In horses, the pathogenesis of laminitis is multifactorial and involves perturbations in metabolic, enzymatic, inflammatory, and vascular homeostasis within the laminar dermis.1–3 Eicosanoids are lipid-derived mediators that are widely distributed in mammalian tissues4 and have wide-ranging biological actions with respect to inflammatory and cardiovascular regulation.5–7 Evaluation of the possible role of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) in laminitis induced in horses by nasogastric administration of black walnut heartwood extract

Objective—To provide insights into the role of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) in the developmental stages of laminitis induced in horses by ingestion of black walnut heartwood extract (BWHE).

Sample Population—10 adult mixed-breed horses.

Procedures—Horses were separated into 2 groups and were euthanatized at 12 hours after placebo (water) administration (control horses) or after BWHE administration and development of Obel grade 1 laminitis. Blood samples were obtained to determine plasma PGF$_{2\alpha}$ concentrations hourly for the first 4 hours and subsequently every 2 hours after substance administration. Laminar arteries and veins were isolated, and responses to increasing concentrations of PGF$_{2\alpha}$ were measured before and after preincubation of blood vessels with prostanoid and thromboxane receptor antagonists SQ 29,548, SC-19220, and AH 6809.

Results—Plasma PGF$_{2\alpha}$ concentrations increased in horses given BWHE; the WBC count decreased concurrently. In control horses, PGF$_{2\alpha}$ was a potent contractile agonist for laminar veins but not for laminar arteries. In horses given BWHE, PGF$_{2\alpha}$ was similarly selective for laminar veins; however, the magnitude of PGF$_{2\alpha}$-induced vasoconstriction was less than that in control horses. After preincubation with SQ 29,548, laminar veins from control horses responded to PGF$_{2\alpha}$ with a small degree of dilation, whereas laminar veins from horses given BWHE did not.

Conclusions and Clinical Relevance—PGF$_{2\alpha}$ may play a role in the inflammatory and vascular dysfunction associated with the prodromal stages of laminitis. Prostanoids such as PGF$_{2\alpha}$ may be viable targets for the prevention of acute laminitis in horses. (Am J Vet Res 2010;71:186–193)

Eicosanoids, therefore, may contribute to the etiology of laminitis in horses, and recently obtained evidence suggests that the enzymes responsible for eicosanoid

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>%T$_{X}$</td>
<td>Percentage of the maximal contractile response to 80mM potassium physiologic saline solution (isotonic replacement of NaCl with KCl)</td>
</tr>
<tr>
<td>BWHE</td>
<td>Black walnut heartwood extract</td>
</tr>
<tr>
<td>DP$_{R}$</td>
<td>Prostaglandin D subtype-1 receptor</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>Effective concentration that produces 50% of the maximum response</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
<tr>
<td>EPR</td>
<td>Prostaglandin E receptor</td>
</tr>
<tr>
<td>EP$_{1R}$</td>
<td>Prostaglandin E subtype-1 receptor</td>
</tr>
<tr>
<td>EP$_{2R}$</td>
<td>Prostaglandin E subtype-2 receptor</td>
</tr>
<tr>
<td>EP$_{3R}$</td>
<td>Prostaglandin E subtype-3 receptor</td>
</tr>
<tr>
<td>FPR</td>
<td>Prostaglandin F$_{2\alpha}$ receptor</td>
</tr>
<tr>
<td>Max:EC$_{50}$</td>
<td>Ratio of maximal contractions to EC$_{50}$ values</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>Physiologic saline (0.9% NaCl) solution</td>
</tr>
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</table>

The authors thank Maria Acevedo-Avila for technical assistance. Address correspondence to Dr. Robertson (tomrob@uga.edu).
production are upregulated during the prodromal stages of this condition.1,2

An in vitro study3 revealed that conduit digital arteries and veins, isolated from horses with early stage laminitis, were less responsive to PGFα, and U-46619 (a thromboxane analog) than were the corresponding vessels from healthy horses. Similarly, maximal contractions of digital arteries and veins from horses with early stage laminitis were less than those for vessels from healthy horses in response to serotonin and norepinephrine exposure.5 However, the importance of conduit-vessel function to the local regulation of blood flow within the laminar tissues in a digit may be limited, and it is likely that more pertinent data may be obtained by examining the small arteries and veins that are important in the minute-to-minute regulation of precapillary and postcapillary resistances in the digit. In vitro exposure to PGFα has been reported to elicit profound contractile responses in small laminar veins, whereas similar-sized arteries from the same region of the laminar dermis are virtually unresponsive to PGFα.10 Furthermore, the venoselective properties of PGFα and other vasoactive factors such as serotonin and endothelin-1 might explain the observation that diverse systemic diseases can result in laminitis.10 In horses, it has been suggested that systemic conditions characterized by endotoxemia and increased circulating concentrations of inflammatory mediators such as PGFα may lead to venoconstriction in laminar vascular beds, thereby contributing to the development of laminitis via an increase in postcapillary resistance.10

Information is lacking regarding the expression or function of FPRs within the laminar dermis. Because of structural similarities among eicosanoids, delineation of specific agonist-receptor interactions in tissues may not be straightforward. For example, PGEα and PGFα are structurally identical except at the C-9 position in the cyclopentane ring, at which PGEα has a keto substituent and PGFα has a hydroxyl group. It is not surprising, therefore, that PGEα and PGFα can activate FPRs and EPRs.11 Results of radioligand-binding studies reveal that PGEα is 30-fold less potent than PGFα at human FPRs,12 and that FPRs and EPRs mediate PGFα and PGEα-induced expression of interleukin-1β in a progenitor testicular cell line.13 Furthermore, PGFα binds to EPRs in pigs,14 both PGFα and PGEα constrict isolated rat aortic ring preparations via activation of thromboxane receptors,15 and PGFα also activates EPs, EPs, and thromboxane receptors in the gastrointestinal tract of mice.16

The purpose of the study reported here was to provide insight into the possible role of PGFα in the development of laminitis. Specifically, we sought to determine whether plasma PGFα concentrations increase during the prodromal stages of laminitis and to compare the vasoactive effects of PGFα in laminar arteries and veins isolated from healthy horses and horses with Obel grade 1 laminitis induced by administration of BWHE. An additional objective was to evaluate the effects of currently available thromboxane and prostanoid receptor antagonists on the responses of laminar vessels to PGFα in horses.

Materials and Methods

Animals—Ten mixed-breed horses ranging in age from 4 to 12 years old (mean, 9 years) were used in the study. Each horse lacked clinical evidence of lameness, and survey radiographs of the forelimb digits revealed no radiographic abnormalities. All study protocols were approved by the University of Georgia Institutional Animal Care and Use Committee.

BWHE extract—Heartwood shavings were obtained from a mature black walnut tree and stored in 500-g aliquots at −20°C. The day before the study began, 1 kg of shavings was added to 6 L of water at room temperature (approx 22°C) and gently agitated for 24 hours. The resulting solution was then separated from the shavings by straining the liquid through cheesecloth.

Experimental protocol—Horses were alternately assigned to 1 of 2 groups. Horses in the control group (n = 5) received 6 L of water via nasogastric tube. Horses in the BWHE group (n = 5) received BWHE via nasogastric tube. Each horse was evaluated prior to intubation and every hour thereafter for attitude, heart rate, respiratory rate, capillary refill time, hoof temperature, digital pulse quality, and evidence of lameness consistent with Obel grade 1 laminitis. Blood samples were obtained via a jugular catheter immediately before intubation was performed (time 0) and at 1, 2, 3, 4, 6, 8, 10, and 12 hours afterward.

Control horses were euthanatized 12 hours after intubation, following collection of the final blood sample; BWHE-administered horses were euthanatized as soon as clinical signs of Obel grade 1 laminitis were detected (ie, weight shifting and bounding digital pulses without evidence of lameness at a walk) or at 12 hours after intubation if signs of Obel grade 1 laminitis had not developed by that time. Euthanasia was performed by use of a penetrating captive bolt, as approved by the AVMA Panel on Euthanasia.17

EIA for plasma PGFα concentration—Blood samples were divided into 3 ice-chilled, vacuum-evacuated tubes containing EDTA, 1 of which was used for determination of WBC count. The other samples were placed on ice for 10 minutes and then immediately centrifuged at 400 g for 10 minutes at 4°C. Plasma from the centrifuged tubes was frozen at −80°C until assayed for PGFα concentration.

Prior to determination of PGFα concentrations, plasma samples were subjected to methanol extraction, with 1 mL of plasma added to 9 mL of methanol. The samples were then centrifuged for 10 minutes at 400 × g, and the supernatant was decanted and concentrated by vacuum centrifugation.8 The concentrated supernatants were stored at −80°C until reconstituted to a 2× concentration in EIA buffer immediately before the EIA was performed, in accordance with the manufacturer’s instructions. Plasma PGFα concentrations were determined by use of a commercially available EIA kit.8 Briefly, 50 µL of each standard or methanol-extracted plasma sample was placed in a 96-well plate precoated with mouse monoclonal antibody. Thereafter, 50 µL of PGFα tracer

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and PGF$_{2\alpha}$ antiserum was added into each well, and the plate was incubated for 18 hours at room temperature. After the plate was washed with wash buffer, 200 µL of Ellman reagent containing acetylcholinesterase was added. Plates were read spectrophotometrically at a wavelength of 412 nm, and concentrations of PGF$_{2\alpha}$ were calculated from a standard curve generated through measurement of known concentrations of PGF$_{2\alpha}$. The detection limit of the assay was 11 pg/mL.

**Isolation of laminar vessels**—Laminar arteries and veins were isolated as described elsewhere. Briefly, 2 full-thickness segments of the dorsal aspect of each forelimb hoof were placed in ice-cold PSS containing the following compounds: NaCl, 118mM; NaHCO$_3$, 24mM; MgSO$_4$, 1mM; NaH$_2$PO$_4$, 0.435mM; glucose, 5.56mM; CaCl$_2$, 1.8mM; and KCl, 4mM, and the PSS was gassed with 21% O$_2$ and 5% CO$_2$ (pH, 7.40 ± 0.01). On the stage of a high-powered microscope, the lamellar portion of the dermis of each hoof segment was shaved until only a thin layer covered the laminar vascular bed. Laminar arteries and veins (2 to 3 cm distal to the coronary band; 200- to 800-µm internal diameter; 1 to 2 mm in length) were isolated by use of microfine surgical instruments and mounted on small vessel myographs for the measurement of contractile properties. The blood vessels were bathed in PSS, and the bath temperature was raised to and maintained at 37°C for 1 hour while the vessels equilibrated. Laminar arteries and veins were then stretched to produce equivalent transmural pressures of 3.1 and 1.9 kPa, respectively.$^{18}$

**Evaluation of blood vessel contractile responses**—All vessels were exposed for 2 minutes to 80mM PSS in which the NaCl had been isotonically replaced with KCl. Exposures were conducted 3 times, 15 minutes apart, to establish the maximal contractile response to a depolarizing stimulus. Concentration-response curves were then obtained for PGF$_{2\alpha}$ (1nM to 10µM) or phenylephrine (1nM to 100µM) by cumulative addition of each agonist to the PSS bathing the vessels. Similar experiments were performed in which laminar veins were preincubated with each of the following antagonists 10 minutes prior to commencement of phenylephrine or PGF$_{2\alpha}$ concentration-response
curves: SQ 29,548 (thromboxane receptor antagonist, 50µM), SC-19220 (prostaglandin EP receptor antagonist, 50µM), or AH 6809 (EPR, EPRI, EPRII, and DPRI antagonist, 50µM). The concentration of each antagonist was based upon published reports23-26 of their efficacy in isolated blood vessels.

**Statistical analysis**—Data are reported as mean ± SEM. Contractile responses were calculated as %Tc, for each vessel. The data were analyzed by means of repeated-measures ANOVA by use of a commercial software program. Differences between means were identified with a Student t test and Bonferroni correction for multiple comparisons between means, in which the error mean square term from the ANOVA was used. Plasma PGFα concentrations for the 2 treatment groups at time 0 were compared with an unpaired Student t test. Because of the variability in magnitude of the increases of plasma PGFα concentrations in the BWHE group, repeated-measures ANOVA with appropriate corrections for multiple comparisons did not yield differences at any time point between mean values for the 2 treatment groups. Accordingly, for each horse, the plasma PGFα concentration at each posttreatment time point was expressed as percentage change from time 0, the cumulative values for the 1- through 8-hour posttreatment points were summed, and the means and SEs of these values were also derived for the 5 horses in each treatment group. The statistical difference between the mean values of these 2 groups was analyzed with an unpaired Student t test. A value of P < 0.05 was deemed significant for all analyses23,24.

**Results**

**Animals**—All 5 horses that received BWHE had increased intensity of digital pulses, and 4 of these horses exhibited weight shifting and were lame within 12 hours after BWHE administration. Digital pulse intensity was unremarkable in the control horses, and none of this group had any clinical signs of lameness.

**Plasma PGFα concentrations**—Mean ± SEM plasma PGFα concentrations in the control and BWHE-administered horses at time 0 were 252 ± 28 pg/mL versus 294 ± 75 pg/mL, respectively. Administration of BWHE resulted in a sustained increase in the plasma PGFα concentration in the BWHE group (Figure 1). The cumulative percentage changes in PGFα concentrations in the control and BWHE-administered horses differed significantly (−191 ± 26% and 301 ± 124%, respectively). Qualitatively, it appeared that the increases in plasma PGFα concentrations in the BWHE-administered horses were highest 2 to 4 hours after administration of BWHE, which was 1 hour prior to the time when the WBC count in blood samples was at its lowest point22 or had decreased by at least 30% (data not shown).

**Responses of laminar arteries and veins to PGFα or phenylephrine exposure**—Laminar veins were significantly more sensitive to PGFα exposure with respect to the concentration of agonist required to initiate a contractile response. Laminar veins also contracted to a greater degree (when expressed as %Tc) than did laminar arteries (Figures 2 and 3; Tables 1 and 2). Laminar veins of BWHE-administered horses were significantly less sensitive to PGFα and phenylephrine exposure than were veins from control horses, whereas laminar arteries of BWHE-administered horses were significantly more sensitive to PGFα than to phenylephrine.

Table 1—Mean ± SEM effects of preincubation with prostaglandin receptor antagonists SC-19220, AH 6809, or SQ 29,548 on contractile responses to PGFα, (1nM to 10µM) exposure for laminar veins and arteries obtained from horses approximately 12 hours after nasogastric administration of water (control; n = 5 horses) or BWHE (5).

<table>
<thead>
<tr>
<th>Horses</th>
<th>Treatment</th>
<th>No. tested</th>
<th>Max (%Tc)</th>
<th>ECα (nM)</th>
<th>Max:ECα</th>
<th>No. tested</th>
<th>Max (%Tc)</th>
<th>ECα (nM)</th>
<th>Max:ECα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>PSS</td>
<td>8</td>
<td>112 ± 11</td>
<td>1,585 ± 216</td>
<td>7.1 ± 0.8</td>
<td>10</td>
<td>27 ± 4†</td>
<td>4,444 ± 526</td>
<td>0.62 ± 0.12*</td>
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<tr>
<td></td>
<td>SQ 29,548</td>
<td>10</td>
<td>24 ± 6†</td>
<td>1,175 ± 166</td>
<td>2.0 ± 0.5†</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SC-19220</td>
<td>9</td>
<td>98 ± 16</td>
<td>2,377 ± 341†</td>
<td>4.1 ± 0.6†</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>AH 6809</td>
<td>10</td>
<td>12 ± 4†</td>
<td>1,056 ± 161</td>
<td>1.1 ± 0.3†</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BWHE</td>
<td>PSS</td>
<td>9</td>
<td>194 ± 16†</td>
<td>1,318 ± 266</td>
<td>14.7 ± 1.7†</td>
<td>11</td>
<td>50 ± 6‡</td>
<td>6,144 ± 529*</td>
<td>0.81 ± 0.7†</td>
</tr>
<tr>
<td></td>
<td>SQ 29,548</td>
<td>10</td>
<td>−21 ± 6†</td>
<td>Approx 0‡</td>
<td>Approx 0‡</td>
<td>NA</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td></td>
<td>SC-19220</td>
<td>10</td>
<td>104 ± 15†</td>
<td>1,220 ± 204</td>
<td>8.5 ± 1.2†</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>AH 6809</td>
<td>10</td>
<td>−8 ± 4‡</td>
<td>Approx 0‡</td>
<td>Approx 0‡</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Indicated variable value differs significantly (P < 0.05) between veins and arteries within each horse group. †Indicated variable value differs significantly (P < 0.05) between control horses and horses given BWHE.

Max = Maximal contraction. NA = Not applicable. ND = Not determined.

Table 2—Mean ± SEM effects of preincubation with prostaglandin receptor antagonists SC-19220, AH 6809, or SQ 29,548 on contractile responses to phenylephrine (1nM to 10µM) exposure for laminar veins from the dorsal aspect of hooves obtained from horses approximately 12 hours after nasogastric administration of water (control; n = 5 horse or BWHE (5).

<table>
<thead>
<tr>
<th>Horses</th>
<th>Treatment</th>
<th>No. tested</th>
<th>Max (%Tc)</th>
<th>ECα (nM)</th>
<th>Max:ECα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>PSS</td>
<td>6</td>
<td>205 ± 18</td>
<td>186 ± 14</td>
<td>121 ± 15</td>
</tr>
<tr>
<td></td>
<td>SQ 29,548</td>
<td>6</td>
<td>206 ± 14</td>
<td>196 ± 16</td>
<td>110 ± 9</td>
</tr>
<tr>
<td></td>
<td>SC-19220</td>
<td>6</td>
<td>224 ± 12</td>
<td>173 ± 16</td>
<td>134 ± 16</td>
</tr>
<tr>
<td></td>
<td>AH 6809</td>
<td>6</td>
<td>194 ± 14</td>
<td>468 ± 49</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>BWHE</td>
<td>PSS</td>
<td>4</td>
<td>148 ± 16</td>
<td>324 ± 46</td>
<td>46 ± 6</td>
</tr>
<tr>
<td></td>
<td>SQ 29,548</td>
<td>4</td>
<td>164 ± 15</td>
<td>355 ± 42</td>
<td>47 ± 5</td>
</tr>
<tr>
<td></td>
<td>SC-19220</td>
<td>4</td>
<td>132 ± 14</td>
<td>407 ± 43</td>
<td>32 ± 5</td>
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<tr>
<td></td>
<td>AH 6809</td>
<td>4</td>
<td>98 ± 13</td>
<td>479 ± 53</td>
<td>20 ± 6</td>
</tr>
</tbody>
</table>

See Table 1 for key.
Figure 4—Mean ± SEM contractile responses of laminar veins isolated from control horses (A and C) and horses given BWHE (B and D) in response to increasing concentrations of PGF$_{2\alpha}$ (A and B) or phenylephrine (PE; C and D) with (circles) or without (squares) preincubation of blood vessels with SQ 29,548. *Differences between mean values at the indicated point are significant ($P < 0.05$).

Figure 5—Mean ± SEM contractile responses of laminar veins isolated from control horses (A and C) and horses given BWHE (B and D) in response to increasing concentrations of PGF$_{2\alpha}$ (A and B) or phenylephrine (C and D) with (diamonds) or without (squares) preincubation of blood vessels with SC-19220. See Figure 4 for remainder of key.
responded to PGF2α to a significantly greater degree than did arteries from control horses.

Effects of antagonists on contractile responses of laminar veins to PGF2α—Preincubation of laminar veins from control and BWHE-administered horses with the putative thromboxane receptor antagonist SQ 29,548 (50 µM) significantly reduced contractile responses to PGF2α when compared with responses of laminar veins when exposed to PGF2α alone (Figure 4). After preincubation of laminar veins from control horses with SQ 29,548, addition of PGF2α resulted in a slight dilation. In contrast, laminar veins isolated from BWHE-administered horses and preincubated with SQ 29,548 did not dilate upon subsequent exposure to PGF2α. The SQ 29,548 had no significant effect on vasoconstrictor responses to phenylephrine in laminar veins from control or BWHE-administered horses.

Preincubation of laminar veins from control horses with the EP1R antagonist SC-19220 (50 µM) significantly reduced maximal contractile responses to PGF2α when compared with responses of laminar veins exposed to PGF2α alone (Figure 5). The inhibitory effect of SC-19220 was much less pronounced in laminar veins isolated from BWHE-administered horses. Preincubation with SC-19220 had no significant effect on the vasoconstrictor responses to phenylephrine in laminar veins from control or BWHE-administered horses.

Preincubation of laminar veins from control and BWHE-administered horses with the EP2R, EP3R, and DP1R antagonist AH 6809 (50 µM) significantly reduced contractile responses to PGF2α when compared with responses of laminar veins exposed to PGF2α alone (Figure 6). The AH 6809 caused a slight rightward shift in the phenylephrine concentration-response curve for laminar veins from control horses, although the maximal response was not different from that in laminar veins exposed to phenylephrine alone. In contrast, preincubation with AH 6809 significantly reduced the maximal contractile response to phenylephrine in laminar veins from BWHE-administered horses.

Discussion

In the present study, an increase in plasma PGF2α developed during the prodromal stages of BWHE-induced laminitis in horses. This increase in plasma PGF2α concentration was transient, with the peak in plasma PGF2α concentrations coinciding with the reported nadir in WBC counts in BWHE-administered horses. We also determined that laminar veins were exquisitely sensitive to PGF2α, responding with robust and sustained constrictor responses, whereas laminar arteries were virtually unresponsive to PGF2α. Taken together, these findings supported the concept that the prodromal stages of laminitis involve a systemic inflammatory response that results in the production of inflammatory mediators, such as PGF2α, which may have profound effects upon the vasculature of the laminar dermis. Specifically, increases in circulating concentrations of eicosanoids may elicit vasoconstriction within the digit, thereby contributing to the pathogenesis of
laminitis by increasing postcapillary resistance in the laminar dermis.

In addition to their effects on laminar veins, eicosanoids may induce effects through other cells in the hoof that express the appropriate receptors. For example, there is evidence that cultured human melanocytes and murine keratinocytes express receptors for PGE and PGF. Although there is evidence for the existence of receptors for epidermal growth factor in hoof wall tissue of horses, we are unaware of studies in which the presence of eicosanoid receptors on these cells was detected.

Exposure to PGF elicited significantly greater contractile responses in laminar veins than in laminar arteries from control or BWHE-administered horses. This venoselective constrictor effect does not appear to be specific for PGF because laminar veins are generally more sensitive and contract to a greater degree than laminar arteries in response to various physiologically relevant vasoactive agonists. The inherent sensitivity of laminar veins to vasoactive agonists is consistent with the recently proposed hypothesis that the equine digit may be predisposed to venoconstriction and thereby to the development of laminitis. The degree of constriction of laminar veins from BWHE-administered horses to PGF and phenylephrine was significantly less than that measured in laminar veins from control horses. This apparent reduction in efficacy that reported for serotonin and phenylephrine in laminar veins from BWHE-administered horses and is consistent with a suggested dysfunction in laminar vein reactivity at Obel grade 1 laminitis. A similar reduction in maximal contractile responses to PGF has also been reported for conduit digital arteries and veins isolated from horses in which Obel grade 1 laminitis was induced by carbohydrate overload (ie, excessive ingestion of carbohydrates). However, the reduction in maximal contraction detected in digital arteries and veins in the other study (approx 80% to 90%) was far greater than that detected in laminar veins in the present study (approx 45%). Moreover, the EC values reported for conduit arteries and veins in the other study greatly exceeded those determined for laminar veins in the present study, indicating that laminar veins may be far more sensitive to PGF than are digital arteries or veins. These findings, coupled with the fact that conduit digital arteries respond robustly to PGF, whereas laminar arteries do not, emphasize the importance of studying the physiologically relevant small laminar arteries and veins and the potential problems that may arise from extrapolating data obtained with conduit vessels to the control of blood flow within the digit.

Purportedly, SQ 29,548 is a selective antagonist of thromboxane receptors. However, preincubation of laminar veins, from either control horses or those with BWHE-induced Obel grade 1 laminitis, with SQ 29,548 ablated vasoconstrictor responses to PGF, while having no effect on responses to phenylephrine. This finding suggested that PGF may elicit its vasoconstrictor effects in laminar veins via activation of thromboxane receptors and is consistent with the results of another study in which PGF-mediated constriction of rat isolated aortic ring preparations was mediated via activation of thromboxane receptors. However, it is also possible that SQ 29,548 may not be specific for thromboxane receptors, and part of its inhibitory action on PGF may be attributable to nonspecific blockade of other prostanoid receptors. The fact that SQ 29,548 did not reduce the contractile responses of laminar veins to phenylephrine in the present study is, however, supportive of the antagonistic actions of this compound being confined to thromboxane receptor activation.

When laminar vessels were preincubated with SQ 29,548, PGF elicited a slight vasodilation in laminar veins from control horses, whereas PGF induced a slight vasoconstriction in laminar veins from BWHE-administered horses. These findings are consistent with a typical PGF-activatable vasodilator pathway existing in laminar veins that is compromised in Obel grade 1 laminitis. Because the primary aim of the present study was to determine the vasoconstrictor effects of prostanoids, we did not address the vasodilatory effects of these compounds. Additional studies in which the vasodilator effects of eicosanoids are evaluated may provide insight into alterations in vascular function that occur in laminitis.

Preincubation of laminar veins from control horses with the putative EP antagonist SC 19220 significantly reduced maximal contractile responses to PGF by approximately 50%. In contrast, SC 19220 inhibited maximal contractile responses to PGF in veins from BWHE-administered horses to a much lesser extent. Together, these results are consistent with the concept that contractile effects of PGF are mediated, in part, via activation of EP receptors and that this pathway may be downregulated in laminitis (ie, the lack of inhibition by SC 19220 in laminar veins from BWHE-administered horses may have been caused by downregulation of these receptors). Ascription of eicosanoid actions to their respective classically defined receptor subtypes should be done with caution because PGE and PGF activate their respective receptor counterparts (ie, EPs and FP receptors, respectively) and PGF can also activate EP, EP, and thromboxane receptors in some tissues. As with SQ 29,548, preincubation with SC 19220 did not inhibit the contractile responses of laminar veins to phenylephrine, which is supportive of the effects of this compound being confined to eicosanoid receptors.

The putative EP, EP, EP, and DP antagonist AH 6809 ablated contractile responses to PGF in laminar veins isolated from both control and BWHE-administered horses. However, this compound also markedly reduced the contractile responses of these vessels to phenylephrine, which raises concerns regarding the selectivity of this compound for eicosanoid receptors. The apparent lack of specificity of this compound renders interpretation of its effects on PGF in laminar veins moot. Consequently, we feel that this compound should be used with caution. One obvious pharmacological intervention that we were not able to use in the present study was to block FP receptors with specific receptor antagonists. Although putative FP-selective antagonists have been described, they were not commercially available at the time of this study. However, our data would suggest that, similar to other vascular prepara-
tions, PGF$_{2\alpha}$ predominantly acts via thromboxane receptors and that there may be minimal FP receptors present in lamarin veins.

The increase in plasma PGF$_{2\alpha}$ concentration that was evident during the prodomal stages of BWHE-induced laminitis is consistent with a systemic inflammatory response. The observation that equine lamarin veins were exquisitely sensitive to PGF$_{2\alpha}$ is consistent with the concepts that systemic inflammatory events in horses may result in venoconstriction within the digit and that eicosanoids may act as conduits in these responses. The finding that a vasodilator pathway, which can be activated by PGF$_{2\alpha}$, appeared to be compromised in Obel grade 1 laminitis is supportive of vascular perturbations within the lamarin dermis being key events in the development of laminitis. It is hoped that the future development of more specific and chemically distinct prostanoid receptor antagonists will prove helpful in further delineating the roles of eicosanoids in the pathogenesis of laminitis.

References


