Turk. J. Vet. Anim. Sci.
30 (2006) 347-352
© TÜBİTAK

Research Article

The Importance of Refractoriness in Ovine Antral Digestive and Interdigestive Motility

Krzysztof Waldemar ROMAŃSKI*
Department of Animal Physiology, University of Agriculture, Wrocław - POLAND

Received: 17.05.2004

Abstract: The aim of this work was to find possible differences between the effects of anticholinergic drug administration during the duodenal phase 1 or phase 2a of migrating myoelectric complex on antral spike burst amplitude in fasted and non-fasted sheep. Eight adult rams possessing bipolar electrodes in the antrum and small intestine were used in the study. Feeding increased significantly the spike burst amplitude range from 60-110 to 80-160 µV. The effects of hexamethonium (2 mg/kg), atropine (0.1 mg/kg) and pirenzepine (0.5 mg/kg) given i.v. during phase 1 of the migrating myoelectric complex (expressed as per cent of control) in non-fasted sheep were 68 ± 18, $P < 0.05$, 69 ± 20, $P < 0.05$, 45 ± 18, $P < 0.01$, respectively. The effects of the same doses of these drugs given during phase 2a of the migrating myoelectric complex in non-fasted sheep were 54 ± 13, $P < 0.01$, 41 ± 12, $P < 0.0001$, 33 ± 11, $P < 0.0001$, respectively. In non-fasted animals, the anticholinergic drugs exerted a more pronounced effect than in fasted animals. These effects were partially dose-dependent. The effects of drug combinations were not additive. It is concluded that the effects of anticholinergic substances given during phase 1 or 2a of migrating myoelectric complex and under various feeding conditions on ovine antral spike burst amplitude can vary, partially due to the intensity of the refractory period.

Key Words: Sheep, abomasal antrum, myoelectric activity, feeding, anticholinergic drugs

Introduction

The gastric antrum is the most active part of the stomach and its motility is closely related to the small intestine, i.e. also to the migrating myoelectric complex (MMC), the principal motility pattern in this area (1). In ruminants, the abomasal antrum is also the most active part of the stomach and its motility is correlated with duodenal function, at least in part, but phase 3 MMC seems to be absent in this region (2). There is increasing evidence that the modulatory influences of controlling mechanisms may vary during different phases of the MMC cycle, also in sheep (3,4). Thus, antral motility should be studied along with the recording of duodenal motility to be aware of the duodenal MMC phase. The abomasal motility undergoes cholinergic control in sheep (5) although the effect of some cholinergic antagonists is controversial. The results obtained by Ruckebusch et al. (6) suggested that hexamethonium can inhibit antral motility completely while other studies did not confirm this finding (7). It was recently shown that the responses to food and anticholinergic drugs given during phases 1-2a MMC can be different from those given during phase 2b MMC (7). Thus, it was decided to assess whether any difference could be detected in the antral myoelectric response to various feeding conditions and to anticholinergic drugs given during phase 1 in comparison to phase 2a MMC in conscious adult sheep.

Materials and Methods

Eight rams of the Polish Merino breed weighing 38-45 kg each were used. They were fasted for 24 h and underwent surgery to implant bipolar platinum electrodes into the antrum and small intestine for MMC recognition. The procedure was similar to that described elsewhere (7). In brief, lateral laparotomy was performed under general and local anesthesia for electrode implantation. Marked electrode wires were exteriorized, soldered to a plug and connected with the recorder. At least 7 days were allowed for recovery. During this period feeding gradually returned to normal, preoperative rates. Drinking water was not limited except during the

*E-mail: romanski@ozi.ar.wroc.pl

experiments. A total of 624 experiments were performed, including 312 control experiments (performed on 48 h fasted or, separately, on non-fasted animals) during which the animals were not fed, and 312 experiments with feeding or drug administration during phase 1 MMC. In separate experiments, food or drugs were given during phase 2a MMC (in 48 h fasted or non-fasted animals). During these experiments, 250 g of the grain mixture was given in the course of the feeding procedure. 0.15 M NaCl at a rate of 2.0 ml/min was injected during control experiments. Anticholinergic drugs were administered into the jugular vein through the indwelling thin polyethylene catheter over 1-5 min: atropine sulfate (At, Polfa) 0.002, 0.02 or 0.1 mg/kg of body weight, pirenzepine dihydrochloride (Pi, Sigma) 0.02, 0.1 or 0.5 mg/kg bw, hexamethonium bromide (Hx, Sigma) 2.0 (moderate dose) or 5.0 mg/kg bw (high dose). The drug combinations used were as follows: At 0.1 + Hx 1.0, At 0.1 + Hx 2.0, Pi 0.1 + Hx 1.0 and Pi 0.1 + Hx 2.0 mg/kg bw. The gastrointestinal myoelectric activity was recorded continuously throughout the experiments, lasting 2-3 h each. The multichannel electroencephalograph (Reega Duplex TR XVI, Alvar Electronic, Montreuil, France) with a time constant 0.01 s and a speed of paper 2.5 mm/s was used for electromyography. During control experiments at least 1 full normal MMC cycle or 2 consecutive phases 3 MMC were recorded. The experiments were randomized as far as the drug administration and the dose applied were concerned. The anticholinergics were given only once daily and there was at least a 2 day interval between 2 consecutive experiments where the drugs were used (and at least 3 days after the highest dose of Hx). The recordings were visually analyzed and the MMC phases were identified in the small intestine according to the criteria proposed by Code and Marlett (8) with the cleavage of duodenal phase 2 MMC into phase 2a and 2b (9,10) for more precise saline or drug administration. The mean spike burst amplitude was measured for every individual spike burst during the considered periods (2 min – control period and 1 min period during feeding or following drug administration) on the recordings of individual experiments and expressed in μV. Then the median amplitudes of the measured average amplitudes of all spike bursts within these periods were calculated. Thus, one reference median (maximal) value for the control experiment and one median value for an experiment with feeding or drug administration were obtained. Finally, the mean values of the aforementioned medians of spike burst amplitudes were calculated and presented as the percentages of the maximal (during phase 2b MMC) spike burst median amplitudes in the given control group, treated as 100%. Other details concerning the calculation of data were similar to those in the previous report (7) and included the calculation of percentage of slow waves with spikes and duration of spike bursts. The results underwent statistical analysis and were expressed in means ± S.D. Student's t-test for paired values was used (11). The minimal probability (P) level at which compared data were considered significant was 5%.

Results

The means of reference values used for subsequent calculations are listed in Table 1.

In fasted sheep, administration of food during phase 1 or 2a MMC increased antral spike burst amplitude significantly (Figure, upper panel, Tables 2 and 3). Values before and after feeding obtained in this group during phase 1 and compared with phase 2a MMC were significantly different ($P < 0.001$ and $P < 0.01$, respectively). In non-fasted animals, these results obtained during phase 2a MMC were similar, whereas when food was given during phase 1 MMC the results were inconsistent and not significant while the increasing tendency was observed (Table 3).

Injection of Hx at moderate dose decreased antral spike burst amplitude significantly only in non-fasted sheep (Table 2). In fasted animals, however, clear decreasing tendency was observed (Table 3). The effect of high dose of Hx administered during phase 1 or 2a MMC in non-fasted sheep on antral spike burst amplitude, expressed as percentage of maximal control values indicated in Table 1, was also significant (Table 2) as was the effect of high dose of Hx in fasted animals (Table 3). Administration of the lowest dose of At (0.002 mg/kg) during phase 1 or 2a MMC exerted insignificant effects in non-fasted sheep (Table 2). Both higher doses of At evoked a significant response in non-fasted sheep, while in fasted animals a significant effect was observed only when the drug was injected during phase 2a MMC (Figure, lower panel, Tables 2 and 3). In non-fasted animals the highest dose of At (0.1 mg/kg) evoked significantly stronger response during phase 2b than

Table 1. The original mean values (in μV) of maximal average of ovine antral spike burst amplitudes measured during the duodenal phase 2b MMC of the control period of the consecutive experimental groups served as reference values for percentage calculations.

	Food	Hx 2.0	Hx 5.0	At 0.002	At 0.02	At 0.1	Pi 0.02	Pi 0.1	Pi 0.5	Hx 1.0 +At 0.1	Hx 2.0 +At 0.1	Hx 1.0 +Pi 0.1	Hx 2.0 +Pi 0.1
FASTED:													
- phase 1	84 ±17	65 ±31	84 ±25	78 ±18	69 ±23	62 ±18	58 ±27	86 ±21	93 ±28	71 ±31	84 ±26	59 ±27	72 ±21
- phase 2a	79 ±14	74 ±13	96 ±17	84 ±19	75 ±17	76 ±14	89 ±18	103 ±27	90 ±13	84 ±16	86 ±21	68 ±28	83 ±19
NOT FASTED:													
- phase 1	93 ±15	81 ±28	75 ±18	81 ±18	73 ±19	69 ±24	73 ±32	68 ±19	84 ±24	108 ±41	66 ±23	89 ±22	67 ±31
- phase 2a	88 ±10	88 ±16	87 ±11	94 ±23	77 ±17	87 ±19	84 ±16	87 ±18	98 ±18	79 ±25	104 ±38	80 ±24	101 ±33

Values in means ± S.D., n = 6. Abbreviations: Hx – hexamethonium bromide, At – atropine sulfate, Pi – pirenzepine dihydrochloride, doses in mg/kg. Other explanations are in the Materials and Methods.

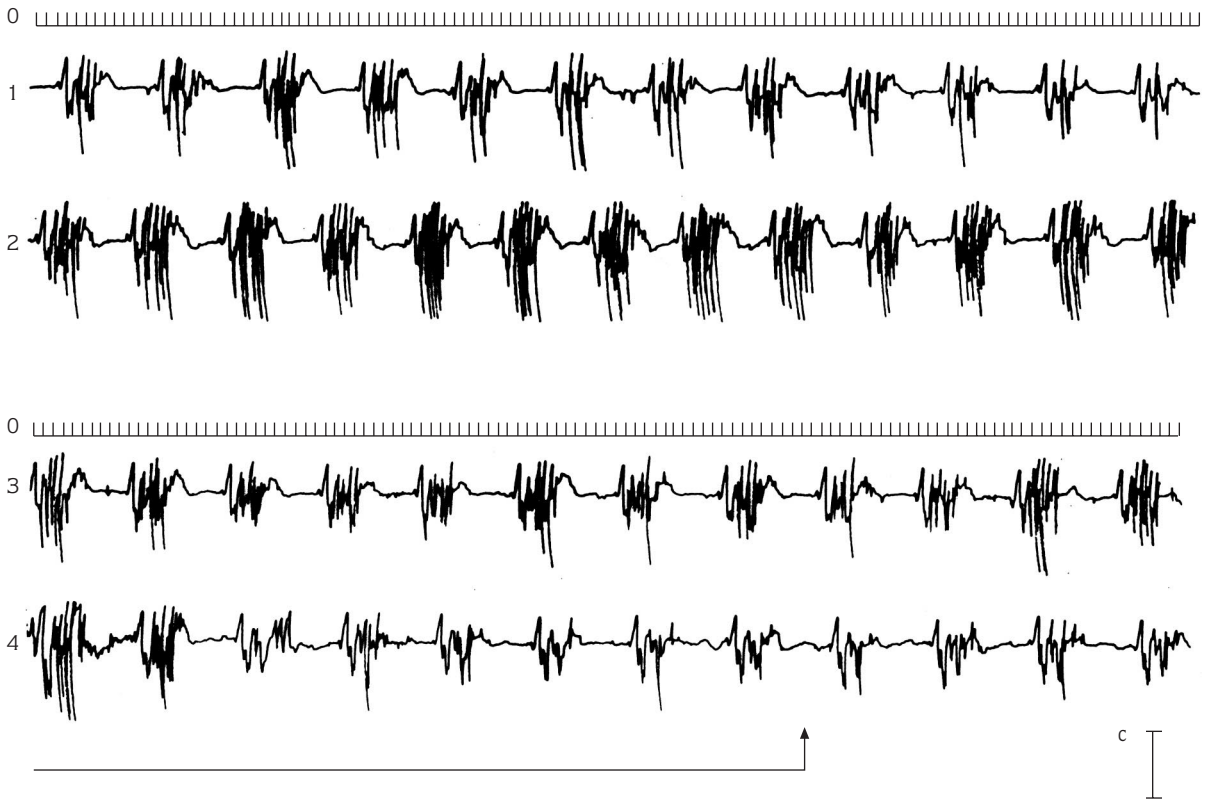

Figure. Antral myoelectric activity in sheep. 0 – time in seconds. Upper panel: 1 – control recording during duodenal phase 2a, 15 min after end of duodenal phase 3 MMC, 2 – antral myoelectrical recording 2 min after the feeding onset; feeding was started during phase 2a of the same MMC cycle in non-fasted sheep. Lower panel: 3 – control recording during phase 2a MMC, 20 min after end of duodenal phase 3 MMC, 4 – termination of slow At injection at the dose 0.1 mg/kg during the same phase 2a MMC in fasted sheep indicated with arrow, c – calibration 100 μV.

Table 2. Spike burst amplitudes before (control) and after feeding or anticholinergic drug administration (treatment) in non-fasted sheep.

		Food	Hx 2.0	Hx 5.0	At 0.002	At 0.02	At 0.1	Pi 0.02	Pi 0.1	Pi 0.5	Hx 1.0+ At 0.1	Hx 2.0 + At 0.1	Hx 1.0+ Pi 0.1	Hx 2.0 + Pi 0.1
Phase 1 MMC	Ct mean ±S.D.	55.7 ±12.9	62.9 ±10.7	53.5 ±6.2	46.3 ±12.1	63.0§ ±12.2	64.0$^{\#}$ ±9.1	52.2 ±11.8	47.2 ±11.6	60.0§ ±15.9	49.3 ±7.9	60.0§ ±8.6	45.3 ±9.0	59.5§ ±10.1
	Tr mean ±S.D.	79.9^{a} ±13.4	41.9^{a} ±10.2	39.4^{a} ±9.2	45.3 ±12.0	33.2^{b} ±8.2	43.7^{a} ±12.7	35.9^{a} ±8.6	22.1^{b,§} ±8.8	26.3^{b} ±11.1	32.0^{a} ±8.5	38.8^{b} ±7.6	31.5 ±9.6	34.2^{b} ±8.2
Phase 2a MMC	Ct mean	57.3$^{\#}$ ±9.4	61.5 ±11.7	69.1 ±19.8	57.6 ±10.3	67.7§ ±10.4	60.1 ±12.6	53.5 ±13.2	66.4^{x,§} ±12.1	62.4§ ±10.4	55.9 ±6.1	56.9 ±10.7	61.3 ±9.8	64.1 ±11.6
	Tr mean ±S.D.	86.6$^{d,\#}$ ±6.9	34.2^{b} ±12.8	35.8^{a} ±16.1	49.2 ±7.2	26.9^{d} ±7.1	23.4^{d,x} ±4.1	31.4^{b} ±7.2	29.5^{c} ±6.4	20.3^{d} ±6.6	37.7^{a} ±9.6	31.4^{c} ±7.0	38.9^{b} ±8.6	36.3^{c} ±6.4

Ct – control, Tr – treatment. At – atropine sulfate, Hx – hexamethonium bromide, Pi – pirenzepine dihydrochloride. Doses in mg/kg, n = 6. Statistical significance: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$ vs. relevant value obtained during Ct period. $^{x}P < 0.05$; $^{y}P < 0.01$; $^{z}P < 0.001$ vs. relevant value obtained during phase 1 MMC. $^{\S}P < 0.05$; $^{\#}P < 0.01$ vs. relevant value obtained in fasted animals. Other explanations as in the Materials and Methods.

Table 3. Spike bursts amplitudes before (control) and after feeding or anticholinergic drug administration (treatment) in fasted sheep.

		Food	Hx 2.0	Hx 5.0	At 0.02	At 0.1	Pi 0.02	Pi 0.1	Pi 0.5	Hx 2.0 + At 0.1	Hx 2.0 + Pi 0.1
Phase 1 MMC	Ct mean ±S.D.	41.3 ±6.8	50.8 ±7.4	55.1 ±7.3	45.2 ±7.4	40.5 ±7.1	42.1 ±5.0	46.6 ±5.4	37.2 ±6.5	47.4 ±7.9	42.2 ±6.3
	Tr mean ±S.D.	80.2^{d} ±9.6	38.8 ±10.3	40.3^{a} ±8.6	30.3 ±12.7	39.0 ±9.9	34.9 ±7.5	39.9 ±7.0	24.0^{a} ±7.1	32.1^{a} ±7.9	30.5 ±10.7
Phase 2a MMC	Ct mean ±S.D.	77.6^{z} ±4.3	52.0 ±7.3	62.4 ±13.3	52.8 ±4.0	53.8^{x} ±5.8	49.4 ±7.2	47.8 ±8.0	42.2 ±7.7	49.3 ±8.2	50.1 ±6.3
	Tr mean ±S.D.	104.3^{d,y} ±6.3	38.2 ±12.8	34.1^{a} ±12.9	37.0^{a} ±12.6	24.6^{c} ±13.1	32.1^{a} ±7.4	29.9^{a} ±7.3	20.9^{b} ±5.2	27.4^{b} ±6.5	30.7^{b} ±6.0

Explanations as in Table 2

during phase 1 MMC ($P < 0.05$). Administration of both lower doses of Pi (0.02 and 0.1 mg/kg) during phase 1 in fasted animals exerted an insignificant response, while in the remaining groups the effects were statistically significant. The highest dose of the drug decreased significantly antral spike burst amplitude in all the experimental groups studied compared with the relevant control value, i.e. also when Pi was given during phase 1 MMC in fasted animals (Tables 2 and 3). Drug combinations involving the moderate dose of Hx were also effective. Its combination with At was effective when the drugs were applied in fasted animals during phase 2a unlike during phase 1 MMC (Tables 2 and 3). In non-fasted sheep, combinations of Hx 2.0 mg/kg with At or Pi were equally effective (Tables 2 and 3). Drug combinations with the lowest Hx dose (1.0 mg/kg) were also effective and the results in non-fasted animals (data presented below) were similar to those in fasted animals (data not shown). The values of antral spike burst amplitude before (control values) and after drug combinations expressed as percentage of maximal control values indicated in Table 1 and comprising the lowest dose of Hx (1.0 mg/kg) plus At (0.1 mg/kg) given during phase 1 or 2a MMC were significantly lower (Table 2). The values of antral spike burst amplitude before and after combinations comprising the Hx (1.0 mg/kg) plus Pi (0.1 mg/kg) given during phase 2a MMC, unlike during phase 1 MMC, were significantly lower (Table 2).

The results consistent of the percentage of slow waves with spikes, and duration of the spike bursts remained roughly insignificant and was excluded from this chapter.

Discussion

The results indicate that there are some significant differences in antral myoelectric response to food given in fasted or non-fasted animals during the less active phases of MMC. Antral motility in sheep is somehow different than that in monogastric animals and the lack of identifable phase 3 MMC is one of the main differences (2,12). However, the efficient antroduodenal coordination related to MMC can be observed as in monogastric animals (1,13). The response to feeding during the duodenal phase 1 was only slightly different than when food was given during phase 2a MMC identified in this region. Both phases are of a rather non-migratory nature and phase 1 is also more strictly linked with phase 3 MMC in dogs (14). The absolute refractory period in the canine small intestine occurs mainly during phase 1 while during phase 2a MMC relative refraction occurs (15). This is apparently true also for the stomach (16). The refractory period has not been defined in sheep, however. The differences of the effects of feeding in fasted and non-fasted animals were of similar magnitude. The effect of feeding in all the groups studied was in most cases highly significant (possibly attenuating the refractory state) but the spike burst amplitude was not as high as it was suggested previously, i.e. up to 600 μV (12). There were at least 2 reasons for this. Firstly, the study was performed during phase 1 or 2a MMC when the antral spiking activity is less intense. Secondly, feeding is usually a very effective stimulus for antral motility but the type of food is important. When roughage food is given, antral myoelectric activity is stronger than when the animals are fed with concentrate (17).

The effects of anticholinergic drugs were also efficient and in this respect the antral motility in ruminants is similar to that in monogastric species (18,19). However, Hx, the nicotinic receptor blocking drug, was not very effective, especially in fasted animals, and its effect did not exhibit a dose-response character. Hx can be expected to be highly effective in the inhibition of gastrointestinal motility (6,20) but the results of other studies remain contrary to this view (7,21). The reported effect of At, the muscarinic blocker, is also controversial (7,22-24). In the present study, At exerted a clear and dose-response effect although it did not inhibit spike bursts entirely. Alternatively, the effect of Pi did not occur in an evident dose-response manner and was also dependent on feeding conditions and MMC phase, similarly to the effects of the other drugs. Pi is a more selective muscarinic blocker with the decreasing affinity to $M_1>M_4>M_3>M_2\sim M_5$ cholinergic muscarinic receptors (25) and is often called an M_1-receptor antagonist (26). This different affinity to various muscarinic receptor subtypes probably results in different motor responses to this drug (10,27,28). It is possible that Pi might block not only the postsynaptic muscarinic receptors but also the autoreceptors, enhancing acetylcholine release (26). Acetylcholine evokes a stimulatory motor response and/or attenuates inhibition. Therefore, the effect of Pi on M_1 cholinergic receptors can be malformed by its meaningful affinity to other cholinergic muscarinic receptor subtypes, especially when the higher Pi dose is applied.

It is concluded that feeding and anticholinergic drug administration affect ovine antral spike burst amplitude depending on the initial degree of excitation, which can be related, at least in part, to the intensity of the refractoriness properties of antral smooth muscle during less active MMC phases.

References

1. Sarna, S.K.: Myoelectrical and contractile activities of the gastrointestinal tract. In: M.M. Schuster, M.D. Crowell, K.L. Koch, eds. Schuster Atlas of Gastrointestinal Motility in Health and Disease. BC Decker Inc., Hamilton 2002, pp. 1-18.
2. Ruckebusch, Y.: Gastrointestinal motor function in ruminants. In: S.G. Schultz, ed. Handbook of Physiology. The Gastrointestinal System. Am. Physiol. Soc., Bethesda 1989, pp.1225-1282.
3. Medhus, A.W., Sandstad, O., Bredesen, J., Husebye, E.: Stimulation of the small intestine by nutrients in relation to phase of the migrating motor complex. Scand. J. Gastroenterol., 2000, 35: 494-500.
4. Plaza, M.A., Arruebo, M.P., Sopena, J., Bonafonte, J.I., Murillo, M.D.: Myoelectrical activity of the gastrointestinal tract of sheep analysed by computer. Res. Vet. Sci., 1996; 60: 55-60.
5. Plaza, M.A., Arruebo, M.P., Murillo, M.D.: Effect of motilin, somatostatin and bombesin on gastroduodenal myoelectric activity in sheep. Life Sci., 1996; 58: 1413-1423.
6. Ruckebusch, Y., Malbert, C.H., Crichlow, E.C.: Hexamethonium: a probe to assess autonomic nervous system involvement in upper gastrointestinal functions in conscious sheep. Vet. Res. Commun., 1987; 11: 293-303.

7. Romański, K.W.: Antral myoelectric activity in sheep: the effect of feeding and anticholinergic drug administration during various phases of migrating myoelectric complex. Acta Vet. Beograd, 2002; 52: 235-248.

8. Code, C.F., Marlett, J.A.: The interdigestive myoelectric complex of the stomach and small bowel of dogs. J. Physiol., 1975; 246: 289-309.

9. Dent, J., Dodds, W.J., Sekiguchi, T., Hogan, W.J., Arndorfer, R.C.: Interdigestive phasic contractions of the human lower esophageal sphincter. Gastroenterology, 1983; 84: 453-460.

10. Romański, K.W.: Characteristics and cholinergic control of the 'minute rhythm' in ovine antrum, small bowel and gallbladder. J. Vet. Med., A, 2002; 49: 313-320.

11. Snedecor, G.W., Cochran, W.G.: Statistical Methods. The Iowa State University Press, Ames 1971.

12. Bueno, L., Fioramonti, J.: Rhythms of abomaso-intestinal motility. In: Y. Ruckebusch, P. Thivend, eds. Digestive Physiology and Metabolism in Ruminants. MTP Press Ltd. Lancaster 1980, pp. 53-80.

13. Gregory, P.C., Miller, S.J., Brewer, A.C.: The relationship between food intake and abomasal emptying and small intestinal transit time in sheep. Br. J. Nutr., 1985; 53: 373-380.

14. Lang, I.M., Sarna, S.K., Condon, R.E.: Generation of phases I and II of migrating myoelectric complex in the dog. Am. J. Physiol., 1986; 251: G201-G207.

15. Sarna, S., Northcott, P., Belbeck, L.: Mechanism of cycling of migrating myoelectric complexes: effect of morphine. Am. J. Physiol., 1982; 242: G588-G595.

16. Sarna, S.K., Daniel, E.E.: Threshold curves and refractoriness properties of gastric relaxation oscillators. Am. J. Physiol., 1974; 226: 749-755.

17. Lester, G.D., Bolton, J.R.: Effect of dietary composition on abomasal and duodenal myoelectrical activity. Res. Vet. Sci., 1994; 57: 270-276.

18. Davenport, H.W.: Physiology of the Digestive Tract. An Introductory Text. Year Book Medical Publishers, Inc. Chicago 1982.

19. Ooms, L.A.A., Weyns, A., Degryse, A., Ruckebusch, Y.: Pharmacology of the (fore)-stomach smooth muscles. In: A.S.J.P.A.M. Van Miert, M.G. Bogaert, M. Debackere, eds. Comparative Veterinary Pharmacology, Toxicology and Therapy. MTP Press Ltd. 1986, pp. 107-121.

20. De Vos, W.C.: Role of the enteric nervous system in the control of migrating spike complexes in the feline small intestine. Am. J. Physiol., 1993; 265: G628-G637.

21. Nakazato, Y., Sekine, H., Isogaya, M., Ito, S., Ohga, A.: Atropine- and hexamethonium-resistant motor response to greater splanchnic nerve stimulation in the dog stomach. J. Auton. Nerv. Syst., 1987; 20: 35-42.

22. Bueno, L., Praddaude, F.: Electrical activity of the gallbladder and biliary tract in sheep and its relationships with antral and duodenal motility. Ann. Biol. Anim. Bioch. Biophys., 1979; 19: 1109-1121.

23. Bueno, L., Ruckebusch, Y.: Effect of anticholinergic drugs on the electrical activity of the antrum and duodeno-jejunum in sheep. J. Vet. Pharmacol. Ther., 1978; 1: 225-232.

24. Eglen, R.M., Hedge, S.S., Watson, N.: Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev., 1996; 48: 531-565.

25. Caulfield, M.P., Birdsall, J.M.: International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev., 1998; 50: 279-290.

26. Ogishima, M., Kaibara, M., Ueki, S., Kurimoto, T., Taniyama, K.: Z-338 facilitates acetylcholine release from enteric neurons due to blockade of muscarinic autoreceptors in guinea pig stomach. J. Pharmacol. Exp. Ther., 2000; 294: 33-37.

27. Dobrilla, G., de Pretis, G., Comberlato, M., Amplatz, S.: Pirenzepine and upper gastrointestinal tract motility in man. Hepato-gastroenterology, 1988; 35: 51-53 (review).

28. Schiavone, A., Sagrada, A., Pagani, F., Giachetti, A.: Role of muscarinic receptor subtypes in the regulation of migrating myoelectric complex in the dog. Gastroenterology, 1989; 96: 116-121.