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

John F. Peroni, James N. Moore, Erik Noschka, Megan E. Grafton, Maria Aceves-Avila, Stephen J. Lewis, and Tom P. Robertson. Predisposition for venoconstriction in the equine laminar dermis: implications in equine laminitis. J Appl Physiol 100: 759–763, 2006. First published November 3, 2005; doi:10.1152/japplphysiol.00794.2005.—Equine laminitis is a crippling condition associated with a variety of systemic diseases. Although it is apparent that the prodromal stages of laminitis involve microvascular dysfunction, little is known regarding the physiology of this vasculature. The aim of the present study was to determine the relative responses of equine laminar arteries and veins to the vasoconstrictor agonists phenylephrine (1 nM–10 μM), 5-HT (1 nM–10 μM), PGF2α, (1 nM–100 μM), and endothelin-1 (1 pM–1 μM). We have determined that laminar veins were more sensitive, with respect to the concentration of agonist required to initiate a contractile response and to achieve EC50, for all agonists tested. EC50 values, for veins and arteries, respectively, were 84 ± 7 vs. 688 ± 42 nM for phenylephrine, 35 ± 6 vs. 224 ± 13 nM for 5-HT, 496 ± 43 vs. 3.0 ± 0.6 μM for PGF2α, and 467 ± 38 μM vs. 70.6 ± 6.4 nM for endothelin-1. Moreover, when expressed as a percentage of the response to a depolarizing stimulus (80 mM potassium), the maximal contractile response of laminar veins exceeded that for the laminar arteries for each agonist. These results indicate that there may be a predisposition for venoconstriction within the vascularature of the equine digit. While this physiological predisposition for venoconstriction may be important in the regulation of blood flow during exercise, it also may help to explain why laminitis can result from a variety of pathological systemic conditions.

Equine laminae arteries; veins

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, results 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 venoconstriction 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 venoconstriction 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 venoconstriction. 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 disarticulated 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 nM 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), PGE2 (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 α1-adrenergic receptor stimulation. Typical experimental records of the responses of laminar vessels to the α1-adrenergic receptor agonist PE (1 nM–10 μM) are shown in Fig. 1. Laminar veins and arteries contracted robustly to PE in a concentration-dependent manner. However, laminar veins were significantly more sensitive to PE (EC50 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 (EC50 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; Fig. 2).

Responses to PGE2. PGE2 elicited robust contractions in laminar veins with an EC50 of 496 ± 43 nM and a maximal response of 181 ± 17% T_K (n = 15 veins from 6 horses). In contrast, PGE2 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 (Fig. 3). The EC50 for PGE2 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 pM and an EC50 of 467 ± 38 pM (n = 19 veins from 8 horses). Laminar arteries were significantly less sensitive to ET-1 (EC50 = 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; Fig. 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...
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 (means ± SE) (C).

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 PGF<sub>2α</sub> (1 nM–100 μM). PGF<sub>2α</sub> elicited robust contractile responses in laminar veins, but not in laminar arteries, which responded poorly to PGF<sub>2α</sub> (means ± SE) (C).
mal responses, than laminar arteries. Moreover, the effective were more sensitive, and produced higher comparative maxi-

Fig. 4. Typical experimental records and mean responses of laminar veins (A) and arteries (B) to endothelin-1 (ET-1; 1 pM–1 μM). Laminar veins were exquisitely sensitive to ET-1, whereas laminar arteries were relatively insen-
sitive to ET-1 (means ± SE) (C).

teries (5). 3) Because cyclooxygenase-2 is upregulated in the laminae during the prodromal stages of equine laminitis, PGF$_{2α}$ was selected as a representative vasoconstrictor pro-
duced by cyclooxygenase enzymes (7, 24, 25). 4) ET-1 is one of the most potent vasoconstrictors identified to date, and administration of an ET-1 receptor antagonist has been re-
ported to reduce vascular resistance in the isolated perfused digit of anesthetized horses after carbohydrate overload (9). Furthermore, ET-1 is increased in equine laminar tissues during acute laminitis (17).

For all agonists tested in the present study, laminar veins were more sensitive, and produced higher comparative maxi-
mal responses, than laminar arteries. Moreover, the effective concentrations of 5-HT, PGF$_{2α}$, and ET-1 required to elicit venoconstriction correlated well with previously reported plasma concentrations of these substances in horses (5, 20, 21). These results are in contrast to those of studies performed using large-conduit digital vessels. In those studies, digital veins were less sensitive than digital arteries to PE (28). Furthermore, the EC$_{50}$ reported for PE in large digital veins was much 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 vessels used in the present study are further exemplified by previous reports of similar potencies for PGF$_{2α}$ and 5-HT in conduit arteries and veins (16). Interestingly, Katz et al. (18) reported 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 veins 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 re-
ponsible 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 in-
creased 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$_{2α}$, may lead to venoconstriction within the laminar tissue and predispose the horse to the develop-
ment 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 venoconstriction 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 venoconstriction 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 experimental-
tially 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$_{2α}$, 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 venoconstriction 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.

REFERENCES


