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Evidence for the involvement of 5-HT_4 receptors in the 5-hydroxytryptamine-induced pattern of migrating myoelectric complex in sheep

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1 The effects induced by 5-hydroxytryptamine (5-HT) on gastrointestinal myoelectric activity in conscious sheep were recorded through electrodes chronically implanted and analysed by computer. The 5-HT receptors and the cholinergic neuronal pathways involved in these actions were investigated.

2 The intravenous (i.v.) administration of 5-HT (2, 4 and 8 μg kg^{-1} min^{-1}, 5 min) induced an antral inhibition concomitant with a duodenal activity front that migrated to the jejunum, followed by a period of intestinal inactivity. This myoelectric pattern closely resembled that observed in the phases III and I of the migrating myoelectric complex (MMC) in sheep. The 0.5 μg kg^{-1} min^{-1} dose evoked the same pattern in only two out of the six animals used. Likewise, the 1 μg kg^{-1} min^{-1} dose similarly affected four of the six animals. In addition, a transient stimulation was observed in the antrum and jejunum when the two highest doses were used.

3 The 5-HT_1 antagonist, methiothepin (0.1 mg kg^{-1}), the 5-HT_2 antagonists, ritanserin (0.1 mg kg^{-1}) and ketanserin (0.3 mg kg^{-1}), the 5-HT_3 antagonists, granisetron (0.2 mg kg^{-1}) and ondansetron (0.5 mg kg^{-1}), as well as the 5-HT_4 antagonist, GR113808 (0.2 mg kg^{-1}), did not modify the spontaneous gastrointestinal myoelectric activity. However, the cholinoceptor antagonists, atropine (0.2 mg kg^{-1}) and hexamethonium (2 mg kg^{-1}), inhibited gastrointestinal activity.

4 When these antagonists were injected i.v. 10 min before 5-HT (2 or 4 μg kg^{-1} min^{-1}, 5 min), only GR113808, atropine and hexamethonium were able to modify the 5-HT-induced actions, all of them being completely blocked by the three antagonists.

5 Our data show that 5-HT initiates a MMC-like pattern in the gastrointestinal area in sheep through 5-HT_4 receptors. Furthermore, these actions are mediated by cholinergic neural pathways involving muscarinic and nicotinic receptors. However, our results do not indicate a role for either 5-HT_1, 5-HT_2 or 5-HT_3 receptors in the 5-HT-induced effects.

Keywords: 5-Hydroxytryptamine; 5-HT_4 receptors; gastrointestinal myoelectric activity; migrating myoelectric complex (MMC)

Introduction

The subtypes of 5-hydroxytryptamine (serotonin, 5-HT) receptors belonging to seven main groups have already been cloned. Four main subtypes have been extensively characterized pharmacologically (Martin & Humphrey, 1994; Hoyer *et al*., 1994). The recently cloned 5-HT_4 receptor (Gerald *et al*., 1995) was first identified in mammalian brain as a receptor which was positively coupled to adenylate cyclase. Subsequently, it was characterized in the gastrointestinal tract and in other tissues such as porcine heart, human atria or sheep pulmonary vein (Bockaert *et al*., 1992; Ford & Clarke, 1993).

In guinea-pig stomach, ileum and colon, 5-HT induces contractions through 5-HT_4 receptors located on cholinergic neurones in the myenteric plexus, facilitating acetylcholine release. However, the 5-HT-induced relaxations in oesophageal muscularis mucosae and ileal strips from rats are mediated by 5-HT_4 receptors located on the smooth muscle cells (Ford & Clarke, 1993; Briejer *et al*., 1995; Kilbinger *et al*., 1995). In man, 5-HT inhibits spontaneous motility in circular muscle strips of the colon through 5-HT_4 receptors (Tam *et al*., 1994) whereas it induces contraction of longitudinal muscle strips of the ileum through 5-HT_{2B} receptors (Borman & Burleigh, 1995). In *in vivo* studies, 5-HT_4 receptors mediate several 5-HT-induced effects such as tachycardia in pigs (Villalón *et al*., 1991), forestomach hypomotility in sheep (Plaza *et al*., 1996c), gastric contractions in dogs (Bingham *et al*., 1995) and gastric emptying in both rats (Hegde *et al*., 1995) and dogs (Gullikson *et al*., 1993). Furthermore, gastrointestinal prokinetic benzamides, probably acting through 5-HT_4 receptors, stimulate gastrointestinal contractile activity in man (Briejer *et al*., 1995) and dogs (Gullikson *et al*., 1993). In the same way, presynaptic 5-HT_4 receptors facilitate fast synaptic transmission in the myenteric plexus that could contribute to the prokinetic effects of substituted benzamides (Pan & Galligan, 1994).

The migrating myoelectric (or motor) complex (MMC) is a basic pattern of mammalian gastrointestinal motility that consists of a phase of irregular spiking activity (phase II) followed by a period of regular spiking activity (phase III) and then a quiescent period (phase I). 5-HT has been proposed as a regulator of the MMC in several species. Thus, its intravenous administration evokes a phase III-like pattern in man (Lördal & Hellström, 1995) and increases both the frequency and propagation velocity of MMC in opossums (Coelho *et al*., 1986) and pigs (Wechsung & Houvenaghel, 1993). MMC frequency is also enhanced by the 5-HT precursor, 5-hydroxytryptophan (5-HTP) in rats (Sagrada *et al*., 1990) whereas it is decreased by neuronal 5-HT depletion in guinea-pigs (Galligan *et al*., 1986) and rats (Piñeiro-Carrero *et al*., 1991). Furthermore, selective destruction of enteric 5-HT neurones in rats (Piñeiro-Carrero *et al*., 1991) and depletion of endogenous 5-HT in dogs (Haga *et al*., 1996) disrupt the MMC pattern. In man (Wilmer *et al*., 1993), dogs (Itoh *et al*., 1991) and rats (Sagrada *et al*., 1990), the involvement of 5-HT_3 receptors in the regulation of the MMC has been revealed by the use of 5-HT_3 antagonists.

In sheep, 5-HT induces an antroduodenal phase III-like pattern and it has been proposed that myenteric 5-hydro-

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xytryptaminergic neurones in the duodenal bulb regulate the gastroduodenal cyclic motor activity (Ruckebusch, 1984; 1989; Ruckebusch & Bardon, 1984). These 5-HT-evoked effects are mimicked by several substituted benzamides (Plaza *et al*., 1992; 1994), suggesting that 5-HT$_4$ receptors could be involved. Consequently, the aims of this study were to characterize the 5-HT receptors mediating the 5-HT-evoked MMC activity in sheep, by using selective 5-HT antagonists and also to examine the possible involvement of cholinergic neuronal pathways in these actions.

Methods

Animal preparation

Animals were handled in accordance with the European Council Legislation 86/609/EEC on experimental animal protection. Six ewes 3–4 years old and weighing 40–50 kg were used. Animals were fasted for 24 h before being surgically prepared for electromyography, according to a previously described technique (Ruckebusch, 1970). Under general anaesthesia with intravenous (i.v.) thiopentone sodium (20 mg kg^{-1}; Pentothal, Abbott, Madrid, Spain), a right flank laparotomy was performed. Eight triplets of 120 μm nickel/chrome electrodes (Microfil Industries, Renens, Switzerland) were implanted in the muscular wall of the abomasal antrum (−5 cm from the pylorus), duodenum (10 and 50 cm from the pylorus) and proximal jejunum (1, 2, 3, 4 and 5 m from the ligament of Treitz). For analgesia, flunixin meglumine (5 mg kg^{-1}; Finadine, Schering-Plough, Madrid, Spain) was administered intramuscularly for 3 days after surgery. The animals were housed in metabolism cages at a controlled room temperature (20°C) on a 12 h light-dark cycle and they were fed *ad libitum* with pelleted lucerne hay.

Myoelectric recordings

Recording sessions began one week after surgery. Electrodes were connected to high-gain amplifiers (Lectromed MT8P, St. Peter Jersey Channel Islands, UK) and then, the amplified spiking activity was filtered by low-pass (50 Hz) and high-pass filters (10 Hz), to select net spike bursts. Simultaneously, a computer-based method (Datasystem EMG 4.0, Panlab, Barcelona, Spain) converted the analogue signal into digital values and stored them in a computer hard disk (PCS 386, Olivetti) with a sampling frequency of 100 samples s^{-1} per channel. The myoelectric activity was integrated as the sum of the absolute values of the signal amplitude minus the background value (5% of the maximal signal amplitude) over 1 min intervals. Data of integrated myoelectric activity were expressed as a percentage relative to the mean value of the control period (phase II) as previously described (Plaza *et al*., 1996d). In order to simplify the results, data showed in this work correspond to those obtained from duodenum at 50 cm from the pylorus and from jejunum at 5 m from the ligament of Treitz. Recordings were reproduced by a printer at an equivalent paper speed of 0.9 cm min^{-1}. However, to analyse specific myoelectric events, the computer programme allowed a recording expansion of up to 1.75 cm s^{-1}.

Experimental procedure

Experiments were started 30 min after a spontaneous duodenal phase III. In the first series of experiments, after a control period of 30 min, 5-HT (0.25, 0.5, 1, 2, 4 and 8 μg kg^{-1} min^{-1}) was administered as a continuous intravenous (i.v.) infusion for 5 min through a chronic silicone catheter (Silastic; id, 0.75 mm, od, 1.65 mm; Dow Corning, Midland, MI, U.S.A) into a jugular vein with a peristaltic pump (Microperpex 2132; LKB, Bromma, Sweden). To test the effect of a longer-lasting infusion, 5-HT was also administered i.v. at 2 μg kg^{-1} min^{-1} for 20 min.

During the second series of experiments, saline or 5-HT (at 2 or 4 μg kg^{-1} min^{-1}, 5 min) was infused i.v. 10 min after i.v. injection of one of the following antagonists: methiothepin (0.1 mg kg^{-1}), ritanserin (0.1 mg kg^{-1}), ketanserin (0.3 mg kg^{-1}), granisetron (0.2 mg kg^{-1}), ondansetron (0.5 mg kg^{-1}), GR113808 (0.2 mg kg^{-1}), atropine (0.2 mg kg^{-1}) or hexamethonium (2 mg kg^{-1}). Doses of 5-HT used in the experiments with antagonists were chosen because they were the smallest doses that evoked a MMC-like pattern in all the animals studied (2 μg kg^{-1} min^{-1}) and induced antral and jejunal stimulation (4 μg kg^{-1} min^{-1}). Doses of antagonists were chosen according to previous studies in sheep (Ruckebusch & Bardon, 1984; Brikas *et al*., 1994; Plaza *et al*., 1996c) and dogs (Itoh *et al*., 1991; Bingham *et al*., 1995). Experiments were performed in each sheep at 4 day intervals. In order to exclude diurnal variations in the studied parameters, all recording sessions started at 10 h 00 min.

Drugs

5-HT creatinine sulphate complex, atropine sulphate and hexamethonium bromide were purchased from Sigma (St. Louis, MO, U.S.A.). Methiothepin mesylate and ritanserin were obtained from Research Biochemicals Incorporated (Natick, MA, U.S.A.). The following drugs were kindly provided: ketanserin tartrate from Janssen (Beerse, Belgium), ondansetron and GR 113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate) (maleate salt) from Glaxo-Wellcome (Greenford, UK) and granisetron from Beecham (Harlow, UK). These compounds were dissolved in sterile saline, except for ketanserin and ritanserin which were dissolved in 10% dimethyl sulphoxide in the stock solution and then dissolved in saline. Drugs were administered in a final volume of 1 ml. Previous administration of these vehicles did not modify the gastrointestinal myoelectric activity.

Statistical analysis

The results are expressed as mean ± s.e.mean. Data expressed as percentages were subjected to arcsine transformation to make them normally distributed before statistical analysis. A one-way analysis of variance (ANOVA) was used to determine the significance of the overall variation in the data. A posterior Scheffé-*F* test was used to analyse multiple comparisons between mean values. Differences with $P<0.05$ were considered statistically significant.

Results

Control studies

Myoelectric activity of the gastrointestinal tract in sheep is organized into cyclic MMCs that recurred every 103.0 ± 5.3 min ($n=24$) in our study. They were characterized by the appearance of a duodenal activity front (phase III) that migrated to the jejunum at 57.4 ± 5.1 cm min^{-1}. Phase III was followed by a period without spiking activity (phase I) lasting 9.6 ± 1.2 min in the duodenum and 15.7 ± 1.4 min in the jejunum (Figure 1, Table 1). Coinciding with the duodenal phases III and I, antral activity decreased to 46.4 ± 2.5% ($P<0.001$) and to 41.9 ± 1.6% ($P<0.001$), respectively. In approximately 30% of MMCs, a stimulation was recorded in some jejunal sites before the appearance of the phase III. In these cases, the propagation velocity of the jejunal phase III significantly ($P<0.001$) decreased to 30.0 ± 2.1 cm min^{-1} ($n=12$).

Effects of 5-HT

After 60 min from the start of a spontaneous phase III, 5-HT was infused for 5 min at 2 μg kg^{-1} min^{-1}. It induced a duodenal activity front followed by a period of quiescence lasting

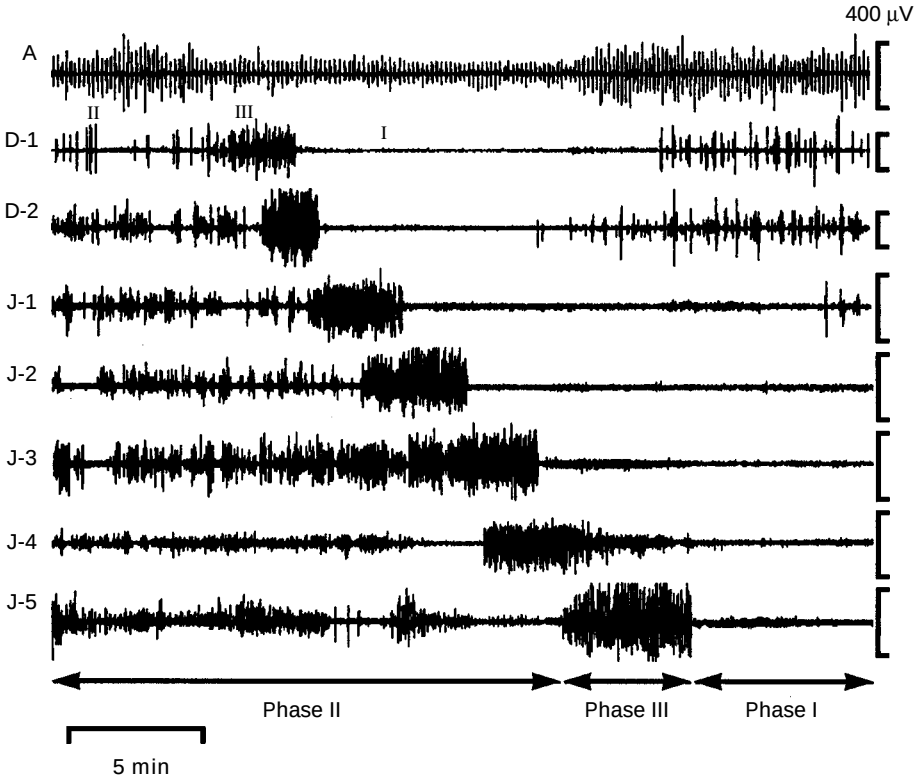

Figure 1 Recording of gastrointestinal myoelectric activity in sheep, showing a spontaneous MMC. Electrodes were implanted in the antrum −5 cm from the pylorus (A), in the duodenum 10 (D-1) and 50 cm (D-2) from the pylorus and in the jejunum 1 (J-1), 2 (J-2), 3 (J-3), 4 (J-4) and 5 (J-5) m from the ligament of Treitz.

Table 1 Myoelectric parameters from spontaneous phase III period of MMC and from the intestinal activity fronts evoked by an intravenous infusion of 5-HT (2 μg kg^{-1} min^{-1}, 5 min) in sheep: comparative influence of several antagonists on the effects induced by this dose of 5-HT

	Dose of antagonist (mg kg^{-1})	*Presence of 5-HT induced activity front*	*Parameters of duodenal activity front* *Duration* (min)	*Integrated activity* (%)	*Propagation velocity* (cm min^{-1})	*Parameters of jejunal activity front* *Duration* (min)	*Integrated activity* (%)	*Propagation velocity* (cm min^{-1})
Spontaneous activity front (phase III)[a]	...	...	2.0±0.1	607.0±20.6*	57.4±5.1	4.8±0.3	855.7±29.5*	43.7±1.9
5-HT								
+Saline	...	Yes	2.1±0.2	507.4±47.6*	51.5±7.2	4.5±0.4	466.0±40.3*	39.9±3.4
+Methiothepin	0.1	Yes	2.4±0.1	884.6±77.1*	55.8±9.0	5.0±0.6	529.5±44.1*	41.0±4.1
+Ritanserin	0.1	Yes	1.9±0.2	558.4±64.2*	50.7±5.3	5.3±0.7	650.6±48.7*	45.3±1.8
+Ketanserin	0.3	Yes	2.3±0.2	649.4±42.8*	59.6±8.1	4.3±0.7	832.3±72.0*	38.1±5.5
+Granisetron	0.2	Yes	1.8±0.2	433.2±39.3*	52.1±4.7	5.0±0.3	795.4±75.2*	47.5±7.2
+Ondansetron	0.5	Yes	1.9±0.1	714.8±74.0*	59.8±6.2	5.2±0.4	814.9±52.2*	41.5±6.4
+GR-113808	0.2	No	...	...	...	...	...	...
+Atropine	0.2	No	...	...	...	..	...	...
+Hexamethonium	2	No	...	...	...	...	...	...

Values are mean±s.e.mean of measurements from six animals, except for [a] (24 animals). Data were obtained 50 cm from the pylorus (duodenum) and 5 m from the ligament of Treitz (jejunum). Propagation velocity was determined between 10 and 50 cm from the pylorus in the duodenum and between 1 and 5 m from the ligament of Treitz in the jejunum. Integrated activity is the sum of the absolute values of myoelectric signal amplitude over 1 min intervals and this is expressed as percentage relative to the mean value of the control period (phase II), considered as 100%. Saline or antagonists were injected intravenously 10 min before 5-HT. *Values of integrated activity significantly ($P<0.001$) different from control.

9.9±2.3 min. The duodenal phase III-like activity migrated to the jejunum, where it was also followed by a phase I-like period of 19.2±3.1 min. Antral activity was inhibited to 58.9±5.6% ($P<0.001$) and to 45.2±1.8% ($P<0.001$) while the activity front and the quiescence period respectively developed in the duodenum. These myoelectric events resembled those observed during phases III and I of the MMC (Figure 2, Table 1). The next spontaneous phase III was recorded 98.7±11.3 min after this premature activity front evoked by 5-HT. This period was significantly ($P<0.01$) larger than that observed when saline instead of 5-HT was administered (49.3±8.6 min). Thus, the interval between the 5-HT-induced phase III-like activity and the next spontaneous phase III as well as the following MMC intervals were similar to that recorded between two spontaneous cycles, suggesting that 5-HT restarted the MMC cycle. When 5-HT was infused at 0.5 and 1 μg kg^{-1} min^{-1}, only two and four sheep from the six animals used, respectively, showed this myoelectric pattern. In the remaining animals, as occurred with the dose of 0.25 μg kg^{-1} min^{-1}, the gastrointestinal myoelectric activity was not modified. In addition to this MMC-like pattern, the doses of 4 and 8 μg kg^{-1} min^{-1} increased antral activity to

132.7±5.5% ($P<0.001$) and to 156.2±6.1% ($P<0.001$), respectively, concomitant with the duodenal activity front. Both doses also evoked a stimulation at several jejunal sites before the appearance of the jejunal activity front. The propagation velocity of the jejunal activity front was significantly ($P<0.01$) decreased to 25.1±2.7 cm min^{-1} ($n=6$). A longer-lasting infusion (20 min) of 5-HT at 2 μg kg^{-1} min^{-1}, evoked similar results to those observed after its infusion for 5 min at the same dose.

Effect of antagonists

The i.v. injection of the 5-HT antagonists, methiothepin (5-HT$_1$, 0.1 mg kg^{-1}), ritanserin (5-HT$_2$, 0.1 mg kg^{-1}), ketanserin (5-HT$_2$, 0.3 mg kg^{-1}), granisetron (5-HT$_3$, 0.2 mg kg^{-1}), ondansetron (5-HT$_3$, 0.5 mg kg^{-1}) and GR-113808 (5-HT$_4$, 0.2 mg kg^{-1}), did not modify spontaneous gastrointestinal myoelectic activity or MMC frequency. When methiothepin, ritanserin, ketanserin, granisetron or ondansetron were administered 10 min before the 5-HT infusion (2 or 4 μg kg^{-1}, 5 min), they did not modify the 5-HT-induced response. However, pretreatment with GR113808 completely blocked the MMC-like pattern evoked by both doses of 5-HT (Figure 3 , Table 1) as well as the transient antral and jejunal stimulations induced by 5-HT (4 μg kg^{-1} min^{-1}, 5 min). Thus, myoelectric activity and MMC frequency remained unchanged with respect to the control period.

The cholinoceptor antagonists atropine (muscarinic, 0.2 mg kg^{-1}) and hexamethonium (nicotinic, 2 mg kg^{-1}) inhibited antral activity for 43.0±7.6 min and for

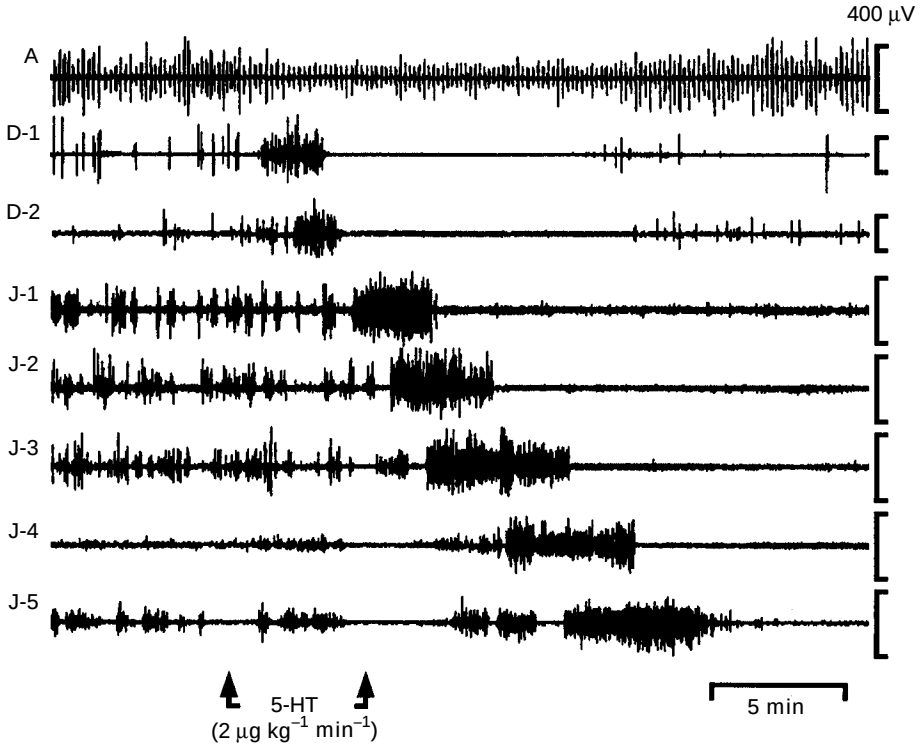

Figure 2 MMC-like pattern evoked by an intravenous infusion of 5-HT for 5 min in sheep. Electrodes were implanted in the antrum −5 cm from the pylorus (A), in the duodenum 10 (D-1) and 50 cm (D-2) from the pylorus and in the jejunum 1 (J-1), 2 (J-2), 3 (J-3), 4 (J-4) and 5 (J-5) m from the ligament of Treitz.

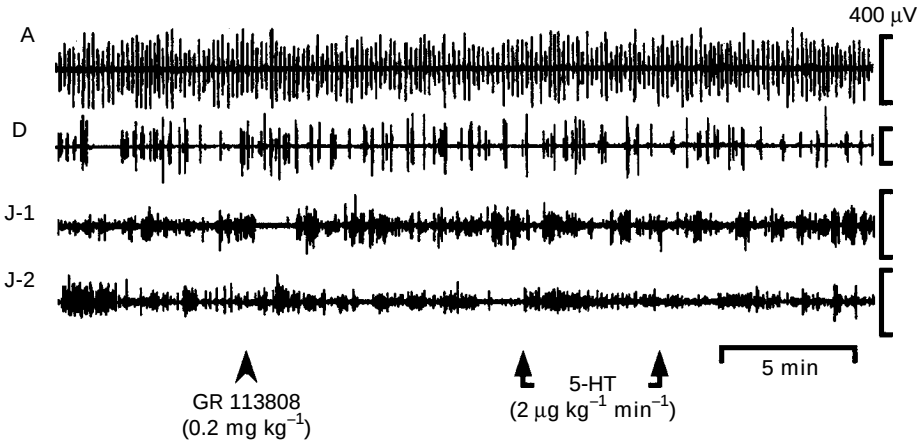

Figure 3 Antagonistic effect of an i.v. injection of GR 113808 on the MMC-like pattern induced by an i.v. infusion of 5-HT for 5 min in sheep. Electrodes were implanted in the antrum −5 cm from the pylorus (A), in the duodenum 50 cm from the pylorus (D) and in the jejunum 1 (J-1) and 5 (J-2) m from the ligament of Treitz.

33.8 ± 7.3 min, respectively. Furthermore, the spiking activity of the duodenum and jejunum was abolished for 58.6 ± 22.3 min and 111.2 ± 17.5 min, respectively, after atropine and for 68.2 ± 18.4 min and 95.6 ± 15.5 min, respectively, after hexamethonium. The appearance of the following MMC was delayed, 179.8 ± 19.8 min after atropine and 143.0 ± 29.8 min after hexamethonium. Both agents blocked the effects induced by 5-HT, because the infusion of 5-HT (2 or 4 μg kg^{-1} min^{-1}, 5 min), 10 min after atropine or hexamethonium, did not change either the gastrointestinal myoelectric activity or the increase in the MMC cycle interval evoked by blocking the cholinoceptors.

Discussion

Our results show that 5-HT evokes a gastrointestinal myoelectric pattern that closely resembles that observed during the phases III and I of the spontaneous MMC in sheep. Furthermore, the 5-HT-induced effects are completely blocked by atropine and hexamethonium as well as by the specific 5-HT$_4$ antagonist GR 113808, but not by 5-HT$_1$, 5-HT$_2$, or 5-HT$_3$ antagonists.

A 5-hydroxytryptaminergic control in the frequency and propagation of MMC has been proposed in pigs, opossums, rats and guinea-pigs (Coelho *et al*., 1986; Galligan *et al*., 1986; Piñeiro-Carrero *et al*., 1991; Wechsung & Houvenaghel, 1993), and a role for 5-HT$_3$ receptors has been suggested in rats because 5-HT$_3$ antagonists decreased MMC frequency in this species (Sagrada *et al*., 1990).

In man (Wilmer *et al*., 1993) and dogs (Itoh *et al*., 1991; Yoshida *et al*., 1991), 5-HT$_3$ receptors are involved in the appearance of the MMCs originating in the stomach because they are suppressed by 5-HT$_3$ antagonists while activity fronts are still present in the jejunum. Similar results have been obtained with the endogenous 5-HT depletion in dogs (Haga *et al*., 1996). With respect to MMCs originating at intestinal level in man, 5-HT triggers a phase III-like activity in the distal duodenum propagating to the jejunum without modifying the antrum and proximal duodenum (Lördal & Hellström, 1995). Furthermore, inhibition of 5-HT neuronal reuptake increases the frequency of intestinal MMC (Gorard *et al*., 1994). However, the 5-HT receptors involved in the regulation of intestinal MMC in man remain unknown. Thus, the cisapride-induced jejunal activity front is not a phase III-like activity because it does not migrate and is followed by an intense phase II activity instead of a true phase I (Coremans *et al*., 1988). In contrast to man, exogenous 5-HT disrupts the intestinal MMC pattern in dogs, being replaced by a simultaneous increase in the spiking activity of the gastrointestinal tract, resembling a phase II activity (Ormsbee *et al*., 1984; Iwanaga *et al*., 1989; Davidson *et al*., 1990). It is likely that these actions are mediated by 5-HT$_2$ and 5-HT$_4$ receptors because they are blocked by 5-HT$_2$ antagonists (Davidson *et al*., 1990) and are reproduced by substituted benzamides and specific 5-HT$_4$ agonists (Yoshida *et al*., 1991; Gullikson *et al*., 1991; 1993).

Our results show that 5-HT induces a MMC-like pattern in the gastrointestinal area in sheep and restarts the MMC cycle, suggesting that 5-HT could participate in the control of this cyclic activity in this species. In the same way, the 5-HT precursor, 5-hydroxytryptophan (5-HTP) increases the MMC frequency (Ruckebusch, 1984; Ruckebusch & Bardon, 1984) and 5-HT causes release of endogenous somatostatin and bombesin, two regulatory peptides that are involved in the origin of MMCs in sheep (Plaza *et al*., 1996a,b). In this species, the activity front corresponding to phase III of MMC starts at the duodenal level while antral activity remains inhibited (Ruckebusch & Buéno, 1977). In addition to 5-HT, somatostatin induces a similar pattern whereas motilin or its agonist erythromycin do not modify the antroduodenal myoelectric activity (Plaza *et al*., 1996a, b). Similarly, somatostatin (Peeters *et al*., 1983) and 5-HT (Lördal & Hellström, 1995) induce a migrating duodenal phase III-like pattern in man. Furthermore, motilin is involved in gastric but not intestinal MMCs (Bormans *et al*., 1987). Thus, with respect to their regulation, the sheep MMCs show more similarities to the human intestinal than gastric MMCs.

We have previously shown that cisapride, metoclopramide and zacopride evoke an antroduodenal phase III-like pattern in sheep (Plaza *et al*., 1992; 1994). These prokinetic benzamides behave as 5-HT$_4$ agonists as well as 5-HT$_3$ antagonists (Ford & Clarke, 1993; Hoyer *et al*., 1994; Briejer *et al*., 1995). However, other 5-HT$_3$ antagonists that do not act as 5-HT$_4$ agonists, such as ondansetron and granisetron (Hoyer *et al*., 1994), were not able to induce a MMC-like pattern in our experimental model. Thus, the benzamide-induced gastrointestinal motor effects in sheep are probably due to their 5-HT$_4$ agonistic properties. Our results are in agreement with this hypothesis because GR113808, a potent and selective 5-HT$_4$ antagonist (Gale *et al*., 1994), completely blocked all myoelectric events induced by 5-HT in the gastrointestinal area. This antagonist was not able to modify the frequency of the spontaneous MMCs. However, it has been shown that GR 113808 is rapidly degraded *in vivo* (Gale *et al*., 1994; Eglen *et al*., 1995) making its use difficult to block long-lasting cycles such as the MMC. In our experimental model, atropine and hexamethonium

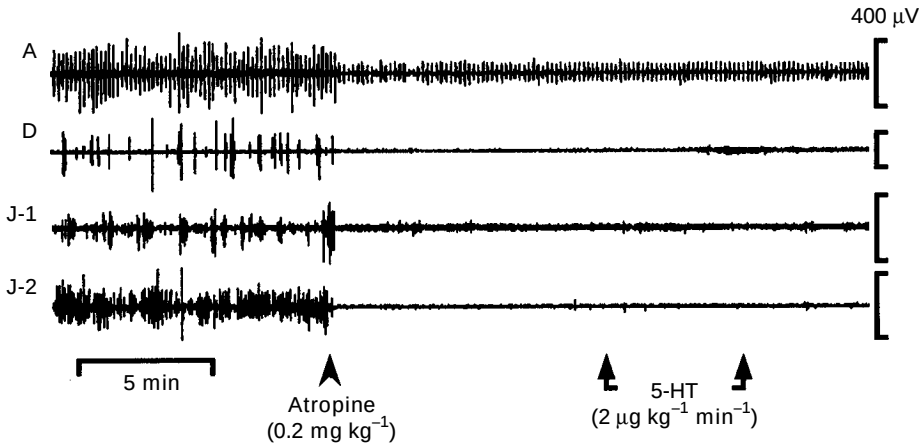

Figure 4 Effect of an i.v. injection of atropine on the intestinal migrating activity front induced by an i.v. infusion of 5-HT for 5 min in sheep. Electrodes were implanted in the antrum −5 cm from the pylorus (A), in the duodenum 50 cm from the pylorus (D) and in the jejunum 1 (J-1) and 5 (J-2) m from the ligament of Treitz.

blocked the MMC-like pattern induced by 5-HT, indicating that this effect is mediated by neuronal pathways involving both muscarinic and nicotinic receptors. Likewise, most of 5-HT$_4$-mediated gastrointestinal excitatory effects are elicited by the release of acetylcholine from myenteric neurones (Ford & Clarke, 1993; Kilbinger *et al*., 1995) and are blocked by atropine (Bockaert *et al*., 1992; Briejer *et al*., 1995).

The initial and transient increases in the antral and jejunal activity recorded in our study with the highest doses of 5-HT were also blocked by GR 113808, atropine and hexamethonium but not by the other 5-HT antagonists. Similar doses of 5-HT induce inhibition of forestomach myoelectric activity in sheep, these effects also being antagonized by the same agents (Plaza *et al*., 1996c). The excitatory antral effects were also induced in sheep by 5-HT$_4$ agonists (Brikas, 1994). Thus, the antral and jejunal stimulation resemble the 5-HT$_4$-mediated prokinetic action observed in the gastrointestinal tract in man (Briejer *et al*., 1995) and dogs (Gullikson *et al*., 1993; Bingham *et al*., 1995). The increase in antral activity was not observed in a spontaneous phase III of MMC in sheep. However, we have recorded a jejunal stimulation just before some spontaneous phase III periods and found that their propagation rate was diminished, as occurs with those MMC-like patterns induced by high doses of 5-HT. Similarly, inhibition of forestomach motility has also occasionally been recorded in association with the spontaneous duodenal phase III in sheep (Plaza *et al*., 1996d). Taken together, these results suggest that in some spontaneous cycles, endogenous 5-HT could be released at a rate slightly higher than that needed to evoke a MMC pattern. In these cases, 5-HT could stimulate jejunal motility and even inhibit forestomach activity.

In conclusion, 5-HT induces a gastrointestinal myoelectric pattern similar to that observed during a spontaneous MMC in sheep. The 5-HT-evoked effects are mediated through 5-HT$_4$ receptors, although spontaneous MMC were not blocked by the 5-HT$_4$ antagonist. However, our results do not support a role for 5-HT$_1$, 5-HT$_2$ or 5-HT$_3$ receptors. Furthermore, these actions are triggered through cholinergic neural pathways involving both muscarinic and nicotinic receptors.

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