been identified in the rat genoma by molecular cloning (for review see Harrison et al 1991). The rank order of potency of α-adrenoceptor antagonists for faecal excretion disclosed by our study (idazoxan > PmP, phentolamine, yohimbine > prazosin) was similar to that reported for α_{2A} binding sites (idazoxan > PmP > prazosin) and different from that for α_{2B} sites (prazosin = idazoxan > PmP) (Gobbi et al 1990). Thus, although caution should by exercised in extrapolating results from in-vitro binding studies to in-vivo functional experiments, our findings do not suggest that the prejunctional α_2-adrenergic receptors modulating faecal excretion are of the α_{2B}-subtype. Further studies with agonists and antagonists more selective for the different subtypes of α_2-receptors must be done to test these hypotheses. The possibility that α-adrenergic antagonists, at least partially, affect faecal excretion by a CNS-mediated component remains open, in view of the well recognized functional link between brain and gut (Fargeas et al 1986).

In conclusion, the present study shows, apparently for the first time, that α_2-adrenoceptor antagonists potently stimulate faecal excretion by rats. It is likely that the rat colon is under tonic inhibition by prejunctional α_2-adrenergic receptors (possibly of the α_{2A}-type), whose blockade by specific antagonists induces faecal excretion by releasing acetylcholine. This view is supported by the ability of the α_2-adrenoceptor agonist clonidine and the muscarinic antagonist atropine to prevent the defecation-promoting action, although the well known constipating properties of these two agents could not be assessed with the model adopted.

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