Characterization of the in vitro responses of equine cecal longitudinal smooth muscle to endothelin-1

Ramaswamy M. Chidambaram, BVSc, PhD; Susan C. Eades, DVM, PhD; Rustin M. Moore, DVM, PhD; Giselle Hosgood, BVSc, PhD; Changaram S. Venugopal, BVSc, PhD

Objective—To characterize the in vitro response of equine cecal longitudinal smooth muscle (CLSM) to endothelin (ET)-1 and assess the role of ET_A and ET_B receptors in those ET-1–induced responses.

Animals—36 horses without gastrointestinal tract disease.

Procedure—To determine cumulative concentration-response relationships, CLSM strips were suspended in tissue baths containing graded concentrations of ET-1 (10^{-9} to 10^{-6}M) with or without BQ-123 (ET_A receptor antagonist); with or without IRL-1038 (ET_B receptor antagonist); or with both antagonists at concentrations of 10^{-9}, 10^{-7}, and 10^{-5}M. To determine the percentage change in baseline tension of CLSM, the areas under the curve during the 3-minute periods before and after addition of each dose were compared. Also, the effects of ET-1 and a combination of selective ET_A and ET_B receptor antagonists on electrically evoked contractions were studied.

Results—ET-1 caused sustained increases in CLSM tension in a concentration-dependent manner. Contractile responses to ET-1 were not significantly inhibited by either BQ-123 or IRL-1038 alone at any concentration; however, responses were significantly inhibited by exposure to the antagonists together at a concentration of 10^{-5}M. Electrical field stimulation did not change the spontaneous contractile activity of CLSM and did not significantly alter the tissue response to ET-1, BQ-123, or IRL-1038.

Conclusions and Clinical Relevance—Results indicated that ET-1 has a contractile effect on equine CLSM that is mediated via ET_A and ET_B receptors. In vitro spontaneous contractions of equine CLSM apparently originate in the smooth muscle and not the enteric nervous system. (*Am J Vet Res* 2005;66:1202–1208)

The **endothelin (ET)** family of peptides consists of 3 distinct isoforms (ET-1, ET-2, and ET-3), each comprised of 21 amino acids and encoded by separate genes.[1] The most abundant and biologically important of these is ET-1. Endothelin-1, a vasoconstrictor peptide, is expressed in a variety of cell types, including endothelial cells, mast cells, macrophages, mesangial cells, and central and peripheral neurons.[1,2] Endothelins have been suggested to play a diverse role in physiologic functions such as production of intestinal secretions, regulation of intestinal motility, maintenance of vasomotor tone, and closure of the ductus arteriosus.[1,3,4] Endothelin-1 exerts its biologic activity by binding to its receptors, ET_A and ET_B, which are members of the 7-transmembrane domain, guanine nucleotide-binding protein (G protein)–coupled rhodopsin-type receptor superfamily.[1,5] The ET_B receptors are classified as ET_{B1} and ET_{B2}, whereas classification of ET_A as ET_{A1} and ET_{A2} is not well established.[5] Generally, ET_A receptors are located on vascular and nonvascular smooth muscles and predominantly mediate contraction. The ET_{B1} receptors are located in the endothelial cells and mediate relaxation via the release of nitric oxide. The ET_{B2} receptors located on nonvascular smooth muscle mediate contraction.[6] The contractile effects of ET-1 are mediated through G-proteins, leading to the activation of phospholipase C, protein kinase C, and other signal transduction pathways.[1,4] Actions of ET-1 on smooth muscle are complex and involve excitatory and inhibitory effects, depending on the type of receptor, type of tissue (vascular and nonvascular), and species involved.[2,7,8]

The contractions and relaxations that result in adequate mixing and propulsion of ingesta in the small and large intestine of dogs, cats, and laboratory mammals are controlled by the visceral smooth muscle cells (myogenic properties) and the **enteric nervous system** (**ENS**; neurogenic properties) and are modulated by the autonomic nervous system as well as by neurocrine and paracrine agents.[9] Myogenic properties of gastrointestinal smooth muscle cells provide the basis for all gastrointestinal tract motility by generating cyclical changes in cell membrane potential that, if conditions are appropriate, give rise to spontaneous, rhythmical contractions.[10] Most of the effects of ETs in the gastrointestinal tract are contractile and occur via the direct actions of ETs on smooth muscle.[6] The involvement of ET-1 in intestinal motor regulation is a result of the presence of both ET_A and ET_{B2} receptors in the gastrointestinal tract.[2,11]

The local neurogenic control for motility is provided by the ENS.[12,13] These neurogenic mechanisms involve cholinergic neurons that cause contraction via action on muscarinic receptors in visceral smooth muscle.[10] Adrenergic neurons predominately inhibit contraction; however, stimulatory input by adrenergic

Received May 31, 2004.
Accepted October 29, 2004.
From the Equine Health Studies Program, Departments of Comparative Biomedical Sciences (Chidambaram) and Veterinary Clinical Sciences (Eades, Moore, Hosgood, Venugopal), School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803-8410.
This manuscript represents a portion of the dissertation submitted by the senior author to the Louisiana State University Graduate School in partial fulfillment of the requirements for a PhD degree.
Supported by a grant from the Louisiana State University School of Veterinary Medicine Equine Health Studies Program.
The authors thank Earnestine Holmes and Catherine Koch for technical assistance.
Address correspondence to Dr. Moore.

nerves has also been demonstrated.[10,14] Many of the excitatory and inhibitory nerves within the ENS lack adrenergic and cholinergic mechanisms and are referred to as **nonadrenergic noncholinergic (NANC)** neurons.[15] Both excitatory and inhibitory NANC neurons appear to play important roles in the control of intestinal contractions.[15] The role of NANC innervation in the gastrointestinal tract has been studied[11,13,16] in a variety of species, including rats, sheep, cats, and horses. It has been determined that nitric oxide and vasoactive intestinal peptide have a function in NANC inhibitory neurotransmission in the equine jejunum and ventral colon.[12,13,17] The nitric oxide released from the NANC system allows receptive relaxation aboral to a bolus during peristalsis.[15] Endothelin-1 is a NANC excitatory neurotransmitter in the urinary bladder, lungs, and CNS of rats and guinea pigs.[18,19] Because ETs have been shown to be localized in the ENS and to have neurotransmitter functions in the urinary bladder and CNS, they are hypothesized to be NANC neurotransmitters in the ENS.[2,20]

In horses, motility disorders of the gastrointestinal tract are associated with a variety of pathologic conditions, including impaction, postoperative ileus, endotoxemia, and peritonitis.[21] Luminal distention associated with obstruction and ischemic injury may increase gastrointestinal tract motility by altering the release of various neurotransmitters, thereby leading to abdominal pain because of increased wall tension during intestinal spasm.[12,22] Alternatively, endotoxemia results in cessation of cecal and colonic contractile activity, thereby potentially inducing abdominal pain because of colonic distention.[23-25] Results of a previous study[26] performed in our laboratory indicated that plasma concentrations of ET-1 increased in horses with gastrointestinal disease, compared with values in clinically normal horses, and that the greatest ET-1 concentrations were identified in horses with strangulating lesions and enterocolitis, which are conditions typically associated with severe endotoxemia. Increased synthesis of ET-1 in the diseased intestinal tissue of horses with colic could result in intestinal ischemia because of ET-1–induced vasoconstriction and alterations in intestinal motility. An in vitro study[27] performed in our laboratory revealed that ET-1 is capable of causing contraction of equine colonic arteries and veins, which could result in colonic ischemia. However, to date, there have been no studies reported to our knowledge regarding the functional role of ET-1 in the visceral smooth muscle of horses. A study on isolated cecal muscle would allow a more definitive evaluation of the presence of ET receptors in cecal smooth muscle and their potential role in cecal motility.

We hypothesized that ET-1 would cause concentration-dependent contraction (mediated by ET_A and ET_B receptors) of **cecal longitudinal smooth muscle (CLSM)**. The purpose of the study reported here was to characterize the in vitro response of equine CLSM to ET-1, compared with responses to carbachol (a well-known intestinal smooth muscle contractile agent), and to assess the role of ET_A and ET_B receptors in the ET-1–induced responses of CLSM by use of specific ET receptor antagonists. Furthermore, we intended to examine the role of ET-1 as an excitatory neurotransmitter in the gastrointestinal tract by use of **electric field stimulation (EFS)**.

Materials and Methods

Animals and tissue collection—The study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Tissues collected from 36 adult healthy horses that were euthanatized for reasons unrelated to any gastrointestinal tract or systemic diseases were used; the tissues were collected immediately after euthanasia by use of overdose of pentobarbital sodium[a] (100 mg/kg, IV). In each horse, a 15-cm-long segment of the cecum was isolated from the cecal base parallel to the ventral taenia; ingesta were gently removed via lavage with saline (0.9% NaCl) solution. The tissue was then placed in a beaker containing oxygenated warm modified Krebs solution. The tissue segments were pinned flat in a dissecting bath containing sufficient warm (37°C) oxygenated Krebs buffer solution, to ensure that tissues were completely immersed; the bathing solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide. For each sample, the intestinal mucosa and submucosa were removed gently by use of sharp dissecting scissors, and strips measuring 2 mm wide and 10 mm long were cut parallel to the taenia to obtain longitudinal muscle sections.

Drugs and reagents—All solutions were prepared on the day of use. Endothelin-1,[b] BQ-123,[c] IRL-1038,[d] carbachol,[e] atropine,[e] guanethidine,[e] and tetrodotoxin[e] were used. The BQ-123 and IRL-1038 are competitive antagonists of ET_A and ET_B receptors, respectively; however, they are not subtype specific. Concentrations of ET-1, BQ-123, and IRL-1038 were based on those physiologic concentrations used in the in vitro evaluation of these agents in equine colonic arteries and veins and guinea pig ileum.[4,27,28] The drugs were dissolved with distilled water according to manufacturer's instructions. The ET-1 was reconstituted in distilled water and stored in aliquots of 10^{-4}M at –80°C. Aliquots were thawed immediately prior to use and further diluted to the desired concentration. Carbachol was dissolved in distilled water immediately before use. The composition of the modified Krebs solution was as follows: NaCl, 118mM; $MgSO_4$, 1.2mM; $CaCl_2$, 2.5mM; KCl, 4.7mM; $NaHCO_3$, 24.9mM; and dextrose (pH, 7.3 to 7.4).

Experimental protocol—One end of each strip was mounted to the floor of the organ bath (10 mL), and the other end was attached to a force-displacement transducer[f] interfaced with a polygraph[g] to record the change in tension. To observe the onset of spontaneous contractile activity, intestinal smooth muscle strips were allowed to accommodate for 45 minutes at 0.5-g tension in organ baths containing modified Krebs solution maintained at 37°C by a circulating water bath and oxygenated with a gas mixture of 95% oxygen and 5% carbon dioxide. Initial tension was set at the optimum tension (2 g) that had been determined in preliminary studies, and tissues were allowed to equilibrate for an additional 60 minutes. Throughout the study, the modified Krebs solution was changed at 15-minute intervals and the tension was not readjusted.

Tissues from 6 of the 36 horses were used in a preliminary investigation to determine the appropriate resting tension and equilibration period for use in experiments to evaluate the effect of ET-1 on CLSM. Various resting tensions (2, 3, or 4 g) were applied to tissues with different equilibration periods (30, 45, or 60 minutes), and **concentration-response (C-R)** relationships of ET-1 (at 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}M) were performed. The equilibration period that resulted in the most reproducible and consistent spontaneous contractile activity was utilized in all subsequent studies. The resting

tension applied to tissues that resulted in near maximal contraction was considered the optimum tension.[29]

Three main experiments were performed (**Appendix**). Tissues from 6 horses (48 strips) were used to determine the C-R relationships of both ET-1 and carbachol (experiment 1); tissues from 18 horses (144 strips) were used to determine the C-R relationships of ET-1 in the presence of ET_A and ET_B receptor antagonists, separately and in combination (experiment 2); and tissues from 6 horses (48 strips) were used for the EFS experiment (experiment 3).

C-R relationships of carbachol and ET-1 on CLSM—After a 105-minute equilibration period, cumulative C-R relationships for ET-1 and carbachol at graded concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}M were performed for each strip. Each dose was added at 3-minute intervals, and changes in tension were recorded.

Effects of BQ-123 and IRL-1038 separately and in combination on ET-1–induced responses of CLSM—During the last 45 minutes of the equilibration period (total incubation, 105 minutes), tissues were incubated separately with ET_A (BQ-123) and ET_B (IRL-1038) receptor antagonists at concentrations of 10^{-9}, 10^{-7}, and 10^{-5}M, and a C-R relationship of ET-1 (10^{-9} to 10^{-6}M) was then determined. Similarly, in other tissue baths, CLSM strips were incubated with each of 3 concentrations of 10^{-9}, 10^{-7}, and 10^{-5}M of the combined antagonists for the last 45 minutes of the equilibration period (total incubation, 105 minutes), and a C-R relationship of ET-1 (10^{-9} to 10^{-6}M) was determined as before.

EFS—The electrodes used for the EFS consisted of a pair of platinum wires fixed at both sides of the CLSM strip. Electric field stimulation (train duration, 1 to 10 seconds; 0.5-second duration pulses at 5 to 30 Hz at 40 to 120 V) was generated by a stimulator[h] and applied to all tissues simultaneously.[13,i] Cecal longitudinal smooth muscle strips were prepared as described and allowed to accommodate for 45 minutes at tension of 0.5 g; tension was then set at 2 g, and strips were equilibrated for an additional 60 minutes. During the last 30 minutes of equilibration and throughout the experimentation, groups of 12 tissue strips (2 strips from each of 6 horses) were incubated with one of the following treatments: atropine (10^{-5}M) and guanethidine (10^{-5}M) to facilitate assessment of NANC neurons[17]; atropine (10^{-5}M), guanethidine (10^{-5}M), and a combination of ET_A (BQ-123; 10^{-5}M) and ET_B (IRL-1038; 10^{-5}M) receptor antagonists; a combination of ET_A (BQ-123; 10^{-5}M) and ET_B (IRL-1038; 10^{-5}M) receptor antagonists; or no drug solutions added. During experimentation, EFS was applied and contractions were recorded for 12 minutes, after which tissues were washed. After reapplication of the aforementioned antagonists (BR-123 and IRL-1038), ET-1 at graded concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}M was added at 3-minute intervals and changes in tension were recorded. After addition of 10^{-6}M ET-1, tetrodotoxin (sodium channel blocker; 10^{-3}M) was added to evaluate the role of nerve conduction in the recorded contractions.

Data analyses and statistical methods—By use of image analysis software,[j] the active change in tissue responses was calculated as the percentage change from baseline tension. To determine the percentage change in baseline tension of CLSM for ET-1 responses, the areas under the curve during the 3-minute periods before and after addition of each dose were compared.[17] The changes in tension due to the action of ET-1 were compared with changes in tension due to the action of carbachol and with changes in tension due to the action of ET-1 in the presence of the ET receptor antagonists.

All data were considered continuous and followed a normal distribution as determined by failure to reject the null hypothesis of normality at $P \leq 0.05$ by use of the Shapiro-Wilk test. The data were summarized as mean ± SEM and evaluated for an effect of antagonist and concentration by use of a mixed linear model that accounted for the random variance of horse. Where there were significant interaction effects at $P \leq 0.05$, predetermined multiple comparisons were made across concentrations within each antagonist group and among groups at specified concentrations by use of least squares means analysis that maintained type I error at 0.05. Data were analyzed by use of computer software.[k]

Results

A resting tension of 2 g was required to elicit a

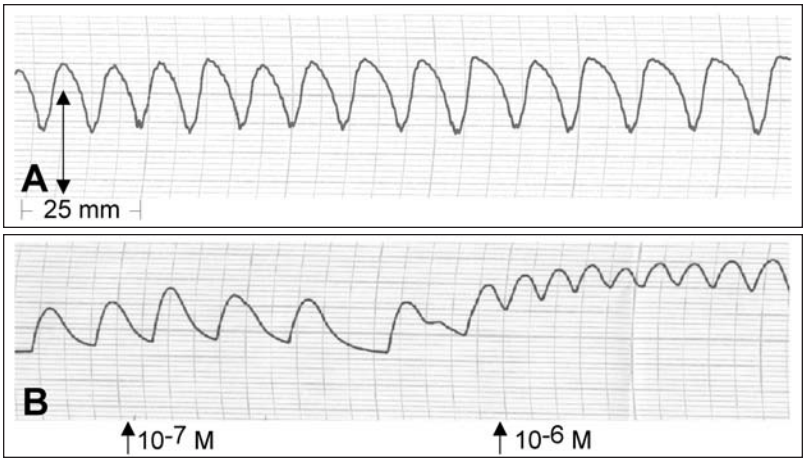

Figure 1—Polygraph recordings (paper speed, 25 mm/min) of the in vitro contractile activity of 2 specimens of equine cecal longitudinal smooth muscle (CLSM). A—Trace recorded from a sample of untreated CLSM held at a resting tension of 2 g. The interval between the top and bottom of the double-headed arrow represents 2 g tension. B—Trace of the contractile response of a sample of CLSM to increasing log molar concentration of endothelin (ET)-1. Arrows denote moment of concentration adjustment.

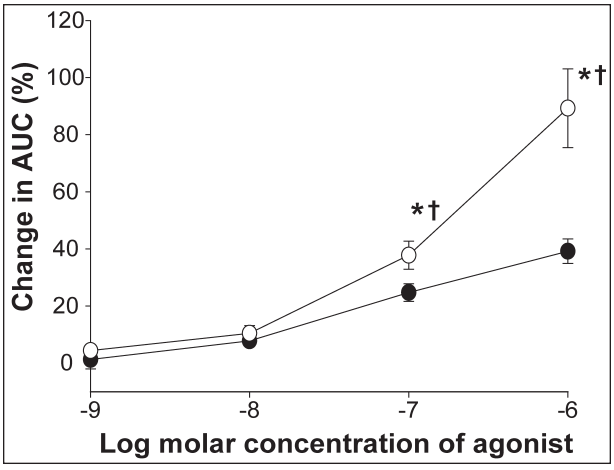

Figure 2—Mean ± SEM percentage change in the area under the curve (AUC) in equine CLSM strips (paper speed, 25 mm/min) in response to changes in log molar concentrations of ET-1 (closed circles) and carbachol (open circles). *Significant difference ($P < 0.001$) between responses to ET-1 and carbachol detected at this log molar concentration. †Significant differences between responses to ET-1 at 10^{-7} and 10^{-6} log molar agonist concentrations.

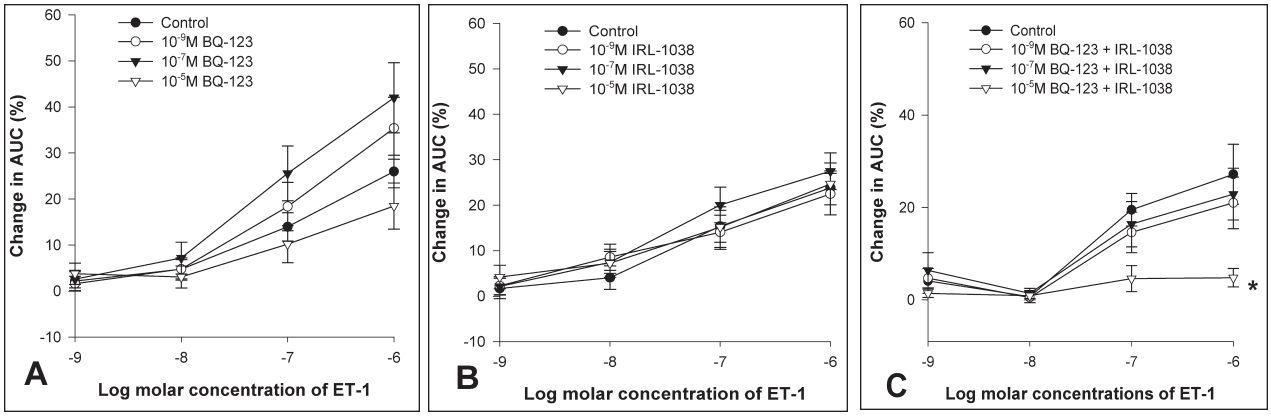

Figure 3—Mean ± SEM percentage change in AUC in equine CLSM strips in response to log molar concentrations of ET-1 before (control) and after incubation of tissues with different concentrations (10^{-9}, 10^{-7}, and 10^{-5}M) of an ET_A receptor antagonist (BQ-123; A), with similar concentrations of an ET_B receptor antagonist (IRL-1038; B), and with similar concentrations of each antagonist in combination (C). When each antagonist was applied alone, there were no significant differences among treatments; contractile responses to ET-1 were significantly ($P < 0.001$) decreased (asterisk) when tissues were incubated with both antagonists at 10^{-5}M concentration, compared with other treatments.

consistent contractile pattern in the CLSM strips. The spontaneous activity of longitudinally oriented smooth muscle was cyclic and occurred at a steady rate of approximately 2 to 3 contractions/min (**Figure 1**). Compared with pretreatment values, the addition of ET-1 caused slow, sustained, and significant ($P < 0.001$) increases in the area under the tension-time curve of equine CLSM at 10^{-7} and 10^{-6}M bath concentrations. The carbachol-induced contractile effects were significantly ($P < 0.001$) greater than those induced by ET-1 at greater concentrations (**Figure 2**).

Preincubation with the ET_A receptor antagonist BQ-123, the ET_B receptor antagonist IRL-1038, or these antagonists in combination did not alter the baseline spontaneous contractions of the CLSM strips. Preincubation of CLSM strips with BQ-123 did not inhibit the contractile response of ET-1 (**Figure 3**). Compared with tissues treated with ET-1 alone, there was a slight, nonsignificant ($P = 0.063$) increase in contractile activity in tissues preincubated with BQ-123 (10^{-7}M). Similarly, preincubation of tissues with IRL-1038 did not abolish the ET-1–induced response. The contractile responses of CLSM tissues preincubated with BQ-123 and IRL-1038 together (each at a 10^{-5}M concentration) to ET-1 at 10^{-7} and 10^{-6}M were significantly lower, compared with control tissues receiving no antagonists.

The spontaneous contractile activity of the CLSM control tissues or tissues incubated with atropine and guanethidine was not significantly affected by EFS. Electric field stimulation did not significantly alter the response of tissues to ET-1 or to the combination of ET_A or ET_B receptor antagonists. Additionally, the addition of atropine, guanethidine, or tetrodotoxin did not alter the contractile activity of CLSM during EFS.

Discussion

The present study has revealed several important findings. In our in vitro experiments, ET-1 induced concentration-dependent contraction of the CLSM of horses that were apparently clinically normal with regard to their gastrointestinal tracts. The muscarinic agent carbachol had significantly greater contractile activity than ET-1 on an equimolar concentration basis. Preincubation of CLSM strips with BQ-123 and IRL-1038 (antagonists of ET_A and ET_B receptors) together at a high concentration (10^{-5}M each) abolished the ET-1–mediated contractile response, whereas these antagonists failed to do so when applied individually to the tissues. This suggests that both ET_A and ET_B receptors mediate ET-1–induced contraction. Electric field stimulation did not alter the spontaneous contractile activity of the CLSM or the responses of the tissue to ET-1 or ET_A and ET_B receptor antagonists. Also, tetrodotoxin did not alter the EFS or spontaneous contractile activity of CLSM, indicating that the contractions detected were not mediated by nerve conduction.

Endothelin-1 caused a slow, sustained, concentration-dependent contractile response in the CLSM layer. Endothelin-1 causes a marked, biphasic contraction in longitudinal and circular smooth muscle of guinea pig ileum and mouse ileum.[30-32] Similar contractile effects of ET-1 have been reported[2] in tissues from the stomach and cecum of rats, and ET-1 receptors have the greatest distribution in guinea pig cecum and stomach tissue.[33] The presence of ETs in enteric neurons has been confirmed, and ET-binding sites in the myenteric and submucosal plexi, mucosa, muscular layers, and vasculature of the colon of rats have been detected.[2] Results of a previous study[26] have indicated that circulating concentrations of ET-1 are increased in horses with gastrointestinal tract disease, compared with concentrations in clinically healthy horses. Plasma concentrations of ET-like immunoreactivity are significantly increased in horses with a variety of gastrointestinal tract disorders, especially in conditions associated with endotoxemia.[26] The synthesis and release of ET-1 and its subsequent actions may represent an unknown physiologic control system in gastrointestinal tract function.

In our study, the magnitude of contraction of the equine CLSM in response to ET-1 was significantly less than that detected in response to the muscarinic agonist carbachol. The parasympathetic system is known to have a dominant effect in the gastrointestinal tract of

most species studied.[9] Because cholinergic receptors are responsible for most of the contractile responses of visceral smooth muscle,[12,22] the contractile response of CLSM tissue to cholinergic agents was expected to be greater in our study. Also, in the CLSM samples evaluated, the magnitude of contraction in response to ET-1 was less than that detected in the vasculature of the large intestine in horses.[27]

The ET receptor antagonists BQ-123 and IRL-1038 used in our study are specific, competitive, ET receptor antagonists that block ET_A and ET_B receptors, respectively.[33,34] Therefore, the ET receptors present in the CLSM preparations studied may be of ET_A and ET_{B2} receptor types. Preincubation of tissues with either BQ-123 or IRL-1038 did not significantly alter the ET-1–induced changes in CLSM tension. It can be speculated that when tissues were incubated with ET_A or ET_B receptor antagonists, only 1 type of ET receptor was blocked, and the other receptor type remained unblocked; subsequently, ET-1–induced contractile effects were not prevented. However, the ET-1–mediated contractile response was almost completely abolished when CLSM strips were incubated with both antagonists combined (each at a 10^{-5}M concentration). Therefore, both ET_A and ET_B receptors appear to mediate ET-1–induced contraction in the CLSM of horses. Similarly, in specimens of guinea pig ileum, a combination of BQ-123 and PD-145065 blocked the response to ET-1, whereas treatment with each antagonist alone had a minimal effect.[35]

The spontaneous activity of the longitudinally oriented cecal smooth muscle from the horses used in our study was cyclic and occurred at a steady rate of approximately 2 to 3 contractions/min. Because these spontaneous contractions were not affected by tetrodotoxin, atropine, or guanethidine, these contractions were not generated by stimulation of enteric nerves or adrenergic or muscarinic receptors.[10] It is possible that the release of neurotransmitters was not blocked at the concentrations of these agents that we used. However, because these concentrations are commonly used to investigate neurogenic contractions in the ENS, it is more likely that myogenic mechanisms within the CLSM are responsible for the spontaneous activity of this tissue.[12,17] Regular spontaneous contractions of longitudinal smooth muscle of the equine ileum and ventral colon occur similarly at a rate of 10 and 1 contraction/min, respectively, and are not affected by tetrodotoxin.[13] This decreasing frequency of spontaneous contractions between tissues of the ileum and the right ventral colon is consistent with the gradient of intrinsic frequency of periodic depolarizations (electric control activity) responsible for myogenic control of gastrointestinal tract motility.[36] Because the contractile effects of ET-1 in the CLSM tissues evaluated in the present study were also not altered by the addition of tetrodotoxin, the contractile action of ET-1 probably resulted from direct effects on the visceral smooth muscle. This is consistent with the actions of ET-1 on guinea pig cecal smooth muscle.[6]

In vitro, ET-1 has a concentration-dependent contractile effect on equine CLSM. Analysis of the results of our study also revealed that ET-1 mediates its contractile effects through binding to both ET_A and ET_B receptors on cecal smooth muscle cells. The observed effects of ET-1 on CLSM contraction may suggest that alterations in cecal motility might be associated with changes in ET-1 synthesis and release during naturally acquired gastrointestinal tract disorders. Results of a study[26] of horses with naturally occurring gastrointestinal tract disease have indicated that plasma concentrations of ET-1 are increased, compared with findings in clinically healthy horses, and are greatest in inflammatory and ischemic conditions that are accompanied by endotoxemia. However, endotoxemia is accompanied by decreased contractile activity in the cecum.[23] Therefore, the results of the present study suggest that the effects of endotoxemia cannot be attributed to increased ET-1 concentration. In contrast, contraction of the intestinal wall during intestinal obstruction and distention may be a consequence of the synthesis and release of ET-1. Further studies are needed to determine the role of ET-1 in the smooth muscle responses of diseased or damaged gastrointestinal tissues of horses.

a. Beuthanasia-D, Schering-Plough Animal Health Corp, Union, NJ.
b. Endothelin-1, American Peptide Co, Sunnyvale, Calif.
c. BQ-123, American Peptide Co, Sunnyvale, Calif.
d. IRL-1038, American Peptide Co, Sunnyvale, Calif.
e. Sigma-Aldrich Inc, St Louis, Mo.
f. Model 7D polygraph, Grass Instruments, Quincy, Mass.
g. Chart recorder model 25-60, Grass Instruments, Quincy, Mass.
h. Grass S48 stimulator, Astro-Med Inc, Grass Instruments Division, West Warwick, RI.
i. Stimu-Splitter II, Med-Lab Instruments, Loveland, Colo.
j. Sigma Scan Pro 4.0, Jandel Scientific Software, San Rafael, Calif.
k. PROC UNIVARIATE and PROC MIXED programs, SAS for Windows 95, version 7.0, SAS Institute Inc, Cary, NC.

References

1. Rubanyi GM, Polokoff MA. Endothelins: molecular biology, pharmacology, physiology, and pathophysiology. *Pharmacol Rev* 1994;46:325–415.

2. Takahashi K, Jones PM, Kanse SM, et al. Endothelin in the gastrointestinal tract. Presence of endothelinlike immunoreactivity, endothelin-1 messenger RNA, endothelin receptors, and pharmacological effect. *Gastroenterology* 1990;99:1660–1667.

3. Coceani F, Kelsey L, Seidlitz E. Evidence for an effector role of endothelin in closure of the ductus arteriosus at birth. *Can J Physiol Pharmacol* 1992;70:1061–1064.

4. Shahbazian A, Holzer P. Regulation of guinea pig intestinal peristalsis by endogenous endothelin acting at ET(B) receptors. *Gastroenterology* 2000;119:80–88.

5. Fagan KA, McMurtry IF, Rodman DM. Role of endothelin-1 in lung disease. *Respir Res* 2001;2:90–101.

6. Clozel M, Gray GA, Breu V, et al. The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. *Biochem Biophys Res Commun* 1992;186:867–873.

7. Bolger GT, Liard F, Krogsrud R, et al. Tissue specificity of endothelin binding sites. *J Cardiovasc Pharmacol* 1990;16:367–375.

8. Eglen RM, Michel AD, Sharif NA, et al. The pharmacological properties of the peptide, endothelin. *Br J Pharmacol* 1989;97:1297–1307.

9. Christensen J. Colonic motility. In: Wood D, ed. *Handbooks of physiology—gastrointestinal tract: motility and circulation, part II*. Bethesda, Md: American Physiology Society, 1989;939–974.

10. Sarna SK. Physiology and pathophysiology of colonic motor activity. *Dig Dis Sci* 1991;36:827–862.

11. Yoshinaga M, Chijiiwa Y, Misawa T, et al. EndothelinB receptor on guinea pig small intestinal smooth muscle cells. *Am J Physiol* 1992;262:G308–G311.

12. Malone ED, Kannan MS, Brown DR, et al. Adrenergic,

cholinergic, and nonadrenergic-noncholinergic intrinsic innervation of the jejunum in horses. *Am J Vet Res* 1999;60:898–904.

13. Van Hoogmoed LM, Rakestraw PC, Snyder JR, et al. Evaluation of nitric oxide as an inhibitory neurotransmitter in the equine ventral colon. *Am J Vet Res* 2000;61:64–68.

14. Luckensmeyer GB, Keast JR. Activation of alpha- and beta-adrenoceptors by sympathetic nerve stimulation in the large intestine of the rat. *J Physiol* 1998;510:549–561.

15. Sanders KM, Ward SM. Nitric oxide as a mediator of non-adrenergic noncholinergic neurotransmission. *Am J Physiol* 1992; 262:G379–G392.

16. Venkova K, Krier J. A nitric oxide and prostaglandin-dependent component of NANC off-contractions in cat colon. *Am J Physiol* 1994;266:G40–G47.

17. Rakestraw PC, Snyder JR, Woliner MJ, et al. Involvement of nitric oxide in inhibitory neuromuscular transmission in equine jejunum. *Am J Vet Res* 1996;57:1206–1213.

18. Donoso MV, Salas C, Sepulveda G, et al. Involvement of ETA receptors in the facilitation by endothelin-1 of non-adrenergic non-cholinergic transmission in the rat urinary bladder. *Br J Pharmacol* 1994;111:473–482.

19. Fernandes LB. Phosphodiesterase inhibitors and endothelin as modulators of respiratory neurotransmission. *Clin Exp Pharmacol Physiol* 1996;23:980–982.

20. Inagaki H, Bishop AE, Escrig C, et al. Localization of endothelinlike immunoreactivity and endothelin binding sites in human colon. *Gastroenterology* 1991;101:47–54.

21. Blikslager AT, Bowman KF, Levine JF, et al. Evaluation of factors associated with postoperative ileus in horses: 31 cases (1990–1992). *J Am Vet Med Assoc* 1994;205:1748–1752.

22. Gerring EL. Effects of pharmacological agents on gastrointestinal motility. *Vet Clin North Am Equine Pract* 1989;5:283–294.

23. Eades SC, Moore JN. Blockade of endotoxin-induced cecal hypoperfusion and ileus with an alpha 2 antagonist in horses. *Am J Vet Res* 1993;54:586–590.

24. King JN, Gerring EL. Antagonism of endotoxin-induced disruption of equine bowel motility by flunixin and phenylbutazone. *Equine Vet J* 1989;7:38–42.

25. King JN, Gerring EL. Antagonism of endotoxin-induced disruption of equine gastrointestinal motility with the platelet-activating factor antagonist WEB 2086. *J Vet Pharmacol Ther* 1990;13:333–339.

26. Ramaswamy CM, Eades SC, Venugopal CS, et al. Plasma concentrations of endothelin-like immunoreactivity in healthy horses and horses with naturally acquired gastrointestinal tract disorders. *Am J Vet Res* 2002;63:454–458.

27. Venugopal CS, Holmes EP, Koch CE, et al. In vitro pharmacologic effect of two endothelin-1 antagonists on equine colonic arteries and veins. *Am J Vet Res* 2001;62:154–159.

28. Allcock GH, Battistini B, Fournier A, et al. Characterization of endothelin receptors mediating mechanical responses to the endothelins in the isolated stomach strip of the rat. *J Pharmacol Exp Ther* 1995;275:120–126.

29. Meiss RA. Mechanical properties of gastrointestinal smooth muscle. In: Wood D, ed. *Handbooks of physiology—gastrointestinal tract: motility and circulation, part I*. Bethesda, Md: American Physiology Society, 1989;273–329.

30. Lin WW, Lee CY. Biphasic effects of endothelin in the guinea-pig ileum. *Eur J Pharmacol* 1990;176:57–62.

31. Miasiro N, Karaki H, Matsuda Y, et al. Effects of endothelin ET(B) receptor agonists and antagonists on the biphasic response in the ileum. *Eur J Pharmacol* 1999;369:205–213.

32. Okabe H, Chijiiwa Y, Yoshinaga M, et al. Direct contractile effect of endothelin-1 on isolated circular smooth muscle cells of guinea pig caecum. *Digestion* 1995;56:21–24.

33. Kuwahara K, Kitsukawa Y, Suzuki Y, et al. Effects of several endothelin receptor antagonists on gastrointestinal transit of guinea pigs. *Dig Dis Sci* 2000;45:960–966.

34. Bolger GT, Liard F, Garneau M, et al. Characterization of intestinal smooth muscle responses and binding sites for endothelin. *Can J Physiol Pharmacol* 1992;70:377–384.

35. Miasiro N, Karaki H, Paiva AC. Heterogeneous endothelin receptors mediate relaxation and contraction in the guinea-pig ileum. *Eur J Pharmacol* 1995;285:247–254.

36. Sellers AF, Lowe JE, Brondum J. Motor events in equine large colon. *Am J Physiol* 1979;237:E457–E464.

Appendix appears on the next page.

Appendix

Outline of the experimental design and number of horses and tissues assigned to each of 3 experiments to characterize the in vitro response of equine cecal longitudinal smooth muscle (CLSM) to endothelin (ET)-1 and to assess the role of ET_A and ET_B receptors in those ET-1–induced responses.

No. of horses	No. of tissue strips used per horse	Experiment	Pretreatment	Treatment concentration-response (at 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6}M)
6	4	1	None	Carbachol
6	4	1	None	ET-1
6 for each antagonist dose (n = 18)	2	2	None	ET-1
6 for each antagonist dose (18)	2	2	ET_A receptor antagonist BQ-123 (at 10^{-9}, 10^{-7}, or 10^{-5}M)	ET-1
6 for each antagonist dose (18)	2	2	ET_B receptor antagonist IRL-1038 (at 10^{-9}, 10^{-7}, or 10^{-5}M)	ET-1
6 for each antagonist dose (18)	2	2	Both BQ-123 and IRL-1038 each at 10^{-9}, 10^{-7}, or 10^{-5}M	ET-1
6	2	3	None	ET-1
6	2	3	Atropine and guanethidine (10^{-5}M each)	ET-1
6	2	3	Both BQ-123 and IRL-1038 (10^{-5}M each)	ET-1
6	2	3	Atropine, guanethidine, and both BQ-123 and IRL-1038 (10^{-5}M each)	ET-1

Experiment 1 = Assessment of concentration-response relationships of carbachol and ET-1 on equine CLSM. Experiment 2 = Investigation of the effects of BQ-123 and IRL-1038 separately and in combination on ET-1–induced responses of equine CLSM. Experiment 3 = Investigation of the effects of ET-1 and a combination of selective ET_A and ET_B receptor antagonists on electrically evoked contractions (result of electrical field stimulation) of the CLSM of horses.