In vitro effects of cisapride, metoclopramide and bethanechol on smooth muscle preparations from abomasal antrum and duodenum of dairy cows

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The objective of this study was to investigate the effects of cisapride (CIS), metoclopramide (MET) and bethanechol (BET) on contractility parameters from smooth muscle preparations of the abomasal antrum and proximal duodenum of cows. Smooth muscle preparations were harvested shortly post-mortem from 42 healthy dairy cows, and concentration–response curves were performed by cumulative application of the drugs. Cisapride and MET did not have any significant effect on the contractility parameters studied, while BET induced a significant, concentration-dependent increase in basal tone (BT), mean amplitude (A_{mean}), and area under the curve (*AUC*) in smooth muscle preparations from the abomasal antrum, but not from the duodenum. The effect of BET on BT was more pronounced in specimens with longitudinal orientation while the maximal obtainable effect (V_m) in A_{mean} was more pronounced in circular-oriented preparations. Atropine (1×10^{-5} M) significantly inhibited the effect of BET, whereas pre-incubation with hexamethonium or tetrodotoxin (TTX) had no effect, suggesting that the effect was mediated by cholinergic receptors on the smooth muscle. The results may be relevant to diseases or disorders associated with gastric emptying and gastric hypomotility. Further investigations are warranted to investigate the potential ability of BET to enhance abomasal emptying of adult dairy cows.

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INTRODUCTION

Displacement of the abomasum represents a common disease in dairy cows, which has led to a major economic problem in the dairy industry in recent years (Geishauser *et al.*, 1996; Eicher *et al.*, 1999). Several risk factors, that are involved in the development of displacement of the abomasum, have been reported. They include individual factors such as breed (Constable *et al.*, 1992; Eicher *et al.*, 1999), age (Willeberg *et al.*, 1982; Constable *et al.*, 1992; Varden, 1979; Stengärde & Pehrson, 2002), yearly milk production, and periparturient factors like retained placenta and ketosis (Geishauser, 1995; Shaver, 1997; Varden, 1997; Rohrbach *et al.*, 1999; Geishauser *et al.*, 2000), metritis (Shaver, 1997; Rohrbach *et al.*, 1999), twins (Stengärde & Pehrson, 2002), and hypocalcaemia (Massey *et al.*, 1993; Shaver, 1997; Rohrbach *et al.*, 1999). Adequate feeding regimens during the periparturient period are important in the prevention of displacement of the abomasum (Geishauser, 1995; Eicher *et al.*, 1999; Stengärde & Pehrson, 2002). Abomasal atony has been described to be a prerequisite for the development of displacement of the abomasum, followed by gas accumulation in the abomasal fundus, and subsequent displacement of the abomasum (Constable *et al.*, 1992; Roussel *et al.*, 1994). However, mechanisms of motility disorders with abomasal displacement are poorly understood. Prokinetic drugs may be useful for the treatment or prevention of abomasal atony. Prokinetic compounds are defined as agents that restore, normalize, and facilitate motility of the gastrointestinal (GI) tract (McCallum, 1991). Cisapride (CIS), metoclopramide (MET) and bethanechol

(BET) have been reported to stimulate GI motility with their mechanisms being dissimilar.

Cisapride, a substituted benzamide, is a prokinetic agent that stimulates GI motility in a number of animal species (Washabau & Hall, 1995). It increases the release of acetylcholine from postganglionic nerve endings of the myenteric plexus in the gut and may also antagonize the inhibitory action of 5-hydroxytryptamine (5-HT) on the myenteric plexus (Dowling, 1995). In horses, CIS induced a concentration-dependent increase in the contractile amplitude of smooth muscle preparations of the jejunum *in vitro* (Nieto *et al.*, 2000a). *In vivo*, CIS produced an increase in myoelectric activity of the stomach and small intestine (King & Gerring, 1988), as well as of the caecal body and caecal base. In cattle, CIS (7.5×10^{-5} M) did not have any effect on longitudinal smooth muscle preparations from the proximal colon *in vitro* (Steiner *et al.*, 1992). Likewise, CIS failed to affect myoelectric activity of the ileum, caecum and proximal colon in healthy experimental cows, when given at a dose of 0.08 mg/kg, intravenously (i.v.) (Steiner *et al.*, 1995a).

Besides its anti-dopaminergic activity (Washabau & Hall, 1995), MET increases acetylcholine release from postganglionic nerve endings and enhances the sensitivity of cholinergic receptors of the GI smooth muscles to acetylcholine. Inadequate cholinergic stimulation is involved in a number of GI motility disorders (Burrows, 1983). Unfortunately, MET is able to cross the blood–brain barrier and may provoke side-effects such as involuntary muscle spasms, motor restlessness and aggression (Dowling, 1995). Its concentration-dependent increase in contractile activity has been reported for equine smooth muscle *in vitro* (Nieto *et al.*, 2000b). However, when given at high doses to healthy horses, MET provoked a weak and unspecific stimulatory motor effect in the area of the ileo-caeco-colonic junction (Ruckebusch & Roger, 1988), whereas no effect on jejunal or pelvic flexure motility was evident *in vitro* (Sojka *et al.*, 1988). Metoclopramide (1×10^{-4} M) failed to evoke any effect in preparations from the proximal colon of cattle, producing no increase in spontaneous contractile activity. In healthy cattle, MET did not exert any measurable effect on caecocolic myoelectric activity, when given at a dose of 0.15 mg/kg, intramuscularly (i.m.) (Steiner *et al.*, 1995b).

Bethanechol, an acetylcholine receptor agonist, induces contraction of smooth muscle cells by direct stimulation of muscarinic M_2 receptors (Roussel *et al.*, 1994). In healthy horses, BET hastened gastric emptying (Ringger *et al.*, 1996) and increased myoelectric activity of the ileum, caecum and right ventral colon (Lester *et al.*, 1998). *In vitro*, BET (5 or 10×10^{-5} M) caused a significant concentration-dependent increase of the contraction amplitude of smooth muscle preparations from the proximal colon (Steiner *et al.*, 1992). In healthy cows, BET has been shown to increase the myoelectric activity of the ileocaecocolic area (Steiner *et al.*, 1995b), and increased myoelectric activity of the abomasum and duodenum at a dose of 0.07 mg/kg, subcutaneously (s.c.) (Roussel *et al.*, 1994).

To our knowledge, *in vitro* studies on the effect of CIS, MET and BET on abomasal and duodenal smooth muscle preparations of cattle have not been published. Therefore, the objective of this study was to investigate possible effects of CIS, MET and BET on abomasal antrum and duodenum of healthy, adult cows *in vitro*.

MATERIALS AND METHODS

Collection and preparation of tissue samples

Lactating dairy Simmental × Red Holstein cross-bred cows were used. Specimens of the abomasal antrum were harvested within 20 min after death by stunning at the slaughterhouse. Whole mount muscle preparations without mucosa were obtained from the pyloric antrum of the abomasum and from the duodenum 10 cm aboral to the pylorus by dissecting one rectangular piece of 6 × 15 cm dimensions at each location. Tissue samples were immediately rinsed with cooled (8 °C) modified Krebs' solution (KS) containing 117 mM NaCl, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM of $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose and stored in 8 °C KS pre-oxygenated for 1 h with 95% O_2 and 5% CO_2 during transportation (15 min) from the slaughterhouse to the laboratory. The tissue was pinned flat in a dissecting dish containing oxygenated KS and preparations were cut parallel to the longitudinal muscle fibres or parallel to the circular muscle fibres with a final size of 20–25 × 2 mm. The remaining tissues were stored in oxygenated KS at 5 °C. The muscle preparations (AL, antrum longitudinal; AC, antrum circular; DL, duodenum longitudinal; and DC, duodenum circular) were suspended in eight individual organ baths (ML0186, LSi LETICA®; Panlab s.l., Barcelona, Spain) containing 50 mL KS (37 °C) each, constantly oxygenated with carbogen (95% O_2, 5% CO_2). The preparations were distally attached to a hook and connected to an isometric force transducer (MLT0201, LSi LETICA®) proximally. Preparations were allocated to the organ baths in random manner and were allowed an equilibration period of 1 h. The mechanical activity of the muscle strips was amplified (ML119; ADInstruments GmbH, Spechbach, Germany) and recorded using the data acquisition system PowerLab® (ADInstruments GmbH). The sampling rate was set at 10 samples/sec. Muscle tension was preset to 2 g in two steps during the equilibration time. Following the experiments the tissues were dried and weighed.

Construction of concentration–response curves

Preparations from eight to 10 cows were used for the experiments. Basal tone (BT), frequency, mean amplitude (A_{mean}) and area under the curve (*AUC*) were analysed after the equilibration time for a 5-min period. These values were defined as predrug (baseline period) and were used for further comparative analysis. The effects of CIS, MET and BET were studied by establishing cumulative concentration–response curves for each compound. Corresponding solvent controls were performed for each location and muscle fibre orientation (longitudinal, circular). Each compound was tested by cumulative addition of half-log increments in concentration at 5-min intervals, using 0.1 mL boluses. Cisapride was tested at concentrations ranging from 1×10^{-10} to

3×10^{-6} M and MET at concentrations from 1×10^{-9} to 1×10^{-4} M. Bethanechol was tested at concentrations ranging from 1×10^{-10} to 3×10^{-3} M. At the end of each trial, the organ baths were flushed, and 1×10^{-6} M carbachol was added to each organ bath to test the ability of the preparations to exert a contractile response.

To investigate possible identification of receptors involved in the effect of BET on muscle preparations from the abomasum in longitudinal orientation, an effective concentration of BET (3×10^{-6} M) was used. Pre-incubation with either atropine (1×10^{-5} M), hexamethonium (1×10^{-6} M), tetrodotoxin (TTX) (1×10^{-6} M) or distilled water for 20 min was performed before BET (3×10^{-6} M) was added and contractility recorded for another 10 min. Again, at the end of each trial, the organ baths were flushed and 1×10^{-6} M carbachol was added to test the ability of the preparations to exert a contractile response after activation of cholinergic receptors. For each trial, four preparations from the same animal were used and compounds were assigned to the organ baths in random order.

Drugs

Cisapride monohydrate [Janssen, Dr E. Graeub AG, Berne (distributor), Switzerland], metoclopramide monohydrochloride, bethanechol chloride (carbamyl-β-methylcholine chloride), atropine sulphate, hexamethonium chloride (Sigma, Steinheim, Germany), tetrodotoxin citrate (Tocris, Bristol, UK).

Parameters, data analysis and statistics

The following parameters were analysed to describe contractility parameters for each concentration: BT, A_{mean}, *AUC* and frequency. Basal tone of the muscle has been measured because an increase in this parameter can be independent of A_{mean} of contractions or frequency of contractions. In addition to an increase or decrease in BT, changes in frequency of contractions or A_{mean} indicate changes in contractility due to the drugs used. The variables were calculated electronically by using the software Chart™ included in the PowerLab system. All results were expressed as percentage of the corresponding predrug measurement.

Statistical analysis was performed using SYSTAT® (SPSS Inc., Chicago, IL, USA) and NCSS® (Number Cruncher Statistical Systems, Kaysville, UT, USA). Data were subjected to descriptive and comparative analyses. Data of concentration–response curves are presented as mean and standard error (SEM), and data of the blocking experiments are given as median and 25 and 75% percentiles. Wilcoxon signed rank test was used to compare predrug between solvent and drug. In case of no significant difference in predrug between specimens used for solvent and drug, further calculations were performed. Differences within the concentration–response curves were analysed by the Friedman test. If Friedman analysis revealed significant differences for compound and the corresponding control, Wilcoxon signed rank test was used to test for differences within concentrations. Friedman analysis was also used to test the effect of possible antagonists among groups. If significant differences were found, Wilcoxon signed rank test was used to test for differences in concentrations between groups, and the level of significance was adjusted for repeated sampling, according to Bonferroni. *P*-values < 0.05 indicate significant differences.

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

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

where V_m is the maximal attainable response, C the compound concentration, K the half-effective concentration (EC_{50}, i.e. the concentration yielding half of the maximum effect), and the exponent α describes the shape 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

Spontaneous activity was seen in approximately 90% of the specimens of either duodenum and abomasum after the pre-incubation period. Therefore, only specimens showing spontaneous activity were used for this study. Furthermore, experiments were considered for statistical evaluation only if carbachol evoked a contractile response in the specimen at the end of the experiment.

Basal tone

Metoclopramide and cisapride

Concurrent significant differences with rising concentrations of CIS or MET and repeated administration of the corresponding solvent controls were evident for several locations and orientations. In all locations investigated in this study, no significant differences between compound and solvent were evident for BT (data not shown). Data for CIS and MET obtained from specimens of abomasal antrum in longitudinal orientation at a concentration of 3×10^{-6} M are shown in Table 1 in comparison with BET.

Bethanechol

In longitudinal preparations of the duodenum, BET decreased the BT concentration-dependently with the effect being statistically significant ($P < 0.001$). However, within individual concentrations, significant differences between BET and corresponding solvent control were not found.

Bethanechol produced a concentration-dependent significant increase of BT in longitudinal (AL) and circular (AC) preparations of the antrum ($P < 0.001$) as illustrated in Fig. 1. Representative original tracings of the concentration–response

Table 1. Effects of cisapride, metoclopramide and bethanechol on the basal tone (BT), mean amplitude (A_{mean}), area under the curve (*AUC*) and frequency of smooth muscle preparations of bovine abomasal antrum (longitudinal orientation)

	Cisapride			Metoclopramide			Bethanechol		
Parameter	Median	25%	75%	Median	25%	75%	Median	25%	75%
BT	0.14	0.003	0.33	0.19	0.05	0.64	5.21	1.03	8.50
A_{mean}	1.93	0.94	3.12	1.28	1.02	1.35	2.05	1.20	5.93
AUC	1.84	1.17	3.14	1.23	1.01	1.34	2.04	1.20	5.92
Frequency	1.10	1.02	1.21	1.04	0.91	1.09	0.95	0.82	1.00

The effects are given at concentration of 3×10^{-6} M. Data are expressed as median values and interquartile range (25% and 75% quantiles) ($n = 8$).

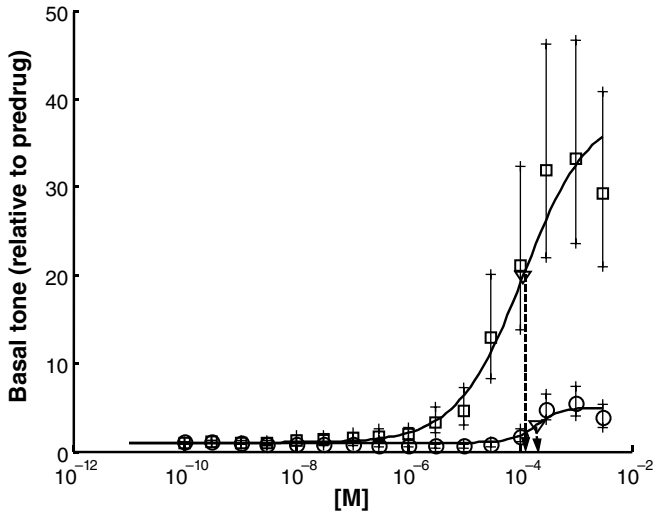

Fig. 1. Concentration–response curves of the basal tone (BT) of bethanechol expressed relative to baseline in longitudinal antrum (squares) ($n = 8$) and circular antrum (circles) ($n = 8$). Bethanechol was added at 5-min intervals at the end of the equilibration period. Arrows indicate estimated EC_{50} values. Values are mean ± SEM.

of BET are shown in Fig. 2. In circular preparations of the antrum, the effect was less pronounced when compared to AL with V_m about 10 times higher in AL compared with AC (Fig. 1 and Table 2). In addition, BT also altered significantly over repeated administration of solvent. However, at a concentration higher than 1×10^{-6} M, the increase in BT was significantly higher for BET compared with the solvent control (data not shown). Concentration–response curves including the estimated EC_{50} for both orientations of the abomasal antrum are given in Fig. 1.

Amplitude of contractions (A_{mean})

Cisapride and metoclopramide

Within duodenal preparations or specimens from antrum, CIS, MET or repeated administration of solvent produced no significant concentration-dependent effects on A_{mean}. Data for CIS and MET obtained from specimens of abomasal antrum in longitudinal orientation at a concentration of 3×10^{-6} M are shown in Table 1 in comparison with BET.

Bethanechol

A significant increase in A_{mean} was obtained in both preparations of antrum (AL, $P < 0.001$; AC, $P < 0.001$), whereas no significant effect was observed in duodenal specimens. In specimens of the antrum the effect was more pronounced on AC ($V_m = 14.63$) when compared with AL ($V_m = 3.27$) as shown in Fig. 3. EC_{50} values are given in Table 2. V_m was obtained at a concentration of 3×10^{-4} M and a concentration-dependent decrease occurred at higher concentrations in specimens of abomasal antrum in circular orientation. In specimens of longitudinally oriented antrum, V_m was obtained at a concentration of 3×10^{-4} M, and a reduction in V_m was obtained at higher concentrations (Fig. 3). In longitudinally oriented preparations from the antrum, repeated application of solvent resulted in a significant increase in A_{mean} ($P = 0.006$). However, for concentrations higher than 1×10^{-6} M the increase in A_{mean} was significantly higher for BET compared with the control.

Area under the curve

Cisapride and metoclopramide

In all locations of the bovine GI tract investigated in this study, CIS or MET did not alter *AUC* significantly in the concentrations tested. Data for CIS and MET obtained from specimens of longitudinal abomasal antrum at a concentration of 3×10^{-6} M are shown in Table 1 in comparison with BET.

Bethanechol

In preparations of antrum (AC and AL), a significant and concentration-dependent increase in *AUC* ($P < 0.001$) was obtained, while no effect was obtained in duodenal preparations (data not shown). The maximal effect was more pronounced in circular preparations compared with longitudinal preparations of the antrum with the maximum obtainable effect being 4.5 times higher in specimens from AC (Table 2). Despite the effect of BET on AL being significant ($P = 0.001$), a significant increase in *AUC* was also seen in the solvent control ($P = 0.007$).

Frequency of contractions

Frequency was not significantly altered by administration of CIS, MET and BET for any of the locations, orientations or parameters investigated. Data for CIS, MET and BET obtained from specimens

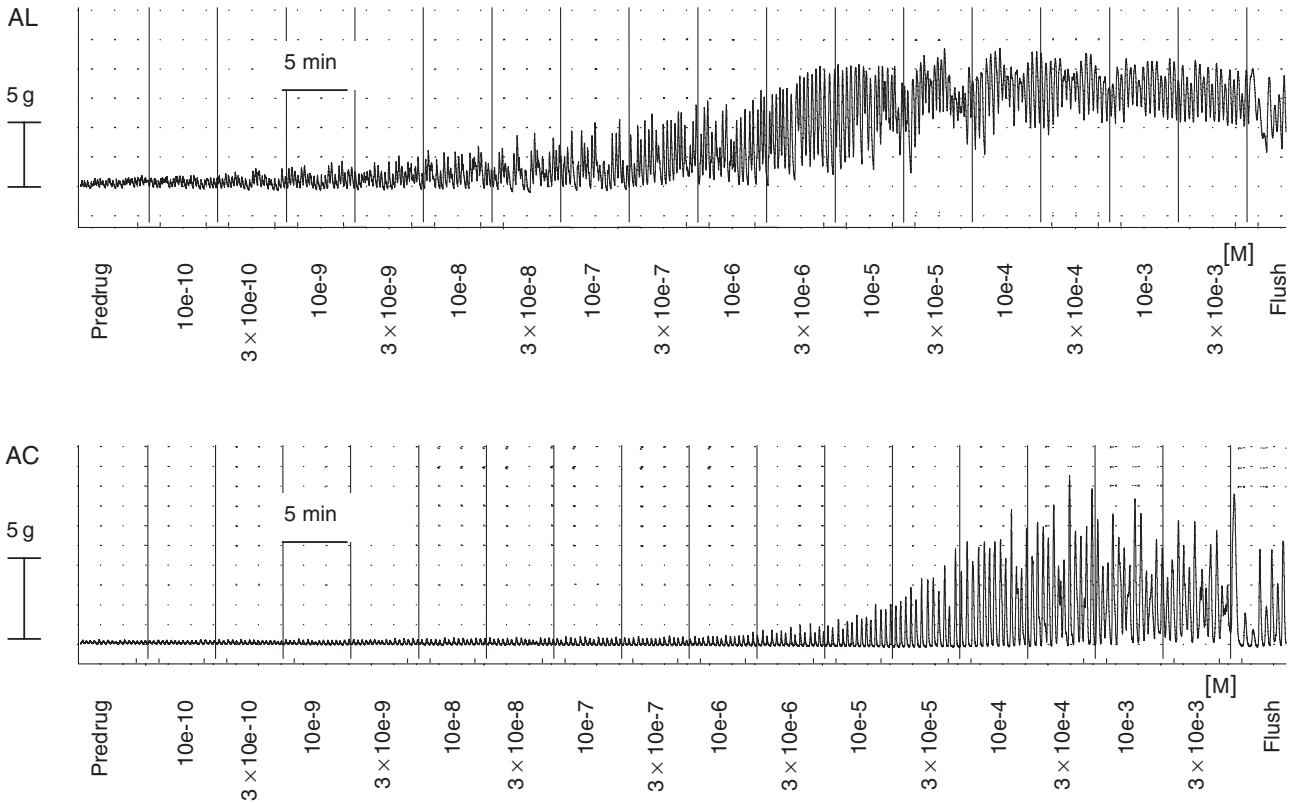

Fig. 2. Tracings of the concentration–response curves of bethanechol in specimens from longitudinal antrum (AL) and circular antrum (AC). Vertical lines represent borders of the 5-min intervals. Flush = flushing; 1 g ≡ 250 μV.

Table 2. Effect of bethanechol on the basal tone (BT), mean amplitude (A_{mean}), and area under the curve (*AUC*) of smooth muscle preparations from the bovine abomasal antrum. EC_{50}, their lower and upper CL (95% confidence limits), were calculated by applying empirical fitting method described under statistics in 'Material and methods' to the concentration–response data

Location	Compound	Parameter	*n*	V_m	EC_{50} (M)	Lower CL (M)	Upper CL (M)
AL	Bethanechol	BT	8	37.94	1.15×10^{-4}	1.45×10^{-5}	9.07×10^{-4}
AC	Bethanechol	BT	8	3.99	2.02×10^{-4}	6.66×10^{-5}	7.00×10^{-4}
AL	Bethanechol	A_{mean}	8	3.27	3.86×10^{-6}	8.00×10^{-9}	1.86×10^{-3}
AC	Bethanechol	A_{mean}	8	14.63	1.94×10^{-5}	8.43×10^{-6}	4.46×10^{-5}
AL	Bethanechol	*AUC*	8	3.27	3.85×10^{-6}	8.08×10^{-9}	1.83×10^{-3}
AC	Bethanechol	*AUC*	8	14.64	1.94×10^{-5}	8.42×10^{-6}	4.46×10^{-5}

AL, antrum longitudinal; AC, antrum circular; *n*, number of trials; V_m, calculated maximum effect attainable (estimates are given relative to predrug).

of longitudinal abomasal antrum at a concentration of 3×10^{-6} M are shown in Table 1.

Atropine, hexamethonium and tetrodotoxin

Significant differences were evident among the groups atropine, hexamethonium, TTX and control for all parameters tested (BT, $P = 0.005$; A_{mean}, $P = 0.005$; *AUC*, $P = 0.006$; frequency, $P = 0.02$) (Table 3). Bonferroni corrected follow-up tests revealed significant differences in the effect of BET (3×10^{-6} M) after pre-incubation with atropine (1×10^{-5} M) when compared with solvent pre-incubation for all parameters tested (BT, $P = 0.005$; A_{mean}, $P = 0.005$; *AUC*, $P = 0.005$; frequency, $P = 0.005$). Pre-incubation of hexamethonium (1×10^{-6} M) or TTX (1×10^{-6} M) revealed no significant alteration to the effect of BET (3×10^{-6} M) for all parameters investigated (Table 3).

DISCUSSION

The main finding of this study was that BET evoked a significant, concentration-dependent increase of BT, A_{mean} and *AUC* in longitudinal and circular smooth muscle preparations from the abomasal antrum of adult dairy cows *in vitro*. This effect was more pronounced in preparations with longitudinal orientation and was mediated by binding to muscarinic receptors situated on

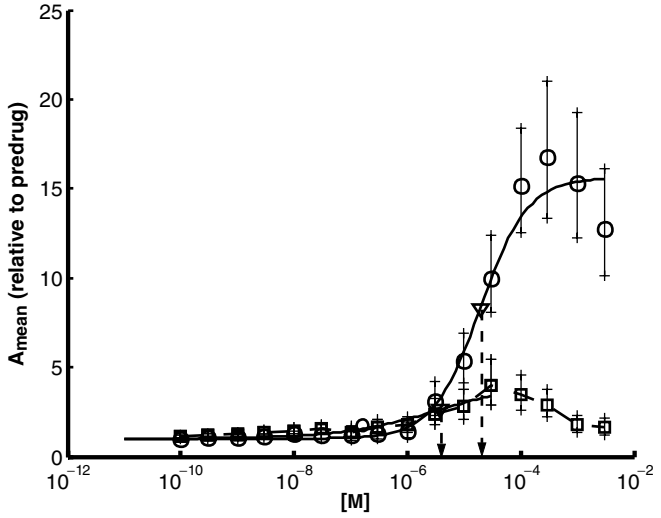

Fig. 3. Concentration–response curves of the mean amplitude (A_{mean}) of bethanechol expressed relative to the predrug in longitudinal antrum (squares) ($n = 8$) and circular antrum (circles) ($n = 8$). Bethanechol was added at 5-min intervals at the end of the equilibration period. Arrows indicate estimated EC_{50} values. Values are mean ± SEM.

the smooth muscle cell, as atropine significantly inhibited the effect. Neither hexamethonium, nor TTX was effective in inhibiting the effect of BET indicating that the response was not nerve mediated. Both CIS and MET did not reveal any significant effect on contractility of smooth muscle preparations from the bovine abomasal antrum and duodenum.

The majority of the preparations from the abomasal antrum exhibited spontaneous contractility, which is in accordance with the results of the study on physiological contractility patterns of the abomasal antrum (Zulauf *et al.*, 2002). Spontaneous motility was demonstrated in approximately 75% of the specimens from abomasal antrum *in vitro* and a wide variability of the spontaneous activity patterns of the preparations was found (Zulauf *et al.*, 2002). The present experiments revealed that neither CIS nor MET evoked a significant effect on smooth muscle preparations from duodenum and abomasal antrum of dairy cows under the experimental conditions used in this study. Similar results were found, when the effect of these compounds was investigated on smooth muscle preparations from the bovine proximal colon *in vitro* (Steiner *et al.*, 1992) and on myoelectric activity of this area *in vivo* (MET: 0.15 mg/kg, i.m.; CIS: 0.08 mg/kg, i.v.) (Steiner *et al.*, 1995b). Furthermore, MET (0.1 mg/kg, s.c.) did not alter myoelectric activity of the antroduodenal area in experimental yearling cattle *in vivo* (Roussel *et al.*, 1994). Comparing the *in vitro* results obtained from specimens from cattle with those from horses, dissimilarities between species are obvious: in the equine jejunum, MET increased the contractile activity of the circular layers (Nieto *et al.*, 2000b) and CIS induced a concentration-dependent increase in the amplitude of contractions (Nieto *et al.*, 2000a).

In this study, BET evoked significant and concentration-dependent increases in BT, A_{mean} and *AUC* in specimens of abomasal antrum, while there was no effect on any parameter in duodenal preparations. The effects were dependent on location and fibre orientation of the preparations. In longitudinally orientated smooth muscle preparations of abomasal antrum, the increase in BT was more pronounced, compared with circularly oriented specimens, while the maximal effect in the latter was more pronounced considering the increase in A_{mean}. The potency of BT was shown to be lower in longitudinal preparations for BT when compared to preparations with circular orientation while the contrary was obtained for A_{mean} and *AUC*. An effect on frequency was not found in any of the preparations of the antroduodenal area. Geishauser *et al.* (1998) reported no effect in frequency and amplitude of phasic contractions in preparations from displaced abomasum when compared with controls. Therefore, the BT might be a parameter of interest with respect to displacement of the abomasum and its possible treatment. Bethanechol (5 and 10×10^{-5} M) evoked a consistent contractile effect on smooth muscle preparations from the proximal colon *in vitro* (Steiner *et al.*, 1992). *In vivo*, BET (0.07 mg/kg, s.c.) significantly increased the number of caecocolic spikes and the number of propagated spike sequences in healthy animals (Steiner *et al.*, 1995b). In recent *in vivo* studies with healthy horses, BET increased ileocaecocolic myoelectric activity and hastened gastric emptying significantly (Ringger *et al.*, 1996; Lester *et al.*, 1998). Similar effects of BET on GI motility have been reported in other species (Schuurkes & Van Nueten, 1984; Summers & Flatt, 1988; Wood & Cheung, 1991). In contrast to our findings, BET (0.07 mg/kg, s.c.) increased myoelectric activity in the duodenum, but not in the antrum in an *in vivo* study with yearling cattle (Roussel *et al.*, 1994). When BET (0.07 mg/kg, s.c.) was administered in combination with MET

Table 3. Basal tone, mean amplitude, area under the curve and frequency of smooth muscle preparations from bovine abomasal antrum (longitudinal orientation) after preincubation with atropine (1×10^{-5} M), hexamethonium (1×10^{-6} M), TTX (1×10^{-5} M), or solvent

	Atropine			Hexamethonium			TTX			Solvent			
Parameter	Median	25%	75%	Median	25%	75%	Median	25%	75%	Median	25%	75%	*P*
BT	0.35*	0.09	0.49	0.65	0.34	1.15	0.94	0.56	1.19	1.16	0.87	1.43	0.005
A_{mean}	0.61*	0.46	0.74	0.97	0.78	1.34	0.9	0.77	1.07	1.10	0.9	1.6	0.005
AUC	0.60*	0.46	0.74	0.98	0.78	1.34	0.9	0.76	1.17	1.13	0.9	1.6	0.006
Frequency	0.91*	0.72	1.0	1.05	0.88	1.3	1.04	0.81	1.14	1.05	1.03	1.13	0.02

Data are expressed as median values and interquartile range (25 and 75% quantiles) ($n = 10$). *P*, results of Friedman analysis, group comparison; TTX, tetrodotoxin; BT, basal tone; A_{mean}, mean amplitude; *AUC*, area under the curve.
*Significantly different from the corresponding solvent control.

(0.1 mg/kg, s.c.), the myoelectric activity was increased in both duodenum and antrum. Two possible reasons may explain this difference. On the one hand, the number of receptors may be age-dependent with muscarinic receptors being less frequently expressed in yearlings than in adult cows. On the other hand, it may be hypothesized that more complex mechanisms than binding of BET to muscarinic receptors situated on smooth muscle cell membranes are involved in the increased antegrade propagated spike activity of the duodenum *in vivo*.

The finding that the effect of BET was mediated by muscarinic receptors situated on the smooth muscle cells was expected, as BET is well known as a direct muscarinic agonist, that has been reported to bind primarily to muscarinic M_2 receptors (Kilbinger & Weihrauch, 1982; Megens *et al.*, 1991). The non-selective muscarinic antagonist atropine inhibited the effect of BET in specimens of abomasal antrum. Despite the non-selective action of atropine, M_2 receptor blockade may explain the inhibition of the BET-induced effect but an additional effect on receptors other than muscarinic cannot be excluded because complete inhibition was not obtained. Muscarinic receptors in intestinal smooth muscle mediate multiple responses including adenylate cyclase inhibition (M_2), activation of a non-selective cationic current (M_2), and calcium store release and contraction (M_3). Differences in potency of muscarinic agonists in longitudinal smooth muscle of guinea-pig intestine have been reported from an *in vitro* study by Okamoto *et al.* (2002). The concentration required to obtain the maximal effect (V_m) was 33 times higher for BET compared with carbachol for activation of non-selective cationic current. Antagonists of M_1, M_2 and M_3 have been reported to inhibit carbachol-induced contractions in human isolated colon (Kerr *et al.*, 1995). Zholos and Bolton (1997) reported that M_2 receptor antagonists produced a concentration-dependent parallel shift of the carbachol concentration–effect curve in guinea-pig ileal smooth muscle, and all M_3 selective antagonists reduced the maximal response of the carbachol curve. Furthermore, only a weak effect in calcium-mobilizing activity was obtained with BET, supporting a link between M_3 receptor activation, calcium store release and contraction (Okamoto *et al.*, 2002). This may also explain why carbachol evokes a contractile response when used at the end of the experiment while BET showed no effect on duodenum. In addition, the ratio of M_2 and M_3 receptors in abomasal antrum may be different from duodenum. However, further evaluation of this hypothesis requires additional investigation.

Geishauser *et al.* (1998) reported a reduced sensitivity to acetylcholine in specimens from displaced abomasa *in vitro*. In addition, mechanisms other than cholinergic seem to be involved in displacement of the abomasum. Nitrergic inhibitory mechanisms predominate over excitatory mechanisms (Geishauser *et al.*, 1998).

We conclude from the results of this study that, in bovine abomasal smooth muscle preparations, BET increased the spontaneous contractility in a concentration-dependent manner *in vitro* under the experimental conditions used in this study. Furthermore, significant regional differences of the response to BET were evident in the antroduodenal area. The latter finding, that BET increased most parameters of contractility of the abomasal antrum but not of the duodenum of dairy cows, provides useful information on the potential benefits of this compound for induction and support of antroduodenal motility in adult dairy cows. However, it is important to be aware that possibly other mechanisms are involved in the development of abomasal displacement. Conclusions as to the effect of BET in healthy adult cows and animals suffering from a disturbance of abomasal emptying *in vivo* remain hypothetical and warrant further investigation.

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