Modification of spontaneous contractility of smooth muscle preparations from the bovine abomasal antrum by serotonin receptor agonists

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The effects of serotonin (5-hydroxytryptamine, 5-HT), a 5-HT_2-receptor agonist, α-methyl-5-hydroxytryptamine (α-M-5-HT) and RS-67506, a 5-HT_4 receptor partial agonist, on spontaneous contractility of bovine abomasal smooth muscle preparations were investigated *in vitro*. Preparations from the abomasal antrum of freshly slaughtered healthy dairy cows were cut parallel to the longitudinal fibres, suspended in isolated organ baths, and concentration–response curves were performed by cumulative application of the 5-HT receptor agonists. Blockade of 5-HT_2-induced response was tested with atropine and hexamethonium. Serotonin evoked a significant increase in the area under curve (*AUC*), whilst the 5-HT_2 receptor agonist α-M-5-HT significantly increased the *AUC* and resting tone (RT). RS-67506 induced a significant increase in *AUC* and RT and a significant decrease in the maximum force. The effect of α-M-5-HT was mediated by a muscarinic cholinergic pathway, as the effect of α-M-5-HT was inhibited in the presence of atropine but not hexamethonium. It is concluded that 5-HT_2 and 5-HT_4 receptors are present in the bovine abomasal antrum. Muscarinic receptors are involved in the increase in RT seen after 5-HT_2 receptor stimulation.

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INTRODUCTION

Within the past three decades, displacement of the abomasum (DA) has developed into a major economic problem in cattle. Left displacement of the abomasum (LDA) is a problem leading to a decrease in milk production, high treatment costs, increased culling rates (Martin *et al.*, 1978; Bartlett *et al.*, 1995), and causes considerable discomfort to the animal. Many studies have been conducted to identify the aetiological factors of DA. Many risk factors have been identified. They include animal-specific factors like breed (Constable *et al.*, 1992; Eicher *et al.*, 1999; Stengärde & Pehrson, 2002), high milk production (Constable *et al.*, 1992), and body size (Stöber & Saratsis, 1974); periparturient factors such as twin birth (Rohrbach *et al.*, 1999), hypocalcaemia (Massey *et al.*, 1993), ketosis (Geishauser *et al.*, 1997; Rohrbach *et al.*, 1999), endotoxemia (e.g. due to metritis or retained placenta) (Vlaminck *et al.*, 1985; Rohrbach *et al.*, 1999), and management factors, such as high concentrate diet (Coppock *et al.*, 1972), negative energy balance in the prepartum phase (Cameron *et al.*, 1998), and low content of neutral detergent fibre (Stengärde & Pehrson, 2002). Several authors have hypothesized that many of these factors lead to diminished appetite, followed by decreased ruminal filling, as a common predisposing factor (Constable *et al.*, 1992; Stengärde & Pehrson, 2002). Although the role of individual factors is disputed, there is general consent to the hypothesis that a prerequisite for DA is a disturbance of normal abomasal motility, followed by accumulation of gas in the fundus region and subsequent displacement of the organ (Svendsen, 1970; Geishauser, 1995).

Serotonin (5-hydroxytryptamine, 5-HT) is concerned with gastrointestinal tract (GIT) motility in several species, ranging from drosophila and snails to man (Peroutka, 1994). It is the neurotransmitter with the highest number of specific receptor subtypes identified (Hedge & Eglen, 1996), and these receptors have been identified in most segments of the GIT of mammals (Taniyama *et al.*, 2000). Although 5-HT receptors have been highly conserved throughout evolution, as evidenced by molecular cloning (Peroutka, 1994), important differences exist between species in the function of individual receptor types and subtypes (Briejer *et al.*, 1995; Hedge & Eglen, 1996).

In sheep, several authors have studied the effect of 5-HT receptor agonists and antagonists on GI motility *in vivo* by means of electromyography. One study led to the conclusion that 5-HT exhibits an inhibitory control function in the central nervous system (CNS) while having excitatory effects on the tone of the rumen wall when administered peripherally (Sorraing *et al.*, 1985). All four 5-HT receptor subtypes (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) investigated in another study seemed to regulate ruminoreticular motility at a central level (Brikas *et al.*, 1994). The effects of 5-HT agonists on myoelectric activity of the forestomach and antroduodenal area were investigated (Plaza *et al.*, 1996). The authors concluded that 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors were all involved in regulation of the small intestinal migrating myoelectric complex (MMC) and on forestomach hypomotility occasionally recorded concomitantly to duodenal phase III activity. In goats, however, the 5-HT_1 receptor agonist MDL-72832 increased the rumen contraction rates (van Miert & van Duin, 1998). In a subsequent study, Plaza *et al.* (1997) found evidence for the involvement of 5-HT_4 receptors in the 5-HT-induced MMC pattern. Cholinergic neural pathways mediated this induced MMC. No evidence for effects induced by 5-HT_1, 5-HT_2 or 5-HT_3 receptors was found. The effects of specific 5-HT_3 and 5-HT_4 receptor agonists and their respective antagonists on abomasal myoelectrical activity were also studied (Brikas, 1994). The findings suggest that in sheep, 5-HT_4 receptor-agonists act peripherally and are excitatory, while 5-HT_3 agonists act centrally and are inhibitory. In goats, however, the peripheral 5-HT_4 receptor agonist 5-methoxy-tryptamine decreased rumen contraction rates (van Miert & van Duin, 1998). The literature demonstrating the involvement of 5-HT receptors in abomasal motility and/or abomasal displacement in cattle is not available.

To our knowledge, *in vitro* studies on the effects of 5-HT on abomasal smooth muscle preparations of cattle have not been published. In a recent study, spontaneous physiological contractility patterns of bovine abomasal smooth muscle preparations of healthy cows *in vitro* have been described (Zulauf *et al.*, 2002). There was a very high inter-individual variability in the contractility patterns and in differences of spontaneous contractility between preparations from the abomasal antrum compared with the abomasal body. However, no significant differences among different dairy breeds were found. The effects of 5-HT and cisapride on circular smooth muscle preparations from the equine jejunum were studied (Nieto *et al.*, 2000). It was concluded that the stimulatory effects of 5-HT and cisapride were mediated through a noncholinergic pathway. The effect of 5-HT was mediated via 5-HT_2 and 5-HT_3 receptors, while the effects of cisapride were mediated by 5-HT_2 receptors only. Furthermore, 5-HT_4 receptor mediated stimulatory effects in the equine ileum and pelvic flexure have been demonstrated recently (Weiss *et al.*, 2002).

The purpose of the present study was to investigate effects of different 5-HT receptor agonists on longitudinally oriented smooth muscle preparations from the abomasal antrum of healthy cows. Hexamethonium and atropine were used in order to localize these receptors.

MATERIALS AND METHODS

Collection and preparation of tissue samples

Collection and preparation of tissue samples have been described in detail (Zulauf *et al.*, 2002). Briefly, specimens were collected from lactating dairy Simmental × Red Holstein cows routinely slaughtered at the slaughterhouse of Bern, Switzerland. Full thickness abomasal wall specimens were harvested from the pyloric antrum. Tissue samples were immediately rinsed with cooled (5 °C) modified Krebs' solution (KS). The mucosa was removed with a pair of fine Metzenbaum's scissors and discarded. Samples were rinsed again with 5 °C KS and stored in 5 °C KS, preoxygenated for 1 h with 95% O_2 and 5% CO_2, during a 15 min transportation to the laboratory. Preparations were cut parallel to the longitudinal muscle fibres. The circular fibres were not removed, and the myenteric plexus was, therefore, preserved. The remaining abomasal wall tissue was stored in cooled oxygenated KS in a refrigerator at 5 °C for possible subsequent use. Preparations were 15–20 mm in length and had a width of 2 mm. The preparations were suspended in individual organ baths containing 75 mL of KS (at 37 °C), and oxygenated with 95% O_2 and 5% CO_2. The preparations were connected to an isometric force transducer (Kraft-Weg-Aufnehmer K 30; Hugo Sachs Elektronik, March/Freiburg, Germany). The mechanical response was amplified (2 Kanal-Brücken-Verstärker Typ 301, Hugo Sachs Elektronik) and recorded on a personal computer at a sampling rate of 10 samples per sec and channel, using the data acquisition program Windaq® (Akron, OH, USA). The preparations were allowed to equilibrate in the organ bath for 1 h. Then the organ baths were flushed and 1 ***g*** of tension was applied to each preparation followed by an additional ***g*** 15 min later. After another 15 min, experiments were started according to the experimental protocols described below. If a preparation did not elicit any spontaneous activity at 15 min after the application of the second ***g*** of tension, it was replaced by a new preparation recovered from the refrigerator.

Study design

The study consisted of three independent experiments. Material from eight cows was used for experiments 1 and 2 and from 10 cows for experiment 3.

Experiments 1 and 2 were performed to demonstrate effects of 5-HT, and 5-HT_2- and 5-HT_4 receptor agonists on spontaneous contractility, studied by establishing cumulative concentration–response curves. All compounds were dissolved in distilled water, and were added to the organ baths as 0.5 mL boluses every 5 min. Each compound was stored in a stock solution of 10^{-4} M, refrigerated at 5 °C and diluted to the desired concentration on the day of the experiment. Before adding to the organ baths, solutions were allowed to equilibrate to room temperature. Each

agonist was tested at concentrations ranging from 10^{-10} to 3×10^{-6} M by cumulative addition of half-log increments at 5-min intervals. Each experiment was started with a 5 min recording of the spontaneous contractility, defined as the baseline period (BP). At the end of the experiments, the organ baths were flushed, and 10^{-6} M carbachol (carbamylcholin chloride; Sigma, Steinheim, Germany) was added to each organ bath to test the ability of the preparations to exert a contractile response after activation of cholinergic receptors. For each trial, 2 or 4 preparations from the same cow were used, and compounds were assigned to the organ baths in random order. In experiment 1, the effect of 5-HT (Sigma) and the effect of α-methyl-5-hydroxytryptamine (α-M-5-HT) (Tocris, Bristol, UK) on spontaneous contractility were tested with time and solvent (distilled water) as controls. In experiment 2, the effect of the specific 5-HT_4 receptor partial agonist RS-67506 (Tocris) was tested in the same manner with distilled water as control.

Experiment 3 was intended to characterize the possible location of the 5-HT_2 receptors. For this purpose, an effective concentration of α-M-5-HT (2×10^{-7} M) was used. In a cross-over design, baseline contractility patterns were recorded for 5 min followed by flushing the organ baths and adding α-M-5-HT, and recording was continued for another 5 min. Alternatively, the experiment was started with the 5 min recording of the α-M-5-HT-effect and recording of baseline contractility patterns after thoroughly flushing the organ bath. The preparations were preincubated with either 10^{-6} M atropine (atropine sulphate, Sigma) or 10^{-6} M hexamethonium (hexamethonium chloride, Sigma) for 30 min ($n = 10$ for each compound). α-M-5-HT was added, and the response was recorded for 5 min. Again, at the end of the experiment, the organ baths were flushed and 10^{-6} M carbachol was added to test the ability of the preparations to exert a contractile response after activation of cholinergic receptors. To obtain reproducible results, flushing of the organ baths was always (experiments 1–3) carried out in the same way: 10 s flushing followed by 1 min pause, this procedure was repeated four times.

At the end of each experiment, the weight (in mg) of each preparation was measured after removing moisture from the strips with two pieces of absorbing paper.

Data analysis

The following variables were determined separately for each concentration, each 5 min interval, and each preparation: frequency of contractions per minute (FR), resting tone (RT), maximum force (MF), and area under curve (*AUC*) of force vs. time.

For experiments 1 and 2, RT was analyzed using the software included in the playback software of Windaq®. Frequency, MF, and *AUC* were calculated, using the custom designed software (Labor V000810 ©; Oberli Engeneering GmbH, Hasle-Rüegsau, Bern, Switzerland, http://www.obeng.ch). All results were expressed as percentage of the corresponding BPs. In experiment 3, only the change in RT was calculated. All results of experiments 3 were expressed as absolute values and were corrected for the weight of the preparation.

Data analysis was carried out using statistical software (SYSTAT® 7.0 for Windows®; SPSS®, Inc., Chicago, IL, USA). Data were subjected to descriptive analysis. None of the variables under study were derived from normally distributed populations. Several transformations were investigated. However, data could not be transformed to meet assumptions of normality. Therefore, the median, 25 and 75% quantiles were calculated and non-parametric tests were used. Friedman analysis (experiment 1) and Wilcoxon signed rank test (experiment 2) were used for comparison of BPs among subjects for each trait separately. Friedman test was used to evaluate the effects of repeated application (distilled water), time, and rising concentration (agonists) on contractility within subjects. For experiment 3, Friedman analysis was used to compare among groups. If significant differences were found, Wilcoxon signed rank test was used, and the level of significance was adjusted for repeated sampling, according to Holm (1979). The level of significance was set at $P < 0.05$.

Concentration–response curves were calculated from the log concentration–effect curves using a Hill equation and estimation by the least squares method using MatLab Simulation Software (Matlab, Release 13; The MathWorks, Inc., Cambridge, MA, USA, 2002). The equation for the Hill function is:

$$\text{Response} = V_m C^\alpha (C^\alpha + K^\alpha)^{-1},$$

with V_m being the maximal attainable response, C is concentration, K is the half-effective concentration (EC_{50}, i.e. the concentration yielding half of the maximum effect), and the exponent α describes the slope of the function (Hill coefficient). Statistical significance of any comparisons made on the basis of this model (e.g. testing to see if the Hill coefficient equals 1) were made using the Wald Statistic. Confidence bounds presented for parameters in the Hill model are also based upon the Wald Statistic (Portier *et al.*, 1993).

RESULTS

Experiment 1

Representative original tracings of time control and concentration–response curve for α-M-5-HT are depicted in Figs. 1 & 2, respectively. Median values, 25 and 75% percentile, and *P*-values for RT and *AUC* are given in Tables 1 & 2, respectively. Significant differences of BPs among subjects were not found for any of the variables. Neither time nor solvent had a significant effect on spontaneous contractility. Serotonin significantly increased *AUC* ($P < 0.001$); however, the maximum effect was not reached at the highest concentration given and V_m and EC_{50} were calculated being 1.14 and 8.05×10^{-9} M, respectively (Fig. 3a, Table 3). The variables RT, MF, and FR were not significantly affected by administration of serotonin. The 5-HT_2 receptor agonist α-M-5-HT significantly increased RT

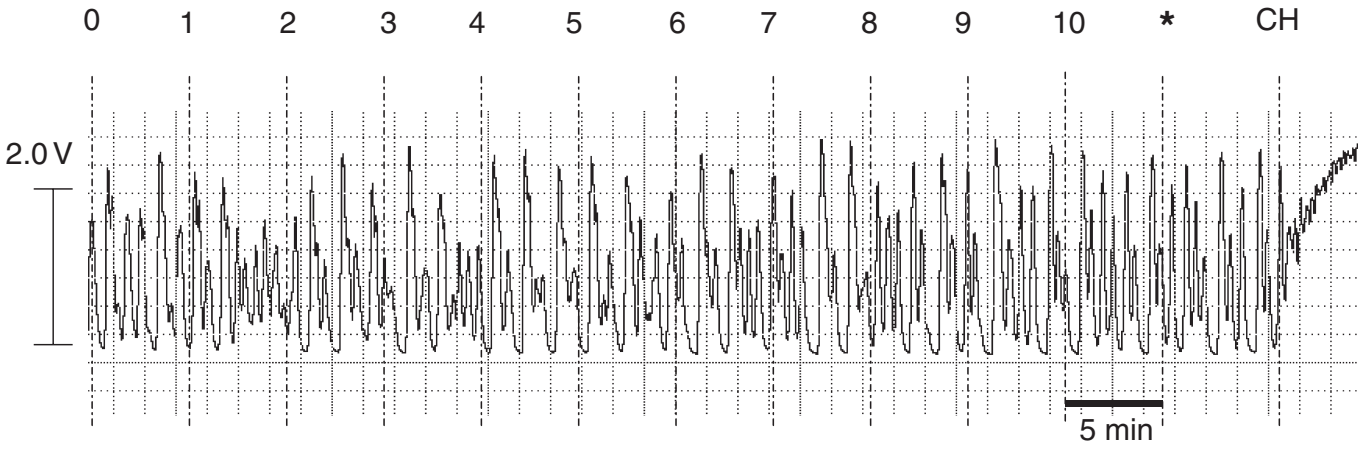

Fig. 1. Original tracing of time control of experiment 1. Vertical interrupted lines represent borders of the 5-min intervals. 0 = beginning of the baseline period; 10 = beginning of tenth 5-min interval. *, Flushing; CH, application of 10^{-7} M carbachol.

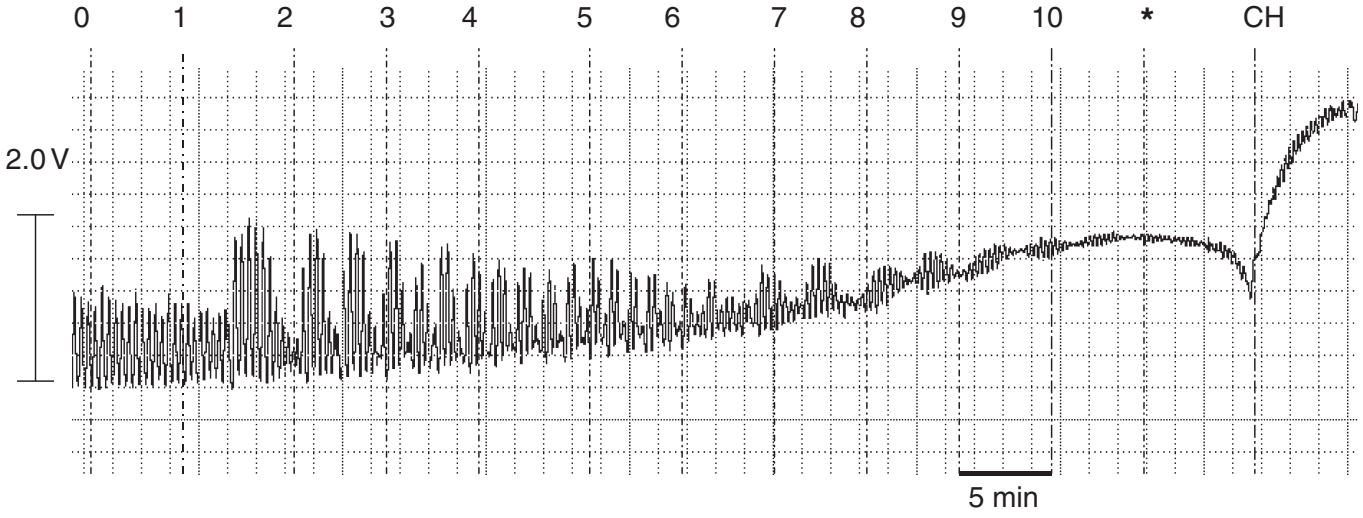

Fig. 2. Original tracing of α-M-5-HT of experiment 1. Vertical interrupted lines represent borders of the 5-min intervals. 0 = beginning of the control period; 1–10 = cumulative application of α-M-5-HT starting with 10^{-10} M (1) and rising at half-log increments to 3×10^{-6} M (10); *, flushing; CH, application of 10^{-7} M carbachol.

($P = 0.016$) and *AUC* ($P < 0.001$). V_m and EC_{50} are illustrated in Fig. 3a,b and estimates are given in Table 3. 5-HT and α-M-5-HT increased *AUC* in specimens of bovine abomasal antrum concentration-dependently with V_m being more pronounced by 5-HT (Fig. 3b). The variables MF and FR (data not shown) were not significantly affected by administration of α-M-5-HT.

Experiment 2

Median values, 25 and 75% percentiles, and *P*-values for RT are given in Table 1 and for *AUC* in Table 2. Significant differences of BPs between subjects were not found for any of the data. The solvent did not have a significant effect on spontaneous contractility. RS-67506 significantly increased RT ($P = 0.001$) and *AUC* ($P = 0.024$) and significantly decreased MF ($P = 0.019$); V_m and EC_{50} are given in Table 3 and are shown in Fig. 3a,b. The 5-HT_4 agonist resulted in a concentration-dependent increase in RT that was more potent but the maximal response was less pronounced when compared with the 5-HT_2 agonist (Fig. 3a). FR was not significantly affected by administration of RS-67506 (data not shown).

Experiment 3

Significant differences of RT were evident among the groups control, α-M-5-HT and atropine ($P = 0.013$; Fig. 4). Holm corrected follow-up tests revealed significant differences between control and α-M-5-HT ($P = 0.005$) and between α-M-5-HT and atropine ($P = 0.016$), while a difference between control and atropine was not detected ($P = 0.233$). Among the groups control, α-M-5-HT, and hexamethonium, RT also showed a significant difference ($P = 0.029$; Fig. 4). Holm corrected

Table 1. The results for resting tone (relative to BP) from experiments 1 and 2 ($N = 8$), shown as median values and interquartile range (25 and 75% quantiles)

			10^{-10}			3×10^{-10}			10^{-9}			3×10^{-9}			10^{-8}			3×10^{-8}			10^{-7}			3×10^{-7}			10^{-6}			3×10^{-6}			
Exp	Comp	0-value	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	*P*
1	Time	1.00	0.99	0.90	1.01	0.92	0.56	0.99	0.96	0.85	1.02	0.80	0.41	0.96	0.74	0.62	0.91	0.91	0.74	1.19	0.89	0.48	1.09	0.93	0.69	1.29	1.10	0.81	1.77	1.18	0.95	2.21	0.51
1	Solvent	1.00	1.25	0.95	1.29	1.27	0.92	1.70	1.19	0.96	1.65	1.25	0.91	2.19	1.23	1.01	2.26	1.43	1.05	2.94	1.61	0.89	2.47	1.66	1.15	2.72	1.38	1.09	3.17	1.59	1.06	2.93	0.14
1	5-HT	1.00	1.01	0.77	1.08	0.75	0.65	1.36	1.04	0.47	1.74	0.93	0.49	1.89	1.05	0.43	2.44	1.19	0.61	2.49	1.24	0.65	2.36	1.81	1.39	2.61	1.95	1.11	3.93	2.82	1.22	4.28	0.23
1	α-M-5-HT	1.00	1.59	1.11	2.65	1.56	1.28	3.34	1.52	1.36	3.97	1.43	1.01	5.06	1.82	1.23	7.74	2.05	1.37	8.03	2.94	1.22	10.71	3.52	2.16	14.64	4.30	2.69	15.19	4.87	3.30	26.00	0.016
2	Solvent	1.00	0.95	0.85	1.04	0.99	0.71	1.24	0.93	0.65	1.27	0.99	0.67	1.40	1.24	0.59	1.60	0.96	0.56	1.67	1.30	0.61	1.68	1.31	0.36	1.65	1.15	0.36	1.64	1.34	0.53	1.51	0.92
2	RS 67506	1.00	1.07	0.96	1.20	1.01	0.95	1.36	1.31	1.06	.165	1.47	1.18	1.64	1.62	1.48	1.67	1.73	1.48	1.91	1.97	1.60	2.15	1.70	1.62	1.82	1.96	1.46	2.03	1.85	1.51	2.07	0.001

BP, baseline period; *P*, results of Friedman analysis over time (Time), repeated application of solvent (Solvent), or rising concentration (agonists); Exp, experiment; Comp, compound; 5-HT, serotonin; α-M-5-HT, α-methyl-5-HT, 5-HT_2-agonist; RS-67506, 5-HT_4-agonist; 10^{-10}, etc., concentration of added compounds in (M).

Table 2. The results for *AUC* (relative to BP) from experiments 1 and 2 ($N = 8$), shown as median values and interquartile range (25 and 75% quantiles)

			10^{-10}			3×10^{-10}			10^{-9}			3×10^{-9}			10^{-8}			3×10^{-8}			10^{-7}			3×10^{-7}			10^{-6}			3×10^{-6}			
Exp	Comp	0-value	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	*P*
1	Time	1.00	0.95	0.94	1.01	0.97	0.93	1.01	1.05	0.94	1.35	1.02	0.94	1.15	1.00	0.91	1.28	1.00	0.95	1.21	1.03	0.92	1.41	0.99	0.94	1.20	0.99	0.89	1.26	0.99	0.90	1.16	0.66
1	Solvent	1.00	1.11	1.07	1.17	1.25	1.12	1.49	1.14	1.02	1.58	1.19	0.99	1.66	1.39	0.91	1.71	1.32	0.87	1.53	1.23	0.84	1.68	1.26	0.97	1.66	1.31	0.99	1.63	1.27	1.04	1.63	0.58
1	5-HT	1.00	1.13	1.03	1.31	1.24	1.14	1.52	1.41	1.26	1.54	1.20	1.12	1.52	1.29	1.12	1.48	1.28	1.21	2.05	1.48	1.31	2.34	1.38	1.28	2.40	1.77	1.55	2.99	1.69	1.48	3.54	<0.001
1	α-M-5-HT	1.00	1.28	1.12	1.63	1.36	1.04	1.52	1.38	1.11	1.68	1.46	1.30	1.56	1.45	1.32	1.59	1.47	1.36	1.58	1.68	1.37	1.76	1.50	1.37	1.80	1.69	1.43	2.00	1.94	1.59	2.19	<0.001
2	Solvent	1.00	1.17	1.10	1.26	1.05	1.04	1.19	1.32	1.17	1.41	1.29	1.04	1.56	1.20	1.00	1.52	1.23	1.00	1.57	1.18	0.95	1.67	1.26	0.99	1.70	1.42	0.92	1.78	1.42	0.98	2.27	0.60
2	RS 67506	1.00	0.91	0.85	1.27	0.96	0.78	1.21	1.00	0.98	1.57	1.24	1.01	1.62	1.28	0.98	1.70	1.30	0.98	2.60	1.28	1.01	2.69	1.25	0.95	3.06	1.22	0.94	3.20	1.16	0.96	3.34	0.024

BP, baseline period; *AUC*, area under curve; *P*, results of Friedman analysis over time (Time), repeated application of solvent (Solvent), or rising concentration (agonists); Exp, experiment; Comp, compound; 5-HT, serotonin; α-M-5-HT, α-methyl-5-HT, 5-HT_2-agonist; RS-67506, 5-HT_4-agonist; 10^{-10}, etc., concentration of added compounds in (M).

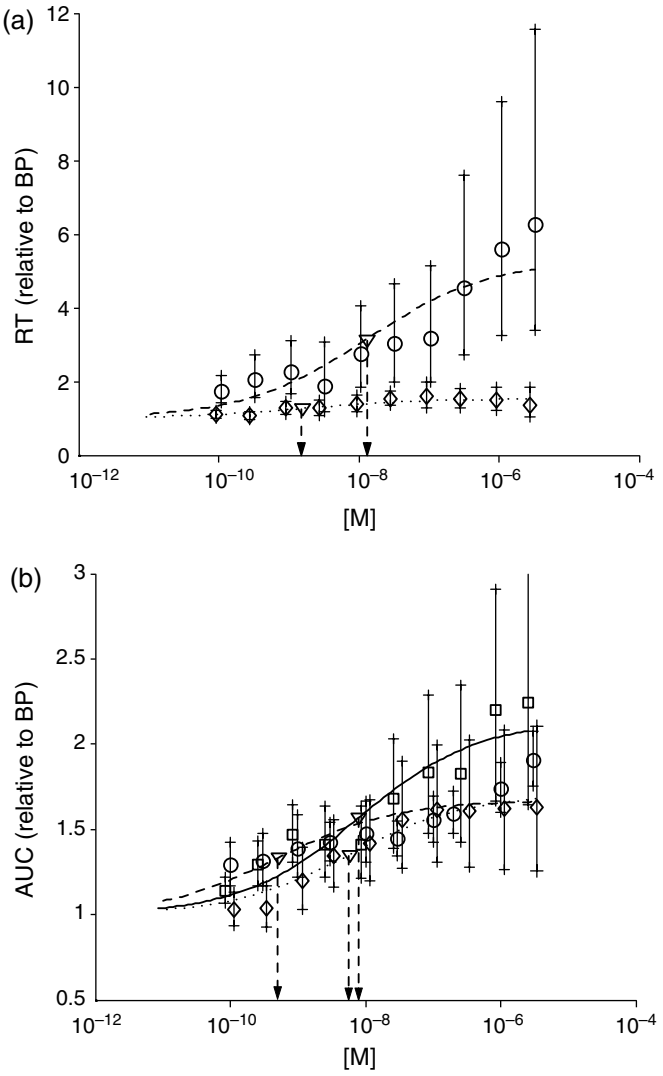

Fig. 3. Concentration–response curves of resting tone (RT) (A) and area under curve (*AUC*) (B), relative to the baseline period (BP) were estimated for serotonin (5-HT, □, solid line), α-M-5-HT (5-HT_2-agonist, ○, dashed line), and RS 67506 (5-HT_4-agonist, ◇, dotted line) in specimens from bovine abomasal antrum. EC_{50} values (marked by arrows) and maximal effect (V_m) were carried out as described in 'Materials and methods'. $n = 8$.

follow-up tests revealed significant differences between control and α-M-5-HT ($P = 0.014$). No significant difference occurred between α-M-5-HT and hexamethonium and between control and hexamethonium.

DISCUSSION

The main findings of this study were that, in spontaneously active longitudinal smooth muscle preparations from the bovine abomasal antrum, 5-HT evoked a significant increase in *AUC*, the 5-HT_2 receptor agonist α-M-5-HT and the 5-HT_4 receptor agonist RS-67506 induced a significant increase in *AUC* and RT, while MF was significantly decreased by RS-67506 only. The effect on RT obtained by α-M-5-HT was less potent when compared with RS-67506 but the maximal effect was more pronounced. The effect of α-M-5-HT was mediated by a cholinergic pathway, as it was significantly blocked by atropine but not by hexamethonium. As a specific 5-HT_2 agonist was used in this study, blocking with a 5-HT_2-antagonist was not performed.

In a recent study on physiological contractility patterns of abomasal smooth muscle specimens *in vitro*, it was demonstrated that spontaneous contractility was characterized by a high inter-individual variability (Zulauf *et al.*, 2002). Therefore, the results were expressed as percentages of the BPs rather than as absolute values. The design of this study was such as to detect a change of contractility with increasing concentrations of receptor agonists, repeated application, or time and not in inter-individual differences. Even with this technique of data analysis, the variability of results was considerable. In experiment 3 this approach could not be maintained, because repeated flushing of the organ bath disturbed RT too much to base results on a BP. Consequently, absolute values corrected for weight were used for data analysis in experiment 3. Preparations oriented parallel to the longitudinal rather than parallel to the circular smooth muscle fibres were used in this study. Analysis of spontaneous contractility patterns of preparations from the bovine abomasal antrum revealed that maximal amplitude was significantly higher in longitudinal as compared with circular layers (Zulauf *et al.*, 2002). It was judged from these results, that contractility of the longitudinal smooth muscle fibres might have a major input on motility of the bovine abomasal antrum. Preparations

Table 3. Effects of 5-HT, α-methyl-5-HT, and RS 67506 on the area under curve (*AUC*) and resting tone (RT) in specimens from bovine abomasal antrum. EC_{50}s, their lower and upper CL (95% confidence limits), were calculated by applying empirical fitting method to the concentration–response data, as described under statistical analysis in the section 'Data analysis'. *N* represents the number of experiments for each compound and V_m represents the calculated maximum effect attainable

Compound	Trait	*N*	V_m	EC_{50} (M)	Lower CL (M)	Upper CL (M)
5-HT	*AUC*	8	1.14	8.05×10^{-9}	3.41×10^{-10}	1.90×10^{-7}
α-Methyl-5-HT	*AUC*	8	0.67	5.29×10^{-10}	2.08×10^{-11}	1.34×10^{-8}
RS-67506	*AUC*	8	0.71	5.89×10^{-9}	1.69×10^{-10}	2.06×10^{-7}
α-Methyl-5-HT	Resting tone	8	4.40	1.30×10^{-8}	1.88×10^{-10}	9.04×10^{-7}
RS-67506	Resting tone	8	0.53	1.60×10^{-9}	6.35×10^{-13}	4.03×10^{-6}

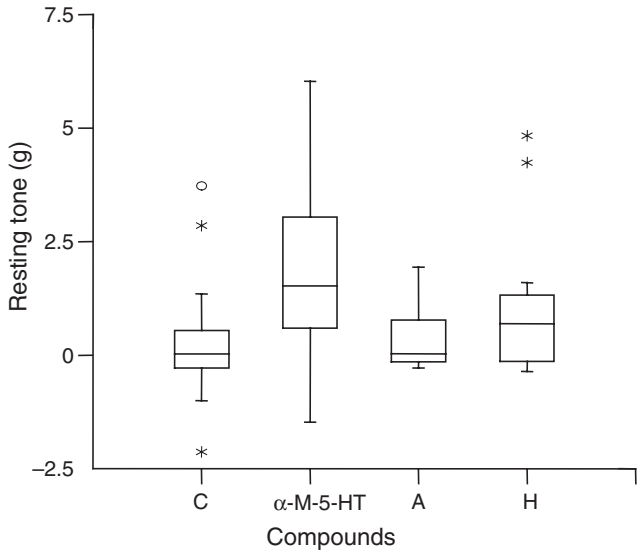

Fig. 4. Box and whiskers plot representation of change in resting tone in response to EC_{50} of α-methyl-5-HT before (α-M-5-HT) and after incubation with either atropine (A) or hexamethonium (H). Change over time acts as a control (C). The length of each box represents the range of the central 50% of the values, with the box hinges at the first and third quartiles. The line within the box shows the median value. The whiskers show the range of values within the inner fences [hinge ± 1.5 × (hinge − median)]. Values between the inner and the outer fences [hinge ± 3 × (hinge − median)] are plotted with asterisks, values outside the outer fences are plotted as empty circles. $n = 10$. BP, baseline period; C, control ($n = 20$); α-M-5-HT, 5-HT_2 agonist ($n = 20$); A, atropine sulphate ($n = 10$); H, hexamethonium ($n = 10$).

showing spontaneous contractility were used for this study, because in both excitatory and inhibitory effects of the applied receptor agonists were of interest. Spontaneous contractility was most consistently present in longitudinal preparations from the antrum. In monogastric species, specific 5-HT receptors are not distributed evenly along the GIT (Eberle-Wang *et al.*, 1994; Takada *et al.*, 1999). Thus, effects demonstrated for the bovine abomasal antrum may not be extrapolated to other parts of the bovine abomasum.

Statistical analysis revealed a significant effect of serotonin on one variable (*AUC*) of spontaneous contractility only. Serotonin receptor subtypes are characterized in other species, and either excitatory or inhibitory effects can be mediated by specific agonists. Thus, simultaneous binding to excitatory and inhibitory receptors may explain the lack of a significant effect on RT. In longitudinal smooth muscle preparations with the myenteric plexus intact from the guinea-pig ileum, the inhibitory effect of 5-HT was found to be mediated by 5-HT_1 receptors (Taniyama *et al.*, 1991), while the excitatory effect was mediated by 5-HT_2 receptors (Takemura *et al.*, 1999). α-M-5-HT is a 5-HT_2 receptor agonist (Baxter *et al.*, 1995), acting on 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, with the highest affinity for 5-HT_{2B} receptors. Serotonin and its related molecules are reported to have high affinity to 5-HT_{2B} and very low affinity to 5-HT_{2A} receptors (Peroutka, 1994). Based on the results of our study, it is not possible to define which subtype of the 5-HT_2 receptor may be involved in the contractile response. In other species, 5-HT_2 receptors have been found to mediate excitatory responses in different regions of the GIT, such as in the guinea-pig stomach (Takemura *et al.*, 1999), rat pylorus (Eberle-Wang *et al.*, 1994), rat ileum (Briejer *et al.*, 1997), dog colon (Prins *et al.*, 1997), and horse jejunum (Nieto *et al.*, 2000). In an electromyographical study on conscious sheep (Plaza *et al.*, 1996), α-M-5-HT evoked a dose dependent increase in antral activity and a dose-dependent inhibition of the myoelectrical activity of both the reticulorumen and the omasum. This is in agreement with our findings that specific activation of 5-HT_2 receptors does have an excitatory effect on spontaneous contractility of smooth muscle preparations from the bovine abomasal antrum. Experiment 3 is intended to further characterize the location of the identified 5-HT_2 receptor within the pyloric part of the bovine abomasal wall. Our findings contradict all the published literature on the location of 5-HT_2 receptors. In horse jejunum (Nieto *et al.*, 2000), in guinea-pig stomach (Takemura *et al.*, 1999), in rat pylorus (Eberle-Wang *et al.*, 1994), and in rat ileum (Briejer *et al.*, 1997), atropine- and tetrodotoxin-independent responses to α-M-5-HT were observed, suggesting that the 5-HT_2 receptor is located on the smooth muscle cell membrane. Our results, however, suggest a cholinergic pathway mediating the contractile effect of 5-HT_2 receptor activation in smooth muscle preparations from the bovine abomasal antrum.

The compound used to investigate the motor-modifying effect of 5-HT_4 receptor activation, RS-67506, is a highly specific but only partial agonist with an intrinsic activity of 0.6 compared with 5-HT (Eglen *et al.*, 1995). This compound was selected rather than a more effective but less specific one, such as a benzamide, for example, because the specificity of an agonist was considered to be vital. The effects of activation of 5-HT_4 receptors are in agreement with many *in vivo* and *in vitro* experimental studies (Taniyama *et al.*, 2000; Weiss *et al.*, 2002) and clinical studies (Briejer *et al.*, 1995) in other species. In conscious sheep, 5-HT_4 agonists were found to stimulate propulsive motility in the abomasum and induce the small intestinal MMC (Brikas, 1994; Plaza *et al.*, 1996). In monogastric animals, stimulation of 5-HT_4 receptors stimulated gastric emptying in rats (Hedge *et al.*, 1995) and dogs (Gullikson *et al.*, 1993). This effect has also been shown *in vitro* in the antrum and corpus of the guinea-pig stomach (Takada *et al.*, 1999) and in the antrum of the canine stomach (Mine *et al.*, 1997). Cisapride, a prokinetic benzamide, no longer available for clinical use, was postulated to act as a 5-HT_4 agonist and 5-HT_3 antagonist (Taniyama *et al.*, 1991). However, it has been shown to act on receptors other than 5-HT_{1-4} in dogs (De Ridder, 1993) and on 5-HT_2 receptors in the horse jejunum (Nieto *et al.*, 2000).

It is concluded from the results of this study that, in bovine abomasal smooth muscle preparations, spontaneous contractility can be modified *in vitro* under the experimental conditions used, by application of serotonin, the 5-HT_2 receptor agonist, α-M-5-HT, and the specific 5-HT_4 agonist RS 67506, suggesting the presence of contractile 5-HT_2 and 5-HT_4 receptors in this

tissue. As the increase in RT after application of α-M-5-HT was inhibited by atropine, a cholinergic mechanism is involved in the signaling pathway of α-M-5-HT. However, further experiments are required to characterize location of the 5-HT_2 receptor in abomasal antrum of cattle and to investigate the presence of other 5-HT receptors, such as 5-HT_1 receptors, in this area.

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