Characterization and comparison of the responses of equine digital arteries and veins to endothelin-1

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Objective—To compare the responses of equine digital arteries (EDAs) and equine digital veins (EDVs) to endothelin-1 (ET-1) and determine the role of the endothelium and type of receptors involved in the modulation and mediation of those responses, respectively.

Sample Population—5 to 9 palmar digital vessels/experiment from 28 healthy horses.

Procedure—Rings of dissected vessels were mounted under tension between force transducer wires in organ baths containing Krebs-Henseleit solution at 30°C. Responses of EDAs and EDVs (with intact [+e] or denuded [–e] endothelium) to cumulative concentrations of ET-1 (10^{-10} to 3×10^{-7} M) were compared. For (+e)EDAs and (+e)EDVs precontracted with a thromboxane-mimetic (U44069; 10^{-8} M) and (–e)EDAs and (–e)EDVs, responses to an ET_B receptor agonist (S6c; 10^{-10} to 3×10^{-7} M) were evaluated. Responses to ET-1 (10^{-7} M) in (–e)EDAs and (–e)EDVs were evaluated after incubation with an ET_A receptor antagonist (BQ-123; 3×10^{-7} M), an ET_B receptor antagonist (BQ-788; 3×10^{-7} M), or vehicle solution.

Results—Endothelin-1 induced a concentration-dependent contraction of endothelium-intact and -denuded EDAs and EDVs; EDVs were more sensitive. Neither vessel type relaxed in response to S6c, although 2 of the (–e)EDAs contracted mildly. Whereas BQ-123 inhibited the (–e)EDA and (–e)EDV responses to ET-1, BQ-788 had no effect.

Conclusions and Clinical Relevance—Endothelin-1 induced digital vasoconstriction (marked constriction in veins). This action was unaffected by endothelium and mediated predominantly by ET_A receptors. These findings suggest ET-1 can induce selective digital venoconstriction. (*Am J Vet Res* 2003;64:1438–1443

Although the pathophysiologic characteristics of equine acute laminitis are unknown, results of most research studies[1-6] indicate involvement of some vascular anomaly that results in decreased blood flow, ischemia, and reperfusion injury to the dorsal laminar tissue. Increased postcapillary resistance associated with digital venoconstriction has been proposed to be the initiating factor in the development of decreased blood flow and onset of clinical signs of acute laminitis.[1,7] **Endothelins (ETs)** are a family of 21-amino acid vasoactive peptides; this group includes ET-1, ET-2, ET-3, and vasoactive intestinal contractor.[8] There are 2 types of G-protein-coupled ET receptors classified as ET_A and ET_B receptors.[9,10] The ET_A receptors are expressed on vascular smooth muscle cells, and their stimulation results in vasoconstriction. The ET_B receptors are found on vascular endothelial and smooth muscle cells; on endothelial cells, these receptors mediate vasodilation through the release of **nitric oxide (NO)**, and on smooth muscle cells, they mediate vasoconstriction.[11]

At present, ET-1 is the most potent endogenous vasoconstrictor that has been identified[11-13] and is implicated in a number of diseases, including cardiovascular and pulmonary disease, renal hypertension, and cerebral ischemia.[14,15] Endothelin-1 is the only ET produced by vascular endothelial cells, although it is also produced by the cells of other tissues, including brain, kidney, intestine, and adrenal glands.[16-18] It is believed to be continuously released in low concentrations via a constitutive pathway to interact with endothelial-derived relaxing factors, such as NO, and maintain normal local vascular flow.[19,20] Release of ET-1 in high concentrations via a regulated pathway is induced by factors such as activated platelets, endotoxin, thrombin, various cytokines and hormones, hypoxia, and ischemia.[12,20] Endothelin-1 is a potent vasoconstrictor of both arteries and veins in various vascular beds and raises pulmonary capillary pressure in rats through selective venoconstriction.[21] It has been suggested that venoconstriction may be a key event in the developmental phase of acute equine laminitis.[1] The expression of ET-1 is increased in connective tissue of hooves of horses with laminitis, compared with that in similar samples obtained from healthy horses.[17] Additionally, plasma concentrations of ET-1 are increased in horses with naturally acquired gastrointestinal disease[22] (which often leads to the onset of acute laminitis[6,23,24]) and those with experimentally induced laminitis.[a] We hypothesized that ET-1 would constrict **equine digital veins (EDVs)** more effectively than it would constrict **equine digital arteries (EDAs)**; such selective vasoconstriction of the venous side of the digital circulation could be an important mediator in the pathogenesis of acute laminitis. The purpose of the study reported here was to compare the responses of EDAs and EDVs to ET-1 and determine the role of the endothelium and type of receptors involved in the modulation and mediation of those responses, respectively.

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Materials and Methods

Animals—The EDAs and EDVs were collected from the forelimbs of 28 mixed-breed healthy adult horses euthanatized at an abattoir. The method of euthanasia involved stunning by use of a captive bolt followed by exsanguination and was conducted according to European Union regulations under the supervision of a veterinarian. Forelimbs were removed within 10 minutes of euthanasia. The limbs and feet were examined for gross abnormalities before tissue collection; any limbs with evidence of gross abnormalities were not included in the study. After removal of each forelimb, the digital artery was cannulated at the level of the metacarpophalangeal joint, and 180 mL of ice-cold modified Krebs-Henseleit solution (Krebs solution) was infused through a 16-gauge, 52-mm catheter.[b] The skin was then reflected to expose the digital artery and coronary venous plexus that were dissected from the limb and placed in ice-cold Krebs solution and transported to the laboratory. The vessels were cleared of connective tissue and cut into rings (3 to 4 mm long). Vessel rings that were not used immediately were stored in oxygenated Krebs solution at 4°C for 18 to 24 hours prior to use.

Experimental procedure—To remove the endothelium of selected rings, the intimal surface of the vessel was gently rubbed with a wooden cocktail stick[25]; the effectiveness of this technique has been confirmed microscopically and functionally.[26] Each vessel ring was placed between 2 parallel wires in a 10-mL organ bath containing Krebs solution that was aerated with 95% oxygen and 5% carbon dioxide and maintained at 30°C. One of the wires was fixed, and the other was connected to an isometric force transducer[c]; the signal was fed to a 2-channel pen recorder[d] via a bridge amplifier.[e] For each of the experiments, a basal resting tension of 3 and 2 × g was applied to arteries and veins, respectively, with an equilibration period of at least 1 hour before the onset of the experiment. These resting tensions had been determined to be optimal[27,28] because of their similarity to diastolic vascular tone measured in vivo; furthermore, the greatest degree of contraction could be obtained at these values. Six vessel rings could be examined at 1 time; pairs of vessels obtained from 1 horse could be compared simultaneously when direct comparison was required in an experiment. One cumulative concentration response curve was obtained per vessel ring. In experiments involving ET_A and ET_B antagonists, adjacent vessel rings from the same horse were used as the vehicle control and treated vessels.

All vessels were constricted initially with **depolarizing Krebs solution (DKS)** that contained 118 mM potassium chloride to evaluate smooth muscle function. At their peak tension, vessels were washed with Krebs solution to return the tension to baseline values; if they constricted to < 50% of their resting tension, the vessels were discarded. After the vessels returned to baseline tension readings, a thromboxane-mimetic U44069[f] (9, 11-dideoxy-9α, 11α-epoxymethano-prostaglandin $F_{2\alpha}$; 30 nM) was added to the organ bath to constrict the blood vessels. This drug was chosen because it induced stable increases in vessel tone against which vasodilators could be assessed. Once the increase in tension had stabilized, carbachol[f] was added either in a cumulative (10^{-8} to 10^{-5} M) or single concentration (1 μM) to confirm the presence or absence of a functional endothelium. Cumulative concentrations were used in the experiments to evaluate EDV and EDA responses to ET-1 and assess the effect of the endothelium on those digital vascular responses; single concentrations were used in the experiments to evaluate EDV and EDA responses to an ET_B agonist and assess the effect of an ET_A antagonist, an ET_B antagonist, or vehicle solution on those digital vascular responses to ET-1. Carbachol activates cholinoceptors on the endothelium, which results in the release of NO and relaxation of the U44069-induced tone (ie, reduction in tension readings). For the EDAs and EDVs, the presence of functional endothelium was confirmed if the U44069-induced tension reading was changed by at least 40 and 50%, respectively, in response to 1μM of carbachol. The endothelium was considered adequately denuded if tension readings in U44069-constricted vessels changed by < 10% in response to carbachol treatment. It had been previously determined that the concentration of U44069 used induced approximately 80% of the maximum increase in tension that could be induced by U44069 in equine tissue of this size.[26] The concentration of carbachol used had also been shown to cause maximal inhibition of the U44069-induced tone in this size and type of tissue; further increases in the concentration of carbachol had been found to cause an increase in tension in some tissues.[26] All vessels were then washed with drug-free Krebs solution to return them to baseline tension. At this time, the ET_A or ET_B antagonist (BQ-123[g] or BQ-788,[h] respectively) or vehicle (distilled water or ethanol for BQ-123 and BQ-788, respectively, if appropriate for the experiment) was added to the organ baths. The tissue and antagonist or vehicle were incubated for 30 minutes prior to addition of the agonist. The concentrations of BQ-123 and BQ-788 used were selected on the basis of results of a pilot study conducted in our laboratory in which the responses of endothelium-denuded (–e)EDAs and (–e)EDVs (n = 2; preconstricted with a submaximal concentration of ET-1 [10^{-7} M]) to cumulative concentrations of BQ-123 or BQ-788 (10^{-8} to 3×10^{-6} M) or vehicle consisting of distilled water were evaluated. For BQ-123, a concentration-dependent reduction of ET-1 induced tone was obtained and at 3×10^{-7}M (the concentration chosen for the main study); BQ-123 reduced ET-1 induced tone by 80 to 100% of the maximum reduction seen with this compound. Because BQ-788 failed to cause relaxation of the ET-1 preconstricted tissues over the entire range of concentrations tested, the same concentration as that selected for BQ-123 was chosen for the main study.

Comparison of the responses to ET-1—Initially, **cumulative response curves (CRCs)** were obtained to ET-1[f] (10^{-10} to 3×10^{-7} M; final bath concentration) for paired endothelium-intact (+e)EDAs and EDVs (n = 6). The increase in tension was expressed as a percentage of the DKS response in that same vessel ring and plotted against $\log_{10}$ ET-1 concentration.

The effect of the endothelium on the digital vascular responses to ET-1—The responses of paired (+e)EDAs and (–e)EDAs (n = 9) and (+e)EDVs and (–e)EDVs (9) to cumulative CRCs of ET-1 (10^{-10} to 3×10^{-7} M; final bath concentration) were then compared.

Comparison of the responses to an ET_B agonist—Cumulative CRCs were obtained to an ET_B agonist (**sarafotoxin**[f] [**S6c**]; 10^{-10} to 3×10^{-7} M) for paired (–e)EDAs and (–e)EDVs (n = 6). The CRCs for S6c (10^{-10} to 3×10^{-7} M) were also obtained in (+e)EDAs and (+e)EDVs (n = 6) that had been preconstricted with U44069.

The effect of an ET_A antagonist, an ET_B antagonist, or vehicle solution on the digital vessels' response to ET-1—Responses of unpaired (–e)EDA (n = 5) or (–e)EDV (7) to a single concentration of ET-1 (10^{-7} M) were obtained for vessels incubated with either an ET_A antagonist (BQ-123; 3×10^{-7} M), an ET_B antagonist (BQ-788; 3×10^{-7} M), or a vehicle (ethanol) 30 minutes prior to the addition of ET-1.

Statistical analyses—Data were expressed as an increase in tension (percentage of the DKS-induced tone)

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and plotted against log agonist (ET-1) concentration. Cumulative concentration response curves were fitted by means of a computerized nonlinear curve-fitting program.[i] The logistic equation used to fit the curves was:

$$\text{Increase in tension} = E_{max} \cdot D^n/(D^n + EC_{50}^{\,n})$$

where E_{max} represents the maximum response, D is the concentration of drug used, n is the Hill slope (a measure of the gradient of the relationship between concentration and response) of the response curve, and EC_{50} is the concentration of the drug at which the response was half-maximal. The EC_{50} values were expressed as geometric means with 95% **confidence intervals (CIs)**; E_{max} values were expressed as arithmetic means ± SEM. The number of horses from which the vessel segments were derived was expressed as n. The EC_{50} and E_{max} values were compared with either a paired Student t test[h] or an ANOVA and a Bonferroni t test.[j] Values of $P \leq 0.05$ were considered significant.

Results

Comparison of the responses to ET-1—The cumulative addition of ET-1 caused a concentration-dependent contraction of (+e)EDAs and (+e)EDVs (**Fig 1**). In both vessel groups, there was a noticeable latent period before the onset of a prolonged contraction. There were no significant differences between E_{max} values of (+e)EDAs and (+e)EDVs (E_{max} value, 124.0 ± 9.9% and 199.0 ± 41.3% DKS, respectively), but the EC_{50} values were significantly ($P = 0.018$) different. Endothelin-1 was a more potent vasoconstrictor of (+e)EDVs (mean EC_{50} value, 1.35×10^{-8}; 95% CI, 0.77 to 1.92×10^{-8}) than (+e)EDAs (mean EC_{50} value, 3.67×10^{-8}; 95% CI, 2.43 to 4.92×10^{-8}).

The effect of the endothelium on the digital vascular responses to ET-1—Responses to ET-1 of (–e)EDAs and (–e)EDVs were not significantly different from those of (+e)EDAs and (+e)EDVs, respectively (**Fig 2 and 3**).

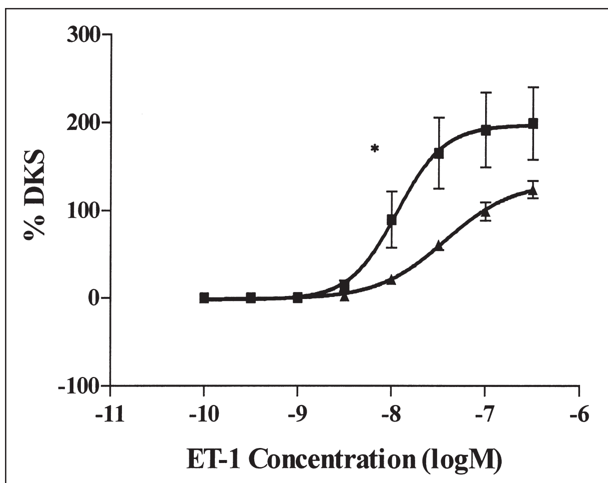

Figure 1—Cumulative concentration response curves to endothelin-1 (ET-1) of 6 paired equine digital arteries (EDAs; triangles) and veins (EDVs; squares) in which the endothelium is intact (+e) and expressed as percentage of vessel response to depolarizing Krebs solution (DKS). Each datum point represents mean value ± SEM. *Significant ($P \leq 0.05$) difference in EC_{50} values between (+e)EDAs and (+e)EDVs (see text for EC_{50} values of these concentration response curves).

Comparison of the responses to an ET_B agonist—The cumulative addition of S6c to paired (+e)EDAs and (+e)EDVs that had been preconstricted with U44069 induced no relaxation in either group, whereas the addition of carbachol to the same vessels caused relaxation of 60.1 ± 6.2% and 110.7 ± 8.4% in the EDAs and EDVs, respectively. The (–e)EDVs had almost no constrictive response to cumulative addition of S6c (E_{max} value, 5.7 ± 1.8% DKS), whereas E_{max} values for 2 of the 6 (–e)EDAs evaluated were 101.9% and 40.6% DKS, respectively. The S6c-induced constrictive responses of the remaining 4 (–e)EDAs were minimal (mean E_{max} value, 9.7 ± 1.9% DKS). There was no significant difference between E_{max} values of (–e)EDAs and (–e)EDVs.

The effect of an ET_A antagonist, an ET_B antagonist, or vehicle solution on the digital vessels' response to ET-1—The ET_A antagonist BQ-123 completely inhibited the responses of (–e)EDAs to ET-1,

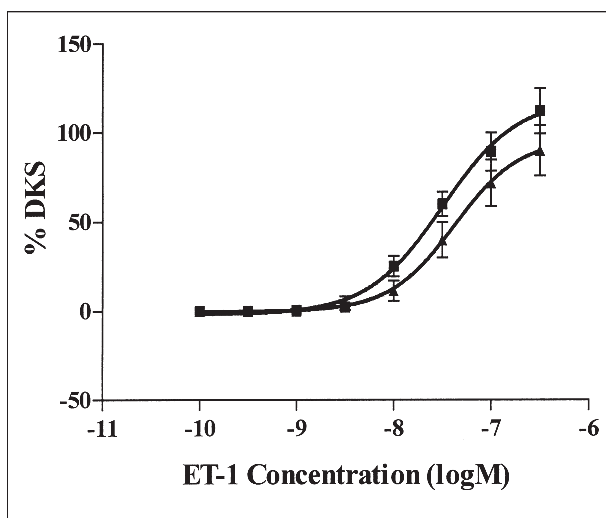

Figure 2—Cumulative concentration response curves to ET-1 of 9 paired (+e)EDAs (squares) and endothelium-denuded (–e) EDAs (triangles) expressed as percentage of vessel response to DKS. Each datum point represents mean value ± SEM.

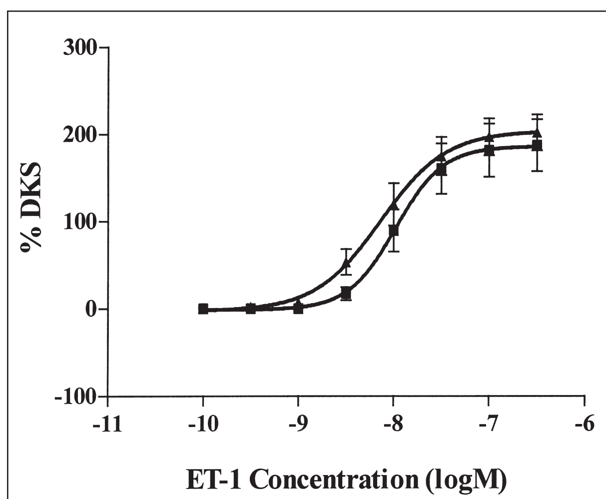

Figure 3—Cumulative concentration response curves to ET-1 of 9 paired (+e)EDVs (squares) and (–e)EDVs (triangles) expressed as percentage of vessel response to DKS. Each datum point represents mean value ± SEM.

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but there was no significant difference between the constrictive responses of the (–e)EDAs that had been pretreated with the ET_B antagonist BQ-788 (E_{max} value, 56.7 ± 21.9% DKS) and those of the (–e)EDAs that had been pretreated with vehicle solution (E_{max} value, 35.6 ± 10.9% DKS). The responses of (–e)EDVs to ET-1 were significantly inhibited by BQ-123 (E_{max} value, 8.9 ± 6.9% DKS), compared with those of the vehicle-treated (–e)EDV group ($P = 0.001$) and the group incubated with BQ-788 ($P = 0.005$). The responses to ET-1 were not significantly different between (–e)EDVs that had been pretreated with vehicle solution (E_{max} value, 106.9 ± 11.5% DKS) and those in (–e)EDVs that had been pretreated with BQ-788 (E_{max} value, 117.2 ± 19.7% DKS).

Discussion

The release of ET-1 from endothelial cells was detected initially in cultured porcine endothelial cells[16] and has since been shown to be released from cultured equine pulmonary artery endothelial cells.[29] Endothelin-1 induces vasoconstriction in equine vessels such as colonic arteries and veins[30] and pulmonary arteries.[k] In the study reported here, ET-1 induced concentration-dependent vasoconstriction of both EDAs and EDVs in vitro. The EDVs were significantly more sensitive to ET-1 than were EDAs, with a shorter latent period before the onset of contraction (data not shown). To the authors' knowledge, there has been only 1 other study[31] to investigate the effects of ET-1 on equine digital vasculature in vitro, in which the responses of arteries and veins from normal and endotoxin-infused horses were evaluated.[31] In that study, ET-1 was observed to cause a profound and prolonged concentration-dependent contraction of both arteries and veins, in which there was a latent period prior to constriction. Those data[31] indicated that compared with arteries, there was a trend for the veins to be more sensitive and have greater constrictive responses to ET-1, although this was not significant. In further support of the results of our study, it has been reported that infusion of ET-1 at increasing concentrations into the digital circulation of conscious healthy horses results in a dose-dependent decrease in blood flow.[l] The reason for the increased sensitivity of EDVs to ET-1 cannot be directly determined from the results of our study. As removal of the endothelium did not modulate the responses of either EDVs or EDAs to ET-1, it is unlikely that the difference is associated with the vascular endothelium. Compared with EDAs, it is possible that EDVs possess a greater density of ET receptors on smooth muscle or that the receptors are more tightly coupled to the contractile response, but this remains to be elucidated.

Endothelin-1 may be a primary factor involved in the vascular changes in acute laminitis; its actions may be enhanced because of damage to the vascular endothelium that results in reduced levels of endothelium-derived relaxing factors or through synergistic actions with other vasoactive mediators present. In addition, it is also possible that factors initiating laminitis increase the expression of ET receptors on vascular smooth muscle, thus enhancing the sensitivity of vessels to ET-1 during the developmental phases of this disease. In our study, the removal of the endothelium had no effect on the responses of EDVs or EDAs to ET-1. Baxter[31] also concluded this from findings of a study in which the ET-1 responses of vessels collected from healthy horses and horses infused with low-dose endotoxin were compared. It has also been reported[m] that the ET-1 responses of digital vessels collected from horses with experimentally induced laminitis and pretreated with an NO synthase inhibitor were not different from the responses of untreated vessels. The lack of modulation of the vasoconstrictive tone by the endothelium supported 2 conclusions. One was that the basal NO production by the digital vascular endothelial cells was too low in the model system used in our study (and in other studies) to affect ET-induced vasoconstriction. The relationship between NO and ET-1 and their combined effects on vascular tone have been studied extensively. Experiments conducted in vitro have shown that NO attenuates contraction induced by ET-1 in porcine pulmonary arteries and veins[32] and reverses constriction induced by ET-1 in human internal mammary arteries.[33] Nitric oxide has also been reported to inhibit ET-1 production in cultured human umbilical vein endothelial cells[34,35] and in vivo in species such as dogs and humans.[35,36] In the vessel ring model, endothelial-intact vessels may be too small to produce significant amounts of NO in response to ET-1 given exogenously.

The second conclusion supported by the data was that there were no functional receptors for ET-1 located on equine digital vascular endothelial cells. It is known that when ET-1 directly activates endothelial ET_B receptors, NO formation is stimulated and results in relaxation.[36,37] In the study reported here, the cumulative addition of a selective ET_B receptor agonist, S6c, to (+e)EDAs and (+e)EDVs that had been preconstricted with U44069 resulted in no relaxation in either group, indicating that ET_B receptors were not located on the vascular endothelium. These results are supported by findings of another study[m] that indicate that the ET-1 responses of digital arteries and veins collected from horses with experimentally-induced laminitis and pretreated with an NO synthase inhibitor were not different from responses of untreated vessels. Overall, it is possible that the endothelium of the digital vasculature may not act as a protective mechanism and other factors, such as the sensory-motor nerves, provide more protection. Similar conclusions have been found with another vasoconstrictive mediator, 5-hydroxytryptamine (serotonin).[28] However, it must be acknowledged that the experiments reported here used large vessels; it is possible that in small-resistance vessels in vivo, endothelium-dependent modulation of vasoconstriction induced by ET-1 could be important, because resistance vessels and the large conductance and capacitance vessels may behave differently.

Additionally, in our study, the (–e)EDVs had almost no constrictive response to the cumulative addition of S6c, which indicated that there were no functional ET_B receptors on the digital vascular smooth muscle. Because 2 out of 6 (–e)EDAs did have a moderate constrictive response to S6c, it is possible that

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either the (–e)EDAs from these 2 horses had ET_B receptors on their vascular smooth muscle or ET_A receptors were being nonselectively stimulated. The lack of involvement of ET_B receptors in the responses of the equine digital blood vessels (particularly those of the EDVs) to ET-1 can be further verified by the lack of inhibitory effect of BQ-788 (a selective ET_B receptor antagonist) on the responses to ET-1 of both (–e)EDAs and (–e)EDVs. The concentration of BQ-788 used in our study was selected on the basis of results of a pilot study in which the responses of (–e)EDAs and (–e)EDVs (preconstricted with a submaximal concentration of ET-1 [10^{-7} M]) to cumulative concentrations of BQ-788 (10^{-8} to 3×10^{-6} M) or vehicle consisting of distilled water were evaluated. In the pilot study, the (–e)EDAs and (–e)EDVs did not relax in response to BQ-788 or the vehicle at any of the concentrations or time points, indicating that ET-1 was able to induce long-lasting constriction in vitro that was unaffected by time and that ET_B receptors were likely not present on the smooth muscle in the vessels tested. The reported pA_2 value (a measure of the affinity of the antagonist for the receptor involved) of BQ-788 for ET_B receptors in rabbit pulmonary arteries is 8.4.[38,39] Therefore, at the concentration used in our study (3×10^{-7} M), some inhibitory effect of BQ-788 should have been evident if ET_B receptors were responsible for the vasoconstrictor responses to ET-1. Overall, the data suggested that there were no ET_B receptors located on the digital vascular endothelium and ET_A receptors appeared to be responsible for most of the vasoconstrictor effects of ET-1 in the vessels examined in the study reported here. In comparison, the selective ET_A receptor antagonist used in our study completely inhibited the responses of (–e)EDAs to ET-1 and significantly inhibited the responses of (–e)EDVs to ET-1. The concentration of BQ-123 had been chosen on the basis of results from a pilot study conducted in our laboratory (data not shown) that found (–e)EDAs and (–e)EDVs that had been preconstricted with ET-1 (10^{-7} M) relaxed in a concentration-dependent manner in response to BQ-123, indicating the likelihood that there were ET_A receptors located on the smooth muscle. The single concentration of BQ-123 used in our study was also chosen, because BQ-123 has been shown to concentration-dependently inhibit contractions induced by ET-1 in other species, with pA_2 values for bovine, guinea pig, and rat aortae reported to be 6.6,[40] 7.4,[41] and 6.9,[42] respectively. In our study, 3×10^{-7} M of BQ-123 completely abolished the constrictive responses of the EDAs to ET-1, whereas the responses of the EDVs were significantly but not completely inhibited. These results were consistent with the greater sensitivity of EDVs than EDAs to ET-1 and the likely competitive nature of the interaction between ET-1 and BQ-123 at ET_A receptors. Overall, the antagonist data collected in the study reported here supported the conclusion that the ET_A receptor is the predominant receptor in EDAs and EDVs. Furthermore, evidence was obtained indicating that ET-1 is a potent vasoconstrictor of the equine digital vasculature (with approx 3-fold selectivity for veins) and the endothelium does not modulate the responses of arteries or veins to ET-1. Because venoconstriction has been proposed to be the initial hemodynamic factor in models of acute laminitis, endothelin may have an important role in the pathogenesis of this disease.

[a]Holm AS, Eades SC, Moore RM. Evaluation of jugular and digital venous plasma endothelin-1 concentrations in horses before and after black walnut extract administration, in *Proceedings*. 7th Annu Int Equine Colic Res Symp 2002;126–127.

[b]Vycon, Ecouen, France.

[c]Model HSE 30, isometric force transducer, Hugo Sachs Electronics, March-Hugstetten, Germany.

[d]Linseis 650 dual channel pen recorder, Linton Instruments, Diss, Norfolk, UK.

[e]Model HSE 301, bridge amplifier, Hugo Sachs Electronics, March-Hugstetten, Germany.

[f]Sigma Chemical Co, Poole, Dorset, UK.

[g]BQ-123: Cyclo[D-Asp-Pro-D-Val-Leu-D-Trp], RBI, Sigma-Aldrich Co, Poole, Dorset, UK.

[h]BQ-788:2,6-Dimethylpiperidinecarbonyl-g-Methyl-Leu-Nin-(Methoxycarbonyl)-D-Trp-D-Nle N-[N-[N-[(2,6-Dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine, sodium salt, RBI, Sigma-Aldrich Co, Poole, Dorset, UK.

[i]GraphPad Prism, version 3.00 for Windows, GraphPad Software, San Diego, Calif.

[j]SPSS, version 9.0 for Windows, Chicago, Ill.

[k]Benamou AE. Endothelin in the pulmonary circulation of the horse: in vitro and in vivo investigations into its vasoconstrictive action. PhD thesis, Department of Veterinary Medicine, University of Liege, Belgium, 1999.

[l]Holm AS, Eades SC, Venugopal CS, et al. Endothelin-1 induces vasoconstriction in the normal equine digit (abstr). *J Vet Intern Med* 2000;14:369.

[m]Holm AS, Venugopal CS, Eades SC, et al. Effects of black walnut extract administration on in vitro responses of equine palmar digital arterial and venous rings to endothelin-1, acetylcholine and nitroglycerine, in *Proceedings*. 7th Ann Int Equine Colic Res Symp 2002;140–141.

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