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Effects of 5-Hydroxytryptamine Agonists on Myoelectric Activity of the Forestomach and Antroduodenal Area in Sheep

M. A. PLAZA, M. P. ARRUEBO AND M. D. MURILLO

Department of Pharmacology and Physiology, Veterinary Faculty, Miguel Servet 177, 50013-Zaragoza, Spain

Abstract

To increase knowledge of the role of 5-hydroxytryptamine (5-HT) receptors in the regulation of reticulorumino-omasal and antroduodenal myoelectric activity in sheep, the effects of 5-HT agonists on forestomach and antroduodenal myoelectric activity have been investigated in conscious sheep. 5-Carboxamidotryptamine, methysergide, α-methyl-5-HT, 2-methyl-5-HT, cisapride, zacopride or metoclopramide were infused intravenously for 5 min and myoelectric recordings were obtained from electrodes chronically implanted in the reticulum, rumen (dorsal sac), omasal body, abomasal antrum and duodenal bulb.

The integrated activity of the reticular and ruminal spike bursts was modified only by the highest doses of α-methyl-5-HT, 2-methyl-5-HT, metoclopramide and cisapride. A phase III-like activity pattern was recorded in the antroduodenal area with all 5-HT-ergic agents and a dose-dependent inhibition of myoelectric activity was recorded in both reticulorumen and omasum at the same time as the antroduodenal effects. In the forestomach, methysergide alone induced inhibition of ruminal secondary contractions; 5-HT, α-methyl-5-HT, cisapride and metoclopramide, moreover, evoked an initial dose-dependent increase in antral activity.

These results suggest that 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors are involved in the regulation of the migrating myoelectric complex in sheep and in the genesis of forestomach hypomotility that is occasionally recorded concomitantly with the spontaneous duodenal phase III in sheep. 5-HT_4 receptors also have a prokinetic action in the antral area.

In the gastrointestinal tract of mammals, 5-hydroxytryptamine (5-HT) is mainly synthesized and stored in enterochromaffin cells in the intestinal mucosa (Erspamer & Asero 1952). 5-HT has, furthermore, been proposed as a neurotransmitter in interneurons of the myenteric plexus (Gershon & Erde 1981). Four distinct groups of 5-HT receptor, some with subtypes, are now pharmacologically characterized: 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), 5-HT_3 and 5-HT_4. Genes encoding four new 5-HT receptors have, furthermore, been described: 5-HT_5 (5-HT_{5A} and 5-HT_{5B}), 5-HT_6 and 5-HT_7, but they have yet to be fully characterized in intact tissues (Hoyer et al 1994; Martin & Humphrey 1994). Several 5-HT receptors that do not comply with the criteria of this classification were not, however, included and are considered as 'orphans' (Hoyer et al 1994).

In the enteric nervous system, 5-HT induces direct responses through different receptors: hyperpolarization (5-HT_{1A}), long-lasting slow depolarization ('orphan' 5-HT_{1P}) or transient fast depolarization (5-HT_3 and 5-HT_4). The putative 5-HT_{1P} receptor, furthermore, mediates slow excitatory postsynaptic potentials whereas prejunctional 5-HT_{1A} receptors mediate suppression of fast excitatory postsynaptic potentials by inhibition of acetylcholine release. In in-vitro studies, the involvement of several 5-HT receptors in 5-HT-induced actions on gastrointestinal smooth muscle in several species has been reported. Thus, they mediate contraction in rat stomach fundus (5-HT_{2B}), guinea-pig oesophagus (5-HT_2), stomach (5-HT_3 and 5-HT_4), ileum (5-HT_{2A}, 5-HT_3 and 5-HT_4), proximal ascending colon (5-HT_4) and distal colon (5-HT_3, 5-HT_4 and the orphan, formerly 5-HT_1-like). The 5-HT-induced relaxation in guinea-pig ileum and proximal colon is a consequence of the orphan receptor, whereas in the colon in man and in the rat oesophagus and ileum it is through 5-HT_4 receptors. Finally, 5-HT_{1D} and 5-HT_4 receptors are involved in the peristaltic reflex in the guinea-pig ileum (Elswood & Bunce 1992; Buchheit & Buhl 1993; Ford & Clarke 1993; Hoyer et al 1994; Martin & Humphrey 1994; Woollard et al 1994; Briejer et al 1995).

Serotonergic mechanisms have been proposed in the control of the migrating myoelectric complex in several species. Administration of 5-HT triggers a duodenal phase III in man (Lördal & Hellström 1995) and 5-HT increases the frequency and propagation velocity of the migrating myoelectric complex in opossums (Coelho et al 1986) and pigs (Wechsung & Houvenaghel 1993), these effects being reproduced by inhibition of the selective 5-HT re-uptake in man (Gorard et al 1994). On the other hand, depletion of endogenous 5-HT in dogs (Haga et al 1996) and selective destruction of enteric 5-HT neurons in rats (Piñeiro–Carrero et al 1991) disrupted the migrating myoelectric complex pattern.

The 5-HT_3 receptor is involved in initiation of the vomiting reflex (Andrews et al 1988) and in the cyclic appearance of gastric phase III contractions in dogs (Itoh et al 1991; Yoshida et al 1991) and man (Wilmer et al 1993). Substituted benzamides that behave as 5-HT_4 receptor agonists, on the other hand, stimulate gastrointestinal contractile activity and increase gastric emptying in man (Johnson 1989; Fraser et al 1993), dogs (Yoshida et al 1991; Gullikson et al 1991, 1993) and rats (Lördal et al 1988; Hegde et al 1995). In the rat, however, 5-HT_3-receptor blockade seem to be an important feature of the ability of benzamides to stimulate gastric motility (Briejer et al 1995).

Correspondence: M. A. Plaza, Departamento de Fisiología, Facultad de Veterinaria, Miguel Servet 177, 50013-Zaragoza, Spain.

In the guinea-pig the orphan 5-HT receptor that mediates both relaxation in the ileum and contraction in the distal colon is located in the smooth muscle cells. The same location has been reported for the gastrointestinal 5-HT_2 receptors in a similar way to that for 5-HT_4 receptors mediating relaxation in the colon in man and in rat oesophagus and ileum. The remaining effects of 5-HT on the gastrointestinal tract are mediated through receptors located on myenteric neurons, by modulating the release of acetylcholine or other neurotransmitters (Elswood & Bunce 1992; Buchheit & Buhl 1993; Ford & Clarke 1993; Hoyer et al 1994; Martin & Humphrey 1994; Woollard et al 1994; Briejer et al 1995).

In sheep, serotonergic mechanisms regulate reticuloruminal cyclic motor activity (Ruckebusch & Ooms 1983; Sorraing et al 1984) and are involved in the origin of antroduodenal migrating myoelectric complex pattern (Ruckebusch 1984; Ruckebusch & Bardon 1984). Few studies have been performed on the involvement of 5-HT receptors in sheep and they were restricted to the reticuloruminal and abomasal activity (Brikas 1994; Brikas et al 1994). Consequently, the aim of this study was to increase knowledge of 5-HT receptors involved in the regulation of reticuloruminal, omasal and antroduodenal myoelectric activity in conscious sheep, by using selective 5-HT agonists. Preliminary reports have been published in abstract form (Plaza et al 1992, 1994a, b).

Materials and Methods

Animal preparation

Six ewes, 40–50 kg and 3–4 years old, were housed in metabolic cages at a controlled room temperature (20°C) in a 12-h light-dark cycle. Food (pelleted lucerne hay) was freely available except during recording of myoelectric activity. The sheep were conditioned to the recording conditions before the collection of data.

After 24-h fasting, animals were surgically prepared for electromyography according to a previously described technique (Ruckebusch 1970). Under premedication with xylazine (Rompun; Bayer, Barcelona, Spain; 0·2 mg kg^{-1} i.m.) and ketamine (Ketolar; Parke-Davis, Barcelona, Spain; 0.5 mg kg^{-1} i.m.) a left and right flank laparotomy was performed under paravertebral and local anaesthesia with lidocaine (Xilocaina; Ovejero, León, Spain; 40 mg kg^{-1}). Five triplets of 120-μm nickel/chrome electrodes (Microfil Industries, Renens, Switzerland) were implanted in the muscle wall of the reticulum, rumen (dorsal sac), omasal body, antrum and duodenal bulb (-5 and 10 cm from the pylorus, respectively).

Myoelectric recordings

The spiking activity was amplified by an electroencephalograph (Reega X; Alvar, Paris, France) with a time constant of 0·1 s, and recorded at a paper speed of 3.5 cm min^{-1}. Simultaneously, a computer-based method (Datasystem EMG 4·0, Panlab, Barcelona, Spain) converted the analogue signal into digital values and stored them on a computer (PCS 386, Olivetti) hard disk; the sampling frequency was 100 samples s^{-1} per channel. Two spike bursts were considered independent when the interval between them was greater than 1 s. The sum of the amplitude values (integrated activity), the duration of activity in each spike burst, and the interval between these spike bursts (period), were evaluated in the reticulum and rumen. For the omasum, antrum and duodenum the myoelectric activity was integrated over 1-min intervals because it was the most stable parameter in the control period (Plaza et al 1996c). In order to remove the background signal, an amplitude of 5% was considered as zero (baseline). Duration and period data were expressed in seconds whereas the integrated activity was given as a percentage relative to the mean value of the control period as previously described (Plaza et al 1996c).

Drugs

The effects induced by intravenous administration of 5-HT at 2·6, 5·2, 10·3 and 20·7 nmol kg^{-1} min^{-1} (1 to 8 μg kg^{-1} min^{-1}) were compared with those evoked by several 5-HT agonists (Hoyer et al 1994): 5-carboxamidotryptamine (5-HT_1 agonist) at 15·7, 31·3, 62·6 and 125·3 pmol kg^{-1} min^{-1} (5 to 40 ng kg^{-1} min^{-1}); methysergide (partial 5-HT_1 agonist and 5-HT_2 antagonist) at 10·7, 21·3, 42·6 and 85·2 nmol kg^{-1} min^{-1} (5 to 40 μg kg^{-1} min^{-1}); α-methyl-5-HT (5-HT_2 agonist) at 6·5, 13·1, 26·1 and 52·2 nmol kg^{-1} min^{-1} (2 to 16 μg kg^{-1} min^{-1}); and 2-methyl-5-HT (5-HT_3 agonist) at 13·1, 26·1, 52·2 and 104·5 nmol kg^{-1} min^{-1} (4 to 32 μg kg^{-1} min^{-1}). We also used substituted benzamides that behave as 5-HT_4 agonists and 5-HT_3 antagonists in gastrointestinal motility models (Briejer et al 1995): cisapride at 10·3, 20·7, 41·3, 82·6 and 165·3 nmol kg^{-1} min^{-1} (5 to 80 μg kg^{-1} min^{-1}); metoclopramide at 74·3, 148·7, 297·4 and 594·7 nmol kg^{-1} min^{-1} (25 to 200 μg kg^{-1} min^{-1}); and zacopride at 144·4, 288·9 and 577·7 pmol kg^{-1} min^{-1} (50 to 200 ng kg^{-1} min^{-1}). For all these agents, preliminary results showed that lower doses than those used in this study did not modify gastrointestinal myoelectric activity in sheep.

Serotonin creatinine sulphate complex and metoclopramide monohydrochloride were purchased from Sigma (St Louis, MO, USA). 5-Carboxamidotryptamine , α-methyl-5-HT and 2-methyl-5-HT, all in the maleate form, were obtained from Research Biochemicals Incorporated (Natick, MA, USA). Zacopride hydrochloride was donated by Delalande (Rueil-Malmaison, France), methysergide maleate by Sandoz (Basel, Switzerland) and cisapride by Janssen (Beerse, Belgium). Methysergide was dissolved in 10% dimethylsulphoxide and cisapride in distilled water acidified with tartaric acid as stock solution (pH $\geq$ 3) and then dissolved in saline. The remaining agents were dissolved in sterilized saline. Drugs were administered in a final volume of 1 mL saline. Previous administration of these vehicles did not modify the gastrointestinal myoelectric activity.

Experimental procedure

Electromyographic recordings began 2 weeks after surgery. To standardize the myoelectric parameters in the control period from the six ewes used in this study, recordings were performed during two whole migrating myoelectric complexes. In the experiments where drugs were given, food was removed 1 h before the beginning of recordings and the control period started 15–20 min after a spontaneous duodenal phase III. Then, after a control period of 30 min, 5-HT or its agonists were given as a continuous infusion for 5 min. Drugs were administered in a random order through an in-dwelling silicone catheter (Silastic; 0·75 mm i.d., 1·65 mm o.d.; Dow Corning, Midland, MI, USA) into the jugular vein with a peristaltic

Table 1. Integrated activity of reticuloruminal spike bursts and duration of ruminal secondary spike bursts after intravenous infusion of 5-HT agonists for 5 min in sheep.

Agonist	Dose (nmol $kg^{-1}min^{-1}$)	Primary cycle (% of activity)		Secondary cycle	
		Reticulum	Rumen	% of activity	Duration (s)
α-Methyl 5-HT	52·2	114·8 ± 3·4*	121·3 ± 5·3*	156·7 ± 11·5*	3·3 ± 0·3*
2-Methyl 5-HT	52·2	106·5 ± 1·4*	98·4 ± 1·8	106·6 ± 4·0	2·1 ± 0·1
	104·5	125·7 ± 2·0*	93·5 ± 2·9	97·3 ± 4·3	2·3 ± 0·1
Cisapride	82·6	86·3 ± 3·3†	104·2 ± 5·2	112·6 ± 7·9	2·5 ± 0·2
	165·3	89·2 ± 2·5†	98·8 ± 4·3	137·9 ± 9·2‡	3·5 ± 0·3‡
Metoclopramide	594·7	83·6 ± 2·1*	126·5 ± 7·8†	107·4 ± 3·6	2·4 ± 0·1

Results are means ± s.e.m. from 6 sheep. Integrated activity data are expressed as a percentage relative to the mean value of the control period and were subjected to an arcsine transformation before statistical analysis. Duration of spike bursts from ruminal secondary contractions in the control period was 2·2 ± 0·06 s. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$.

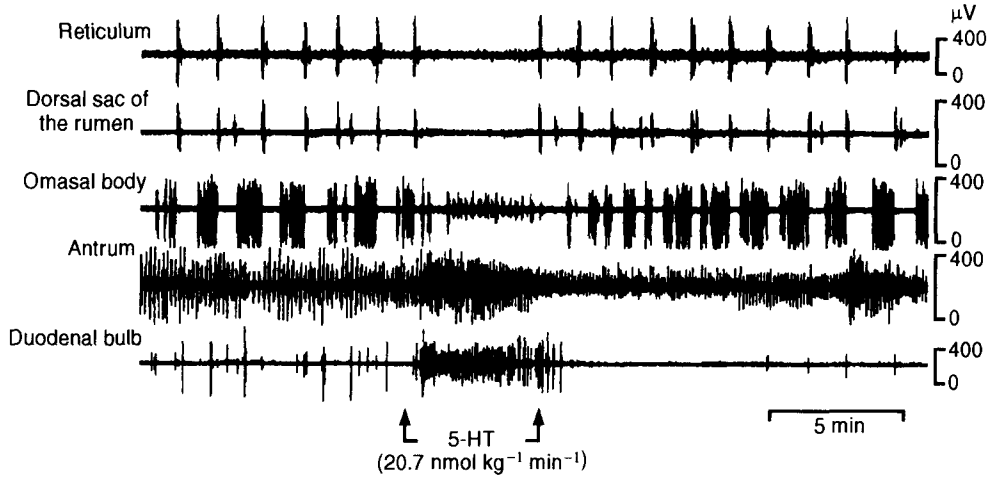

FIG. 1. Effects of an intravenous infusion of 5-hydroxytryptamine on myoelectric activity of the reticulum, the dorsal sac of the rumen, the omasal body, the antrum and the duodenal bulb in sheep.

pump (Microperpex 2132; LKB, Bromma, Sweden). For a given agent, each dose was tested once in each animal and experiments were performed at 2-day intervals at least. In order to exclude diurnal variations in the studied parameters, all recording sessions started at 10·00 h.

Statistical analysis

The results are expressed as mean ± s.e.m. As rumination modifies the forestomach parameters studied, values corresponding to rumination periods were eliminated from further analysis. Data expressed as percentages were subjected to arcsine transformation to make them normally distributed before statistical analysis. A one-way analysis of variance was used to determine the significance of differences among mean values. A posterior Scheffé F-test was used to analyse multiple comparisons. Differences with $P < 0.05$ were considered statistically significant.

Results

Control studies

In the control period, reticuloruminal myoelectric activity was characterized by coordinated cyclic spike bursts, occurring every 76.3 ± 1.3 s, that started in the reticulum and were propagated to the rumen (primary cycle). In approximately half the reticuloruminal primary cycles, a second spike burst started in the rumen (secondary cycle) and appeared at 179.4 ± 6.8 s intervals. Myoelectric activity of the omasal body occurred at almost every reticuloruminal primary cycle and consisted of either a long spike burst or a group that ceased with the onset of the next cycle.

Antroduodenal area recordings exhibited migrating myoelectric complex cycles recurring at 2·2 ± 0.1 h intervals. Duodenal integrated activity increased to 423·8 ± 51·2% ($P < 0.001$) in the activity front corresponding to phase III of the migrating myoelectric complex and was followed by a period of inactivity (phase I). Antral activity decreased to 68·5 ± 3·7% ($P < 0.001$) and 56·4 ± 2·0% ($P < 0.001$) coinciding with duodenal phases III and I, respectively.

Effects of 5-HT and its agonists

In relation to reticuloruminal primary cycles, the administration of 5-HT or its agonists did not modify the duration of reticular or ruminal spike bursts (3·4 ± 0·03 s and 4·2 ± 0·06 s, respectively). Integrated activity of reticular spike bursts was, however, either increased by α- and 2-methyl-5-HT or reduced by cisapride and metoclopramide. The last parameter was increased in the rumen by α-methyl-5-HT and metoclopramide. The integrated activity and duration (2·2 ± 0·06 s) of spike bursts of ruminal secondary contractions were increased

Table 2. Minimum dose (nmol kg^{-1} min^{-1}) of 5-HT or its agonists, infused intravenously for 5 min, inducing inhibition of reticuloruminal primary and secondary cycles, a decrease in myoelectric activity of the omasum and antrum and duodenal phase III-like activity in sheep.

Agonist	Primary cycle	Secondary cycle	Omasum	Antrum	Duodenum
5-Hydroxytryptamine	5·2	10·3	5·2	2·6	2·6
5-Carboxamidotryptamine	0·03	0·03	0·03	0·02	0·02
Methysergide	–*	10·7	–*	10·7	10·7
α-Methyl-5-HT	13·1	26·1	52·2	6·5	6·5
2-Methyl-5-HT	52·2	52·2	104·5	13·1	13·1
Cisapride	86·6	165·3	82·6	10·3	10·3
Metoclopramide	594·7	594·7	594·7	74·3	74·3
Zacopride	0·6	0·6	0·3	0·3	0·3

*Without effect on the corresponding parameter.

Table 3. Effects of intravenous infusion for 5 min of 5-hydroxytryptamine (5-HT) or its agonists on the period of reticuloruminal primary and secondary cycles and on omasal integrated activity in sheep.

Agonist	Dose (nmol kg^{-1} min^{-1})	Reticuloruminal cycles		Omasum
		Primary	Secondary	
5-Hydroxytryptamine	2·6	71·1 ± 2·6	176·6 ± 23·9	97·0 ± 10·7
	5·2	107·5 ± 4·1*	218·4 ± 28·1	73·6 ± 8·8‡
	10·3	149·3 ± 11·8*	365·1 ± 55·8†	56·1 ± 7·8*
	20·7	205·5 ± 34·8*	485·3 ± 82·4*	45·8 ± 5·9*
5-Carboxamidotryptamine	0·016	78·9 ± 3·4	188·9 ± 28·9	98·2 ± 8·1
	0·031	87·5 ± 4·7†	288·8 ± 46·2‡	72·3 ± 8·7‡
	0·063	104·1 ± 8·8*	366·6 ± 63·8†	65·5 ± 6·5†
	0·125	142·6 ± 13·5*	390·0 ± 86·9*	58·7 ± 5·9*
Methysergide	10·7	72·8 ± 2·3	263·0 ± 54·1‡	102·4 ± 9·0
	21·3	74·1 ± 3·6	369·7 ± 73·2†	98·0 ± 10·0
	42·6	73·5 ± 3·5	518·4 ± 104·3*	104·8 ± 8·8
	85·2	81·2 ± 3·0	919·0 ± 165·5*	96·7 ± 10·1
α-Methyl-5-HT	6·5	75·9 ± 4·1	163·0 ± 16·3	106·7 ± 7·6
	13·1	89·6 ± 5·3‡	228·2 ± 24·3	95·9 ± 8·1
	26·1	103·9 ± 9·8*	332·8 ± 61·7†	78·8 ± 9·2
	52·2	154·6 ± 25·5*	553·0 ± 94·6*	61·1 ± 7·5†
2-Methyl-5-HT	13·1	77·7 ± 2·7	198·4 ± 24·9	103·7 ± 9·4
	26·1	79·0 ± 3·7	202·5 ± 30·8	106·8 ± 8·0
	52·2	123·4 ± 18·9*	329·7 ± 49·0†	95·1 ± 11·4
	104·5	248·9 ± 37·9*	676·7 ± 116·0*	71·2 ± 6·1‡
Cisapride	10·3	76·5 ± 3·9	208·8 ± 24·5	97·4 ± 10·6
	20·7	72·2 ± 2·2	219·5 ± 23·2	102·5 ± 8·1
	41·3	80·8 ± 3·5	186·6 ± 13·9	86·7 ± 9·6
	82·6	99·2 ± 5·2†	242·3 ± 25·2	71·5 ± 10·4‡
	165·3	119·4 ± 6·0*	336·7 ± 59·1†	54·6 ± 7·6*
Metoclopramide	74·3	78·9 ± 3·5	172·6 ± 15·3	102·0 ± 11·6
	148·7	73·9 ± 2·9	176·5 ± 17·7	97·5 ± 8·1
	297·4	79·4 ± 2·2	198·4 ± 18·1	85·7 ± 9·6
	594·7	106·1 ± 3·6*	315·6 ± 37·4†	65·1 ± 8·3‡
Zacopride	0·14	71·7 ± 4·1	183·1 ± 14·0	81·4 ± 11·7
	0·29	72·2 ± 3·8	207·4 ± 37·8	68·5 ± 7·2‡
	0·58	273·8 ± 38·0*	542·5 ± 88·7*	53·7 ± 3·9*

Values represent means ± s.e.m. from 6 sheep. In the control period, reticuloruminal primary and secondary cycles recurred every 76·3 ± 1·3 and 179·4 ± 6·8 s, respectively. Omasal integrated activity data are expressed as a percentage relative to the mean value of the control period and were subjected to an arcsine transformation before statistical analysis. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$.

by both α-methyl-5-HT and cisapride. All these effects on the reticulorumen were observed only at the highest doses and always after the end of the infusion period (Table 1). The remaining agents tested did not affect integrated activity or duration of reticuloruminal spike bursts.

An increase in the interval between spike bursts in both reticuloruminal primary and secondary cycles and inhibition of omasal integrated activity were induced by 5-HT or its agonists (Fig. 1 and Table 2). With methysergide, however, the only effect observed in the forestomach was a delay in the appearance of ruminal secondary contractions. All these effects were dose-dependent (Table 3). The order of agonist potency in inhibiting the whole forestomach activity was: 5-carboxamidotryptamine ≫ zacopride ≫ 5-HT > α-methyl-5-HT > 2-methyl-5-HT > cisapride ≫ metoclopramide (Table 2).

Methysergide and zacopride reduced duodenal integrated activity when administered at the lowest dose. The remaining doses and agents tested evoked a duodenal activity front followed by a quiescent period. Inhibition of antral activity was, furthermore, also recorded in close association with the duo-

Table 4. Effects of intravenous infusion for 5 min of 5-HT or its agonists on antroduodenal integrated activity in sheep.

Agonist	Dose (nmol kg^{-1} min^{-1})	Antrum activity front	Antrum inactivity	Duodenum activity front
5-Hydroxytryptamine	2·6	53·6 ± 3·8*	56·8 ± 2·4*	562·1 ± 83·0*
	5·2	68·4 ± 5·4*	36·0 ± 2·1*	594·9 ± 73·0*
	10·3	129·9 ± 5·7*	43·4 ± 2·4*	616·8 ± 53·2*
	20·7	148·3 ± 6·2*	44·9 ± 1·8*	793·2 ± 50·0*
5-Carboxamidotryptamine	0·016	62·0 ± 4·1*	51·3 ± 1·6*	476·8 ± 54·7*
	0·031	56·7 ± 3·5*	39·1 ± 1·6*	644·3 ± 64·4*
	0·063	54·9 ± 3·9*	40·9 ± 2·1*	817·9 ± 72·9*
	0·125	41·4 ± 2·9*	40·5 ± 2·3*	758·0 ± 84·2*
Methysergide	10·7	71·9 ± 4·6*	46·1 ± 2·5*	19·7 ± 6·3*
	21·3	69·5 ± 3·4*	57·0 ± 3·6*	459·2 ± 48·6*
	42·6	56·3 ± 4·3*	49·7 ± 2·2*	547·7 ± 32·1*
	85·2	53·8 ± 3·9*	48·3 ± 2·7*	516·0 ± 56·0*
α-Methyl 5-HT	6·5	66·0 ± 4·3*	54·3 ± 2·2*	405·7 ± 39·5*
	13·1	55·4 ± 3·7*	46·7 ± 3·3*	492·3 ± 62·1*
	26·1	75·7 ± 6·3†	54·7 ± 3·6*	597·8 ± 52·7*
	52·2	118·2 ± 5·1‡	70·1 ± 4·5*	670·7 ± 84·9*
2-Methyl 5-HT	13·1	65·2 ± 4·6*	43·1 ± 3·8*	455·5 ± 31·9*
	26·1	57·4 ± 3·6*	59·6 ± 2·8*	511·5 ± 89·6*
	52·2	50·0 ± 4·4*	45·5 ± 3·1*	569·9 ± 46·4*
	104·5	51·3 ± 3·2*	50·7 ± 1·4*	642·6 ± 55·5*
Cisapride	10·3	115·8 ± 5·9	52·9 ± 3·8*	487·0 ± 53·9*
	20·7	123·7 ± 5·5†	45·5 ± 4·4*	734·8 ± 66·3*
	41·3	146·0 ± 4·5*	56·9 ± 4·3*	580·3 ± 39·8*
	82·6	149·3 ± 6·8*	65·6 ± 5·4†	689·8 ± 66·8*
	165·3	158·2 ± 7·1*	73·6 ± 5·0‡	827·9 ± 87·3*
Metoclopramide	74·3	110·2 ± 4·2	43·7 ± 4·0*	339·8 ± 63·3*
	148·7	128·1 ± 6·1†	48·9 ± 4·7*	529·5 ± 33·3*
	297·4	137·0 ± 6·0*	54·8 ± 5·3*	573·8 ± 55·2*
	594·7	147·2 ± 7·4*	65·8 ± 4·3*	649·2 ± 48·5*
Zacopride	0·14	104·0 ± 5·3	92·7 ± 4·8	74·8 ± 16·9
	0·29	64·2 ± 3·6*	40·8 ± 2·7*	504·4 ± 55·8*
	0·58	44·6 ± 2·6*	36·0 ± 1·4*	427·1 ± 45·9*

Values represent means ± s.e.m. from 6 sheep. Data are expressed as a percentage relative to the mean value of the control period and were subjected to an arcsine transformation before statistical analysis. Antral values concomitant with the duodenal activity fronts and with their subsequent quiescent periods were denoted activity front and inactivity, respectively. When an activity front is not induced in the duodenum, the results correspond to the values within the 5-HT infusion period (5 min). *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$.

denal effects. This pattern of myoelectric activity resembled that observed during phases III and I of a spontaneous migrating myoelectric complex in sheep (Fig. 1, Tables 2 and 4). The order of agonist potency in inducing a phase III-like pattern was: 5-carboxamidotryptamine ≫ zacopride ≫ 5-HT > α-methyl-5-HT > cisapride = 2-methyl-5-HT > methysergide > metoclopramide (Table 2). The inhibition of forestomach myoelectric activity was always closely associated with the duodenal activity front.

The administration of 5-HT, α-methyl-5-HT, cisapride or metoclopramide evoked an initial dose-dependent increase in antral activity. This excitatory effect was followed by a transient antral inhibition coinciding with the duodenal quiescence period (Fig. 1, Table 4). Thereafter, except for 5-HT, a subsequent episode of antral stimulation was recorded.

Discussion

Our results show that agonists at 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors mimicked the 5-HT-induced effects on both forestomach and antroduodenal area in sheep.

Serotonergic mechanisms have been involved in the control of the migrating myoelectric complexes in monogastric species. Thus, the intravenous administration of 5-HT induces a duodenal migrating activity front in man (Lördal & Hellström 1995) and fed dogs (Björck et al 1988) as well as an increase of migrating myoelectric complex frequency in pigs (Wechsung & Houvenaghel 1993) and opossums (Coelho et al 1986). Inhibition of 5-HT re-uptake also increases the frequency of jejunal migrating myoelectric complexes in man (Gorard et al 1994). Endogenous 5-HT depletion, on the other hand, suppresses gastric migrating myoelectric complexes in dogs (Haga et al 1996) and selective destruction of 5-HT enteric neurons disrupts jejunal migrating myoelectric complexes in rats (Piñeiro-Carrero et al 1991). In ruminants, 5-HT inhibits the occurrence of cyclic reticuloruminal contractions in goats (Veenendaal et al 1980) and sheep (Ruckebusch & Ooms 1983; Sorraing et al 1984, 1985) and omasal myoelectric activity (Plaza et al 1994a). It also induces an antroduodenal phase III-like pattern of migrating myoelectric complex in sheep (Ruckebusch 1984; Ruckebusch & Bardon 1984). These gastrointestinal 5-HT-induced actions are triggered by 5-HT_4 receptors and are mediated through neural pathways involving muscarinic and nicotinic receptors (Plaza et al 1992, 1994a, 1994b). Exogenous 5-HT also induces the release of somatostatin and bombesin, two regulatory peptides that are also involved in the genesis of migrating myoelectric complexes in this species (Plaza et al 1996a, b). Thus, 5-HT participates in the control of gastrointestinal motor pattern in sheep and in other mammals.

In our study, 5-HT inhibited myoelectric activity of the whole forestomach and induced an antroduodenal phase III-like pattern. Other authors have reported that 5-HT induces an additional increase in the reticuloruminal tone associated with a continuous low-amplitude spike activity (Ruckebusch & Ooms 1983; Sorraing et al 1985). Although these excitatory effects have been ascribed to peripheral 5-HT_2 and 5-HT_4 receptors (Brikas et al 1994), these actions were observed when 5-HT was injected as a bolus or at higher doses than those used in our study. Indeed, in preliminary experiments we have recorded a similar increase in reticuloruminal activity with doses higher than 20.7 nmol kg^{-1} min^{-1}. These effects are, nevertheless, not observed under physiological conditions and hence could be pharmacological actions.

To clarify the involvement of serotonergic receptors in the control of gastrointestinal motility in sheep, we administered agonists at the 5-HT receptors that have been pharmacologically characterized. The selective 5-HT_1 agonist 5-carboxamidotryptamine (Hoyer et al 1994) reproduced the myoelectric events observed after the administration of low doses of 5-HT. This finding suggests that this receptor subtype mediates the 5-HT effects. Methysergide, considered a 5-HT_2 antagonist, also acts as a partial agonist at 5-HT_1 receptors in several tissues (Hoyer et al 1994). Thus, the phase-III-like pattern induced by methysergide could be a result of its action on 5-HT_1 receptors. Methysergide has been shown to block the 5-HT-induced inhibition of the reticuloruminal primary cycles in both sheep (Ruckebusch & Ooms 1983; Sorraing et al 1984, 1985) and goats (Veenendaal et al 1980). In addition, this agent antagonized the decrease in the myoelectric omasal activity induced by 5-HT in sheep (Plaza et al 1994a). Consequently, except for the ruminal secondary contractions, methysergide would act as a 5-HT_2 antagonist rather than as a 5-HT_1 agonist in the forestomach. These results suggest the possibility that the 5-HT_1 receptors located in both forestomach and antroduodenal area in sheep might be different. In the methysergide-induced inhibition on the ruminal secondary contractions, other receptors or mechanisms could be involved.

α-Methyl-5-HT and 2-methyl-5-HT, specific 5-HT_2 and 5-HT_3 receptor agonists respectively (Hoyer et al 1994), on the other hand, also reproduced the myoelectric events observed after 5-HT infusion. These results indicate that, in addition to 5-HT_1 receptors, 5-HT_2 and 5-HT_3 receptors are also involved in the control of gastrointestinal motility in sheep. The substituted benzamides cisapride, metoclopramide and zacopride, considered to be 5-HT_4 agonists and 5-HT_3 antagonists (Ford & Clarke 1993; Hoyer et al 1994; Briejer et al 1995) were, moreover, also able to mimic all effects evoked by 5-HT in sheep. The benzamide-induced effects observed in our study can probably be ascribed to the 5-HT_4 receptor because 5-HT_3 antagonists devoid of 5-HT_4 agonistic properties (Hoyer et al 1994), such as ondansetron, ICS 205-930 (Plaza et al 1992, 1994a, b) or granisetron (Brikas 1994; Brikas et al 1994), failed to modify gastrointestinal myoelectric activity in sheep.

The excitatory antral effects induced by both cisapride and metoclopramide resembled the increase in the gastrointestinal motility observed after the administration of benzamide derivatives in man (Fraser et al 1993) and dogs (Gullikson et al 1991, 1993; Yoshida et al 1991), this action being ascribed to a prokinetic action through 5-HT_4 receptors. The substituted benzamide zacopride has also been reported as a prokinetic agent acting through 5-HT_4 receptors (Briejer et al 1995). In our experimental model, zacopride mimicked the 5-HT-evoked effects except the excitatory antral action. We, however, used zacopride at very small doses, which could be insufficient to show its pharmacological prokinetic action. α-Methyl-5-HT, on the other hand, is able to induce the stimulatory antral effect when infused at the highest dose. This agent, in addition to its 5-HT_2-agonistic activity, shows pharmacological properties as a weak 5-HT_4 agonist (Ford & Clarke 1993; Hoyer et al 1994), which could explain its prokinetic effect. The antral inhibition observed during the periods of spontaneous duodenal phase III in sheep has been ascribed to an extrinsic nervous inhibitory reflex triggered by the increased duodenal contractions (Ruckebusch & Buéno 1977; Buéno & Fioramonti 1980). For benzamides cisapride and metoclopramide, this antral inhibition could be overlapped by their prokinetic properties.

Inhibition of reticuloruminal and antral motility has also been observed when the proximal duodenum develops continuous maximum spiking activity in response to moderate duodenal distension in sheep. These inhibitory effects are mediated by 5-HT_1, 5-HT_2 and 5-HT_3 receptors (Brikas et al 1993) through a vagal reflex involving tension receptors (Ruckebusch 1989). In our study, low doses of 5-HT or its agonists induced an antroduodenal phase III-like pattern without modifying forestomach activity. This higher sensitivity of the antroduodenal area to serotonergic agents, compared with that of the forestomach, is in agreement with the independent motility coordination in both ruminant digestive areas (Ruckebusch 1989). A slight reduction in omasal activity associated with the spontaneous duodenal phase III has, however, been reported, and a related delay in reticuloruminal cycles has also been occasionally observed in sheep (Plaza et al 1996c). This forestomach hypomotility pattern is also observed with high doses of serotonergic agents. These findings suggest that endogenous 5-HT is spontaneously released to evoke the antroduodenal migrating myoelectric complex, and only if this release reaches the forestomach threshold it is able to trigger the spontaneous inhibition of this area.

In conclusion, 5-HT is a mediator in the control of the reticuloruminal and omasal cycles and in the regulation of the antroduodenal events associated with the migrating myoelectric complex in sheep. 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors are, furthermore, also involved in these actions.

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