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Regulatory role of 5-HT and muscarinic receptor antagonists on the migrating myoelectric complex in rats

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Abstract

The 5-HT_3 and 5-HT_4 receptor antagonists alosetron and piboserod, and the muscarinic receptor antagonists PNU-171990A (2-(diisopropylamino)ethyl 1-phenylcyclopentanecarboxylate, hydrochloride) and PNU-174708A (2-(diisopropylamino)ethyl 1-phenylcyclohexanecarboxylate) were studied by electromyography, defining the migrating myoelectric complex (MMC) after i.v. administration in conscious rats. Alosetron prolonged the MMC cycle length from 16.6 to maximally 30.4 min at the dose 0.5 mg kg^{-1}. Piboserod promptly abolished MMC pattern and prolonged cycle length from 16.5 to >60 min at 0.5 mg kg^{-1}. PNU-171990A and PNU-174708A had no effect on basal cycle length up to a dose of 20 mg kg^{-1}. In controls, saline did not change the MMC pattern, while L-hyoscyamine at the same dose, 20 mg kg^{-1}, prolonged cycle length from 17.6 to 29.0 min. None of the drugs affected duration or propagation velocity of phase III of MMC. Blockade of 5-HT_4 receptors seems to exert a powerful inhibitory effect on motility, 5-HT_3 receptor blockade is less efficient and muscarinic receptor blockade has low efficacy.

Keywords: Antimuscarinic; 5-HT receptor; Gastrointestinal motility; Muscarinic receptor; (Rat)

1. Introduction

Anticholinergic drugs are considered to reduce intestinal motor activity through inhibition of muscarinic receptors. Several subtypes of muscarinic receptors are defined, five of which have been cloned (M_1–M_5) (Caulfield, 1993; Eglen et al., 1996; Hegde and Eglen, 1999). Muscarinic M_1 receptors prevail in neuronal tissue, while the heart contains a homogeneous population of M_2 receptors and exocrine glands M_3 receptors. M_4 receptors are found in the striatum, the cortex and rabbit lung tissue, and M_5 receptor mRNA has a general distribution in the central nervous system (Wess, 1996). Emerging evidence suggest that M_2 receptors mediate smooth muscle contraction directly via inhibition of adenylyl cyclase and indirectly by inhibiting β-adrenoceptor-mediated relaxation. Other contractile mechanisms involving M_2 receptors, such as activation of non-specific cationic channels and inactivation of potassium channels, may also be operative (Eglen et al., 1996; Hegde and Eglen, 1999). From a therapeutic standpoint, combined blockade of M_2 and M_3 receptors would seem ideal to achieve inhibition of intestinal muscle contraction. PNU-171990 has been shown to have no selectivity for any muscarinic receptor subtype, however, it shows functional tissue selectivity (Modiri et al., 2002). A new generation of anticholinergic drugs, represented by the muscarinic receptor antagonists zamifenacin and darifenacin, have been claimed effective in patients with irritable bowel syndrome (Scarpignato and Pelosini, 1999).

Serotonergic receptors seem important for regulation of motility. 5-HT_3 receptor antagonists inhibit cholinergic transmission regulating gut motility (Lördal and Hellström, 1999). In line with this, ondansetron has been shown to slow colonic transit in healthy volunteers (Gore et al., 1990; Steadman et al., 1992). A similar compound, alosetron,

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normalises perturbed intestinal motility and slows propulsion in vivo in the rat (Clayton et al., 1999) and selectively inhibits migrating myoelectric complex (MMC) frequency in vitro in the mouse (Bush et al., 2001). Alosetron is also considered to alleviate symptoms in pain-predominant irritable bowel syndrome (Scarpignato and Pelosini, 1999). A new approach to the importance of 5-HT_3 receptor antagonists is that these drugs may inhibit afferent nervous links from the gut, thereby considered to be able to ameliorate symptoms in pain-predominant irritable bowel syndrome (Kozlowski et al., 2000).

5-HT_4 receptor agonists, such as cisapride, facilitate acetylcholine release from the myenteric plexus (Tonini et al., 1989), thereby enhancing propulsive intestinal motor activity. In the clinical setting, cisapride decreases intestinal transit time and increases the number of bowel movements per week as compared to baseline (Passaretti, 1987; Pace et al., 1995) and decreases severity and frequency scores for abdominal pain and distension in irritable bowel syndrome (Passaretti, 1987; Van Outryve et al., 1991). Another 5-HT_4 receptor partial agonist/antagonist, tegaserod, is of possible value by stimulating orocaecal transit in constipation-predominant irritable bowel syndrome (Prather et al., 2000).

The aim of the present study was to compare effects of the novel antimuscarinics PNU-174708A (2-(diisopropylamino)ethyl 1-phenylcyclohexanecarboxylate) and PNU-171990A (2-(diisopropylamino)ethyl 1-phenylcyclopentanecarboxylate, hydrochloride) (Modiri et al., 2002), as well as L-hyoscyamine, to the 5-HT_3 and 5-HT_4 receptor antagonists alosetron and piboserod (De Ponti and Tonini, 2001) on the migrating myoelectric complex (MMC) in fasted conscious rats.

2. Material and methods

The Northern Stockholm Animal Experimentation Council (Dnr. 193/99) approved the study.

2.1. Preparation of rats for electromyography

The methods for measurement and analysis of MMC has been described earlier (Lördal and Hellström, 1995; Bränström and Hellström, 1996; Lördal et al., 1998). In this experiment, rats were anaesthetized with pentobarbital 50 mg kg^{-1} intraperitoneally (Apoteksbolaget, Umeå, Sweden) and through a midline incision three bipolar stainless steel electrodes SS-5T (Clark Electromedical Instruments, Reading, UK) were implanted into the muscular wall of the small intestine 5 (D), 15 (J_1) and 25 (J_2) cm distal to the pylorus (Fig. 1). All animals were supplied with a jugular vein catheter for administration of drugs. The electrodes and catheter were tunnelled subcutaneously to exit at the back of the animals' neck. After surgery the animals were housed singly and allowed to recover for at least 7 days before experiments were undertaken. During recovery, the rats were trained to accept experimental conditions. Experiments were then carried out in conscious animals after an 18-h fasting period in wire-bottomed cages with free access to water. During the experiments the rats were placed in Bollman cages. The electrodes were connected to an electroencephalogram preamplifier (7P5B) operating a Grass Polygraph 7B (Grass Instruments, Quincy, MA, USA). The time constant was set at 0.015 s and the low and high cut-off frequencies were set at 10 and 35 Hz, respectively.

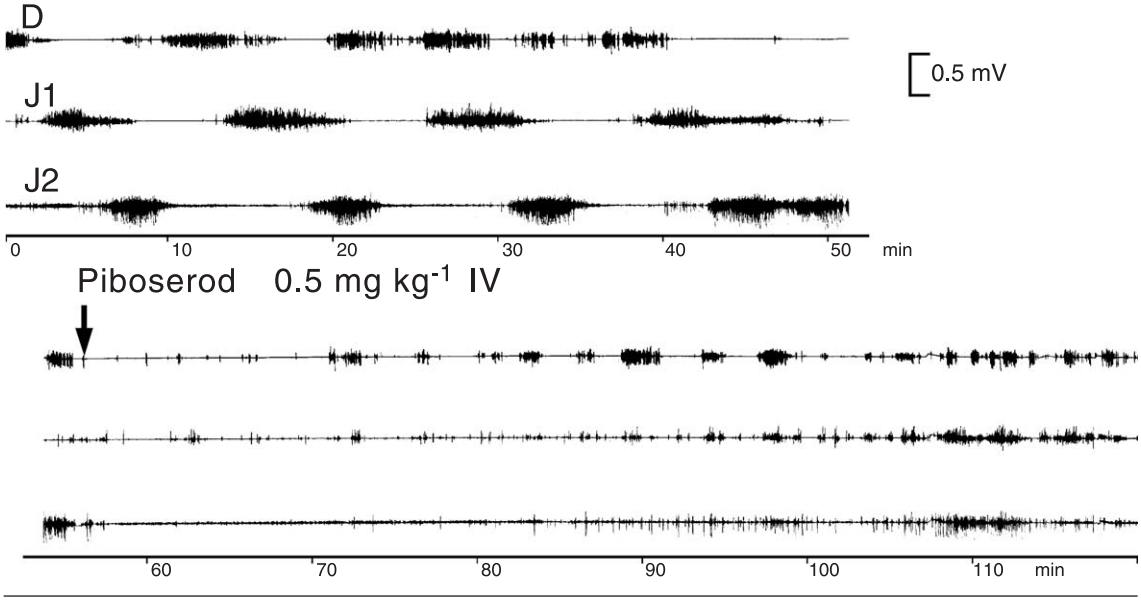

Fig. 1. Electromyographic original recording of 120 min continuous recording time from upper to lower panel showing the effect of piboserod on the migrating myoelectric complex. Phase III (maximal amplitude) of the migrating myoelectric complex propagates through all recording levels in the duodenum and jejunum; 5 (D), 15 (J_1) and 25 (J_2) cm distal to the pylorus.

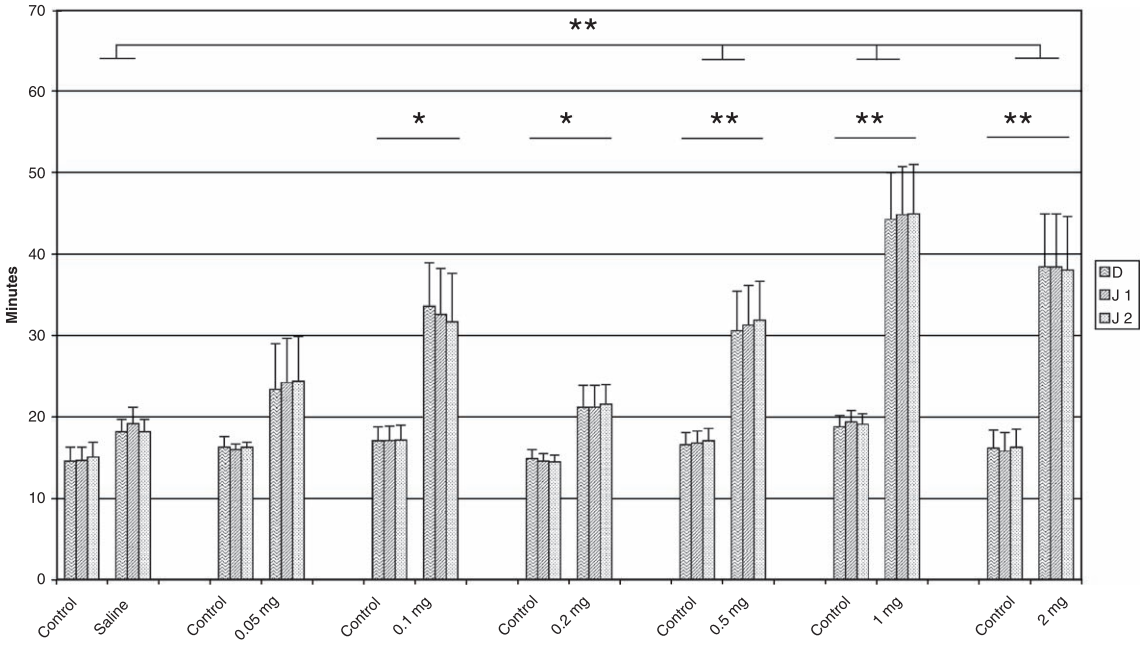

Fig. 2. Diagram showing the effect of increasing doses of alosetron on the MMC cycle length. *$P<0.05$, **$P<0.01$.

2.2. Design of electromyography studies

The animals were randomised to either treatment with the 5-HT_3 receptor antagonist alosetron or the 5-HT_4 receptor antagonist piboserod or the non-selective tissue specific antimuscarinics PNU-174708A or PNU-171990A, using saline as negative control and hyoscyamine as positive control. Each dose of the drugs was given to eight animals in randomised order.

Experiments started with control recording of basal myoelectric activity with four activity fronts propagated through all three recording sites during a 60-min period. The drugs were then given as bolus injections i.v. in a volume of 0.1 ml 100 g^{-1} body weight, immediately after the fifth activity front of MMC had passed the first electrode site, during 1 min.

In a first and second series of experiments, alosetron and piboserod at 0.05 ($n=8$), 0.1 ($n=8$), 0.2 ($n=8$), 0.5 ($n=8$), 1 ($n=8$) and 2 ($n=8$) mg kg^{-1} body weight were given.

In a third and fourth series, PNU-174708A and PNU-171990A at doses of 1 ($n=8$), 2 ($n=8$), 5 ($n=8$), 10 ($n=8$) and 20 ($n=8$) mg kg^{-1} were given.

In a fifth and sixth series, L-hyoscyamine at doses of 5 ($n=8$), 10 ($n=8$) and 20 ($n=8$) mg kg^{-1} and saline were given as positive and negative controls, respectively.

2.3. Data processing and statistical analysis

The main feature of the MMC, the activity front or phase III, was identified as a period of clearly distinguishable intense spiking activity. The amplitude should be at least twice that of preceding baseline, and propagating aborally through the whole recording segment and followed by a period of quiescence, phase I of MMC. Phase II was characterised as a period of irregular spiking preceding the activity front. Prolonged periods of more than 30 min with scattered spike potentials but no discernible cyclic activity were considered as periods of disrupted MMC activity. Periods with no detectable spike potentials were considered

Table 1
Effects of increasing doses of alosetron and piboserod on characteristics of phase III of MMC

Characteristics of phase III	Alosetron (mg kg^{-1} min^{-1})						Piboserod (mg kg^{-1} min^{-1})					
	0.05	0.1	0.2	0.5	1	2	0.05	0.1	0.2	0.5	1	2
Duration (min)												
D	0.9 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	1.6 ± 0.5	1.4 ± 0.2	1.6 ± 0.3	2.1 ± 0.3	1.5 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	1.0 ± 0.2
J_1	2.3 ± 0.4	3.9 ± 0.7	2.0 ± 0.4	2.0 ± 0.4	3.5 ± 0.8	2.9 ± 0.7	3.4 ± 0.5	3.1 ± 0.7	1.4 ± 0.3	3.5 ± 1.0	2.9 ± 0.8	1.7 ± 0.1
J_2	4.0 ± 0.4	4.2 ± 0.3	3.4 ± 0.5	3.4 ± 0.4	3.8 ± 0.5	2.3 ± 0.5	3.4 ± 0.7	4.0 ± 1.5	2.6 ± 0.3	2.7 ± 0.8	3.1 ± 0.5	3.8 ± 0.5
Propagation velocity (cm min^{-1})												
$D–J_1$	3.7 ± 0.7	3.1 ± 0.4	3.4 ± 0.5	4.2 ± 0.9	5.1 ± 1.4	2.4 ± 0.2	3.8 ± 1.4	1.8 ± 0.0	2.9 ± 0.3	2.0 ± 0.4	2.6 ± 0.3	3.4 ± 0.2
$J_1–J_2$	2.9 ± 0.4	4.1 ± 2.0	7.0 ± 4.5	2.4 ± 0.3	2.7 ± 0.3	3.0 ± 0.7	1.7 ± 0.3	1.7 ± 0.1	3.5 ± 0.5	6.5 ± 3.1	3.0 ± 0.6	2.6 ± 0.1

Mean values and S.E.M. ($n=8$).

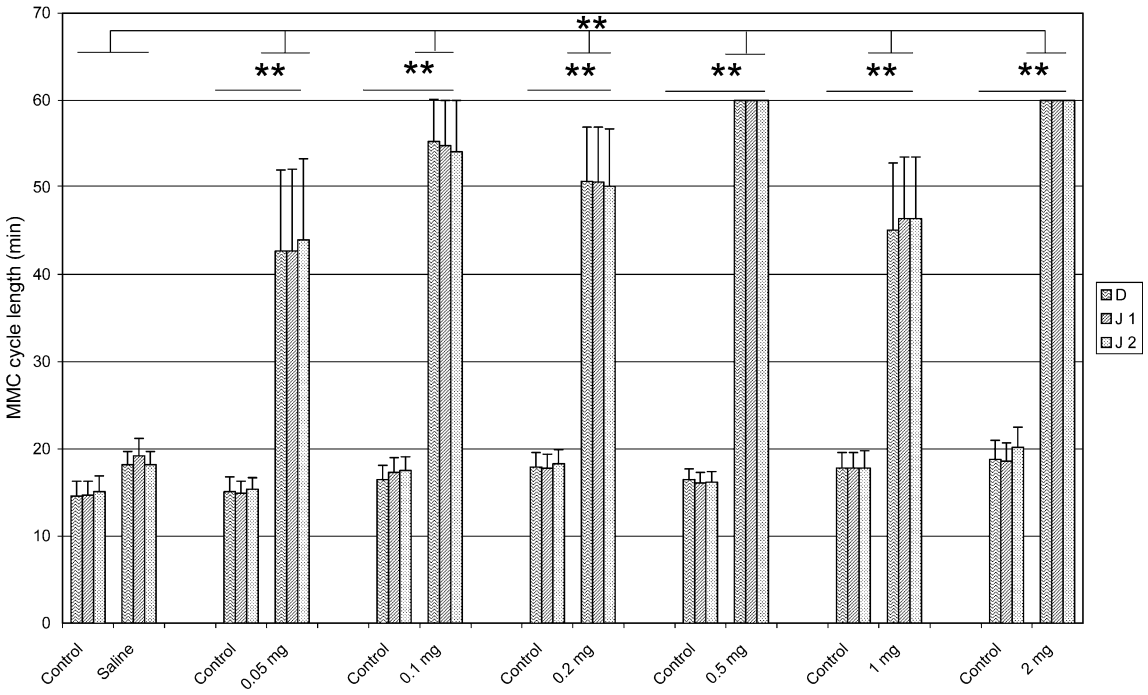

Fig. 3. Diagram showing the effect of increasing doses of piboserod on the MMC cycle length. *$P<0.05$, **$P<0.01$.

as quiescence. Calculations of characteristics of MMC, such as MMC cycle length, duration and propagation velocity of phase III of MMC were accomplished using an evaluated computer program (Bränström and Hellström, 1996).

Data are expressed as means ± standard error of the mean (S.E.M.) of *n* experiments. Data were statistically compared against control recordings using Student's *t*-test. $P<0.05$ in two-tailed tests was considered statistically significant.

2.4. Drugs and chemicals

The pharmacological compounds were recently synthesized and freshly supplied by the Department of Medicinal Chemistry, Pharmacia, Uppsala, Sweden. The PNU compounds showed the following properties: PNU-174708A and PNU-171990A, respectively (binding with Quinuclidinyl benzilate methyl chloride in guinea pig

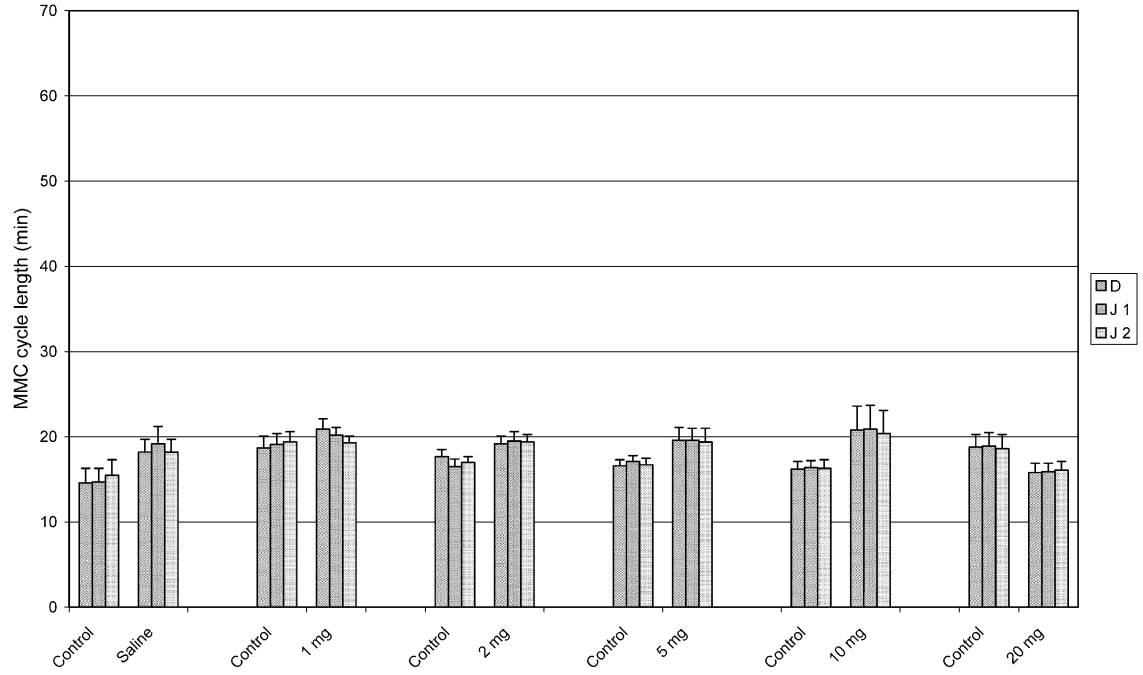

Fig. 4. Diagram showing no effect of PNU-174708A on the MMC cycle length.

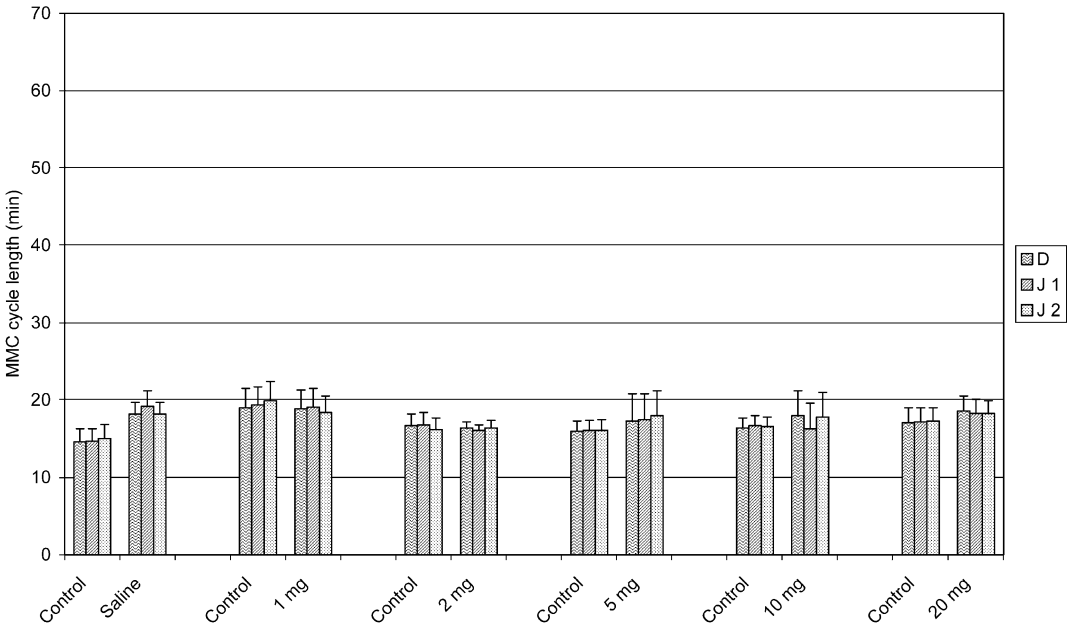

Fig. 5. Diagram showing no effect of PNU-171990A on the MMC cycle length.

tissue) Ki (nM), Cerebral cortex 3.5 respectively 2.2; heart muscle 9.2 respectively 14.4; parotic gland 29 respectively 12.5. Functional studies of small intestine in vitro with carbachol, Kb (nM) 6.4 respectively 3.3 (Modiri et al., 2002; and on file, Pharmacia, Stockholm, Sweden).

On a weekly basis, the PNU-174708A and PNU-171990A compounds were dissolved in saline solution to final concentrations of 0.1 or 1 mg ml^{-1}, while alosetron and piboserod were dissolved in saline to final concentrations of 0.01 or 0.1 mg ml^{-1}. All substances were kept at 5 °C before further dilution and use.

3. Results

Under baseline fasting conditions, all rats exhibited a regular fasting myoelectric pattern with recurring MMCs that were propagated through the intestinal segment under study.

3.1. Effects of the 5-HT_3 and 5-HT_4 receptor antagonists alosetron and piboserod

After the control period, injection of the 5-HT_3 receptor antagonist alosetron already at a dose of 0.5 mg kg^{-1} prolonged the MMC cycle length from 16.6 ± 1.5 min during the control period to 30.6 ± 4.8 min ($P < 0.01$), with no further increase using doses up to 2 mg kg^{-1} (Fig. 2, Table 1).

The 5-HT_4 receptor antagonist piboserod was even more potent and at a dose of 0.5 mg kg^{-1} completely abolished the MMC and prolonged the cycle length from 16.5 ± 1.2 min to the whole observation time of 60 min ($P < 0.01$) (Figs. 1 and 3).

3.2. Effects of the muscarinic receptor antagonists PNU-174708A and PNU-171990A

After control recordings for 60 min, injection of either PNU-174708A or PNU-171990A at increasing doses from 1

Table 2
Effects of increasing doses of PNU-174708A and PNU-171990A on characteristics of phase III of MMC

Characteristic of phase III	PNU-174708A (mg kg^{-1} min^{-1})					PNU-171990A (mg kg^{-1} min^{-1})				
	1	2	5	10	20	1	2	5	10	20
Duration (min)										
D	1.5 ± 0.2	1.4 ± 0.1	2.0 ± 0.3	2.2 ± 0.2	2.1 ± 0.1	1.5 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.7 ± 0.3	2.1 ± 0.2
J_1	3.9 ± 0.6	4.0 ± 0.8	4.0 ± 0.3	3.9 ± 0.5	3.8 ± 0.6	3.8 ± 0.3	3.4 ± 0.3	3.6 ± 0.2	3.8 ± 0.5	3.0 ± 0.4
J_2	5.5 ± 0.5	4.9 ± 0.5	6.1 ± 0.5	4.4 ± 0.4	4.7 ± 0.5	5.0 ± 0.5	5.0 ± 1.0	3.6 ± 0.4	3.7 ± 0.4	4.5 ± 0.8
Propagation velocity (cm min^{-1})										
$D–J_1$	2.6 ± 0.3	2.6 ± 0.2	2.4 ± 0.3	2.7 ± 0.1	2.5 ± 0.2	3.8 ± 0.8	3.5 ± 0.4	3.8 ± 0.7	2.8 ± 0.4	2.2 ± 0.2
$J_1–J_2$	2.0 ± 0.3	1.9 ± 0.3	1.4 ± 0.3	2.0 ± 0.2	2.0 ± 0.3	2.2 ± 0.3	2.2 ± 0.2	2.2 ± 0.3	1.8 ± 0.3	2.6 ± 0.5

Mean values and S.E.M. ($n = 8$).

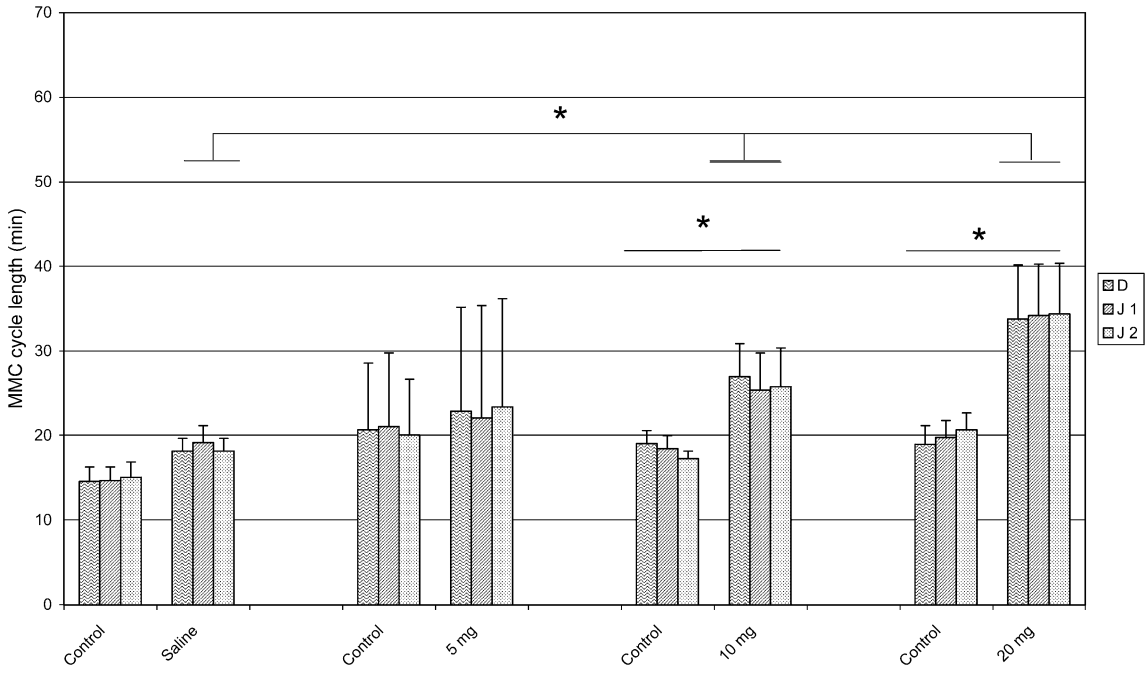

Fig. 6. Diagram showing the effect of saline and increasing doses of L-hyoscyamine on the MMC cycle length. *$P = 0.05$.

to 20 mg kg^{-1} had no effect on the MMC cycle length in the duodenum or jejunum. These compounds neither affected the duration or propagation velocity of phase III of MMC in the duodenum or jejunum (Figs. 4 and 5, Table 2).

3.3. Effects of the muscarinic receptor antagonist L-hyoscyamine or saline

With increasing doses of L-hyoscyamine from 5 to 20 mg kg^{-1}, the MMC cycle length was prolonged from 17.6 ± 1.2 to 31.4 ± 6.5 min ($P = 0.05$). L-hyoscyamine had no effect on the duration or propagation velocity of phase III of MMC. Saline had no effect on the fasting myoelectric activity and did not significantly affect the MMC cycle length (Fig. 6, Table 3).

Table 3
Effects of saline and increasing doses of L-hyoscyamine on characteristics of phase III of MMC

Characteristics of phase III	Saline	Hyoscyamine (mg kg^{-1} min^{-1})		
		5	10	20
Duration (min)				
D	1.7 ± 0.2	2.1 ± 0.5	3.3 ± 0.8	2.9 ± 0.5
J_1	4.1 ± 0.4	2.4 ± 0.4	2.7 ± 0.4	3.8 ± 0.5
J_2	4.5 ± 0.4	3.8 ± 0.4	4.2 ± 0.6	3.7 ± 0.4
Propagation velocity (cm min^{-1})				
J_1–J_2	3.0 ± 0.4	2.2 ± 0.3	2.6 ± 0.3	2.4 ± 0.3
J_2–J_3	2.6 ± 0.3	2.1 ± 0.2	1.9 ± 0.3	1.7 ± 0.2

Mean values and S.E.M. ($n = 8$).

4. Discussion

Our present data evaluating the effects of 5-HT and muscarinic receptor blocking agents on the MMC shows that the 5-HT_3 and 5-HT_4 receptor antagonists alosetron and piboserod exerted prominent inhibitory actions on small bowel motility registered as a profound inhibition of the recycling MMC pattern. Conversely, the smooth muscle selective muscarinic receptor antagonists PNU-174708A and PNU-171990A had no effect on the MMC pattern up to a dose of 20 mg kg^{-1}. A specific effect in our results is indicated by the additional findings using L-hyoscyamine as a positive control, which, as anticipated, had an inhibitory effect on the MMC, whereas saline expectedly had no effect.

Our previous research using the MMC in the rat (Lördal and Hellström, 1999) as an experimental model for the study of gastrointestinal motility confirms the present findings of 5-HT as an important regulator of gut motility. This was evidenced both through dose-dependent physiological stimulation with 5-HT and pharmacological inhibition with 5-HT_3 and 5-HT_4 receptor antagonists. Serotonin-dependent motility was also shown to be atropine-sensitive indicating muscarinic mechanisms to be involved in the motility response to 5-HT (Lördal and Hellström, 1999). In addition, 5-HT-dependent mechanisms seem to be operative also in dogs (Johansen et al., 1998) and humans in a similar fashion (Lördal and Hellström, 1995; Lördal et al., 1998).

The above findings are confirmed by studies in vitro on the MMC in small and large bowel of the mouse (Bush et al., 2001). In agreement with our findings, alosetron was found to cause dose-dependent inhibition ot hte MMC. Assuming an even distribution of alosetron (Mw 294.4) as

well as piboserod (Mw 369.5) to total body water of the mouse, the minimal effective dose of 0.5 mg kg^{-1} of both drugs was calculated to correspond to concentrations of 2.5 and 2 μmol/l, respectively. A similar concentration range (0.1–1.5 μmol/l) was found to be inhibitory on the murine MMC in vitro (Bush et al., 2001).

Atropine has been used for many years in the treatment of irritable bowel syndrome but has not been therapeutically effective. Lately this has been overcome by b.i.d. slow-release preparations of hyoscyamine sulfate, e.g. SR Levbid (Schwarz Pharma, WI, USA). In our investigation we used L-hyoscyamine, which is the most active component of the racemic mixture of the belladonna alkaloids in atropine. Atropine has been shown to have inhibiting effects on MMC in e.g. humans (Mellander et al., 1995) and sheep (Plaza et al., 1997).

The observation that antimuscarinics were not very efficient for the inhibition of MMC is in agreement with previous observations in the rat (Al-Saffar, 1984). The fact that antimuscarinics were less effective in our model was surprising due to earlier observations that PNU-171990 and PNU-174708 administered orally to male mice at doses of 50, 150 and 450 mg kg^{-1} had effects on intestinal transit in the charcoal propulsion test. PNU-171990 showed inhibition of transit time already at 150 mg kg^{-1} and even more at 450 mg kg^{-1}. PNU-174708 was less potent than PNU-171990 in the test, and showed effect only at 450 mg kg^{-1} (unpublished observation, Pharmacia). The high doses needed in this test are due to the oral administration route and that the substances have a high first passage metabolism of about 95% in the liver (data on file, Pharmacia, Stockholm, Sweden). In our MMC studies, we used i.v. administration which will result in higher serum concentration and thus, a lower dosage will be needed. The PNU-171990 has recently been reported to show no subtype selectivity for muscarinic receptors, however it shows tissue selectivity for urinary bladder compared to salivary gland (Modiri et al., 2002) and the PNU-174708 shows a somewhat different selectivity (see Materials and Methods). The antimuscarinics may still have an effect on motility, but seem to require at least a 20-fold higher dose than that used in the present study. Anyhow, a cholinergic mechanism may still be operative in rats since L-hyoscyamine was able to prolong the MMC cycle length at doses below those of the antimuscarinics used. However, the above findings seem at variance with the common practice of using anticholinergics as a treatment for irritable bowel syndrome, mainly of the pain-predominant type (Drossman and Thompson, 1990; Drossman, 1999).

The experimental model using the conscious rat with monitoring of intestinal myoelectric activity, registered as MMC, seems relevant for the objective aimed at in this study. Here, the MMC motility pattern represents a surrogate marker for motility in the gut assumed to be of importance for causing symptoms typical of irritable bowel syndrome. Electromyography of the rat small bowel with analysis of the MMC offers a convenient model for the study of basal and stimulated motor activity. The myoelectric activity of the gut is also known to correlate to the true motor activity (Berkson et al., 1932) responsible for contractions with pressure increases that promote transit through the gut (Al-Saffar et al., 1984; Hellström and Johansson, 1989). The fact that alosetron profoundly inhibited myoelectric activity together with the clinical finding that this drug may be effective in irritable bowel syndrome (Thumshirn et al., 2000; Camilleri, 2000) speaks in favour of inhibition of motility as a target in the development of drugs against irritable bowel syndrome.

Smooth muscle spasmogens, such as cholinergics and tachykinins, are considered capable of inducing painful challenges to the gut (Kellow et al., 1999). Much effort has been raised in order to prove the importance of gut motility and high luminal pressures as an important mechanism in the development of irritable bowel syndrome (Thompson et al., 1999). So far, studies have been hampered by the inaccessibility of the gut to luminal pressure sensors, which may be impossible to position throughout the gut lumen of a sick person, and the inability of myoelectric techniques to register pressures within the gut lumen. Even though such knowledge would be attractive, it seems yet impossible to evaluate the whole gastrointestinal tract through some few recording points along the upper part of the gastrointestinal tract. Under these circumstances, recordings of the MMC seems a reasonable surrogate marker (Kellow et al., 1999) that might be used in order to disclose an inhibitory action on gut motility, believed to be of value for a therapeutic response against spastic conditions of the gut, such as irritable bowel syndrome. We have found 5-HT receptor blockade to be of great importance for inhibition of gut motility, and possibly also a therapeutic response in irritable bowel syndrome. The 5-HT_3 receptor antagonist alosetron suggests usability of smooth muscle relaxing agents in the treatment of irritable bowel syndrome (Maxton et al., 1996; Talley and Spiller, 2002). A drawback here is the development of ischemic colitis during treatment with alosetron (Friedel et al., 2001; Talley, 2001) which has caused withdrawal of the compound from the market. However, this action has been discussed to be reversed (McCarthy, 2002). Another track in the development of drugs against irritable bowel syndrome would thus be 5-HT_4 receptor antagonism, such as achieved by piboserod.

This study indicates that 5-HT receptors, of which primarily 5-HT_4, seem of major importance for inhibition of MMC in the rat. For comparison, the inhibitory action of antimuscarinics seems weaker and muscarinic receptor antagonists are needed at much higher doses than 5-HT receptor antagonists in order to achieve inhibition of small bowel motility.

Acknowledgements

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