In vitro evaluation of the contractile response to endothelin-1 of the circular and longitudinal myometrial layers of the uterine horn of nongravid mares

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Objective—To characterize the in vitro response of circular and longitudinal myometrial layers of the uterine horn (CMLH and LMLH, respectively) of horses to endothelin (ET)-1 by use of specific ET_A (BQ-123) and ET_B (IRL-1038) receptor antagonists.

Sample Population—Uteruses from 10 nongravid mares in anestrus.

Procedure—Muscle strips from the CMLH and LMLH were suspended in tissue baths and connected to force-displacement transducers interfaced with a polygraph. Strips were incubated for 45-minute intervals with no antagonist (control specimens), and 3 concentrations (10^{-9}, 10^{-7}, and 10^{-5}M) of BQ-123, IRL-1038, or BQ-123 and IRL-1038 before concentration-response curves to ET-1 were generated. Contractile response to cumulative concentrations of ET-1 (10^{-9} to 10^{-6}M) was quantified by measuring change in the area under the curve (AUC) for the 3-minute period after each ET-1 dose.

Results—ET-1 caused concentration-dependent contraction of the CMLH and LMLH specimens. Application of BQ-123 decreased AUC values for both layers. Application of IRL-1038 increased the AUC value for LMLH specimens but did not affect the CMLH value. The combination of BQ-123 and IRL-1038 decreased the AUC value for LMLH tissue and increased that for CMLH tissue.

Conclusions and Clinical Relevance—ET-1 causes contraction of the CMLH and LMLH in nongravid horses. In both layers, ET_A receptors mediate contraction but the role of ET_B receptors remains unclear. In the LMLH, ET_A receptors have a dominant role; the presence of another receptor or receptor subtype within this layer is suggested. These findings support a physiologic role for ET-1 in uterine contractility. (*Am J Vet Res* 2005;66:1094–1100)

Endotoxemia (ie, gram-negative sepsis or endotoxic shock) is a systemic disorder that originates from the host response to gram-negative bacteria, resulting in the release of several autacoids including cytokines, arachidonates, and endothelium-derived substances.[1] Horses develop endotoxemia secondary to gastrointestinal tract disorders, pleuropneumonia, retained placenta, and metritis.[1] Mares exposed to endotoxin during gestation have a 32% to 60% pregnancy loss rate secondary to embryonic resorption or preterm fetal expulsion.[2-5] This rate is 4-fold greater than the 8% to 15% annual rate of pregnancy loss reported for the horse-breeding industry and represents a source of considerable financial loss.[4] Although a correlation between endotoxemia and pregnancy loss exists, the exact role of endotoxin and its mechanism of action with respect to myometrial contractility in horses have not been defined.

Plasma concentrations of endothelin-like immunoreactivity are significantly increased in horses with naturally acquired gastrointestinal tract disease and are greatest in horses with conditions typically associated with signs of endotoxemia, such as strangulating obstruction, inflammatory bowel disease, and peritonitis.[6] Messenger RNA for **endothelin (ET)**-1 has been detected in specimens of human endometrial stroma, umbilical vein, and avascular amnion and rat uterus.[7-10] In vitro studies in humans and primates have revealed that ET-1 causes receptor-mediated vasoconstriction of uterine blood vessels, placental vessels, and vessels of the amnion; prostaglandin $F_{2\alpha}$ synthesis by the endometrium; and tonic contraction of the myometrium in vitro.[10] Results of pharmacologic studies have indicated that there are at least 3 distinct ET receptors. The first type of receptor, designated ET_A receptors, mediates the ET signal to induce vasoconstriction, cellular proliferation, and nonvascular smooth muscle contraction.[10-13] The second type of receptor, designated ET_B receptor, has 2 subtypes, ET_{B1} and ET_{B2}.[10,14-16] The ET_{B1} receptor is primarily expressed on endothelial cells of various tissues and is involved in the release of endothelium-derived vasorelaxing factors such as nitric oxide.[10,14-16] The ET_{B2} receptors are located principally on nonvascular smooth muscle where they mediate contraction.[10,14-16] Both ET_A and ET_B receptors have been identified in the myometrium of nongravid humans, guinea pigs, rabbits, and rats; cultured human myometrial cells; and the myometrium of gravid humans, rats, guinea pigs, and monkeys.[10-12,14,17] More recently, ET_B receptors have been shown to be involved in the elimination of ET-1 by mediating the reuptake of ET-1 by endothelial cells.[18,19] A third type of endothelin receptor, designated ET_C receptors, has recently been suggested, but little is known about this receptor type with regard to uterine contraction.[10,20,21]

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The ET_A receptor and both ET_B receptor subtypes are known to be functionally coupled to phospholipase C, which induces phosphoinositide breakdown via 1 or more G proteins in human and rat myometrium.[11,12,22,23] Through this mechanism, in vitro work has shown that ET-1 effectively increases intracellular concentration of calcium, increases myosin phosphorylation, and stimulates the force and frequency of contractions in human myometrial cells, isolated nongravid and late-gravid rat uterus, and isolated myometrium of nongravid and late-gravid humans.[11,12,22,23] However, the contractile response to ET-1 differs between the circular and longitudinal muscle layers of the uterus in gravid humans, gravid and nongravid rats, and nongravid rabbits.[24-27] The contractile response varies depending on the location within the uterine organ; for example, the response of the corpus differs from that of the isthmus in gravid uteruses of humans and baboons.[28-30]

Overall, little information is available regarding the physiologic and pharmacologic interaction of ET-1 with equine myometrial smooth muscle.[31] Therefore, the purpose of the study reported here was to characterize the in vitro response of the **circular and longitudinal myometrial layers of the uterine horn** (**CMLH** and **LMLH**, respectively) of nongravid horses to ET-1 by use of specific ET_A (BQ-123) and ET_B (IRL-1038) receptor antagonists. On the basis of data in the scientific literature, we hypothesized that ET-1 would cause a concentration-dependent contractile response in equine myometrial smooth muscle, the CMLH would have a greater contractile response to ET-1 than the LMLH, and incubation with BQ-123 and IRL-1038 separately or in combination would inhibit the contractile response of both muscle layers to ET-1.

Materials and Methods

Tissue collection—The study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Ten healthy mares were euthanatized by use of an overdose of pentobarbital sodium[a] (100 mg/kg, IV) for reasons unrelated to reproductive disease; the uterus of each horse was removed. On the basis of examination of the ovaries postmortem, time of year, and serum progesterone concentrations, each mare was determined to be in anestrus at the time of euthanasia. Immediately after euthanasia, each uterus was placed into a dissection tray containing modified Krebs solution aerated with 95% oxygen and 5% carbon dioxide at 37°C. The uterus was opened, the serosa of the uterine horn was dissected free of excess connective and adipose tissue, the endometrium was removed, and tissues of both the LMLH and CMLH were cut to yield 4-mm-wide and 12-mm-long strips. The serosa was left intact to maximize the number of fibers and prevent damage to muscle fibers of the LMLH, as reported by Rigby et al.[31]

Drug and reagent preparation—All solutions were prepared on the day of the study. Endothelin-1,[b] ET_A receptor antagonist (BQ-123),[c] and ET_B receptor antagonist (IRL-1038)[d] were used. The ET receptor antagonists were dissolved 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 at room temperature (20° to 22°C) immediately prior to use and then further diluted to the desired concentration. 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, 20mM (pH, 7.3 to 7.4).

Experimental protocol—One end of each muscle strip was fixed with 5-0 silk suture to the floor of an organ bath. The other side of the strip was attached with 5-0 silk suture to a force-displacement transducer[e] interfaced with a polygraph[f] and chart recorder[g] to measure isometric tension. The tissues were equilibrated for 45 minutes in organ baths containing modified Krebs solution that were maintained at 37°C by a circulating bath and oxygenated with a gas mixture of 95% oxygen and 5% carbon dioxide. During this period, the bath solution was replenished with fresh modified Krebs solution at 15-minute intervals.

On the basis of findings of our preliminary studies involving 8 muscle strips (4 CMLH strips and 4 LMLH strips) from 9 reproductively normal mares not used for the present study, the maximum length-tension relationship was determined by use of the technique described by Rigby et al.[31] As a result, a resting tension of 2.0 g was applied to each tissue group.[31] Tissue groups (CMLH and LMLH) were incubated with respective ET antagonists at 3 bath concentrations (10^{-9}, 10^{-7}, and 10^{-5}M). Three separate experimental runs were conducted (1 run/antagonist concentration) with the antagonist concentration being randomly assigned to each run. After completion of an experimental run, tissues were discarded and the baths were cleaned. New muscle strips cut as described from the same uterus were placed as described into clean tissue baths. The first bath in the group was used as a control and received no ET antagonist, the second bath received BQ-123, the third bath received IRL-1038, and the fourth bath received BQ-123 and IRL-1038. Antagonists were added to the appropriate baths each time the fluid was changed (15-minute intervals) for a total exposure time of 45 minutes. After 45 minutes of antagonist exposure, separate cumulative **concentration-response (CR)** curves for ET-1 were performed by adding graded concentrations of ET-1 to yield a final bath log molar concentration of 10^{-9} to 10^{-6}M at 3-minute intervals. Contractile activity was measured during the 3-minute period after the addition of ET-1. The value measured for the **area under the curve (AUC)** in the 3-minute period preceding each CR curve for ET-1 was considered baseline tension and was subtracted from the AUC measured at every ET-1 concentration to account for spontaneous contractile activity present before the beginning of the CR curve.

Carbachol (final bath concentration of 10^{-4}M) was applied to each muscle strip 6 minutes after the last dose of ET-1 (final bath concentration of 10^{-6}M). Strips that did not contract with the carbachol were discarded. The response to carbachol was considered to be the maximum contraction because of the persistent plateau detected in the tissue groups after carbachol exposure.

Statistical analyses—The contractile response to graded log molar concentrations of ET-1 was quantified by measuring the AUC by use of image analysis software.[g] The contractile responses CMLH specimens to ET-1 after incubation with ET receptor antagonists at 3 separate log molar concentrations (10^{-5}, 10^{-7}, and 10^{-9}M) were compared separately with the response of the tissues without the antagonist (control tissue group). Similar statistical comparisons were made involving LMLH specimens.

By use of a Shapiro-Wilk statistical analysis, the AUC representative of the contractile response was considered continuous and was found to follow a normal distribution with failure to reject the null hypothesis of normality at $P < 0.05$. Data were expressed as mean ± SEM and graphed for each muscle type and location (CMLH or LMLH) and by antagonist concentrations (10^{-9}, 10^{-7}, and 10^{-5}M).

The fixed effect of ET-1 concentration before and the response after antagonist exposure was evaluated for both CMLH and LMLH at 3 antagonist concentrations and antagonist combinations. A mixed-effect linear model was used that also included the random variance of tissue source (horse) and accounted for the repeated measurements of each tissue source. Where there was significant interaction of ET-1 concentration and antagonist type at a value of $P < 0.01$ (a conservative type I error chosen to reduce overall type I error because many models were evaluated), comparisons were made across ET-1 concentrations within each antagonist and between antagonists at different ET-1 concentrations by use of least squares means, while maintaining type I error at 0.05; thus, significant differences were identified if the value of P was < 0.05.

The fixed effect of muscle layer (circular vs longitudinal) on the contractile response was evaluated at each antagonist concentration, for each antagonist type, and for ET-1 concentration by use of a mixed-effect linear model that also included the random variance of tissue source (horse) and accounted for the repeated measurements of each tissue source. Where there was significant interaction of muscle layer at a value of $P < 0.01$ (a conservative type I error was chosen to reduce overall type I error because many models were evaluated), comparisons were made across muscle layers and ET-1 concentrations by use of least square means, maintaining type I error at 0.05; thus, significant differences were identified if the value of P was < 0.05. Analyses were performed by use of computer software.[h]

Results

Our preliminary studies (involving tissues from 9 reproductively normal mares not used for the present study) to determine the appropriate tension for reproducible responses to ET-1 revealed that an initial tension of 2.0 g was optimum to elicit a consistent contractile pattern to ET-1. Therefore, in the present study, the initial tension for muscle strips from both the CMLH and LMLH was applied at 2.0 g. This optimum tension also allowed us to record spontaneous activities of these muscles. The spontaneous activity of the CMLH smooth muscle was cyclic and occurred at a mean rate of 1 contraction every 11 minutes, whereas that of the LMLH smooth muscle was cyclic and occurred at a mean rate of 1 contraction every 8 minutes. The spontaneous activity was random and brief during the generation of the CR curve and did not occur in every horse. Additionally, the activity never occurred more than once during any CR curve for an individual horse and never at the higher ET-1 concentrations. Because of the random and brief nature of the activity, the effect of the spontaneous activity occurring during the CR curve was considered to be negligible with regard to the mean AUC (n = 10) reported for each tissue group at each ET-1 concentration. Endothelin-1 caused a concentration-dependent contractile response of both the CMLH and LMLH, indicated by a dose-dependent increase in AUC along with an increase in amplitude of contractions. Significant differences in the contractile response to ET-1 and the ET antagonists were detected between the CMLH and LMLH specimens, compared with the response of their specific controls. Direct comparisons between CMLH and LMLH tissues were not made.

Responses of CMLH tissue—The muscle strips had a concentration-dependent contractile response to graded concentrations of ET-1 (10^{-9} to 10^{-6}M). Incubation with either the ET_A or ET_B receptor antagonist alone or in combination (at concentrations of 10^{-9} or 10^{-7}M) did not yield any significant change in the contractile response to ET-1, compared with the control specimen (no antagonist). When the concentration of ET_A receptor antagonist was increased to 10^{-5}M at ET-1 concentrations of 10^{-8} and 10^{-7}M, the contractile response of the muscle strips to ET-1 was significantly decreased, compared with that of the control specimen (**Figure 1**). The contractile response of the muscle

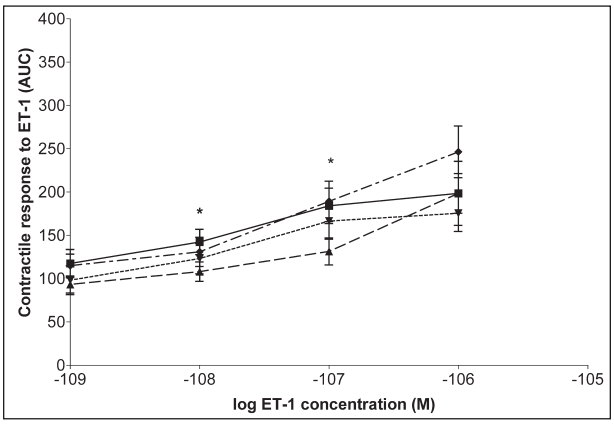

Figure 1—Concentration-response curves for specimens of the circular smooth muscle layer of the uterine horn (CMLH) obtained from 10 nongravid mares after specimens were incubated for 45-minute intervals in the absence of an endothelin (ET)-1 receptor antagonist (squares), in the presence of an ET_A receptor antagonist (BQ-123, 10^{-5}M; triangles), in the presence of an ET_B receptor antagonist (IRL-1038, 10^{-5}M; inverted triangles), and in the combined presence of BQ-123 and IRL-1038 (10^{-5}M; diamonds) and exposed to graded concentrations of ET-1 (10^{-9} to 10^{-6}M). Contractile response is indicated as the area under the curve (AUC); values are reported as mean ± SEM. *Contractile response of specimens treated with BQ-123 was significantly ($P < 0.05$) different from that of control specimens at this ET-1 concentration.

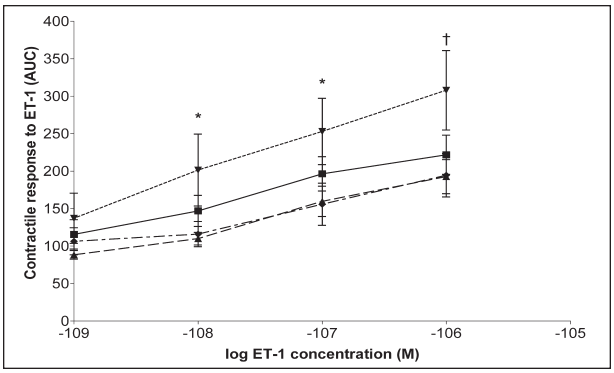

Figure 2—Concentration-response curves for species of the longitudinal smooth muscle layer of the uterine horn (LMLH) obtained from 10 nongravid mares after specimens were incubated for 45-minute intervals in the absence of an ET-1 receptor antagonist (squares), in the presence of an ET_A receptor antagonist (BQ-123, 10^{-5}M; triangles), in the presence of an ET_B receptor antagonist (IRL-1038, 10^{-5}M; inverted triangles), and in the combined presence of BQ-123 and IRL-1038 (10^{-5}M; diamonds) and exposed to graded concentrations of ET-1 (10^{-9} to 10^{-6}M). *Contractile response of specimens treated with IRL-1038 was significantly ($P < 0.05$) different from that of control specimens at this ET-1 concentration. †Contractile response of specimens treated with BQ-123 was significantly ($P < 0.05$) different from that of control specimens at this ET-1 concentration. *See* Figure 1 for remainder of key.

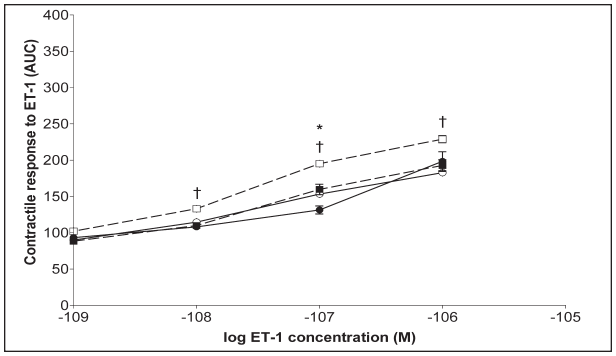

Figure 3—Concentration-response curves for specimens of the LMLH (squares) and the CMLH (circles) obtained from 10 nongravid horses after specimens were exposed to graded concentrations of ET-1 (10^{-9} to 10^{-6}M) in the absence of ET-1 receptor antagonists (control results are the pooled mean ± SEM for 24 tissue samples; open symbols) and in the presence of an ET_A receptor antagonist (BQ-123, 10^{-5}M; closed symbols). *Contractile response of LMLH specimens treated with BQ-123 significantly ($P < 0.05$) different from that of pooled LMLH control specimens at this ET-1 concentration. †Contractile response of CMLH specimens treated with BQ-123 significantly ($P < 0.05$) different from that of pooled CMLH control specimens at this ET-1 concentration. *See* Figure 1 for remainder of key.

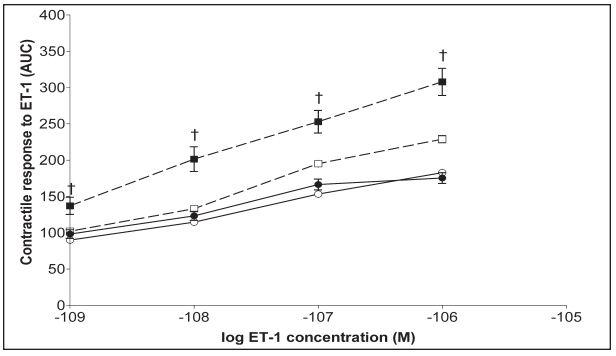

Figure 4—Concentration-response curves for specimens of the LMLH (squares) and the CMLH (circles) obtained from 10 nongravid horses after specimens were exposed to graded concentrations of ET-1 (10^{-9} to 10^{-6}M) in the absence of ET-1 receptor antagonists (control results are the pooled mean ± SEM for 24 tissue samples; open symbols) and in the presence of an ET_B receptor antagonist (IRL-1038, 10^{-5}M; closed symbols). †Contractile response of LMLH specimens treated with IRL-1038 significantly ($P < 0.05$) different from that of pooled LMLH control specimens at this ET-1 concentration. *See* Figure 1 for remainder of key.

strips to ET-1 was not significantly different from that of the control specimen when the concentration of ET_B receptor antagonist was increased to 10^{-5}M. Incubation with the ET_A and ET_B receptor antagonists in combination did not significantly alter the contractile response of the CMLH strips to ET-1, compared with that of the control.

Responses of LMLH tissue—The muscle strips had a concentration-dependent contractile response to graded concentrations of ET-1 (10^{-9} to 10^{-6}M). Incubation with either ET_A or ET_B receptor antagonist alone or in combination (at concentrations of 10^{-9} or 10^{-7}M) did not yield any significant change in contractile response to ET-1, compared with that of the control specimen (no antagonist). When the concentration of ET_A receptor antagonist was increased to 10^{-5}M at ET-1 concentrations of 10^{-8} and 10^{-7}M, the contractile response of the muscle strips to ET-1 was significantly decreased, compared with that of the control specimen (**Figure 2**). When the concentration of ET_B receptor antagonist was increased to 10^{-5}M, the contractile response of the muscle strips to ET-1 at 10^{-6}M was significantly increased, compared with that of the control specimen. Incubation with the ET_A and ET_B receptor antagonists in combination did not significantly alter the contractile response of the LMLH strips to ET-1, compared with that of the control.

Responses of CMLH and LMLH tissues with respect to pooled controls—The contractile responses (ie, AUCs) of the LMLH and CMLH control specimens (n = 24) were pooled for each ET-1 concentration (10^{-9} to 10^{-6}M). Application of the ET_A receptor antagonist at a concentration of 10^{-5}M significantly decreased the contractile response of LMLH to ET-1 at concentrations of 10^{-8}, 10^{-7}, and 10^{-6}M and significantly decreased the contractile response of CMLH to ET-1 at a concentration of 10^{-7}M, compared with responses of their pooled controls (**Figure 3**). Application of ET_B receptor antagonist at a concentration of 10^{-5}M significantly increased the contractile response of LMLH to ET-1 at concentrations of 10^{-8}, 10^{-7}, and 10^{-6}M and had no significant effect on CMLH, compared with responses of their pooled controls (**Figure 4**). Application of ET_A and ET_B receptor antagonists in combination at a concentration of 10^{-5}M significantly decreased the contractile response of LMLH and significantly increased the contractile response of CMLH to ET-1 at concentrations of 10^{-8}, 10^{-7}, and 10^{-6}M, compared with responses of their pooled controls (**Figure 5**).

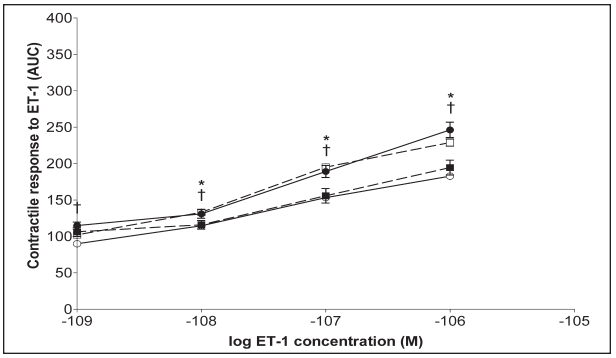

Figure 5—Concentration-response curves for specimens of the LMLH (squares) and the CMLH (circles) obtained from 10 nongravid horses after specimens were exposed to graded concentrations of ET-1 (10^{-9} to 10^{-6}M) in the absence of ET-1 receptor antagonists (control results are the pooled mean ± M; closed symbols). *Contractile response of LMLH specimens treated with BQ-123 and IRL-1038 is significantly ($P < 0.05$) different from that of pooled LMLH control specimens at this ET-1 concentration. †Contractile response of CMLH specimens treated with BQ-123 and IRL -1038 is significantly ($P < 0.05$) is different from that of pooled CMLH control specimens at this ET-1 concentration. *See* Figure 1 for remainder of key.

Discussion

The findings of the present study have provided important physiologic and pharmacologic information regarding the contractile response of the equine nongravid uterine horn to ET-1 and the role of ET receptors in mediating the response to these tissues. Our

data indicate that ET-1 is a potent contractile agent of the CMLH and LMLH in nongravid horses; the results obtained through application of ET_A receptor antagonists to muscle specimens support the existence of ET_A receptors within the CMLH and LMLH in nongravid horses. Furthermore, results obtained through application of ET_B receptor antagonists to muscle specimens support the existence of ET_B receptors within the CMLH and LMLH in nongravid horses. Whereas ET_A receptors mediate contraction in the smooth muscle of the equine nongravid uterine horn, the role of ET_B receptors in that tissue remains unclear. Additionally, the increased contractile response to ET-1 of the CMLH when exposed to ET_A and ET_B receptor antagonists in combination, compared with responses of their pooled controls, suggests the existence of another receptor or a subtype of ET_A for which BQ-123 is not specific or does not block.

Endothelin is a potent contractile agent of the CMLH and LMLH of horses. Endothelin is a ubiquitous peptide that is locally produced and released by the endometrium of rats, humans, and rabbits under the influence of oxytocin and uterine steroids.[7,8,10,12] Endothelin-1 has also been shown to be synthesized by the corpus luteum of rats, the endometrium and placenta of humans, and uterine tissues and cells of rats and humans.[7,8,10,12,19,32] All smooth muscle elements of the gravid and nongravid human uterus and placenta have specific binding sites for ET-1, which are involved in the control of myometrial contractile activity.[21,33] Endothelin-1 is considered to be the most potent oxytocic agent in isolated specimens of nongravid and gravid rat uterus and is an important regulator of myometrial activity.[25,32] Similar to findings of the present study, ET-1 is known to induce concentration-dependent contraction that results in increased contraction frequency and basal tone in gravid and nongravid rat uterine tissues, the myometrium (in culture) of nongravid humans, the uterus of gravid and nongravid humans, and specimens of myometrium of gravid rabbits and nongravid rabbits and guinea pigs.[7,11,21,22,34]

In the present study, we chose to separate the smooth muscle of the LMLH and CMLH and study the response of each layer individually because each layer has a different embryologic origin with different pharmacologic properties.[10,24,26,35] The LMLH originates from subserosal connective tissue, and the CMLH develops from the paramesonephric ducts.[10,22,25] Differences in embryologic origin coupled with receptor heterogeneity and sensitivity may account for the differences in electrical, mechanical, and pharmacologic behavior between the CMLH and LMLH.[10,23,24] These differences are most prominent in nongravid rat uterus, where no coordination exists between the layers and regulation of uterine contraction is achieved primarily by the circular layer.[10,24,25,35] When surgically separated, the uterine smooth muscle layers each develop their own pacemaker for action potential generation.[24,25,27,35] In the present study, we identified differences in the rate and pattern of spontaneous contractions between the CMLH and LMLH, as well as differences in the response to ET-1 in the presence of ET_A and ET_B receptor antagonists, which provide further evidence to support physiologic and pharmacologic differences between the layers, as detected in other species.

Receptor antagonist BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]) is a purely competitive antagonist that selectively and completely inhibits 90.7 ± 1.4% of the myometrial response to ET-1 by displacing the contractile agent from the ET_A receptor.[12,22,32] Receptor antagonist BQ-123 at a concentration of 5×10^{-6}M causes dose-dependent inhibition of ET-1–induced inositol phosphate accumulation in the myometrium of gravid and nongravid rats.[21,22] The selective blockade of ET_A receptors with BQ-123 at a concentration of 10^{-5}M, indicated by a decrease in the contractile response of the CMLH and LMLH to graded concentrations of ET-1, supports the existence of ET_A receptors within these myometrial layers. The increased contractile response to ET-1 detected in specimens of the LMLH when ET_B receptors were selectively blocked by application of IRL-1038 further supports the existence of ET_A receptors because when ET_B receptors are blocked, they cannot bind ET-1; therefore, more ET-1 is available to ET_A receptors, resulting in an enhanced contractile response.

In our study, compared with the control, the contractile response to ET-1 increased in the LMLH when ET_B receptors were selectively blocked by application of IRL-1038 and in the CMLH when ET_A and ET_B receptors were both selectively blocked by application of the BQ-123 and IRL-1038 combination; these data support the existence of ET_B receptors within both smooth muscle layers. It is known that ET_B receptors mediate the clearance of circulating ET-1 by binding ET-1 for reuptake by endothelial cells.[18,19] Blockade of ET_B receptors results in enhanced ET-1 activity through the inhibition of ET_B-mediated clearance, causing more ET-1 to be available to ET receptors. If ET_B receptors were not present, then the application of IRL-1038 would have no significant effect. However, ET_B receptor blockade caused an enhanced contractile response to LMLH, possibly indicating that more ET-1 was available to unblocked ET_A receptors as a result of this blockade. The presence of ET_B receptors in uterine smooth muscle is further supported by the fact that mRNA for ET_B receptors has been detected in specimens of nongravid rat uterus, human endometrium, and gravid and nongravid human uterus.[8,13,21,39] The exact role of ET_B receptors in the smooth muscle of the uterine horn of nongravid horses remains unclear. The response of the equine CMLH to selective blockade of ET_B receptors with IRL-1038 suggests that ET_B receptors may not play an important role in the contractile response to ET-1 of the CMLH of nongravid horses. However, the response of the equine LMLH to selective blockade of ET_B receptors with IRL-1038 supports an elimination role for ET_B receptors in the LMLH of nongravid horses.

When we compared the contractile responses of the equine CMLH and LMLH strips in the presence of specific receptor antagonists alone and in combination with respect to their individual pooled controls, it appeared that both muscle layers have ET_A and ET_B receptors. The results support a dominant role for ET_A receptors in medi-

ating ET-1–induced contractile activity in the nongravid equine uterine horn. It has been shown that 75% of the ET-binding sites in the myometrium of nongravid humans have high affinity for ET-1 (ET_A or ET_B receptors) and that ET-1 had 4 times as many binding sites as ET-3 (specific for ET_B and ET_C receptors).[21] The preponderance of ET_A receptor subtypes in human myometrium is similar to findings in other species in that the concentration of ET_A receptors is 9 times as great as that of ET_B receptors in rabbit myometrium and in the preferential expression of ET_A receptor mRNA in the rat uterus.[40] The ET_A receptor has been shown to be 6 times as concentrated as the ET_B receptor in the human uterus.[7] Thus, the dominance of ET_A receptors in the myometrium is well documented in other species; our findings in specimens of equine CMLH and LMLH support these contentions.

Combined use of BQ-123 and IRL-1038 at a concentration of 10^{-5}M significantly decreased the LMLH contractile response to ET-1 and significantly increased the CMLH response to ET-1, compared with responses of pooled control specimens. The response of the LMLH was consistent with the response to ET_A receptor antagonist alone at a concentration of 10^{-5}M. Such a response not only supports the existence of ET_A receptors within the LMLH but also suggests their dominant role in mediating ET-1 induced contraction. The response of the CMLH was not consistent with the response to either ET_A or ET_B receptor antagonist alone at a concentration of 10^{-5}M. Such a response supports the possible existence of another ET receptor or receptor subtype for which neither antagonist is specific. Additionally, it may also suggest that ET_B receptors are present in the CMLH; when blocked, these receptors make more ET-1 available to unblocked receptors within the CMLH.[38] A variable response to BQ-123 was reported for the bovine coronary artery in which BQ-123 at a concentration of 3×10^{-7}M antagonized contractions initiated by ET at concentrations of 10^{-10} and 10^{-9}M but potentiated contractions initiated by ET at concentrations of 3×10^{-8}M and 3×10^{-7}M.[17] The binding of ET-1 to ET_A and ET_B receptors is highly specific (75% to 92% for ET_A and 95% to 98% for ET_B) and highly saturable and expresses high affinity; however, variations in the ratio of ET_A to ET_B receptor expression in the equine myometrium may also account for the contradictory response in the specimens of CMLH.[41]

To date, 2 ET_A receptor subtypes have been detected in human myometrial cells in culture.[11] One subtype has high affinity for ET-1, does not bind BQ-123, and represents 58% of the ET_A receptors in cultured human myometrial cells.[11] The other receptor subtype expresses low affinity for ET-1, binds BQ-123, and represents 42% of the ET_A receptors in cultured human myometrial cells.[11] With regard to the existence of another ET-1 receptor, results of pharmacologic studies support the existence of 3 distinct ET receptors that are responsible for mediating the biological effects of ET peptides.[41] Amino acid homology, conservation of structural motifs, and coupling to second messenger systems are similar among ET_A, ET_B, and ET_C receptors.[18] The greatest difference among these ET receptor subtypes lies in the extracellular domains, which are involved in selective ligand binding and activation and account for the rank order of potencies for individual ET peptides.[41] Given the facts that the ET_C receptor is 200 times as responsive to ET-3 as it is to ET-1 or ET-2 and the ET_A receptor is the predominant receptor in the uterine tissues of most species, the response to ET-1 observed for the equine myometrial smooth muscle is unlikely to be a result of the presence of ET_C receptors and more likely a result of the presence of an ET_A receptor subtype. However, the fact that the contractile response of equine CMLH specimens was reversed in response to ET-1 (10^{-9} to 10^{-6}M) in the presence of ET_A and ET_B receptor antagonists at a concentration of 10^{-5}M supports the suggestion that a receptor subtype of ET_A is present in the equine CMLH.

In the present study, the concentration-dependent contractile effect of ET-1 on the LMLH and CMLH of nongravid horses has been determined. By use of ET_A and ET_B receptor antagonists, we have provided evidence to support the presence of ET_A and ET_B receptors within the myometrial smooth muscle of the equine uterus and identified a significant difference in pharmacologic behavior between CMLH and LMLH in nongravid horses. We have also raised a question with respect to the existence of another ET-1 receptor or receptor subtype within the equine myometrium. Further work is needed to characterize ET receptors within the equine myometrium, define the role of ET-1 in uterine pathophysiology, and determine the role of ET receptors in preterm fetal expulsion secondary to endotoxemia in 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. Model 7D polygraph, Grass Instruments, Quincy, Mass.
f. Chart recorder model 25-60, Grass Instruments, Quincy, Mass.
g. Sigma Scan Pro 4.0, Jandel Scientific Software, San Rafael, Calif.
h. SAS, version 8.2, SAS Systems Inc, Cary, NC.

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