Predisposition for vasoconstriction in the equine laminar dermis: implications in equine laminitis

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Submitted 5 July 2005; accepted in final form 2 November 2005

Equine laminitis is a crippling disease in which the integrity of the digital laminae is compromised, leading to loss of support of the distal phalanx within the keratinized hoof wall (16). One of the most perplexing aspects of laminitis is that this condition is not primarily the result of insult or injury to the digit, but more often is associated with “systemic” conditions, such as colitis, intestinal ischemia, grain overload, placenta retention, and grazing on lush pasture (23). Because progression of laminitis is similar, irrespective of the initial causative systemic condition, it is likely that a common pathway(s) or mechanism(s) exists that can be activated by a variety of disparate systemic conditions that, in turn, result in the development of laminitis.

Although the precise mechanisms underlying the pathogenesis of laminitis remain obscure, it is apparent that the early stages of laminitis are associated with dysfunction of the laminar microvasculature. Alterations in blood flow have been reported, at both the level of the digital arteries and the laminar microvasculature, during the prodromal stages of experimentally induced laminitis (1, 12–15, 22). Moreover, it is apparent that an active vasoconstriction occurs in laminitis induced either by grain overload or administration of black walnut heartwood extract, which may be responsible for the observed increase in postcapillary pressure (2, 10). Studies regarding the physiological control of vascular tone in the functionally important small laminar arteries and veins are vital to our understanding of both the physiology and pathophysiology of the equine digit. However, due to their unique anatomical location between the hoof wall and the distal phalanx, few studies exist that have detailed the physiological properties of these vessels (19, 21).

Because vasoconstriction has been documented in both experimental models of laminitis (2, 3, 10), the aim of the present study was to determine whether there is a general predisposition of the equine digital microvasculature to vasoconstriction. Therefore, we examined the effects of a variety of vasoconstrictor agents on tension development in laminar arteries and veins isolated from healthy horses. We report here, for the first time, that laminar veins are more sensitive and contract to a comparatively greater degree in response to vasoconstrictor agonists than do laminar arteries. These findings may have important implications for equine laminitis, as they provide a common underlying mechanism by which a variety of clinical conditions can result in the development of laminitis.

MATERIALS AND METHODS

This study involved 16 adult horses, ranging in age from 6 to 14 yr old (mean, 11 yr old). To be included in the study, each horse lacked clinical evidence of lameness, and survey radiographs of the forelimb digits were within normal limits. All protocols were approved by the University of Georgia Institutional Animal Care and Use Committee. The horses were euthanized by using a penetrating captive bolt, as approved by the Report of the American Veterinary Medical Association’s Panel on Euthanasia (4).

Isolation of laminar vessels. Laminar arteries and veins were isolated, as previously described in detail (19, 21). Briefly, the distal portions of both forelimbs were dissected at the level of the metacarpophalangeal joint, with the hooves sectioned with a band saw to isolate two full thickness segments of the dorsal hoof. These segments were placed in ice-cold physiological salt solution (PSS) containing (in mM) 118 NaCl, 24 NaHCO3, 1 MgSO4, 0.435 NaH2PO4, 5.56 glucose, 1.8 CaCl2, and 4 KCl, gassed with 21% O2 and 5% CO2 (pH = 7.40 ± 0.01). On the stage of a high-powered dissecting microscope, the lamellar portion of the dermis was shaved until only a thin layer covered the laminar vascular bed. Laminar...
arteries and veins (2–3 cm distal to the coronary band, 200- to 800-μm internal diameter, 1–2 mm in length) were isolated using microfine surgical instruments and mounted on small vessel myographs (model 500A, Danish Myo Technology). Initially, the vessels were bathed in PSS, and the myograph bath temperature was raised to, and maintained at, 37°C while the vessels equilibrated for 1 h. Laminar arteries and veins were then stretched to produce equivalent transmural pressures of 3.1 and 1.9 kPA, respectively (19, 21). Data were collected for each agonist from two to four arteries and veins from a minimum of six horses. The numbers of vessels and horses used to obtain the data in this study were based on our laboratory’s previous experience with isolated laminar arteries and veins (19, 21).

Experimental protocols. All vessels were given three 2-min exposures to 80 mM KCl-PSS (isotonic replacement of NaCl with KCl), 15 min apart, to establish the maximum contractile response to a depolarizing stimulus. Concentration response curves were then obtained to either the α-adrenergic receptor agonist phenylephrine (PE; 1 nM–10 μM), 5-hydroxytryptamine (5-HT; 1 nM–10 μM), PGF$_{2\alpha}$ (1 nM–100 μM), or endothelin-1 (ET-1; 1 pM–1 μM) by cumulative addition of each agonist.

Data and statistical analyses. Contractile responses were calculated as a percentage of the maximal contractile response to isotonic replacement of NaCl with KCl (%TK) for each vessel. The data are presented as means ± SE. The data were analyzed by repeated-measures ANOVA. Differences between individual means were determined by Student’s modified t-test using the Bonferroni correction for multiple comparisons between means using the error mean square term from the ANOVA. A value of P < 0.05 was deemed to be significant (26, 27).

RESULTS

Responses to α$_1$-adrenergic receptor stimulation. Typical experimental records of the responses of laminar vessels to the α$_1$-adrenergic receptor agonist PE (1 nM–10 μM) are shown in Figure 1. Laminar veins and arteries contracted robustly to PE in a concentration-dependent manner. However, laminar veins were significantly more sensitive to PE (EC$_{50}$ for PE in laminar veins was 84 ± 7 nM, n = 37 veins from 12 horses compared with 688 ± 42 nM for laminar arteries, n = 39 arteries from 12 horses), and PE elicited significantly higher maximum responses in veins than in laminar arteries (maximum responses: 245 ± 21 and 123 ± 8% T$_K$ for laminar veins and arteries, respectively).

Responses to 5-HT. 5-HT elicited robust contractions in laminar arteries and veins, with laminar veins being significantly more sensitive and displaying a significantly higher maximum response than laminar arteries (EC$_{50}$ for 5-HT in laminar veins was 35 ± 6 nM, n = 25 veins from 12 horses compared with 224 ± 13 nM for laminar arteries, n = 36 arteries from 12 horses; maximum responses to 5-HT were 193 ± 18 and 142 ± 14% T$_K$ for laminar veins and arteries, respectively; Figure 2).

Responses to PGF$_{2\alpha}$. PGF$_{2\alpha}$ elicited robust contractions in laminar veins with an EC$_{50}$ of 496 ± 43 nM and a maximal response of 181 ± 17% T$_K$ (n = 15 veins from 6 horses). In contrast, PGF$_{2\alpha}$ was a relatively poor vasoconstrictor agonist in laminar arteries, eliciting a maximal response of 15 ± 8% T$_K$ (n = 16 arteries from 6 horses), which was significantly lower than that observed in laminar veins (Figure 3). The EC$_{50}$ for PGF was also significantly greater for laminar arteries (3.0 ± 0.6 μM).

Responses to ET-1. Laminar veins were exquisitely sensitive to ET-1 with the initiation of contractile responses observed at 3 μM and an EC$_{50}$ of 467 ± 38 pM (n = 19 veins from 8 horses). Laminar arteries were significantly less sensitive to ET-1 (EC$_{50}$ = 70.6 ± 6.4 nM, n = 22 veins from 8 horses), and the maximal constrictor response was significantly less in laminar arteries than in laminar veins (maximum responses: 245 ± 22 and 139 ± 10% T$_K$ for laminar veins and arteries, respectively; Figure 4).

DISCUSSION

To gain insights into the etiology of equine laminitis, it is first necessary to define the physiological regulation of blood flow in the equine digit. To this aim, we recently developed techniques that allow routine isolation and functional examination of laminar microvessels (19, 21). The present study, while being observational in nature, has important implications for the understanding of the physiology of the equine digit and...

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**Figure 1.** Typical experimental records and mean responses of laminar veins (A) and arteries (B) to phenylephrine (PE; 1 nM–10 μM). Laminar veins were significantly more sensitive to PE, and PE elicited higher maximum responses in veins than in laminar arteries (means ± SE) (C). KPSS, NaCl with KCl; %TK, percentage of maximal contractile response to KPSS. Brackets denote concentration.
how this physiology may bias the equine digit to the development of acute laminitis. The results of the present study are consistent with the concept that the vasculature within the equine digit is predisposed to venoconstriction. The observation that a variety of distinct vasoconstrictors elicits more profound contractile responses in laminar veins than in laminar arteries may help to explain why diverse systemic conditions, presumably resulting in increases in similarly diverse humoral factors, all could result in venoconstriction that appears to be an initiating factor in equine laminitis.

Equine laminitis is associated with a diverse array of “systemic” conditions, such as intestinal inflammation or ischemia, grain overload, placenta retention, and grazing on lush pasture (23). The question then arises as to why the microvascular dysfunction is localized to the digit? It has been proposed that the unique microvascular anatomy and adaptations of the laminar circulation “that provide the horse adequate digital hydrostatic pressure and thermoregulation have also predisposed it to vascular disease” (16). This synopsis implies that the laminar microcirculation possesses regulatory mechanisms with respect to the control of digital dermal blood flow, which is unique to the equine digit and, moreover, may result in a general susceptibility of the digit to laminitis.

Horses with experimentally induced laminitis have a significant increase in postcapillary resistance in the laminar microvasculature that is most likely due to venoconstriction at the level of the laminae (2, 10). We postulated that venoconstriction may be a facet of the prodromal stages of laminitis per se, rather than being confined to experimental models of laminitis, and that this may be a common link from systemic conditions to the development of laminitis. Therefore, we selected four constrictor agonists with which to initially assess the vasoreactivity of isolated laminar arteries and veins. The agonists were selected based on the following rationales. 1) PE activates α-adrenergic receptors, which have been shown to induce contraction of equine large digital arteries (6, 11, 22). Moreover, blockade of α-adrenergic receptors elicits vasodilation of the digital circulation in normal horses, consistent with a basal activation of these receptors in the digital circulation (8). 2) Circulating concentrations of 5-HT increase during experimentally induced laminitis (19), and 5-HT induces contraction of equine digital arteries but not equine systemic peripheral ar-

Fig. 2. Typical experimental records and mean responses of laminar veins (A) and arteries (B) to 5-hydroxytryptamine (5-HT; 1 nM–10 μM). Laminar veins were significantly more sensitive to 5-HT, and 5-HT elicited higher maximum responses in veins than in laminar arteries (means ± SE) (C).

Fig. 3. Typical experimental records and mean responses of laminar veins (A) and arteries (B) to PGF2α (1 nM–100 μM). PGF2α elicited robust contractile responses in laminar veins, but not in laminar arteries, which responded poorly to PGF2α (means ± SE) (C).
concentrations of 5-HT, PGF$_2$α, and ET-1 were more sensitive, and produced higher comparative maxi-
mal responses, than laminar arteries. Moreover, the effective dose results of the present study that equine laminar veins are
greater than the values we have obtained with laminar veins (11). These differences in vasoreactivity between large digital vessels and the smaller, physiologically relevant, laminar ves-
sels used in the present study are further exemplified by previous reports of similar potencies for PGF$_2$α and 5-HT in conduit arteries and veins (16). Interestingly, Katz et al. (18) re-
ported that ET-1 was more potent with respect to eliciting contractile responses in digital veins than in digital arteries. However, the EC$_{50}$ for ET-1 in digital veins reported in that study (~14 nM) was ~30-fold higher than the value (~0.5 nM) that we obtained with laminar veins.

The profound differences in contractile properties of large digital vessels and small laminar arteries question the validity of extrapolating findings obtained with conduit vessels (i.e., digital arteries and veins) to the much smaller vessels that regulate blood flow in the laminar tissue. Because changes in the caliber of conduit arteries and veins have little effect on vascular resistance, our understanding of the mechanisms responsible for regulating pre- and postcapillary resistance within the laminar soft tissue will only be improved by studying the small laminar arteries and veins. This is exemplified by our present findings that the vasoconstrictive responses of laminar veins differ not only from those of laminar arteries, but also from digital veins (11, 18, 28).

The results of the present study have several implications regarding equine laminitis. The finding of a generalized increased sensitivity of laminar veins to the vasoconstrictive substances tested may help to explain why dissimilar systemic conditions may lead to the same end result (i.e., acute lamini-
tis). For example, systemic conditions characterized by endo-
toxemia and increased circulating concentrations of inflamma-	ory mediators, such as PGF$_2$α, may lead to vеноconstriction within the laminar tissue and predispose the horse to the development of laminitis. Similarly, conditions that result in increased circulating concentrations of catecholamines could elicit the same response and, thereby, produce the same end result.

The fact that a variety of humoral factors can lead to vеноconstriction within the laminar tissue also has important implications for the development of targeted therapies for the treatment of horses with laminitis. Because a variety of hu-
moral factors could induce vеноconstriction in the digit, it is unlikely that a single pharmacological receptor antagonist would be effective in treating laminitis per se. For example, based on the finding that concentrations of ET-1 are increased in laminar tissues during the prodromal stages of experimentally induced laminitis (9), ET-1 receptor antagonists have been studied as a potential therapeutic intervention to prevent laminitis. The results of that study not only suggest that antagonism of ET-1 by itself is insufficient to prevent develop-
ment of the condition (9), but they also provide support for the hypothesis that a variety of humor factors work in concert to activate the pathway(s) that initiates laminitis. Based on the findings of the present study that equine laminar veins are exquisitely sensitive to 5-HT, PE, and PGF$_2$α, it is feasible that the increase in postcapillary resistance that characterizes acute laminitis may be due to one or more of these humor factors. However, if the responses of laminar veins to contractile agonists were to be mediated by a common signal transduction pathway, then this may represent a viable therapeutic target to alleviate the vеноconstriction in the digit during laminitis.
Studies that address the signal transduction mechanisms involved in the constrictor responses of laminar veins and arteries should prove insightful and may, in time, provide therapeutic targets that may offer effective treatment regimens for laminitis arising from a broad spectrum of clinical conditions.

In summary, the present study has determined that small laminar veins are exquisitely sensitive to contractile agonists compared with their arterial counterparts. The present results are also consistent with the contractile properties of laminar arteries and veins being very different from those of the digital arteries and veins. Studies comparing responses of digital and laminar vessels from the same horses are required to confirm this likelihood. Similarly, studies that define the signal transduction mechanisms that underpin the contractile responses of laminar arteries and veins will likely provide important insights into both the physiology and pathophysiology of the equine digit.

GRANTS

The project was supported by National Research Initiative of the USDA Cooperative State Research, Education and Extension Service Grants 2003-35204-13350 and 2002-35204-12423, Morris Animal Foundation, and the White Fox Farm Research Fund.

REFERENCES


