Contractile response of fescue-naïve bovine lateral saphenous veins to increasing concentrations of tall fescue alkaloids1,2

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ABSTRACT: Various alkaloids found in endophyte-infected tall fescue have been shown to elicit different effects in the grazing animal. As part of an ongoing characterization of vascular response generated by different alkaloids, the objective of this study was to examine the vasoconstrictive potentials of ergonovine (a simple lysergic acid derivative) and α-ergocryptine, ergocristine, and ergocornine (all ergopeptine alkaloids) using bovine lateral saphenous veins (cranial branch) biopsied from fescue-naïve cattle. Segments (2 to 3 cm) of vein were surgically biopsied from healthy crossbred yearling cattle (n = 18; 274 ± 8 kg of BW). Veins were trimmed of excess fat and connective tissue, sliced into 2 to 3 mm sections, and suspended in a myograph chamber containing 5 mL of oxygenated Krebs-Henseleit buffer (95% O2/5% CO2; pH = 7.4; 37°C). Tissue was allowed to equilibrate at 1 g of tension for 90 min before initiation of treatment additions. Increasing doses of each alkaloid (1 × 10⁻⁴ to 1 × 10⁻¹ M) were administered every 15 min after buffer replacement. Data were normalized as a percentage of contractile response induced by a reference dose of norepinephrine (1 × 10⁻⁴ M). Exposure of vein segments to increasing concentrations of ergocryptine, ergocristine, and ergonovine did not result in a contractile response until 1 × 10⁻⁴ M, and ergocornine was even less potent (P < 0.05). Ergonovine had a greater maximal contractile intensity than ergocristine and ergocryptine (P < 0.05), with the 1 × 10⁻⁴ M responses of ergonovine, ergocristine, ergocryptine, and ergocornine reaching maximums of 68.5 ± 4.1, 45.5 ± 4.5, 42.9 ± 4.1, and 57.2 ± 9.9% of the norepinephrine maximum, respectively. The contractile response to increasing concentrations of ergonovine vs. ergocristine, ergocryptine, and ergocornine were opposite from previous evaluations of ergoline (e.g., lysergic acid) and ergopeptine (e.g., ergovaline) alkaloids using this bioassay, where the ergopeptine generated the greater contractile intensity. These data indicate that ergopeptides structurally different only at a single position of the peptide moiety do not exhibit differing contractile responses when considering contractile intensity. This difference may alter the potency when considering ergocornine was less potent than ergocryptine or ergocristine. These alkaloids may need to be considered when evaluating causative agents vasoconstriction associated with tall fescue-induced toxicosis.

Key words: bovine, ergocornine, ergocristine, ergocryptine, ergonovine, vasoconstriction


INTRODUCTION

Peripheral vasoconstriction in grazing animals is a response attributed to alkaloids (Strickland et al., 2009) found in endophyte-infected tall fescue (Lolium arundinaceum). The endophyte (Neotyphodium coenophialum) produces numerous ergot alkaloids (Yates et al., 1985) that have an ergoline ring moiety as a common structural feature. Derivatives of the ergoline ring system can be structurally categorized as ergoline alkaloids (e.g., lysergic acid; Figure 1A), simple lysergic acid derivatives (e.g., ergonovine; Figure 1B), and ergopeptide alkaloids (e.g., ergovaline; Figure 2; Garner et al., 1993). Ergopeptide alkaloids have a tricyclic peptide moiety attached via a carbonyl at the 8 position in the D ring that differs at 2 locations (designated as R1...
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As a result, it was hypothesized that α-ergocryptine, ergocristine, and ergocornine, which also differ only at the R₂ positions, would generate similar contractile responses, but would differ from that of the simple lysergic acid derivative, ergonovine (a 2-propanolamine derivative of lysergic acid). Therefore, the objective of this experiment is to compare the vasoconstrictive potentials of 3 ergopeptine alkaloids that differ only at the R₂ position and ergonovine using lateral saphenous veins biopsied from fescue-naïve cattle.

MATERIALS AND METHODS

Methods used for the biopsies were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animals and Tissues

Lateral saphenous vein (cranial branch) tissue used in this study was collected via surgical biopsy from fescue-naïve Angus × Brangus crossbred heifers (n = 18; 274 ± 8 kg of BW; obtained from the USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR) using methods reported by Klotz et al. (2008). Heifers were maintained in a drylot on a corn silage diet before biopsy. The biopsy consisted of placing the animal in a left lateral recumbency using a tilt table (Spring-O-Matic Inc., Marion, KS), and the biopsy site was clipped free of hair, cleaned (povidone-iodine soap solution), disinfected (70% ethyl alcohol), and locally anesthetized (lidocaine, 2% injectible; The Butler Co., Dublin, OH). A 10-cm incision was made in the tarsal region, slightly above and parallel to the target vein. After identification of the cranial branch of the lateral saphenous vein, ligatures were placed after the division of the lateral saphenous vein into cranial and caudal branches and before the cranial branch merged with a branch of the cranial tibial vein. The isolated venous tissue was excised 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 kept on ice until processed. Immediately after biopsy, heifers received penicillin (Procaine G, 6,600 U/kg of BW; Norbrook Inc., Kansas City, MO) and flunixin meglumine (Flunixinject, 1.1 mg/kg of BW; IVX Animal Health Inc., St. Joseph, MO) and were returned to the drylot for observation. The administration of flunixin meglumine continued for 2 d postbiopsy.

Tissue processing consisted of removal of excess fat and connective tissue and the cleaned segment was sliced into 2- to 3-mm cross-sections. Cross-sections were examined under a dissecting microscope (Stemi 2000-C, Carl Zeiss Inc., Oberkochen, Germany) at...
Figure 2. A) General structure of ergopeptine alkaloids that illustrates the 2 functional groups $R_1$ and $R_2$. B) Chemical structures of ergopeptine alkaloids organized by an isopropyl or methyl group in the $R_1$ position and a methyl benzyl, isopropyl, or isobutyl group at the $R_2$ position.
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Figure 3. Example of typical responses of isolated bovine lateral saphenous vein cross-sections to increasing concentrations of ergonovine, α-ergocryptine, ergocristine, and ergocornine. Venous tissue was biopsied from fescue-naive heifers, and the complete data recording from the myograph includes initial addition of norepinephrine (NE), the addition of the alkaloid standards, and the concluding addition of NE. The spikes that precede compound additions are artifacts generated from buffer replacement and were not included in the data collection and analysis.

Cross-sections of lateral saphenous veins were run in duplicate from each animal for each alkaloid experiment. The uneven distribution of veins across treatments (n = 6 for ergonovine and ergocryptine, n = 5 for ergocristine, and n = 4 for ergocornine) was a result of some experiments being conducted on the same animals and some biopsies yielding unviable tissue (contractile response to 1 × 10^-4 M norepinephrine less than 10 g). After recovery from the 1 × 10^-4 M norepinephrine addition (45 to 60 min) and the reestablishment of the 1 g baseline tension, alkaloid additions occurred in 15-min intervals. Each 15-min interval consisted of a 9-min incubation period, followed by a washout period.

Evaluation of Ergonovine, Ergocryptine, Ergocristine, and Ergocornine
during which duplicate aliquots of buffer minus the alkaloid were incubated with the vein segment for two 2.5-min periods, followed by a final buffer replacement and 1-min incubation before the next alkaloid addition. After the final addition of an alkaloid, the experiment was concluded with a final addition of $1 \times 10^{-4} M$ norepinephrine to verify that the vein cross-sections were still viable at the conclusion of the experiment.

Stock standards of ergonovine maleate (100% purity, E6500, Sigma Chemical Co.), α-ergocryptine (99% purity, E5625, Sigma Chemical Co.), and ergocristine (≥97% purity, E140, Sigma Chemical Co.) were prepared in 100% methanol (EMD Chemicals, Gibbstown, NJ). Ergocornine (>95% purity, E3131, Sigma Chemical Co.) was not soluble in methanol; thus, standards were prepared in 100% ethyl acetate (Fisher Scientific, Fair Lawn, NJ). To achieve a $1 \times 10^{-4} M$ concentration in the incubation buffer, a $2 \times 10^{-2} M$ stock concentration was prepared for each alkaloid. Serial dilutions of this stock made up the remaining standards and 25-μL aliquots of the dilutions were added to the incubation buffer to attain desired treatment concentrations ($1 \times 10^{-10}$ to $1 \times 10^{-4} M$). Using this protocol, organic solvent concentrations in the incubation buffer were kept below 0.5%.

**Data Collection and Analysis**

Isometric contractions were recorded as grams of tension in response to exposure to norepinephrine and the subsequent additions of ergonovine, α-ergocryptine, ergocristine, or ergocornine. Data were digitized and recorded using a Powerlab/8sp and Chart software (Version 5.5; ADInstruments, Colorado Springs, CO). The contractile responses were recorded as the greatest grams of tension detected within the 9-min incubation period. All maximal values measured were corrected by the baseline measured during the interval just preceding the addition of the norepinephrine ($1 \times 10^{-4} M$) reference treatment, thus generating cumulative contractile responses. To compensate for variation of tissue responsiveness due to differences in tissue size or individual animal variation, values were normalized as a percentage of the maximal contraction produced by norepinephrine. Contractile response data are presented as percentage means ± SE of the maximum effect ($E_{\text{max}}$) of a contraction induced by norepinephrine and plotted to illustrate the response of the bovine lateral saphenous vein. Potency of an alkaloid was determined as the molar concentration of the alkaloid producing 50% of the maximum response or effective concentration ($E_{\text{C50}}$) and was calculated from the contractile-response data using the Dose Response Module for Chart (Version 1.0, 2007, ADInstruments).

**Statistical Analysis**

Concentration responses of ergonovine, α-ergocryptine, ergocristine, and ergocornine were compared to determine if the alkaloids elicited different responses. Data for each alkaloid were analyzed as a completely randomized design using the mixed model procedure (SAS Inst. Inc., Cary, NC). Animal was the experimental unit and the model included alkaloid, concentration and alkaloid × concentration effects. ANOVA was conducted, and pairwise comparisons of least squares means (±SEM) were performed if the probability of a greater F statistic was significant for the tested effect. Additionally, the probabilities of linear, quadratic, and cubic effects of alkaloid relative to concentration were determined and evaluated as a test for heterogeneity of slopes (Littell, et al., 2006). Terms and associated interactions that were not significant were removed from model. Effects and comparisons were considered different at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

The understanding of the relative vasoconstrictive strength of the different alkaloids isolated from endophyte-infected tall fescue in an animal model is incomplete. Thus far for the bovine model, lysergic acid, ergovaline, ergotamine, and N-acetyllyotonin have been evaluated using the lateral saphenous vein bioassay (Klotz et al., 2006, 2007, 2008). Additionally, bovine contractile responses have been reported for ergosine, ergotamine, agroclavine (Solomons et al., 1989), and ergonovine (Oliver et al., 1992) using the dorsal pedal vein; lysergamide using the lateral saphenous vein (Oliver et al., 1993); and ergovaline using uterine and umbilical arteries (Dyer, 1993). For α-ergocryptine, ergocristine and ergocornine, there are few contractility data, and none using the bovine model. The current study reports the evaluation of the vasoconstrictive responses of the commercially available ergonovine, α-ergocryptine, ergocristine, and ergocornine alkaloids using a bovine lateral saphenous vein bioassay.

Exposure of lateral saphenous veins from tall fescue-naive heifers to increasing concentrations of ergonovine, α-ergocryptine, ergocristine and ergocornine resulted in dose-dependent contractile responses that were evident in the traces presented in Figure 3. All 4 alkaloids had a cumulative concentration response as there was negligible recovery during the rinse buffer replacements before subsequent alkaloid additions. This is typical of previous reports for ergopeptide alkaloids (Klotz et al., 2007, 2008), but ergonovine was expected to behave more like lysergic acid, which is easily rinsed from the system allowing the vein segments to return to baseline tension (Klotz et al., 2006).

The normalized data were plotted as contractile responses as a percentage of the corresponding norepinephrine maximum against the increasing concentrations of each alkaloid, and best-of-fit polynomial curves were calculated for all alkaloids evaluated (Figure 4). Responses to all 4 alkaloids were quadratic ($P < 0.01$), with positive $x^2$ coefficients, positive slopes, and no detectable contractile response below $1 \times 10^{-7} M$. Al-
Table 1. The EC₅₀ (M) and Eₘₐₓ (% of norepinephrine maximum) values for ergonovine, ergocryptine, ergocristine, and ergocornine in fescue-naïve bovine lateral saphenous veins¹

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>EC₅₀</th>
<th>Eₘₐₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergonovine, n = 6</td>
<td>3.4 × 10⁻⁶± 8.8 × 10⁻⁷</td>
<td>68.5° ± 4.1</td>
</tr>
<tr>
<td>Ergocryptine, n = 6</td>
<td>5.4 × 10⁻⁶± 1.2 × 10⁻⁶</td>
<td>42.9° ± 4.1</td>
</tr>
<tr>
<td>Ergocristine, n = 5</td>
<td>5.6 × 10⁻⁶± 1.3 × 10⁻⁶</td>
<td>45.5° ± 4.5</td>
</tr>
<tr>
<td>Ergocornine, n = 4</td>
<td>4.0 × 10⁻⁶± 2.3 × 10⁻⁵</td>
<td>57.2° ± 9.9</td>
</tr>
</tbody>
</table>

*Means within a column not sharing like superscripts are different (P < 0.05).

¹EC₅₀ = the potency of an agonist expressed as the molar concentration producing 50% of the maximum response. Eₘₐₓ = the maximal contractile response expressed as a percentage of the 1 × 10⁻⁹ M norepinephrine reference addition.

Figure 4. Mean contractile responses of tall fescue-naïve bovine saphenous veins to increasing concentrations of ergonovine (●; n = 6), α-ergocryptine (△; n = 6), ergocristine (■; n = 5), and ergocornine (◊; n = 4). The best-of-fit polynomial lines demonstrate the effect of alkaloid concentration on contractile response for each alkaloid. Line equations are y = 3.2x² + 56.8x + 247.8, r² = 0.98 for ergonovine; y = 2.1x² + 37.5x + 150.9, r² = 0.99 for α-ergocryptine; y = 2.1x² + 37.9x + 164.1, r² = 0.99 for ergocristine; and y = 2.4x² + 43.1x + 186.6, r² = 0.99 for ergocornine. Effects of alkaloid and concentration were both significant (P < 0.01), and the alkaloid × concentration was also significant (P = 0.03).

Though all 4 alkaloids had significant quadratic effects, the slope of ergonovine was greater (P < 0.01) than ergocryptine, ergocornine, and ergocristine, which all had similar slopes. The effects of alkaloid, concentration, and alkaloid by concentration were all significant. The Eₘₐₓ (i.e., contractile intensities at the 1 × 10⁻⁹ M response) are listed in Table 1 for each alkaloid evaluated. Ergonovine had the greatest Eₘₐₓ (68.5 ± 4.1%) when compared with ergocryptine and ergocristine (P < 0.05), but was not different than the Eₘₐₓ for ergocornine (P = 0.29). This was opposite to previous observations of ergoline (e.g., lysergic acid) and ergopeptide (e.g., ergovaline) alkaloids using this bioassay, where the ergopeptide alkaloid generated the greater...
contractile intensity (Klotz et al., 2008). The EC$_{50}$ or potency of ergocornine (Table 1) was approximately 10-fold greater or less potent than ergonovine, ergocristine, or ergocryptine ($P < 0.05$).

The hypothesis that ergopeptines that differ at the R$_2$ position (e.g., ergocryptine, ergocristine, and ergocomnine consisting of the AA leucine, phenylalanine, or valine, respectively), but not the R$_1$, will generate similar contractile responses was strengthened with the comparison of α-ergocryptine, ergocornine, and ergonovine not resulting in different E$_{max}$ values (Table 1) or slopes (Figure 4) in this experiment. This is also in agreement with the comparison of ergovaline and ergotamine by Klotz et al. (2007), which resulted in similar contractile responses. Further, the structural differences between ergonovine and the 3 ergopeptines resulted in different contractile response slopes and E$_{max}$ for ergocryptine and ergocristine. This lends credence to the possibility that the differences in the ergopeptines at the R$_{1}$ group have a greater impact on receptor binding and vasoconstriction than differences at the R$_{2}$ position. This differs from a report by Solomons et al. (1989) where ergotamine and ergosine (Figure 2B; differ only at the R$_{2}$ position) were evaluated using the bovine dorsal pedal vein model. In their model, ergotamine was found to be more potent and generated greater mean maximal contraction than ergosine.

The E$_{max}$ and EC$_{50}$ values for ergovaline and lysergic acid were calculated from data obtained from biopsied fescue-naïve bovine lateral saphenous veins originally reported by Klotz et al. (2008). The inclusion of these ancillary data allows the comparison of structural differences at the R$_{1}$ position to demonstrate differences in contractile response. When considering ergovaline and ergocornine (Figure 2B), which differ at the R$_{1}$ and not the R$_{2}$, the difference in E$_{max}$ is doubled and the EC$_{50}$ is an order of magnitude less for ergovaline (E$_{max}$ = 104.1 ± 6.0% of norepinephrine maximum; EC$_{50}$ = 4 × 10$^{-6}$ ± 1.5 × 10$^{-6}$ M) compared with ergocornine (Table 1). Conversely, when comparing the E$_{max}$ and EC$_{50}$ for lysergic acid and ergonovine, it is evident that the presence of the 2-propanolamide on the ergoline ring of ergonovine. This pseudo-R$_{1}$ group found on the substituent of ergonovine (Figure 1B) is a methyl group compared with the isopropyl group found on the R$_{1}$ of the 3 ergopeptines (Figure 2B). Free rotation of this methyl group at the pseudo-R$_{1}$ position of ergonovine may better accommodate binding at a receptor site than the isopropyl groups of ergocryptine, ergocristine, and ergocornine. An understanding of the relationship an ergot alkaloid has with a receptor and the resultant vascular response may be related to the chemical structure of that alkaloid; hence the type of binding structurally permissible may influence the subsequent signal transduction.

In conclusion, ergonovine is a potent alkaloid that generates an intense contractile response. Ergocristine, α-ergocryptine, and ergocornine did not differ in contractile response, but α-ergocryptine, ergocristine had less E$_{max}$ than ergonovine. Although ergocornine appeared similar to α-ergocryptine and ergocristine, it was less potent as evident by the greater EC$_{50}$. It appears that structural differences in the R$_{2}$ position of ergopeptines do not result in differences when evaluating contractile response. Conversely, the structural difference at the R$_{1}$ position resulting in the AA alanine for ergotamine, ergovaline, and ergonovine or valine for α-ergocryptine, ergocristine, and ergocornine appears to have the potential to influence E$_{max}$ and possibly the potency of ergopeptine alkaloids. These observations on structural differences and how they affect physical response will aid future research concerned with receptor binding of alkaloids and the mechanisms by which alkaloids elicit the observed vasoconstrictive effects on grazing animals.

**LITERATURE CITED**


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