Bovine lateral saphenous veins exposed to ergopeptine alkaloids do not relax\textsuperscript{1,2}

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ABSTRACT: The ergot alkaloid ergovaline has demonstrated a persistent and sustained contractile response in several different vascular models. It was hypothesized that different alkaloids isolated from tall fescue (\textit{Lolium arundinaceum}) will contribute to this contractile response differently. The objective was to compare contractile-response patterns of single additions of the ergoline alkaloids lysergic acid, lysergol, and ergonovine and the ergopeptine alkaloids ergotamine, ergocristine, ergocryptine, ergocornine, and ergovaline (provided as tall fescue seed extract). Lateral saphenous veins were collected from 6 Holstein steers (BW = 397 ± 28 kg) immediately after slaughter, sliced into cross-sections, and suspended in myograph chambers containing oxygenated Krebs-Henseleit buffer (95% O\textsubscript{2}/5% CO\textsubscript{2}; pH = 7.4; 37°C). Treatments were added at 0 min and buffer was replaced in 15-min intervals for a 120-min incubation. In addition to maximum tension and time to reach maximum tension, percent relaxation and rate of relaxation were determined following maximum tension for each treatment.

All compounds tested produced significant contractile responses (\(P < 0.05\)). All ergoline alkaloids reached maximum response in less time (\(P < 0.05\)) than the remaining compounds and began to relax immediately after first buffer change. Lysergic acid had the greatest (\(P < 0.05\)) percent relaxation and ergonovine had the greatest (\(P < 0.05\)) rate of relaxation. The ergopeptine alkaloids ergovaline, ergotamine, ergocristine, ergocryptine, and ergocornine had slower developing contractile responses with a longer (\(P < 0.05\)) interval until maximum tension was achieved compared to the ergoline alkaloids. Maximal responses to all the ergopeptine alkaloids, however, all persisted for the 120-min duration with negligible relaxation occurring. The different classes of alkaloids differed greatly in the type of contractile response generated in the lateral saphenous vein. Persistence of contractile response is thought to be the primary contributing factor to the vasoconstriction observed in animals demonstrating signs of fescue toxicosis, where different ergot alkaloids can contribute differently.

\textbf{Key words:} bovine, contractile response, ergoline alkaloids, ergopeptine alkaloids

INTRODUCTION

Tall fescue (\textit{Lolium arundinaceum}) is commonly infected with the endophytic fungus \textit{Neotyphodium coenophialum} (Bush et al., 1982; Lyons and Bacon, 1984). This fungus produces numerous ergot alkaloids (Lyons et al., 1986) that cause vasoconstriction, a primary sign and cause of symptoms of the fescue toxicosis syndrome, in grazing animals (Strickland et al., 2011). These alkaloids are classified by their chemical structure into 2 groups, ergoline alkaloids and ergopeptine alkaloids.

Ergovaline is the ergopeptine alkaloid produced in the greatest quantity by the endophyte (Yates et al., 1985; Lyons et al., 1986). Some of the other alkaloids produced by \textit{N. coenophialum} are the ergoline alkaloids lysergic acid (LSA), lysergol (LYS), and ergonovine (ERN) and the ergopeptine alkaloids ergotamine (ERT), ergocristine (ERS), ergocryptine (ERP), and ergocornine (ERO; Fig. 1). Previous research has reported an interesting phenomenon in vasculature exposed to ergot alkaloids. Solomons et al. (1989) reported a persistent contractile response to ERT in the bovine dorsal pedal

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\textsuperscript{1}Mention of trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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vein, and Dyer (1993) and Klotz et al. (2007) reported sustained contractile responses to ergovaline in core and peripheral bovine vasculature, respectively. Schöning et al. (2001) demonstrated a near permanent receptor binding of ergovaline, with negligible dissociation in a rat tail bioassay. The studies by Silberstein (1997), Schöning et al. (2001), and Klotz et al. (2007) demonstrated slow dissociation of ergot alkaloids from receptors and may be the cause of vasoconstriction associated with fescue toxicosis. Because N. coenophialum produces numerous ergot alkaloids, it is hypothesized that different contractile response patterns would contribute differently to the vascular signs of fescue toxicosis. The objective of this study was to observe the contractile response patterns of the lateral saphenous vein to single additions of LSA, LYS, ERN, ERT, ERS, ERP, ERO, and a tall fescue seed extract (EXT) using a multimyograph.

**MATERIALS AND METHODS**

Procedures used in this study did not require approval from the University of Kentucky Animal Care and Use Committee because no live animals were used.

**Animals and Tissues**

The cranial branch of the lateral saphenous vein was collected from Holstein steers (n = 6; BW = 397 ± 28 kg) immediately after slaughter at the University of Kentucky abattoir and processed according to the methods of Klotz et al. (2006). Segments (4 to 5 cm in length) of vein were removed and placed in a modified Krebs-Henseleit (oxygenated buffer solution, 95% O₂ + 5% CO₂; pH = 7.4; mM composition = D-glucose, 11.1; MgSO₄, 1.2; KH₂PO₄, 1.2; KCl, 4.7; NaCl, 118.1; CaCl₂, 3.4; and NaHCO₃, 24.9; Sigma Chemical Co., St. Louis, MO) for transport and were kept on ice until processed. The venous segments had excess fat and connective tissue carefully removed and then were sliced into 2- to 3-mm cross-sections and examined under a dissecting microscope (Stemi 2000-C; Carl Zeiss Inc., Oberkochen, Germany) at 12.5x magnification to confirm physical integrity of the tissue and to verify the consistent segment size (Axiovision, version 20; Carl Zeiss Inc.). If abnormalities were found or an inconsistent size was observed, the cross-section was discarded and another segment was tested.

**Myograph Experiments**

Duplicate cross-sections from each animal were horizontally suspended into a tissue bath (DMT610M Multichamber myograph; Danish Myo Technologies, Atlanta, GA) containing 5 mL of continuously gassed (95% O₂ + 5% CO₂) modified Krebs-Henseleit buffer (37°C). The transport buffer was modified for myograph incubations, with the addition of desipramine (3 × 10⁻⁵ M; D3900; Sigma Chemical Co.) and propranolol (1 × 10⁻⁶ M; P0844; Sigma Chemical Co.) to inactivate neuronal uptake of catecholamines and to block β-adrenergic receptors, respectively, as described by Klotz et al. (2006). The baseline tension used to equilibrate the tissue segments was 1 g for 90 min and the buffer solution was replaced at 15-min intervals throughout the entire experiment. The tissue segments were exposed to a 500-μL aliquots of norepinephrine (1 × 10⁻⁴ M) to assure responsiveness and for subsequent normalization of the tissue response data. Viable tissues were washed every 15 min until the original 1-g resting tension (baseline) was achieved.

Ergot alkaloids can be classified by their chemical structure (Evans et al., 2004a,b) into groups (Fig. 1). Ergopeptine alkaloids 1) ergovaline (provided as EXT that was analyzed, validated, and described in detail by
The ergoline alkaloids (for structures see Fig. 1) had a larger initial contractile response than ergopeptine alkaloids, with LSA reaching a maximum \( (P < 0.05) \) in the first 15-min interval and LYS and ERN reaching maximums \( (P < 0.05) \) in the second 15-min interval (Fig. 2). These contractile responses began to immediately decrease after reaching the maximums, with ERN and LYS decreasing \( (P < 0.05) \) towards the baseline value with each subsequent 15-min interval. Vasoactivity of veins exposed to LSA decreased \( (P < 0.05) \) more rapidly than LYS and ERN after reaching maximum response and returned to baseline tension by 45 min (Fig. 2).

Conversely, none of the ergopeptine alkaloids evaluated in the current experiment relaxed back to the baseline value during the whole 120-min incubation (Fig. 3). As for the ergopeptine alkaloids, ERO, ERP, and ERT took 45 min to reach maximum \( (P < 0.05) \) contractile response, and ERS needed 75 min to reach \( (P < 0.05) \) maximum response (Fig. 3). Previous studies using segments of the cranial branch of the lateral saphenous vein of bovines showed that ergovaline and ERT are potent vasoconstrictors (Klotz et al., 2007) and that LSA was not not a potent vasoconstrictor (Klotz et al., 2006), and repetitive additions of ergovaline \( (1 \times 10^{-7} \, M) \) resulted in a significant increase in the contractile response (Klotz et al., 2008) compared to the baseline tension. The data cited suggest that there is a bioaccumulative effect of repeated ergovaline exposures on the saphenous veins exposed in vitro (Klotz et al., 2009). This bioaccumulation is hypothesized to be a result of the sustained contractile response observed in the current study and the irreversible receptor binding of ergovaline reported by Schöning et al. (2001).
It is hypothesized that the sustained contractile response caused by ergopeptine alkaloids could be related to the accumulative ability of some ergot alkaloids. This effect may be explained by the strength of their receptor affinity. Dihydroergotamine mesylate (DHE) is a synthesized ergot alkaloid similar to ERT, and both have similar vasoactivity on the cranial vascular bed; however, they differ in their effect on peripheral blood vessels, where DHE is more potent in veins and ERT more potent in arteries (Müller-Schweinitzer, 1992). Dihydroergotamine mesylate and the other alkaloids have an affinity for norepinephrine, epinephrine, dopamine, and serotonin receptors (Saper and Silberstein, 2006). The observed effect of ergot alkaloids is related to the activity of these receptors, yet the mechanisms of this action are not well defined. The biologic activity of DHE and all ergot alkaloids differ from their plasma concentrations, which means that even at low concentrations their activity may persist for days (Saper and Silberstein, 2006). The possible explanations are the binding effect where DHE has a slow dissociation from the receptor sites and the slow release of DHE back into circulation caused by nonspecific binding to other receptor sites (Saper and Silberstein, 2006). This theory could explain the binding effect of the ergopeptine alkaloids to the vascular tissue, resulting in the sustained contractile response observed during the 2-h incubation period in the current experiment.

Klotz et al. (2009) hypothesized that ergovaline accumulates in vascular tissue, repeatedly exposed in vitro, but that LSA does not accumulate. Ergovaline increased the contractile response in the vascular tissue, but LSA did not cause the same effect, even in repeated exposures and increasing concentrations. The results obtained in the current study observed similar response to LSA exposure, where ergoline alkaloids returned to the baseline tension during the incubation period and the ergopeptine alkaloids caused the saphenous vein to remain in a contracted state. The contractile response of ergovaline was observed in studies using cross-sections of bovine lateral saphenous veins (Klotz et al., 2007), bovine uterine arteries (Dyer, 1993), and rat caudal arteries (Schöning et al., 2001), which similarly showed the tissue did not return to baseline tension. The observed failure of multiple vasculature models from a variety of species to relax after an in vitro exposure to ergovaline is likely due to the high affinity for ergovaline and a slow dissociation, similar to mechanisms discussed above for DHE.

Contractile response data were integrated with time data to test the interaction between each ergot alkaloid and time. These data show that the ergopeptine alkaloids have a lower percent of relaxation and the contractile response was mostly maintained during the 120-min incubation period (Table 1) with a single addition of the alkaloids. The time to minimum occurred faster in vessels exposed to ergopeptine alkaloids, and the time to maximum was greater ($P < 0.05$) for the ergopeptine alkaloids (Table 1). This was due to the fact that vessels exposed to ergoline alkaloids continued to relax during the majority of the incubation while vessels exposed to ergopeptine alkaloids continued to constrict for the majority of the incubation. Ergonovine had the greatest rate of relaxation ($P < 0.05$), ERS had the lowest rate of relaxation, and LSA had the greatest percent of relaxation (Table 1). None of the ergopeptine alkaloids including EXT differed in percent relaxation and only differed slightly in rate of relaxation (Table 1). The rate of relaxation was different between the ergoline alkaloids, where LSA and LYS had similar rates ($P > 0.05$) but ERN had a higher value ($P < 0.05$). Ergocristine had the

![Figure 2](image2.png) **Figure 2.** Contractile response (normalized to the $1 \times 10^{-4}$ M norepinephrine maximum) of lateral saphenous veins to a single additions of $1 \times 10^{-4}$ M ergoline alkaloids ergonovine (ERN), lysergic acid (LSA), and lysergol (LYS) in a 120-min experiment with buffer replacement occurring at 15-min intervals.

![Figure 3](image3.png) **Figure 3.** Contractile response (normalized to the $1 \times 10^{-4}$ M norepinephrine maximum) of lateral saphenous veins to a single additions of $1 \times 10^{-4}$ M ergopeptine alkaloids ergocornine (ERO), ergocryptine (ERP), ergocristine (ERS), and ergotamine (ERT) in a 120-min experiment with buffer replacement occurring at 15-min intervals.
Table 1. Percent relaxation, time to minimum, time to maximum, and rate of relaxation of bovine lateral saphenous veins exposed to ergoline and ergopeptine alkaloids

<table>
<thead>
<tr>
<th>Variable</th>
<th>LSA</th>
<th>LYS</th>
<th>ERN</th>
<th>ERO</th>
<th>ERP</th>
<th>ERS</th>
<th>ERT</th>
<th>EXT</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to reach maximum tension, 2 min</td>
<td>14.30&lt;sup&gt;C&lt;/sup&gt;</td>
<td>25.70&lt;sup&gt;C&lt;/sup&gt;</td>
<td>18.30&lt;sup&gt;C&lt;/sup&gt;</td>
<td>52.00&lt;sup&gt;B&lt;/sup&gt;</td>
<td>66.60&lt;sup&gt;B&lt;/sup&gt;</td>
<td>99.30&lt;sup&gt;A&lt;/sup&gt;</td>
<td>62.70&lt;sup&gt;B&lt;/sup&gt;</td>
<td>65.00&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to reach minimum tension, 3 min</td>
<td>97.60&lt;sup&gt;A&lt;/sup&gt;</td>
<td>90.10&lt;sup&gt;A&lt;/sup&gt;</td>
<td>94.20&lt;sup&gt;A&lt;/sup&gt;</td>
<td>57.90&lt;sup&gt;B&lt;/sup&gt;</td>
<td>38.70&lt;sup&gt;B&lt;/sup&gt;</td>
<td>12.40&lt;sup&gt;C&lt;/sup&gt;</td>
<td>44.90&lt;sup&gt;B&lt;/sup&gt;</td>
<td>41.20&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relaxation, %</td>
<td>119.15&lt;sup&gt;A&lt;/sup&gt;</td>
<td>53.10&lt;sup&gt;B&lt;/sup&gt;</td>
<td>59.72&lt;sup&gt;B&lt;/sup&gt;</td>
<td>13.80&lt;sup&gt;C&lt;/sup&gt;</td>
<td>11.47&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.11&lt;sup&gt;C&lt;/sup&gt;</td>
<td>14.08&lt;sup&gt;C&lt;/sup&gt;</td>
<td>11.44&lt;sup&gt;C&lt;/sup&gt;</td>
<td>4.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rate of relaxation, g/min</td>
<td>0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>0.006&lt;sup&gt;D&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>A–D</sup> Means within a row with different subscripts differ (<i>P</i> < 0.05).

<sup>1</sup>Based on a single 1 × 10<sup>-4</sup> M addition of lysergic acid (LSA), lysergol (LYS), ergonovine (ERN), ergocornine (ERO), ergocryptine (ERP), ergocristine (ERS), or ergotamine (ERT) or 1 × 10<sup>-6</sup> M tall fescue seed extract (EXT).

<sup>2</sup>The interval of time from the addition of the alkaloid until the maximum tension was recorded within the 120-min incubation period.

<sup>3</sup>The interval of time following the point of observed maximum tension until the minimum tension was recorded within the 120-min incubation period.

<sup>4</sup>The percent relaxation was determined by subtracting the baseline-corrected maximum and the minimum tensions and dividing by the maximum tension and multiplying by 100.

<sup>5</sup>The rate of relaxation was determined once a vessel segment reached a maximum tension in the 120-min incubation period, by subtracting the baseline-corrected maximum and minimum tensions, and dividing by the increment of time that it took to reach the minimum tension.

The lowest rate of relaxation (<i>P</i> < 0.05) of all the alkaloids, which is consistent with the observation that it took 99.3 min to reach a maximum response leaving little time for relaxation in the remaining 120-min incubation. These data support previous studies (Klotz et al., 2006, 2008) regarding the theory that ergopeptines have a greater vasoactivity than the ergoline alkaloids, especially LSA.

Tall fescue seed extract was presented separately (Fig. 4) because it could not be added at the same concentration as the other alkaloids evaluated, and although it contains mostly ergovaline (97% of measured alkaloids), it is technically a mixture of different ergot alkaloids. Foote et al. (2012) described the exact alkaloid content of EXT and compared the contractile response produced by EXT to the same concentrations of pure ergovaline in bovine lateral saphenous veins. The contractile responses between the pure ergovaline and EXT did not differ, indicating that the other detected alkaloids were present at levels below those required to induce a biological response in this bioassay. The EXT containing 1 × 10<sup>-6</sup> M ergovaline used in the current study is from the same lot of EXT used in the Foote et al. (2012) study. Although the contractile response over time to EXT (Fig. 4) was not compared directly to the other ergopeptine alkaloids evaluated in the current study, the response did not appear to differ much in magnitude from ERP, ERO, ERS, or ERT (Fig. 3) even though the concentration of EXT was 2-fold less. This is additional evidence that ergovaline is the most vasoactive of the ergopeptine alkaloids produced by <i>N. coenophialum</i>. Furthermore, the response to a 1 × 10<sup>-6</sup> M ergovaline addition through the EXT was not different from 1 × 10<sup>-6</sup> M additions of ERP, ERO, or ERT in the time to maximum response, rate of relaxation, or percent relaxation (Table 1). In the current study, the maximum response to 1 × 10<sup>-4</sup> M EXT was recorded at 65 min. In lower concentrations (10<sup>-10</sup> M), ergovaline was shown to require a minimum of 120 min to reach the maximal contractile response in bovine uterine and umbilical arteries (Dyer, 1993) This time shortened as the concentration increased and even after 3 h of repeatedly changing the bath fluid with fresh Krebs solution the tissue did not begin to relax (Dyer, 1993). In the current study, the lateral saphenous vein began to relax from the maximum (<i>P</i> < 0.05) at the end of the 120-min incubation period. This difference in time of relaxation could be due to the different anatomic origin and vessel type (artery versus vein) of the tissues used in the experiments.

In conclusion, this study indicates that ergot alkaloids classified as ergolines do not have a persistent binding effect and do not cause a sustained contractile response in the cranial branch of the bovine lateral saphenous vein. The contractile response caused by ergolines is highest during the first 15- to 30-min interval followed by constant relaxation during the remaining 120-min incubation period. Obversely, the ergopeptine alkaloids have a sustained contractile response that slowly increased during the first three to five 15-min intervals.
Furthermore, the ergopeptides did not relax markedly during the incubation period. The absence of relaxation by the lateral saphenous vein after the ergopeptine alkaloids were removed from the buffer is an indication of the ability of these toxins to cause vasoconstriction, possibly accumulate, and delay the animal’s recovery from fescue toxicosis. To mitigate the vascular effects of fescue toxicosis, future research should be directed at analyzing the ergot alkaloid receptor affinity mechanism and how to manipulate this effect.

**LITERATURE CITED**


