Characterization of the responses of equine digital veins and arteries to calcitonin gene-related peptide

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Objective—To compare responses of equine digital arteries (EDAs) and veins (EDVs) to human-αcalcitonin gene-related peptide (hαCGRP), evaluate effect of the endothelium, and characterize receptors and sources of endogenous CGRP.

Sample—Palmar digital vessels (5 to 9/experiment) from healthy adult horses killed at an abattoir.

Procedures—Vessel rings were mounted under tension in organ baths containing Krebs-Henseleit solution at 30°C, with relaxation responses examined in vessels preconstricted with a thromboxane-mimetic (3×10^{-8}M). Responses of endothelium-intact (+e) and -denuded (–e) EDAs and EDVs to hαCGRP (10^{-10} to 3×10^{-7}M) were compared. Following incubation with an hαCGRP receptor antagonist ($h\alpha CGRP_{8-37}$; 1μM), responses of EDA(–e) and EDV(–e) to hαCGRP (10^{-7}M) were obtained. Responses of endothelium-intact and -denuded arteries and veins to hαCGRP (3×10^{-7}M) or capsaicin (10^{-5}M) were evaluated as well as responses of endothelium-intact and -denuded EDA and EDV to hαCGRP (10^{-10} to 10^{-6}M) after incubation with endothelin-1 (ET-1; 10^{-12}M).

Results—hαCGRP resulted in nonendothelium, concentration-dependent relaxation in EDAs and EDVs, with greater responses in EDAs. Treatment with $h\alpha CGRP_{8-37}$ had minimal effect on responses to hαCGRP in either vessel type. Capsaicin induced relaxation in both vessel types. There were no differences between responses to hαCGRP for vessels pretreated with ET-1 or vehicle.

Conclusions and Clinical Relevance—Both hαCGRP and capsaicin induced digital vasodilation unaffected by a functional endothelium. This suggested that endogenous CGRP likely emanates from sensory-motor nerves and may contribute to digital vasodilation. (*Am J Vet Res* 2011;72:975–981)

Digital venoconstriction leading to increased postcapillary resistance has been proposed to be the initiating factor that leads to clinical signs of acute laminitis in horses.[1,2] Because vascular tone in small blood vessels is under neuronal and endothelial cell control,[3] dysfunction of either may contribute to the pathogenesis of acute laminitis. It has been proposed by some researchers that peripheral neuropathy contributing to

Abbreviations

CGRP	Calcitonin gene-related peptide
CRC	Concentration response curve
DMSO	Dimethyl sulfoxide
EDA	Equine digital artery
EDA(+e)	Endothelium-intact equine digital artery
EDA(–e)	Endothelium-denuded equine digital artery
EDV	Equine digital vein
EDV(+e)	Endothelium-intact equine digital vein
EDV(–e)	Endothelium-denuded equine digital vein
ET-1	Endothelin-1
hαCGRP	Human-αcalcitonin gene-related peptide
NO	Nitric oxide
pA_2	Negative logarithm of the molar concentration of an antagonist that reduces the effect of a dose of agonist to that of half the dose
SP	Substance P

Received May 29, 2010.
Accepted June 2, 2010.
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Supported by a Clinical Research Fellowship from The Royal Veterinary College and the President's Research Award from the University College Dublin.
Presented in part at the 19th Annual Forum of the American College of Veterinary Internal Medicine, Denver, May 2001, and at the 46th British Equine Veterinary Association Congress, Edinburgh, September 2007.
The authors thank David Byrne, Erin Bennett, Turlough McNally, and Teresa Quinn for technical assistance.
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an alteration of digital blood flow, similar to that observed in human diabetic patients[4] or in humans with Raynaud's phenomenon,[5] could contribute to the development of laminitis in horses. The pathogenesis of Raynaud's phenomenon is unknown, but it has been hypothesized that sensory-motor nerve dysfunction involving depletion of vasodilatory neuropeptides, such as CGRP, contributes to the development of the disease.[3,6,7] Interestingly, investigators in another study[8] detected abnormal morphology of peripheral nerves as well as a reduced number of unmyelinated and myelinated nerve fibers in the digital nerves of horses with laminitis, compared with results for clinically normal horses.

Calcitonin gene-related peptide is part of the calcitonin family and is involved in nociception, neurogenic inflammation, neurotransmission, and regulation of vascular tone.[9–14] In rats and humans, 2 main isoforms of CGRP have been identified as αCGRP (CGRP-I) and βCGRP (CGRP-II),[15] whereas more recently, the cDNA encoding for equine CGRP-I and -II has been cloned and sequenced.[16] Both CGRP-I and CGRP-II are synthesized in the CNS and peripheral nervous system and have similar biological activities in most vascular beds.[15,17] However, CGRP-I predominates in sensory pathways[15] and is considered the most potent and abundant vasodilatory neuropeptide identified.[18,19] It is commonly found in unmyelinated sensory afferent fibers and is usually colocalized with SP.[20–22] Its relaxant effects can be mediated by direct action on vascular smooth muscle receptors or indirectly through the release of endothelial-derived relaxing factors[23] (eg, NO); these relaxant effects differ between vascular beds.[24]

It has been proven in an immunohistochemical study[25] that CGRP and SP often coexist in the same perivascular terminal. In equine tissues, CGRP has been identified with SP in the respiratory tract,[26–30] reproductive tract,[31] synovial joints,[32,33] and feet.[34,35] More specifically to equine digits, CGRP-like immunoreactivity has been detected within the hoof dermis,[32] navicular suspensory apparatus, and distal interphalangeal joint.[35] In addition, CGRP-containing sensory-motor nerves innervating arteries and arteriovenous anastomoses have been identified within equine feet,[36] although their physiologic importance remains to be determined. Receptors for CGRP have been identified within the media and intima of resistance vessels in several species,[37] although to our knowledge, no CGRP-like sensory receptor has been identified in association with CGRP-like immunoreactivity in equine digits, which leads to the suggestion that the CGRP-containing nerve fibers are naked nerve endings.[32] Only 1 published study[38] has been conducted to evaluate functional responses of equine tissues to CGRP. In that study,[38] investigators found that exogenous CGRP had no effect on tracheal smooth muscle.

Because it has been determined that SP has an endothelial-dependent relaxant effect on both EDAs and EDVs[39] and is often colocalized with CGRP, the examination of equine digital vascular responses to CGRP is fundamental to understanding its physiologic importance as a potential counter-regulatory vasodilatory mediator in equine digits. We hypothesized that CGRP could be involved in vascular regulation in equine digits. Therefore, the objectives of the study reported here were to examine the relative potency and efficacy of CGRP as a vasodilator of EDVs and EDAs obtained from clinically normal horses, establish the effect of the endothelium on these responses, determine whether the observed responses were receptor-mediated events, and evaluate a possible source for endogenous CGRP in the equine digital vasculature.

Materials and Methods

Sample—Digital arteries and veins were collected from the forelimbs of the cadavers of mixed-breed healthy adult horses (males and females) killed at an abattoir. Horses were stunned by use of a captive bolt, which was followed by exsanguination; this was conducted under the supervision of a veterinarian and in accordance with European Union regulations. Forelimbs were removed within 10 minutes after stunning and exsanguination, and the limbs and feet were examined. Any limbs with gross pathological lesions were rejected from the study. For each forelimb, the digital artery was cannulated at the level of the metacarpophalangeal joint, and 180 mL of ice-cold modified Krebs-Henseleit solution (ie, Krebs solution) was infused through a 16-gauge, 52-mm catheter.[a] The skin was then reflected to expose the digital artery and coronary venous plexus, which were dissected free, placed in ice-cold Krebs solution, and transported to our laboratory. On arrival at our laboratory, connective tissue was removed from the vasculature, and the arteries and veins were cut into rings of 3 to 4 mm in length. Vessel rings that were not used immediately were stored overnight in oxygenated Krebs solution at 4°C.

Experimental procedure—When necessary, the endothelium was removed by gently rubbing the intimal surface of a vessel with a wooden cocktail stick[40]; this technique has been confirmed microscopically and functionally[41,42] to be an appropriate method. Vessel rings were placed between 2 parallel wires within an organ bath containing Krebs solution maintained at 30°C and aerated with 95% O_2 and 5% CO_2. One wire was fixed in place and the other connected to an isometric force transducer,[b] with the signal sent to a 2-channel pen recorder[c] via a bridge amplifier.[d] Four experiments were conducted, and for each experiment, a basal resting tension of 3 and 2 g was applied to arteries and veins, respectively; tissues were allowed to equilibrate under resting tension for a period of at least 1 hour. These resting tensions had previously been assessed to be optimal for mimicking in vivo diastolic vascular tone and allowing the greatest degree of contraction to be obtained.[43,44] Six vessel rings could be examined at 1 time, with the vessels paired from the same horse when direct comparison was required within a given experiment. Only 1 cumulative CRC was obtained for each vessel ring. Experiments in which antagonists were tested were performed by the use of adjacent vessel rings from the same horse for the control and antagonist-treated vessels.

All vessels were constricted initially with depolarizing Krebs solution (118mM KCl) to evaluate

smooth muscle function; vessels were discarded if they constricted to < 50% of their resting tension. After peak tension was attained, vessels were washed with Krebs solution to return the muscle to the basal resting tension. Following return to basal resting tension, a thromboxane-mimetic (U44069[e] [9, 11-dideoxy-9α, 11α-epoxymethano-prostaglandin $F_{2\alpha}$; 3×10^{-8}M]) was added to the organ bath to provide a stable increase in vessel tension against which vasodilators could be assessed; if the vessel's resultant contractile response to the thromboxane mimetic was < 50% of its response to the depolarizing Krebs solution, then the vessel was discarded. Once the increase in tension had stabilized, a cholinergic agonist (carbachol[e]) was added cumulatively (10^{-8} to 10^{-5}M for experiments 1 and 2) or as a single concentration (1μM for experiments 3 and 4) to confirm the presence or absence of a functional endothelium. We considered a functional or intact endothelium to be present for the EDAs and EDVs if the U44069-induced tension was changed by at least 40% and 50%, respectively, in response to 1μM carbachol. The endothelium was considered adequately denuded if the vessels relaxed < 10% of the U44069-induced tension. The concentration of U44069 used in the study reported here had been previously determined to induce approximately 80% of the maximum increase in tension induced by U44069 in equine tissue of this size.[41] The concentration of carbachol used (1μM) in the study reported here had also been previously found to cause maximal inhibition of the U44069-induced tension in this size and type of tissue[41]; further increases in the concentration of carbachol had been found to cause an increase in tension in some tissues.[41] All vessels were then washed with drug-free Krebs solution to return them to basal resting tension. When applicable, antagonists or the vehicle control treatment used in the experiment was added to the organ baths at this point, and tissues were allowed to incubate for 30 minutes before addition of the vasoconstrictor.

Experiment 1—Digital vascular responses to CGRP and effect of the endothelium on these responses

Following return of the vessel rings to basal resting tension, U44069 (3×10^{-8}M) was added to the bathing solution to increase tension. Once a steady tension was established, CRCs to hαCGRP[e] (10^{-10} to 10^{-6}M) were obtained for paired endothelium-intact and -denuded EDA and EDV from 6 horses. The decrease in tension was expressed as a percentage of the U44069-induced tension in that same vessel ring and plotted against the $\log_{10}$ CGRP concentration.

Experiment 2—Effect of a peptide CGRP-receptor antagonist on EDA(–e) and EDV(–e) responses to CGRP

Responses of EDA(–e)s (n = 5; 1/horse) or EDV(–e)s (n = 5; 1/horse) to a single concentration of hαCGRP (10^{-7}M) were obtained in vessels preconstricted by the addition of U44069 (3×10^{-8}M) before and after a 30-minute incubation with a peptide CGRP-receptor antagonist ($hαCGRP_{8-37}$[e]; 1μM) or the same volume of 1% acetic acid (vehicle control treatment).

Experiment 3—response of EDAs and EDVs to exogenous capsaicin

Concentration response curves to capsaicin[e] (10^{-10} to 10^{-4}M) were obtained for paired endothelium-intact and -denuded EDA and EDV from 9 horses. Responses of paired EDA(–e)s and EDV(–e)s (n = 6; 1 pair/horse) that had been preconstricted with U44069 (3×10^{-8}M) were then obtained to a single concentration of hαCGRP (3×10^{-7}M), a single concentration of capsaicin (10^{-5}M), and a vehicle control treatment (DMSO). The single concentrations of hαCGRP and capsaicin were chosen on the basis of the previous CRCs (experiment 1 and the first portion of experiment 3) in which a ≤ 80% relaxant response in U44069-preconstricted EDAs and EDVs had been elicited. The same volume of the vehicle control treatment (DMSO) was also added to additional vessels at basal resting tension for each horse.

Experiment 4—relaxant responses of EDAs and EDVs to hαCGRP in the presence of a subthreshold concentration of ET-1

Concentration response curves to hαCGRP (10^{-10} to 10^{-6}M) were obtained for paired endothelium-intact and -denuded EDA and EDV from 6 horses in vessels preconstricted with U44069 (3×10^{-8}M) following a 30-minute incubation with ET-1[e] (10^{-12}M) or the same volume of distilled water (vehicle control).

Statistical analysis—Data were expressed as decreases in tension (calculated as a percentage of the U44609-induced tension) and plotted against the $\log_{10}$ CGRP concentration. Cumulative CRCs were fitted to a 1-site model by use of a computerized nonlinear curve-fitting program.[f,g] The logistic equation used to fit the curves was as follows:

$$\text{Decrease in tension} = E_{max} \bullet D^n/(D^n + EC_{50}{}^n)$$

where E_{max} represents the maximum response, D is the concentration of the 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 of the maximum response. The EC_{50} values were expressed as geometric means with 95% confidence intervals, whereas the E_{max} and Hill slope values were expressed as arithmetic means ± SEM. The EC_{50} and E_{max} values were compared by use of a paired Student *t* test[f] or an ANOVA and Bonferroni *t* test,[h] as appropriate. Significance was set at values of $P \leq 0.05$.

Results

Experiment 1—The CRC responses to hαCGRP were summarized (**Table 1**). The addition of hαCGRP induced a nonendothelium, concentration-dependent vasodilation in both EDAs and EDVs (**Figure 1**). Although there were no significant differences between the relaxant responses of the endothelial-intact and endothelial-denuded vessels to hαCGRP, the relaxant responses for both EDAs and EDVs to hαCGRP were consistently less in endothelial-denuded vessels. The relaxant response to hαCGRP was significantly greater in EDAs than in EDVs, both

Table 1—The CRC values for paired endothelium-intact and -denuded EDAs and EDVs (n = 6; 1 pair/horse) to hαCGRP.

Vessel type	EC_{50} (10^{-8}M)	E_{max} (%)	Hill slope
EDA(+e)	0.29 (–1.96 to 2.53)	159.3 ± 19.5*	2.48 ± 0.79
EDV(+e)	1.04 (–0.68 to 2.76)	70.0 ± 13.7	1.10 ± 0.16
EDA(–e)	2.30 (–4.81 to 9.42)	138.9 ± 32.6†	2.21 ± 0.93
EDV(–e)	2.29 (0.22 to 4.37)	37.1 ± 10.8	2.10 ± 0.56

Values for EC_{50} are obtained at a hαCGRP concentration of 10^{-8}M and are expressed as the geometric mean (95% confidence interval); values for E_{max} are expressed as the arithmetic mean ± SEM percentage of the U44609-induced tension; values for Hill slope are expressed as the arithmetic mean ± SEM.

*Value differs significantly ($P \leq 0.05$; 2-way ANOVA and a Bonferroni *t* test) from the value for EDV(+e). †Value differs significantly ($P \leq 0.05$; 2-way ANOVA and a Bonferroni *t* test) from the value for EDV(–e).

EC_{50} = Concentration of the drug at which the response was half of the maximum response. E_{max} = Maximum response.

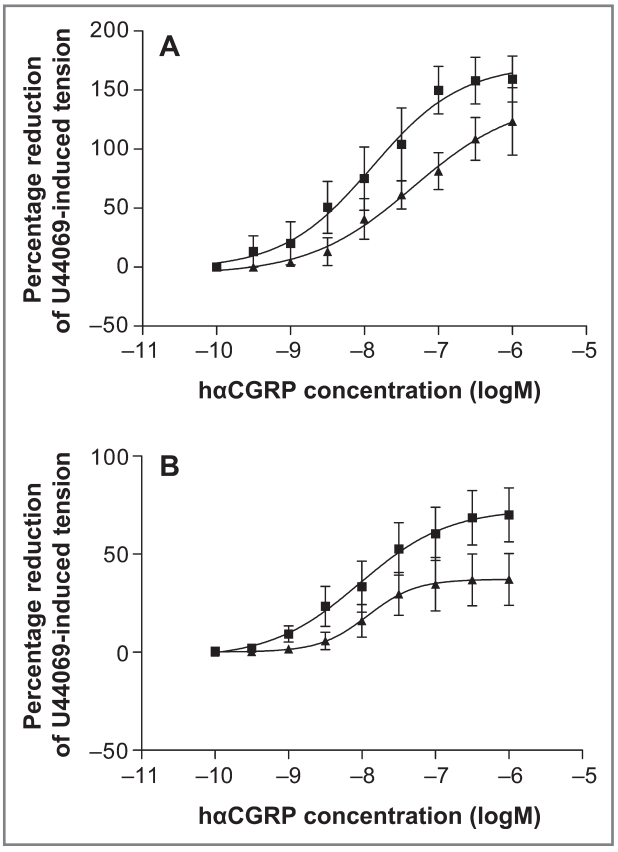

Figure 1—Concentration response curves of paired (n = 6; 1 pair/horse) EDA(+e)s and EDA(–e)s (A) and EDV(+e)s and EDV(–e)s (B) to hαCGRP. Results are expressed as the mean ± SEM percentage of the U44609-induced tension. In both panels, endothelial-intact and endothelial-denuded vessels are indicated by squares and triangles, respectively. Notice that the scales on the y-axis differ between panels.

with an intact endothelium (E_{max} value, 159.3 ± 19.5% of U44069-induced tension vs 70.0 ± 13.7% of U44069-induced tension, respectively; $P = 0.02$) and with a denuded endothelium (E_{max} value, 138.9 ± 32.6% of U44069-induced tension vs 37.1 ± 10.8% of U44069-induced tension, respectively; $P = 0.05$). There were no significant differences between the EC_{50} and Hill slope values for any of the groups.

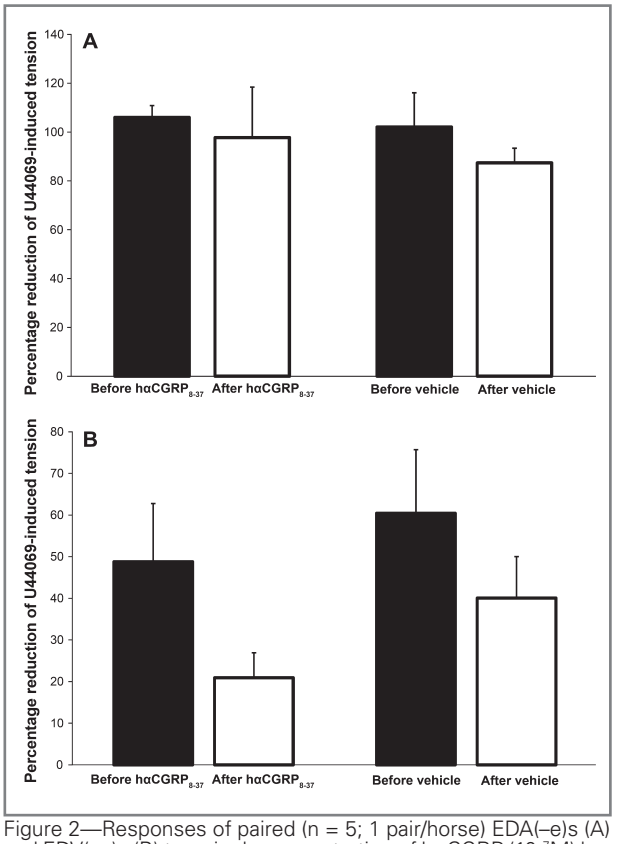

Figure 2—Responses of paired (n = 5; 1 pair/horse) EDA(–e)s (A) and EDV(–e)s (B) to a single concentration of hαCGRP (10^{-7}M) before (black bar) and after (white bar) incubation with $hαCGRP_{8–37}$ (1μM) or the vehicle control treatment (1% acetic acid) for 30 minutes. Results are expressed as the mean ± SEM percentage of the U44609-induced tension. Notice that the scales on the y-axis differ between panels.

Experiment 2—The relaxation responses of EDA (–e) and EDV(–e) to hαCGRP before and after incubation with $hαCGRP_{8–37}$ or the vehicle control treatment did not differ significantly (**Figure 2**).

Experiment 3—In all vessels, capsaicin induced a concentration-dependent, biphasic relaxant CRC; there was a plateau in the response to capsaicin between concentrations of 3×10^{-8}M and 3×10^{-6}M (data not shown). The addition of a single concentration of hαCGRP or capsaicin to U44069-preconstructed EDA(–e)s induced a strong relaxant response (E_{max} value, 103.5 ± 6.0% of U44069-induced tension vs 101.1 ± 1.7% of U44069-induced tension, respectively); both of these values differed significantly ($P = 0.02$) from the minimal relaxant response of U44069-preconstricted EDA(–e)s to the vehicle control treatment (DMSO; **Figure 3**). The addition of a single concentration of hαCGRP or capsaicin to U44069-preconstricted EDV(–e)s also induced a strong relaxant response (E_{max} value, 46.1 ± 10.2% of U44069-induced tension vs 47.3 ± 12.8% of U44069-induced tension, respectively); both of these values differed significantly ($P = 0.02$) from the relaxant response of U44069-preconstricted EDV(–e)s to the vehicle control treatment (DMSO). There were minimal changes in basal resting tension in any of the vessel groups following the addition of DMSO.

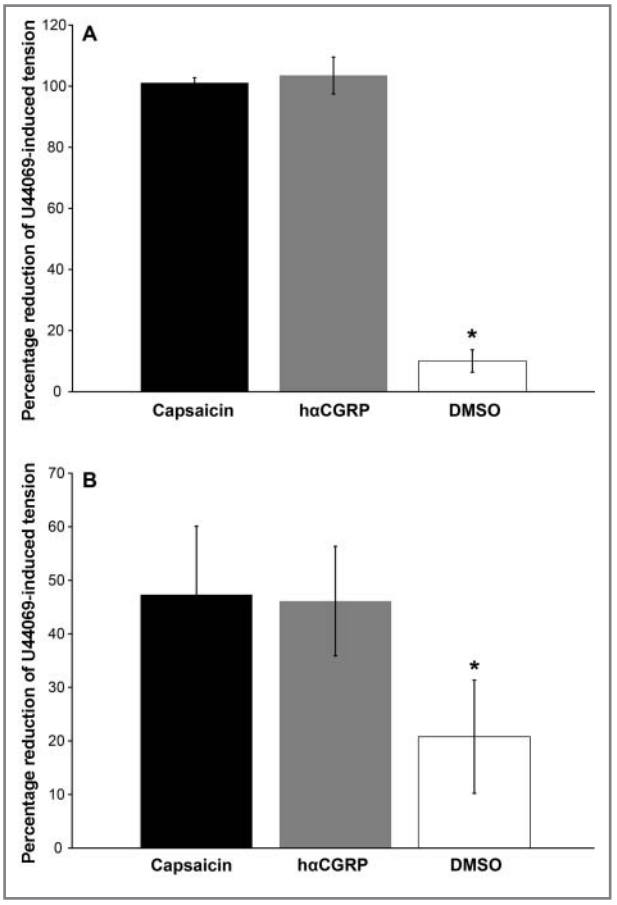

Figure 3—Responses of paired (n = 6; 1 pair/horse) EDA(–e)s (A) and EDV(–e)s (B) to a single concentration of capsaicin (black bar), hαCGRP (gray bar), or the vehicle control treatment (DMSO [white bar]). Results are expressed as the mean ± SEM percentage of the U44609-induced tension. Notice that the scales on the y-axis differ between panels. *Value for the maximum response differs significantly ($P < 0.001$) from the maximum response values for the other treatments.

Experiment 4—We did not detect significant differences between the relaxant responses to hαCGRP for EDA(+e) pretreated with ET-1 or the vehicle control treatment (E_{max} value, 81.3 ± 2.8% of U44069-induced tension vs 74.3 ± 7.4% of U44069-induced tension, respectively) and for EDA(–e) pretreated with ET-1 or the vehicle control treatment (E_{max} value, 78.7 ± 2.1% of U44069-induced tension vs 69.9 ± 7.5% of U44069-induced tension, respectively). There were no significant differences between the relaxant responses to hαCGRP for EDV(+e) incubated with or without ET-1 (E_{max} value, 64.3 ± 4.9% of U44069-induced tension vs 61.2 ± 3.5% of U44069-induced tension, respectively) and for EDV(–e) pretreated with ET-1 or the vehicle control treatment (E_{max} value, 48.9 ± 6.5% of U44069-induced tension vs 58.8 ± 8.9% of U44069-induced tension, respectively). There were no significant differences between the EC_{50} and Hill slope values for any of the groups.

Discussion

To our knowledge, in vitro responses of healthy equine digital vessels to CGRP have not been evaluated previously. The relatively high potency and efficacy of hαCGRP as a vasodilator when exogenously applied to the digital vessels suggested that these responses are receptor-mediated events. Furthermore, the relaxant effects of hαCGRP on EDAs and EDVs were evident in the absence of the endothelium, which suggested that most of the receptors for CGRP are located on the vascular smooth muscle cells and that their activation leads to relaxation of the smooth muscle. However, the CRCs for EDA(+e) and EDV(+e) were to the left of the corresponding curves obtained for the EDA(–e) and EDV(–e) segments. This observation indicated that although the response to CGRP was not an endothelium-dependent event, mediators released from the endothelium may act synergistically with CGRP. It has been suggested that NO may mediate a portion of the vasorelaxant response of some vascular beds to CGRP.[24] However, the lack of synergistic effect of endothelial-derived relaxing factors and CGRP in equine digital vessels was supported by the fact that no significant differences were found between the EC_{50} and E_{max} values for EDAs and EDVs with and without a functional endothelium in the present study. This lack of a significant difference indicated that the influence of the endothelium was variable, both across the range of concentrations tested and among horses, such that significant differences could not be detected with the number of horses included in the present study. Further investigations are necessary to determine the importance of tonic release of mediators (eg, NO, prostaglandin I_2, and endothelial-derived hyperpolarizing factor) from the endothelium on CGRP responses, perhaps by use of inhibitors of these mediators rather than physical removal of the endothelium that may damage the smooth muscle layer and concomitantly affect the direct relaxant effects of CGRP. However, in a preliminary study conducted by our research group on 3 horses (data not shown) in which the responses of paired endothelial-intact and -denuded arteries and veins were compared with responses for hαCGRP after incubation with a NO-synthase inhibitor (L-N^G-nitro-L-arginine methyl ester [300µM]) or a distilled water vehicle, no significant differences were revealed in the responses of the vessels to hαCGRP.

The in vitro responses of digital vessels to CGRP need to be characterized more fully before any firm conclusions can be drawn about the effects of this neuropeptide on equine digital vasculature. The high potency of CGRP suggests its effects are receptor-mediated events; however, the type of receptors involved and where these receptors are located have not been determined. The use of the selective peptide CGRP-receptor antagonist, $h\alpha CGRP_{8-37}$, in the present study did not inhibit the relaxant responses of EDA(–e) or EDV(–e) to hαCGRP. The concentration of $h\alpha CGRP_{8-37}$ used in the study reported here was selected on the basis of the pA_2 values reported for other species[45–50] and on the basis of CRCs obtained for equine digital vessels in response to hαCGRP obtained in the present study. The single concentration of $h\alpha CGRP_{8-37}$ (1µM) used in the present study was 10 times the reported affinity of this antagonist for CGRP-I receptors in other species.[45–50] It is possible that because $\alpha CGRP_{8-37}$ has a higher affinity for the

CGRP-I receptor subtype (pA_2 = 7 to 8)[45–48] than for the CGRP-II receptor subtype (pA_2 = 5.5 to 6.5),[48–50] equine digital vasculature may contain primarily CGRP-II receptor subtypes. This is not considered likely because the responses of the digital vessels to low concentrations of hαCGRP were pronounced, which would make it more likely that the primary receptor in equine digital vasculature is the CGRP-I receptor subtype or an atypical CGRP-I receptor with an extremely low affinity for $hαCGRP_{8–37}$. Interestingly, equine CGRP-I has low homology (51% to 59%[16]) with CGRP-I from other species, whereas equine CGRP-II has greater homology (> 80%) with CGRPs from other species. Although the homology between human CGRP-I and human CGRP-II is high (> 90%), the homology between equine CGRP-I and equine CGRP-II is low (< 56%)[16]; thus, it has been speculated that CGRP-II in horses may have functions that are typically attributable to CGRP-I in other species.[16]

Although researchers have identified CGRP within the perivascular sensory-motor nerves in equine digits,[32] its origin within the equine digital circulation and hence its function as an important endogenous vasodilator are unknown. Sensory-motor nerves can be classified as capsaicin-sensitive or capsaicin-insensitive, with capsaicin-sensitive nerves made up of primarily unmyelinated (C-fibers) and myelinated (Aδ-fibers) nerve fibers that respond to chemical, thermal, and mechanical stimuli by releasing neurotransmitters such as CGRP.[37,51,52] It is known that CGRP is primarily released from perivascular capsaicin-sensitive nerve endings,[53] although in rats, CGRP is also synthesized and stored within arterial endothelial cells.[54] Capsaicin, which acts on the vanilloid VR-1 receptor, can stimulate the release of CGRP (and SP) from capsaicin-sensitive nerves in humans,[55] guinea pigs,[56] and rats.[55] Thus, the in vitro vascular relaxant responses of endothelial-denuded vessels to exogenous hαCGRP and capsaicin observed in the present study supported the hypothesis that endogenous CGRP is released from perivascular sensory-motor nerves. However, the conclusion that capsaicin causes vasodilation through neuronal release of CGRP requires additional evidence for selective antagonists before it can be stated with certainty. In addition, electrical field stimulation in the presence of appropriate antagonists could provide further evidence that may support the hypothesis that endogenous CGRP is released from perivascular sensory-motor nerves.

Analysis of results of the experiments reported here indicated that hαCGRP is a potent and highly effective endothelium-independent vasodilator of both EDAs and EDVs, with significantly higher potency for EDAs. The possibility that relaxant factors released from the endothelium enhance the responses to CGRP warrants further investigation. The antagonist data were inconclusive, and additional functional and binding studies are required before the type and location of the receptors can be elucidated. Similar to the situation for SP, further studies are warranted to investigate the importance of CGRP-mediated vasodilation in protection against digital ischemia during acute laminitis in horses. On the basis of the vasodilatory responses of healthy equine digital vessels to the neuropeptides SP[39] and CGRP (observed in the study reported here), it is likely that the peripheral nervous system is involved in the regulation of digital blood flow, with profound consequences when such regulation does not function properly.

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