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Enhancement of guinea-pig intestinal peristalsis by blockade of muscarinic M_1-receptors

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1 The effects of pirenzepine and hyoscine on the peristaltic reflex were investigated in the guinea-pig isolated small intestine. Peristalsis was induced by raising the intraluminal pressure and the volume of fluid propelled was taken as a measure of the efficiency of peristaltic activity.

2 Low concentrations of pirenzepine (0.1–1 nM) and of hyoscine (0.01 nM) significantly enhanced peristalsis, whereas larger concentrations of both drugs caused inhibition. Pirenzepine was about 6 times less potent than hyoscine in increasing peristalsis, but was about 100 times less potent in inhibiting it.

3 Neither tolazoline (1 μM) nor naloxone (0.3 μM) affected the stimulatory action of pirenzepine on peristalsis.

4 Bicuculline increased the efficiency of peristalsis at concentrations of 1 μM and 10 μM; at 10 nM, bicuculline reduced significantly the increase of peristalsis by pirenzepine. γ-Aminobutyric acid (GABA) did not affect peristaltic activity, but the stimulatory effect of pirenzepine was abolished in the presence of 100 μM GABA.

5 The results indicate that activation of neuronal M_1-receptors causes inhibition of small intestinal peristalsis. Bicuculline-sensitive 'GABAergic' synapses are probably involved in this inhibition.

Introduction

It is generally believed that muscarinic receptor antagonists inhibit gastrointestinal motility (see review by Kosterlitz & Lees, 1964) yet, Kay & Smith (1956) reported that a low dose of atropine enhanced gastric motility in man, whereas a large dose inhibited it. They suggested that the stimulation was probably central. However, the antagonist pirenzepine which does not cross the blood-brain barrier, may also accelerate gastro-intestinal transit time (Jaup *et al.*, 1985) or increase the frequency of antral contractions (Stacher *et al.*, 1982) in man. Pirenzepine has a higher affinity for M_1-muscarinic receptors than for M_2-receptors, whereas atropine or hyoscine do not discriminate between both subtypes (for review see Birdsall & Hulme, 1985). M_1-receptors have been detected on guinea-pig myenteric neurones in functional (Kilbinger & Nafziger, 1985; North *et al.*, 1985) and autoradiographic (Buckley, 1985) studies, but it is not known whether these receptors play a role in intestinal motility.

The purpose of the present work was to study in detail the effects of pirenzepine and hyoscine on peristalsis of isolated segments of the guinea-pig small intestine. Peristalsis was induced by increasing the intraluminal pressure. The amount of fluid propelled during peristalsis served as a sensitive parameter for the efficiency of the peristaltic activity (Holzer & Lembeck, 1979; Sanger & Bennett, 1984).

Methods

Male guinea-pigs (350–500 g body weight) were stunned by a blow to the head and bled. Segments of the proximal part of the small intestine (within 30 cm from the duodeno-jejunal junction), about 6 cm in length, were mounted horizontally in a 100 ml organ bath, which contained Tyrode solution (composition in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and D-glucose 5.6) at 37°C bubbled with a mixture of 95% O_2 and 5% CO_2. Care was taken that the segments developed no loops. The arrangement for eliciting the peristaltic reflex was similar to that described by Holzer & Lembeck (1979; 'arrangement 1'). In brief, the oral end was connected to a Mariotte bottle with two inlet tubes which contained prewarmed (37°C) Tyrode solution; the aboral end was connected to a

mercury valve which prevented back flow of fluid and which was set to open at an outflow pressure above 500 Pa.

During an equilibration period of 45 min, peristalsis was elicited by increasing the intraluminal pressure of the intestinal segment by 500 Pa for 1 min every 10 min. At the end of the equilibration time (45th min) and 15 min later (60th min) peristalsis was elicited by 500 Pa for 2 min and the volume of the fluid expelled during the peristalsis period of 2 min was measured. Then the organ bath was refilled with Tyrode solution at 37°C (control group) or with Tyrode solution containing the substances to be tested. At intervals of 15 min the peristaltic reflex was elicited by 500 Pa for 2 min. Peristalsis was quantified by measuring the amount of fluid propelled which is a reliable parameter for the efficiency of peristaltic activity (Sanger & Bennett 1984). The changes of the fluid volumes expelled are expressed as % of the mean fluid volume expelled during the 2 min peristalsis periods evoked at the 45th and 60th min.

Drugs

The following drugs were used: pirenzepine dihydrochloride monohydrate (Thomae, Biberach, FRG); hyoscine (scopolamine) hydrobromide (EGA-Chemie, Steinheim, FRG); γ-amino-*n*-butyric acid (GABA); (+)-bicuculline; tolazoline hydrochloride (all Sigma, St. Louis, Mo, U.S.A.); naloxone hydrochloride (Goedecke, Freiburg, FRG).

Statistics

Results are expressed as means ± s.e. means. Significance of differences between two mean values was calculated by Student's *t* test. If more than one group of treatments was compared with one control group a modified *t* test according to Bonferroni was used (Wallenstein *et al.*, 1980).

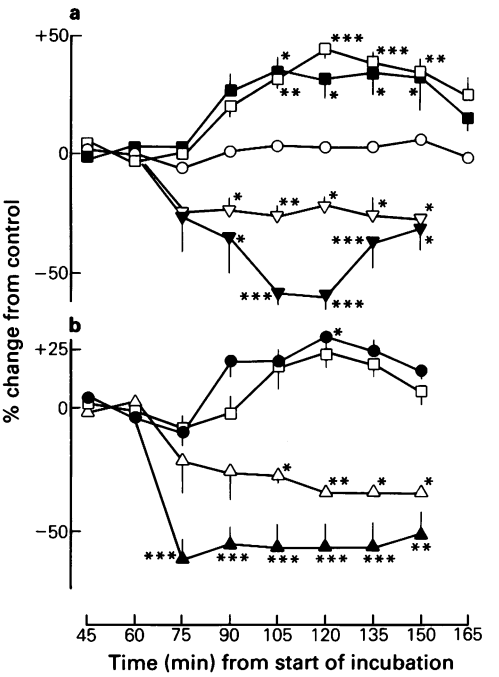

Figure 1 Time course of the effects of pirenzepine (a) and hyoscine (b) on the fluid volume expelled during peristalsis in the isolated small intestine of the guinea-pig. The peristaltic reflex was evoked with 500 Pa for 2 min every 15 min. Pirenzepine or hyoscine, was added to the organ bath after the 62nd min. Concentrations of pirenzepine and hyoscine: (●) 0.01 nM, $n = 3$; (□) 0.1 nM, $n = 13$, and $n = 6$; (■) 1 nM, $n = 5$; (△) 10 nM, $n = 3$; (▲) 100 nM, $n = 6$; (▽) 1 μM, $n = 7$; (▼) 10 μM, $n = 5$; (○) control experiments, $n = 8$. Ordinate scale, change of the volume of fluid ejected during peristaltic activity expressed as % of the mean volume expelled during peristalsis in the 45th and 60th min. Given are means (with s.e. mean shown by vertical lines) of n experiments. Significance of difference from the corresponding value obtained in control experiments at the same time: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (modified *t* test according to Bonferroni).

Results

Effects of pirenzepine and hyoscine on peristalsis

The fluid volume expelled during successive periods of peristalsis remained nearly constant up to 165 min after the set-up of the preparation (Figure 1a). The absolute value of the mean fluid volume expelled in the 45th and 60th min was 16 ± 0.4 ml $2\,min^{-1}$ ($n = 85$). Figure 1 shows the time-course of the effects of pirenzepine and hyoscine. Pirenzepine at concentrations of 0.1 nM and 1 nM caused a significant increase of the fluid volume expelled during the peristaltic reflex. The maximal increase of 43 ± 5% was observed with 0.1 nM pirenzepine and was reached 60 min after its application. Pirenzepine at concentrations of 0.01 nM, 10 nM and 100 nM did not significantly affect the amount of fluid expelled. Higher concentrations reduced the fluid volume propelled, maximally at 10 μM by 60 ± 5%. Hyoscine at a concentration of 0.01 nM increased the volume of fluid ejected during peristalsis. As with pirenzepine, the maximal increase (29 ± 5%) was observed only 60 min after application of 0.01 nM hyoscine (Figure 1b). Concentrations of hyoscine between 10 nM and 10 μM decreased the efficiency of peristalsis. A partial

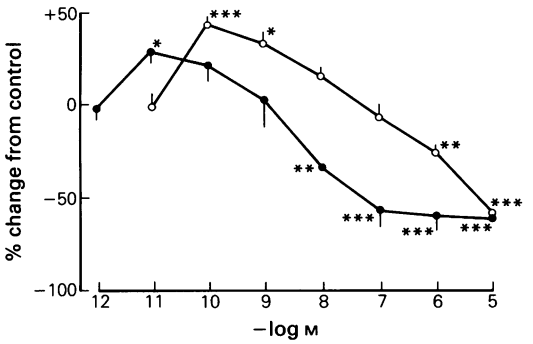

Figure 2 Facilitation and inhibition by hyoscine (●) and pirenzepine (○) of the peristaltic reflex. Shown are the maximal effects, observed 45 or 60 min after addition of the substances to the organ bath fluid. Ordinate scale, percentage change of the volume of fluid expelled during peristalsis. Abscissa scale, negative log M concentrations of pirenzepine or hyoscine. Given are means of 3–13 experiments. Vertical lines indicate s.e. mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs control (see Figure 1).

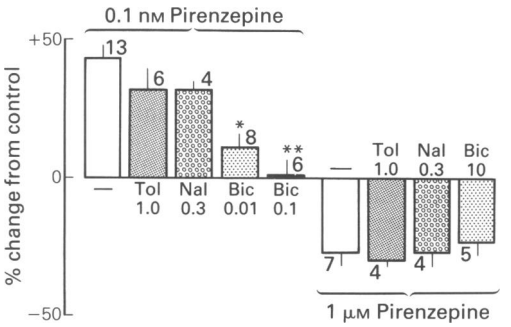

Figure 3 Facilitation and inhibition of intestinal peristalsis by pirenzepine in the presence of various antagonists. Open columns, control experiments with 0.1 nM (left panel) and 1 μM (right panel) pirenzepine. Tolazoline (Tol 1 μM), naloxone (Nal 0.3 μM) and bicuculline (Bic 10 μM) were added to the medium 60 min before the application of pirenzepine. Bicuculline at concentrations of 0.01 and 0.1 μM was added simultaneously with 0.1 nM pirenzepine. Given are the percentage changes of the fluid volume expelled during peristalsis. Vertical lines represent s.e. mean, n is as indicated. Significance of difference from the effect of 0.1 nM pirenzepine alone: $*P < 0.01$; $**P < 0.001$ (modified t test according to Bonferroni).

recovery from the inhibition was seen with concentrations of 10 μM of pirenzepine and hyoscine. Such a transient inhibitory effect on peristalsis has been described by Schaumann (1955) and Barthó *et al.* (1982).

Figure 2 shows the biphasic concentration-response curves for the effects of hyoscine and pirenzepine. The Figure illustrates that equieffective concentrations of hyoscine and pirenzepine in the ascending limb of the curves differed only by a factor of about 6. In contrast, pirenzepine was about 100 times less potent than hyoscine in causing the same degree of inhibition of peristalsis.

Interaction experiments

The twofold effects of pirenzepine on peristalsis were studied in the presence of antagonists of neurotransmitters which are known to inhibit intestinal motility, namely noradrenaline (Kosterlitz & Robinson, 1957), opioids (Gyang *et al.*, 1964) and γ-aminobutyric acid (GABA) (Hobbiger, 1958). As shown in Figure 3, the facilitation and inhibition of peristalsis obtained with 0.1 nM and 1 μM pirenzepine, respectively, remained unchanged in the presence of tolazoline (1 μM) or naloxone (0.3 μM). Tolazoline (1 μM, $n = 6$) or naloxone (0.3 μM, $n = 4$) added alone, did not significantly alter the fluid volume propelled during peristalsis within an observation period of 90 min. On the other hand, bicuculline increased the fluid volume expelled in a concentration-dependent manner (Figure 4). The

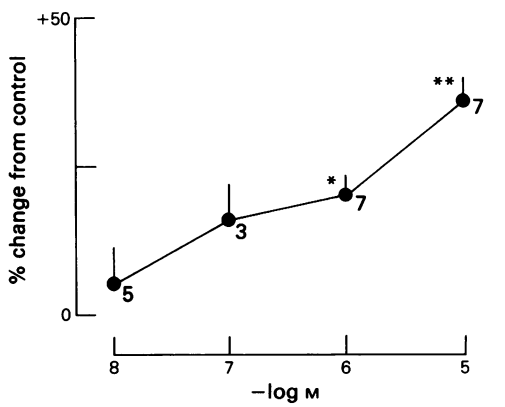

Figure 4 Increase by bicuculline of intestinal peristalsis. Shown are the maximal effects observed 45 or 60 min after addition of bicuculline to the organ bath. Ordinate scale, percentage increase of the fluid volume expelled during peristalsis. Abscissa scale, negative log M concentration of bicuculline. Vertical lines represent s.e. mean, n is as indicated. Significance of difference from the corresponding value obtained in control experiments at the same time: $*P < 0.05$; $**P < 0.001$ (modified t test according to Bonferroni).

maximum increase obtained with 10 μM bicuculline was 36 $\pm$ 3%, and the EC_{50} for the facilitatory effect was 0.3 μM. Concentrations of 10 and 100 nM bicuculline which alone had no effect on the efficiency of peristalsis, significantly reduced the pirenzepine-induced increase in the fluid volume expelled during peristalsis (Figure 3). In contrast, the inhibitory effect of 1 μM pirenzepine was not affected in the presence of 10 μM bicuculline.

The results obtained with bicuculline suggest that endogenous GABA is involved in the peristaltic reflex activity. Therefore, GABA was added as a bolus (100 μl) to the organ bath fluid just before eliciting the peristaltic reflex in the 75th min. Both 10 μM ($n = 4$) and 100 μM ($n = 9$) GABA showed no significant influence on the fluid volume expelled (data not shown).

In some other experiments 100 μM GABA was added to the medium at the start of the incubation period and 62 min later. Such high concentrations of GABA are known to render the intestine insensitive to the effects of GABA (Krantis *et al.*, 1980; Frigo *et al.*, 1987). Figure 5 shows that the fluid volume expelled during peristalsis (75th–150th min) remained unchanged in the presence of GABA. However, the facilitation of peristalsis by 0.1 nM pirenzepine was significantly reduced in the presence of 100 μM GABA.

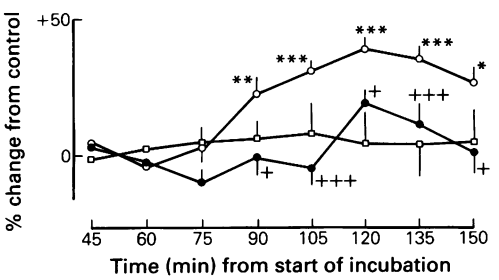

Figure 5 Effect of γ-aminobutyric acid (GABA) on the pirenzepine-induced increase of fluid volume expelled during peristalsis: (○) 0.1 nM pirenzepine added after the 62nd min ($n = 6$); (□) 100 μM GABA, added at the start of the incubation; change of the bath fluid with 100 μM GABA containing Tyrode solution after the 62nd min ($n = 3$); (●) 0.1 nM pirenzepine + 100 μM GABA, added together after the 62nd min. The organ bath contained 100 μM GABA from the start of the incubation time onwards ($n = 8$). Ordinate scale, percentage change of the volume of fluid expelled during peristalsis. Given are means of n experiments. Vertical bars indicate s.e. mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs control experiments in the absence of modifying drugs. $+P < 0.05$; $+++P < 0.001$ vs corresponding values in the presence of 0.1 nM pirenzepine.

Discussion

The present study shows that nanomolar and subnanomolar concentrations of pirenzepine and hyoscine significantly increased intestinal peristalsis, whereas higher concentrations of the antagonists caused the well known inhibition. Peristalsis was not wholly abolished even by micromolar concentrations of hyoscine or pirenzepine. This confirms earlier studies which showed that a substantial noncholinergic component is involved in intestinal peristalsis (Kosterlitz & Watt, 1963; Tonini *et al.*, 1981; Barthó *et al.*, 1982). For the following reasons we suggest that the differential effects of the muscarinic receptor antagonists are mediated by blockade of subtypes of muscarinic receptors. The concentrations of pirenzepine required to cause the same degree of inhibition were 100 times greater than those of hyoscine. This difference corresponds to the difference in antagonistic potencies (pA_2 values) of pirenzepine and hyoscine at the M_2-muscarinic receptors of the smooth muscle of the guinea-pig small intestine (Kilbinger *et al.*, 1984). Thus, the inhibition of peristalsis is likely to be due to blockade of muscular M_2-receptors. On the other hand, pirenzepine was only about 6 times less potent than hyoscine in facilitating peristalsis. Since the pA_2 values for hyoscine and pirenzepine on myenteric M_1-receptors differ by one log unit only (Kilbinger & Nafziger, 1985; North *et al.*, 1985), we suggest that the enhancement of peristaltic movements by both antagonists is mediated by blockade of M_1-receptors.

The maximal enhancement of peristalsis was less marked with hyoscine than with pirenzepine. Moreover, a significant stimulation was seen only with a single concentration of hyoscine but over a ten fold concentration range of pirenzepine. These observations are consistent with the view that hyoscine, in contrast to pirenzepine, does not discriminate between M_1- and M_2-receptors. A small increase in the hyoscine concentration already cuts short the stimulant effect of this drug.

We have recently shown that an increase in intraluminal pressure causes the release of acetylcholine (Schwörer *et al.*, 1987). The present study implies that, under physiological conditions, the released acetylcholine activates M_1-receptors which, in turn, leads to inhibition of peristalsis. A surprising finding was that the maximal facilitatory effect of pirenzepine occurred at a remarkably low concentration (0.1 nM). This concentration is about 30 fold lower than its K_D value determined against exogenous muscarinic agonists on myenteric M_1-receptors (Kilbinger & Nafziger, 1985; North *et al.*, 1985). There is so far no evidence for the existence of another subtype of muscarinic receptor which has a higher affinity to pirenzepine than the M_1-receptor

and which might have mediated the facilitatory effect of pirenzepine on peristalsis. Rather it seems that, for unknown reasons, pirenzepine is more potent in antagonizing the effect of synaptically released endogenous acetylcholine than in blocking the effects of exogenous muscarinic agonists. In this context it should be noted that a comparable finding has been observed in electrophysiological experiments on guinea-pig submucous plexus: the effect of synaptically released endogenous noradrenaline was more efficiently antagonized by α_2-antagonists than the effect of perfused exogenous noradrenaline (North & Surprenant, 1985).

Studies on the lower oesophageal sphincter of the opossum (Gilbert *et al.*, 1984) and on rat jejunum (Micheletti & Schiavone, 1987) have also demonstrated that the activation of a neuronal M_1-receptor causes relaxation of smooth muscle. A muscarinic inhibition of small intestinal motility in the dog *in vivo* was reported by Fox *et al.* (1985). These authors assumed that the inhibition of motility is due to activation of inhibitory presynaptic autoreceptors. However, presynaptic muscarinic autoreceptors on guinea-pig myenteric neurones belong to the M_2-subtype (Kilbinger *et al.*, 1984; North *et al.*, 1985) and are therefore not involved in the facilitatory effects of pirenzepine on peristalsis.

M_1-receptors are present on the soma-dendritic part of cholinergic and non-cholinergic neurones of the guinea-pig myenteric plexus (Kilbinger & Nafziger, 1985; North *et al.*, 1985). The interaction experiments between pirenzepine on the one hand, and tolazoline or naloxone on the other, demonstrate that neither endogenous noradrenaline nor endogenous opioids play a role in the inhibition of peristalsis caused by M_1-receptor activation. The failure of naloxone to increase peristaltic efficiency is in agreement with previous studies which used a similar procedure to evoke peristalsis (Holzer & Lembeck, 1979; Donnerer & Lembeck, 1985). Naloxone increases peristalsis only if a relatively low intraluminal pressure is applied over a long period (Kromer & Pretzlaff, 1979; Barthó *et al.*, 1982).

Low concentrations of bicuculline antagonized the facilitatory effect of pirenzepine on peristalsis. Likewise, pirenzepine failed to stimulate peristalsis in preparations made tachyphylactic to the effects of GABA. Micheletti & Schiavone (1987) demonstrated antagonism by bicuculline of the relaxation of rat jejunum induced by the agonist McN-A-343. The view that GABA neurones are involved in the inhibition of the peristaltic reflex is corroborated by the finding that bicuculline alone concentration-dependently increased peristalsis. Our results are in agreement with those reported by Frigo *et al.* (1987) who elicited the peristaltic reflex in the guinea-pig distal colon by radial distension of the lumen with a balloon. The same concentrations of bicuculline as in the present study increased the velocity of propulsion in the colon. At first sight it was surprising to find that neither exogenous GABA nor desensitization to GABA affected intestinal propulsion. However, a failure of GABA to inhibit peristalsis has also been observed in previous studies (Hobbiger, 1958; Krantis *et al.*, 1980; Frigo *et al.*, 1987), and it has therefore been questioned whether high concentrations of exogenously applied GABA could mimic the localized effect of synaptically released endogenous GABA (Frigo *et al.*, 1987). GABA does not directly contract or relax the smooth muscles of the guinea-pig small intestine, but affects motility via release of acetylcholine (Kleinrok & Kilbinger, 1983) or release of an unknown inhibitory transmitter (Krantis *et al.* 1980). It is possible that this unknown final mediator of the inhibitory GABA action is ATP (Maggi *et al.*, 1984).

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