Effects of larkspur (*Delphinium barbeyi*) on heart rate and electrically evoked electromyographic response of the external anal sphincter in cattle

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Objective—To determine whether larkspur-derived *N*-(methylsuccinimido) anthranoyllycoctonine (MSAL)-type alkaloids alter heart rate and electrically evoked electromyographic (eEMG) response of the external anal sphincter (EAS) in cattle and whether these effects can be reversed by acetylcholinesterase inhibitors.

Animals—12 beef heifers and 4 cows.

Procedures—3 or 4 heifers were used in 1 or 2 of 7 dose-response experiments; heart rate and EAS eEMG response were assessed before and 24 hours after oral treatment with larkspur (doses equivalent to 0.5 to 15 mg of MSAL-type alkaloids/kg). In 3 subsequent experiments, 3 heifers (1 of which was replaced with another heifer in the control experiment) each received 10 mg of MSAL-type alkaloids/kg and were injected IV with physostigmine (0.04 mg/kg), neostigmine (0.04 mg/kg), or saline (0.9% NaCl) solution 24 hours later, prior to assessment. Additionally, EAS eEMG response was measured in 4 cows before and after epidural administration of 2% lidocaine hydrochloride.

Results—Larkspur-treated heifers developed dose-related increases in heart rate and decreases in EAS eEMG response. Twenty-four hours after administration of MSAL-type alkaloids, neostigmine decreased heart rate but did not affect eEMG response, whereas physostigmine did not affect heart rate but caused a 2-fold increase in eEMG response. In cows, epidural anesthesia did not alter eEMG response, suggesting that transdermal stimulation of the EAS pudendal innervation did not occur.

Conclusions and Clinical Relevance—In cattle, cardiac effects and muscle weakness or loss of EAS eEMG response induced by larkspur-derived MSAL-type alkaloids were reversed by neostigmine or physostigmine, respectively. Treatment with anticholinesterase inhibitors may alter the clinical effects of larkspur poisoning in cattle. (*Am J Vet Res* 2009;70:539–546)

Toxic larkspur (*Delphinium*) species have been responsible for large losses to the cattle industry in western North America since the beginning of the 20th century.[1–3] Clinical signs of larkspur poisoning in cattle include bloating, respiratory depression, visible decreases in external anal sphincter tone, tremors in locomotor muscles, failure of voluntary muscular coordination, and collapse to sternal or lateral recumbency.[4,5] Nation et al[4] reported that heart rate increased by 50% following IV injection of a larkspur alkaloid in 2 beef calves.

The toxic effects of larkspur have been attributed to diterpenoid alkaloids that are produced by the plant. In larkspur, a mixture of 10 to 15 norditerpenoid alkaloids, C_{20}-diterpenoid alkaloids, or *bis*-diterpenoid alkaloids composes the total diterpenoid alkaloid content, which can represent 3% of plant dry weight. These alkaloids vary in relative abundance among species of larkspur.[6] Of the diterpenoid alkaloids, the MSAL-type norditerpenoid alkaloids are the most toxic to cattle and they have been used as a predictor of plant toxicity.[7–10] The effective oral dose of MSAL-type alkaloids that causes collapse and

Abbreviations

ED_{50}	Median effective dose
EMG	Electromyography
MDL	7,8-methylenedioxylycoctonine
MH^+	Protonated molecular ion
MLA	Methyllycaconitine
MSAL	*N*-(methylsuccinimido)anthranoyllycoctonine
m/z	Mass-to-charge ratio
nAChR	Nicotinic acetylcholine receptor
RMS	Root mean square

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sternal recumbency in cattle has been estimated to be 11.2 mg/kg.[11] Moreover, these alkaloids have been described as possessing curariform activity and are potent competitive blockers of nAChRs in autonomic neurons and voluntary striated muscle.[12–14]

The most abundant MSAL-type alkaloids found in larkspur species are MLA, nudicauline, and 14-deacetylnudicauline.[15] Of these, MLA is the most thoroughly investigated and, at nanomolar concentrations, is a potent and selective competitive antagonist of α_7-nAChRs.[16–18] Methyllycaconitine has an affinity value (as determined from the inhibition of radioligand binding in competitive binding assays [K_i]) in the nanomolar range at α_7-nAChRs and in the micromolar range at muscle-type, $\alpha_4\beta_2$-, and $\alpha_3\beta_4$-nAChRs.[14] Nudicaline has nanomolar affinity at the α_7-nAChR, and functional studies have revealed that nudicaline and 14-deacetylnudicauline have 50% inhibitory concentration values in the micromolar range in neuromuscular preparations of lizard sciatic nerve and extensor digitorum longus muscle.[13,19] However, to our knowledge, ED_{50} values for early or subclinical physiologic responses of cattle to larkspur alkaloids and quantitative physiologic data on the effects of drugs at reversing the actions of toxic larkspur alkaloids in cattle have not been published. The objective of the study reported here was to determine whether larkspur-derived MSAL-type alkaloids alter heart rate and electrically evoked EMG response of the external anal sphincter in cattle, establish ED_{50} values for larkspur with regard to toxic effects on heart rate and external anal sphincter EMG response, and determine whether these effects can be reversed by acetylcholinesterase inhibitors.

Materials and Methods

Plant material and chemicals—Tall larkspur (*Delphinium barbeyi*) in the early flowering stage was collected during July 2003 near Manti, Utah, (N lat 39° 03.154, W long 111° 30.752′; Poisonous Plant Research Laboratory collections No. 03-12) at an elevation of approximately 3,000 m above sea level. A voucher specimen was deposited at the Utah State University Herbarium (No. 237,494). The plant material was air-dried, ground until sufficiently fine to pass through a 2.38-mm mesh, and mixed by use of a grinder-mixer.[a] After processing, the plant material was stored in plastic bags away from direct light at ambient temperature (approx 9°C) until use. Improved mixed pasture grass hay was similarly ground and used as a control treatment.

Neostigmine was obtained as neostigmine methylsulfate[b] (1.0 mg/mL), a form of neostigmine that is commonly used in veterinary medicine. Neostigmine is an acetylcholinesterase inhibitor that does not effectively cross the blood-brain barrier. Physostigmine was obtained as physostigmine hemisulfate; it is an acetylcholinesterase inhibitor that does effectively cross the blood-brain barrier. Physostigmine and reserpine were both obtained from a commercial source.[c]

Alkaloid analysis—Replicate samples (n = 5) were extracted[15] and analyzed for alkaloid content by use of previously described methods.[20] A reserpine concentration of 500 µg was used as an internal standard. Individual alkaloid concentrations were quantified from peak areas in ion chromatograms generated from their respective protonated ions in reference to calibration curves. These alkaloid concentrations were used for the calculation of doses of MSAL-type alkaloids in the form of dried ground larkspur.

Animals—Twelve mixed-breed beef heifers (mean weight, 596 kg; range, 478 to 700 kg) and 4 cows were used in the study. The heifers (each assigned an identification number) and cows were maintained on alfalfa-grass hay with supplemental minerals for at least 3 weeks before and between dose-response trials; these animals were accustomed to all study procedures.

Dose-response trials and anticholinesterase agent experiments—In a group of 3 of the 12 heifers (heifers 00, 08, and 09), a control trial was performed a minimum of 11 days prior to commencement of the dose-response trials in those cattle. In the control trial, ground dried grass hay was administered via oral gavage at a dose of 1.8 g/kg. This dose was identical by weight to the total ground larkspur material given to obtain a dose that was equivalent to 10 mg of MSAL-type alkaloids/kg.

For the dose-response trials, the 12 heifers were used in groups of 3. For a given trial, each heifer in a group received the same dose of ground larkspur and was monitored during the same week. After each dose-response trial, an interval of a minimum of 21 days elapsed before the heifers underwent another trial at a different dose; however, for 1 heifer, a 10-day interval elapsed between dose-response trials. The duration of the washout period was based on data from previous experiments that indicated that larkspur toxins are rapidly eliminated from the circulation and that residual concentrations of larkspur alkaloids are likely to be eliminated after several days.[21] Additionally, another study by our group revealed that cattle given larkspur eliminate 99% of serum MLA and deltaline after 144 hours.[22] The doses of ground larkspur used for these trials were equivalent to 0.5, 1, 2, 5, 10, 12, and 15 mg of MSAL-type alkaloids/kg of body weight. The MSAL-type alkaloids are the most toxic of those found in larkspur and were used as the basis for calculating doses in the study.

Heifers underwent 1 or 2 dose-response trials as follows: 0.5 mg/kg dose, heifers 06, 09, and 10; 1 mg/kg dose, heifers 07, 06, and 10; 2 mg/kg dose, heifers 00, 07, and 08; 5 mg/kg dose, heifers 01, 04, and 97; 10 mg/kg dose, heifers 05, 12, and 15; 12 mg/kg dose, heifers 01, 04, and 97; and 15 mg/kg dose, heifers 03, 07, and 10.

For the anticholinesterase agent experiments, the 3 heifers that were given a dose of ground dried larkspur that was equivalent to 10 mg of MSAL-type alkaloids/kg (heifers 05, 12, and 15) were treated IV with neostigmine (0.04 mg/kg) at the end of the dose-response trial (ie, after measurements were obtained at the 24-hour time point). Heart rate and evoked EMG response of the external anal sphincter were measured immediately before and after administration of neostigmine. Three weeks later, another experiment was performed with the same 3 heifers. The heifers were monitored before and at 24 hours after administration of a dose of lark-

spur that was equivalent to 10 mg of MSAL-type alkaloids/kg; immediately following the 24-hour assessments, the heifers were treated IV with physostigmine (0.04 mg/kg). Heart rate and evoked EMG response were measured immediately before and after the administration of physostigmine. Finally, 3 weeks after the physostigmine experiment, 2 of the heifers from the physostigmine and neostigmine experiments (heifers 05 and 15) and a replacement heifer (heifer 09) were used in a control treatment experiment; 1 heifer from the physostigmine and neostigmine experiments (heifer 12) was replaced in this control treatment experiment because it became belligerent. The heifers were monitored before and at 24 hours after administration of a dose of larkspur that was equivalent to 10 mg of MSAL-type alkaloids/kg; immediately following the 24-hour assessments, the heifers were treated IV with 0.02 mL/kg of physiologic saline (0.9% NaCl) solution.[d] Heart rate and evoked EMG response of the external anal sphincter were measured immediately before and after the administration of saline solution. Each anticholinesterase agent experiment was initiated in all 3 heifers on the same day; for the control treatment experiment, the replacement heifer was processed on a different day but within the same week as the other 2 heifers. The data collected from the replacement heifer before and after administration of a dose equivalent to 10 mg of MSAL-type alkaloids/kg were included in the analysis of the 10 mg/kg dose-response trial data.

Dose administrations and monitoring procedures—Food was withheld from each heifer overnight (approx 17 hours) prior to the day of each trial or experiment. During each experimental period, each heifer was weighed and restrained in a squeeze chute. Baseline measurements of heart rate and electrically evoked EMG response were recorded immediately prior to the administration of a single larkspur dose. Each dose of the finely ground dried larkspur or finely dried ground pasture grass mixture (control trial) were administered via oral gavage in approximately 11 L of tap water. The heifer was then released to an individual pen as previously described.[11] Twenty-four hours after dose administration, each heifer was again restrained in a squeeze chute and heart rate and electrically evoked EMG responses were monitored.

In each anticholinesterase or control treatment experiment, each heifer was placed in the squeeze chute and monitored at 24 hours after receiving a dose of larkspur equivalent to 10 mg of MSAL-type alkaloids/kg; via a jugular vein, an injection of an anticholinesterase drug or an equal volume of saline solution (as a control treatment) was administered, and assessments were made during a 15-minute period before and after each injection.

If a heifer became recumbent as a result of larkspur toxicosis, neostigmine (0.02 mg/kg, IM) was administered as a reversing agent.[23] All animal work was done under veterinary supervision and with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

Physiologic monitoring—For each heifer, heart rate and electrically evoked external anal sphincter EMG response were simultaneously recorded by use of an analog to digital converter and signals amplified with an amplifier.[e] Assessors were not blinded to the dose or treatment administered. Heart rate was monitored by use of repositionable monitoring electrodes[f] that were cemented in place with a gel-based formulation of cyanoacrylate adhesive.[g] The lead placements were similar to those described by Chen et al,[24] with the exception of the ground electrode, which was attached to the perineum. The heart rate signal was amplified with a gain range limited to ± 500 μV. The heart rate signal was filtered with a mains filter, a 60-Hz notch filter, a 120-Hz low-pass–0.1-Hz high-pass filter, and a digital band-pass filter with a high cut-off frequency of 45 Hz and a low cut-off frequency of 0.1 Hz. The cyclic measurements feature of the software package[h] was used to calculate heart rate (beats/min).

A large human anal canal EMG probe[i] was used to measure electrically evoked EMG responses of the external anal sphincter (ie, the EMG response). The probe was moistened with ultrasound transmission gel[j] prior to use. Muscular contractions were evoked by use of a stimulating bar electrode[e] that was coated with ultrasound transmission gel and placed dorsal to the anus to transdermally stimulate the external sphincter muscle. A stimulus train of fifteen 2.0-millisecond rectangular pulses, each of which had an amplitude of 10 mA and a current frequency of 10 Hz, was delivered. The probe signal was amplified with a gain range limited to ± 1 mV and filtered with a mains filter, a 60-Hz notch filter, a 60-Hz low-pass–0.3-Hz high-pass filter, and a digital band-pass filter with a high cut-off frequency of 45 Hz and a low cut-off frequency of 0.3 Hz. The RMS of the microvolt EMG signal in response to electrical stimulation was calculated from the filtered response during the stimulus period (1.5 seconds) by use of computer software.[25,h]

In a separate experiment with 4 cows, the evoked EMG response in each cow was measured as described, and then a regional nerve block (epidural anesthesia) was performed via administration of an injection of 5 to 10 mL of 2% lidocaine hydrochloride[k] between first and second coccygeal vertebrae. After the nerve block was established, the evoked EMG response was determined again as described.

Data analysis—Data are expressed as the mean ± SE of each physiologic response. The data recordings of heart rate and electrically evoked EMG response in each animal were allowed to form a stable baseline before being selected for analysis (typically after a period of 10 to 15 minutes). After baseline stabilization, the mean heart rate over a 4-minute period was determined and the RMS of 5 electrically evoked EMG responses during that same period were calculated. The estimation of dose-response relationships for the MSAL-type alkaloids was performed by use of computer software.[l] The data were analyzed by use of a sigmoidal dose-response equation (variable slope) with logarithmically transformed doses and in the case of the EMG response after normalization of the EMG response as a percentage of baseline measured in each animal. The statistical analyses of data obtained for individual doses of MSAL-type alkaloids and for neostigmine and physostigmine

data were also done with computer software.[1] Comparisons of the EMG responses with baseline values were made by use of a 1-sample *t* test after normalization of the EMG response as a percentage of baseline measured in each animal. Comparisons between a control mean value to a single treatment mean value were made by use of a paired 2-tailed *t* test. Comparisons between baseline and multiple treatment means were made by use of an ANOVA followed by a Dunnett test or Tukey-Kramer test as indicated. Comparisons between repeated measures were made by use of a repeated-measures ANOVA and a Tukey-Kramer posttest as indicated. In all analyses, a value of $P < 0.05$ was set as the limit for significance.

Results

Plant chemical characteristics—The norditerpenoid alkaloid extract of *D barbeyi* was assessed via mass spectrography (**Figure 1**). The extract of this larkspur collection from the year 2003 contained 13.2 mg of norditerpenoid alkaloids/g of which 7.4 mg represented the MDL-type alkaloids and 5.8 mg represented the MSAL-type alkaloids. Deltaline (MH^+ m/z, 508) and MLA (MH^+ m/z, 683) were the 2 major norditerpenoid alkaloids in the extract. Deltaline composed approximately 73% of the MDL-type alkaloids and 41% of the total alkaloids. Methyllycaconitine composed approximately 88% of the MSAL-type alkaloids and 39% of the total alkaloids. In addition, 14-deacetylnudicauline (MH^+ m/z, 669) represented approximately 8% of the MSAL-type alkaloids, barbinine (MH^+ m/z, 667) represented approximately 4% of the MSAL-type alkaloids, and although nudicauline (MH^+ m/z, 711) could be detected, it was present in an amount that was less than the lower measurable limit.

Effects of larkspur alkaloids on heart rate and electrically evoked EMG response of the external anal sphincter—Baseline data were derived from data collected at the beginning of 27 experimental periods as follows: dose-response trials for doses of larkspur equivalent to 0.5 mg of MSAL-type alkaloids/kg (n = 3 heifers), 1 mg/kg (3), 2 mg/kg (3), 5 mg/kg (3), 10 mg/kg (4), and 12 mg/kg (3); pasture grass hay control trial (3); physostigmine experiment (3); and saline solution control experiment (2 [although 3 heifers were used in this experiment, baseline data from the replacement heifer were included with the baseline data from the 10 mg/kg dose-response trial]). Data collected during the dose-response trial involving a dose equivalent to 15 mg of MSAL-type alkaloids/kg were not included in these calculations because of adverse reactions among the treated heifers. There were no baseline data from the neostigmine experiment because it was performed as a continuation from the 10 mg/kg dose-response trial. From the data analyzed, mean ± SE baseline heart rate and baseline EMG response RMS values were 74 ± 2 beats/min and 187 ± 10 μV, respectively.

Changes in heart rate and electrically evoked EMG response were detectable at 24 hours after the administration of larkspur. Oral administration of larkspur increased heart rate and decreased the evoked EMG response of the external anal sphincter in a dose-de-

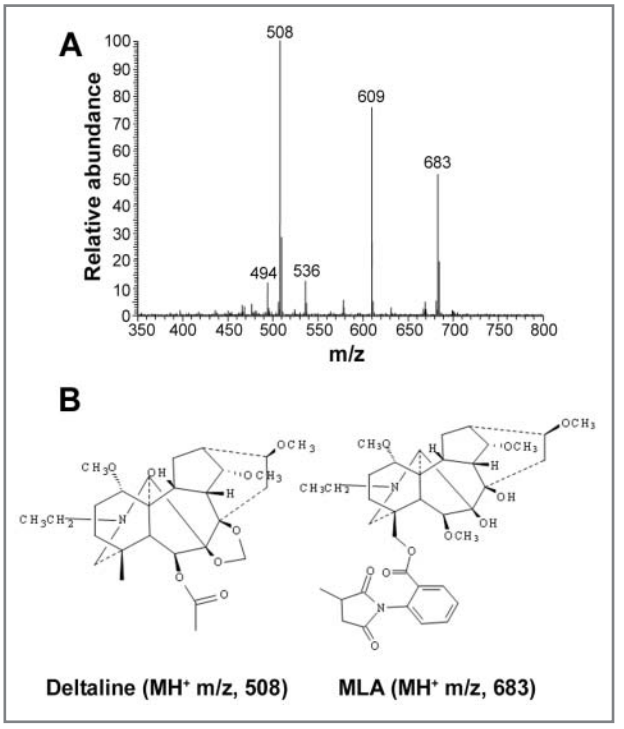

Figure 1—Mass spectrogram of the total alkaloid extract of *Delphinium barbeyi* from Poisonous Plant Research Laboratory collections No. 03-12, Utah State Herbarium voucher No. 237494 (A) and the chemical structures of deltaline and MLA (B). Specific spectrographic peaks include dictyocarpine (MH^+ m/z, 494), deltaline (MH^+ m/z, 508), 14-acetyldictyocarpine (MH^+ m/z, 536), reserpine (an internal standard; MH^+ m/z, 609), and MLA (MH^+ m/z, 683).

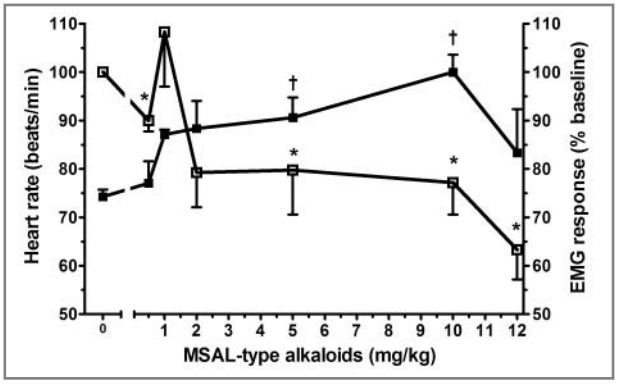

Figure 2—Mean ± SE heart rate (black squares) and electrically evoked EMG response of the external anal sphincter (white squares) in beef heifers at 24 hours after administration (via oral gavage) of ground dried larkspur in doses equivalent to 0.5, 1, 2, 5, 10, 12, and 15 mg of MSAL-type alkaloids/kg. Heifers were monitored before administration of larkspur to determine baseline values. The EMG response is reported as the percentage of baseline measured in each heifer. The 0 mg/kg datum points were derived from baseline data for each heifer and obtained just prior to dose administration; for the EMG response, the 0 mg/kg datum point represents 100% of the response obtained in each heifer just prior to dose administration. Data for the 15 mg/kg dose-response trial were not analyzed because of adverse effects in the recipient heifers. For all doses, each datum point represents the mean values from 3 heifers, except for the 10 mg/kg dose, which represents 9 responses from 4 heifers. *Evoked EMG response value is significantly $P < 0.05$) different from baseline (0 mg/kg) value measured prior to dose administration. †Heart rate is significantly ($P < 0.05$) different from baseline (0 mg/kg) value measured prior to dose administration.

pendent manner (**Figure 2**). Of the 6 larkspur doses investigated that did not cause recumbency, only the doses equivalent to 5 and 10 mg of MSAL-type alkaloids/kg significantly increased the heart rate, compared with baseline values, in the heifers at 24 hours. The maximum increase in heart rate (100 ± 4 beats/min; 9 measurements from 4 animals, prior to the administration of the anticholinesterase agents and saline control experiment) was observed at a dose equivalent to 10 mg of MSAL-type alkaloids/kg. The EMG response was different from baseline after administration of doses equivalent to 0.5, 10, and 12 mg of MSAL-type alkaloids/kg. The estimated ED_{50}s for the heart rate and EMG response were 1.74 mg of MSAL-type alkaloids/kg and 47.57 mg of MSAL-type alkaloids/kg, respectively (95% confidence intervals for heart rate and EMG response were 0.33 to 8.96 mg/kg and 3.45 to 655.7 mg/kg, respectively). No overt signs of intoxication were observed at 8 hours after heifers received a dose equivalent to 12 mg of MSAL-type alkaloids/kg. At 24 hours, the 12 mg/kg dose did induce signs of skeletal muscle weakness, but these were not sufficiently deleterious to warrant discontinuation of experiments.

The highest dose of ground dried larkspur used in the study was equivalent to 15 mg of MSAL-type alkaloids/kg. The 3 heifers treated with this dose of larkspur collapsed to sternal or lateral recumbency after 5 to 8 hours. Immediately after collapse, these heifers were given an injection of neostigmine (0.02 mg/kg, IM) as a rescue treatment. All 3 animals regained a standing position within 15 minutes. Twenty-five hours later, 2 of the heifers were again recumbent and received a second IM injection of neostigmine (0.02 mg/kg); after 35 hours, all affected animals had recovered from larkspur intoxication and were ambulatory. Any physiologic data obtained from these animals prior to collapse were not used for analysis; no data were collected subsequent to collapse because of the heifers' severe instability.

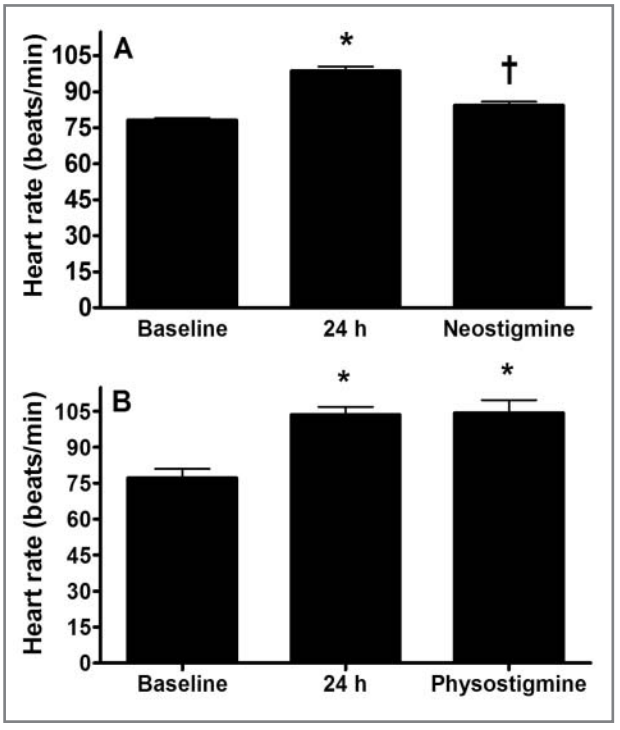

Figure 3—Effect of neostigmine (A) and physostigmine (B) on larkspur-induced changes in heart rate in a group of 3 beef heifers. In 2 experiments (21-day interval), heart rate was assessed in each of the 3 heifers before (baseline) and at 24 hours after administration (via oral gavage) of a dose of ground dried larkspur that was equivalent to 10 mg of MSAL-type alkaloids/kg. In the first experiment, neostigmine (0.04 mg/kg, IV) was administered after data collection at the 24-hour time point; in the second experiment, physostigmine (0.04 mg/kg, IV) was administered after data collection at the 24-hour time point. Data are reported as mean ± SE values. *Value is significantly ($P < 0.05$) different from the baseline value. †Value is significantly different from the baseline value and from the 24-hour value ($P > 0.05$ and $P < 0.01$, respectively).

In the pasture grass hay control trial, ground pasture grass hay was administered orally to 3 heifers at a dose of 1.8 g/kg (identical by weight to the total ground larkspur material in the dose that was equivalent to 10 mg of MSAL-type alkaloids/kg). Heart rate in the pasture grass hay–treated heifers at 24 hours after treatment (78 ± 5 beats/min) did not differ (paired *t* test, $P = 0.105$) from baseline values recorded 24 hours earlier (67 ± 6 beats/min). Likewise, the evoked EMG response was not significantly (1-sample *t* test, $P = 0.363$) different from baseline at the 24-hour time point (153 ± 45% of baseline) recorded 24 hours earlier. The evoked EMG responses of the external anal sphincter were also measured in 4 cows before (baseline) and after epidural injection of an anesthetic agent; after establishment of the regional nerve block, there was no significant difference in EMG response, compared with data recorded prior to nerve block (data not shown). This suggested that transdermal stimulation of the pudendal innervation of the external anal sphincter did not occur.

Effects of anticholinesterase agents on heart rate and EMG responses to administration of larkspur alkaloids—The effects of 2 anticholinesterase agents on

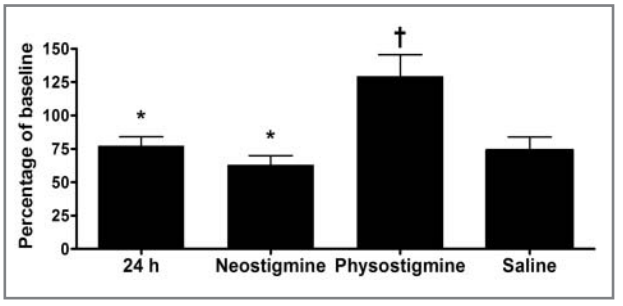

Figure 4—Effect of neostigmine, physostigmine, and saline (0.9% NaCl) solution on larkspur-induced changes in the electrically evoked EMG response of the external anal sphincter in the group of 3 beef heifers in Figure 3. In 3 experiments (21-day intervals), EMG response was assessed in each of the 3 heifers before (baseline) and at 24 hours after administration (via oral gavage) of a dose of dried ground larkspur that was equivalent to 10 mg of MSAL-type alkaloids/kg. In the saline solution control experiment, 1 of the 3 heifers used in the anticholinesterase experiments was replaced. After data collection at the 24-hour time point in the first, second, and third experiments, neostigmine (0.04 mg/kg, IV), physostigmine (0.04 mg/kg, IV), and saline solution (equivalent volume, IV) were administered, respectively. Data are reported as mean ± SE values (n = 3), except for the mean value at the 24-hour time point, which represents the mean value of 9 normalized responses from 4 heifers. †Value for physostigmine is significantly ($p<0.01$) different from each of the other 3 values. *See* Figure 3 for remainder of key.

larkspur alkaloid–induced physiologic responses in heifers were also determined. The 3 heifers that were used in the dose-response trial involving a dose equivalent to 10 mg of MSAL-type alkaloids/kg were used in the anticholinesterase experiments; one of these heifers was replaced with another of the 12 study animals for the final saline solution (control treatment) experiment. In each experiment, heifers were monitored before and 24 hours after administration of the 10 mg/kg dose of MSAL-type alkaloids; after data collection at the 24-hour time point, heifers were administered neostigmine (0.04 mg/kg, IV) in the first experiment, physostigmine (0.04 mg/kg, IV) in the second experiment, and saline solution (equivalent volume, IV) in the third experiment. In the first 2 drug experiments, heart rate was increased, compared with the baseline value, at 24 hours after administration of ground larkspur (**Figure 3**). Heart rate returned to baseline values after the administration of neostigmine but not after administration of physostigmine. In the control treatment trial, the 3 heifers (2 that had been used in the drug experiments and 1 replacement) had a mean baseline heart rate of 73 ± 9 beats/min; this increased to 98 ± 12 beats/min at 24 hours after receiving a dose equivalent to 10 mg of MSAL-type alkaloids/kg. Following IV treatment of these 3 heifers with saline solution, mean heart rate was 91 ± 8 beats/min. This value was significantly different from baseline but not significantly different from the heart rate measured just prior to injection (repeated-measures ANOVA, $P = 0.006$; Tukey-Kramer test [n = 3] of baseline vs 24 hours, $P = 0.01$; Tukey-Kramer test of baseline vs saline solution treatment, $P < 0.05$; and Tukey-Kramer test of 24 hours vs saline solution treatment, $P > 0.05$).

The electrically evoked EMG response of the external anal sphincter was simultaneously recorded with heart rate in the experiments involving neostigmine, physostigmine, and saline solution in the aforementioned heifers. The electrically evoked external anal sphincter EMG response at 24 hours after a dose of larkspur equivalent to 10 mg of MSAL-type alkaloids/kg was decreased, compared with the response measured prior to larkspur administration (**Figure 4**). Intravenous administration of neostigmine or an equivalent volume of saline solution given after collection of data at the 24-hour time point did not alter the sustained decrease in evoked EMG response of the external anal sphincter. However, physostigmine administration induced a significant, compared with the effect of neostigmine, 2-fold increase in evoked EMG response.

Discussion

The tall larkspur used in the present study was chemically similar to other larkspur collections.[26] Of the total norditerpenoid alkaloids extracted, 56% were the MSAL type. The MSAL-type alkaloids were used as the basis for calculating the doses of plant material for administration to the heifers because they are the most toxic of the norditerpenoid alkaloids. For example, the dose of MLA (an MSAL-type alkaloid) that results in recumbency in calves is 25-fold less than that required for deltaline (an MDL-type alkaloid).[2]

Among the heifers used in the present study, there was a dose-dependent increase in heart rate and decrease in the evoked EMG response of the external anal sphincter at 24 hours after oral administration of toxic larkspur containing MSAL-type alkaloids. Because of the toxic effects of these alkaloids at the 15 mg/kg dose, we were unable to measure the maximal effects in our study and were only able to provide an estimate of the ED_{50} values for heart rate and evoked EMG response because of wide confidence intervals. The MSAL-type alkaloids are thought to be competitive antagonists with high affinity and potency at both neuronal-type and muscle-type nAChRs.[13,14] This may explain the effects of these toxins in cattle that are poisoned by larkspur. The inhibition of cholinergic neurotransmission at neuronal-type nAChRs in the intracardiac ganglion could result in tachycardia. Moreover, the decreased evoked EMG response can be attributed to the actions of norditerpenoid alkaloids at muscle-type nAChRs in the external anal sphincter.

The dose of ground larkspur that resulted in clinical signs of larkspur toxicosis and time of onset of the toxic effects determined in the heifers of the present study varied from findings of previous reports.[11,27] In our study in beef cattle, tremors associated with muscle weakness were observed at 24 hours after administration of a dose of ground dried larkspur that was equivalent to 12 mg of MSAL-type alkaloids/kg. Only the largest dose of 15 mg of MSAL-type alkaloids/kg caused collapse and sternal recumbency or resulted in signs of intoxication prior to the 24-hour time point. In studies[11,27] in dairy cattle, signs of intoxication were observed in as little as 5 hours after administration of larkspur and the dose that induced collapse to recumbency was estimated at 11.2 mg/kg. On the basis of these observations, it is tempting to hypothesize that there are breed-specific differences in susceptibility of cattle to larkspur intoxication and to suggest that there is a need for studies of such potential differences.

In the present study, the effects of neostigmine and physostigmine on larkspur-induced physiologic responses (ie, heart rate and evoked EMG response of the external anal sphincter) in cattle were also investigated. Physostigmine has been used in the past to reverse the curare-like effects of larkspur alkaloids in cattle.[27] Neostigmine is a quaternary ammonium derivative of physostigmine that does not as effectively cross the blood-brain barrier and is likely to have fewer CNS-associated adverse effects in field use.[28,29] However, in our study, neostigmine but not physostigmine reduced the larkspur-induced tachycardia at 24 hours after administration of the plant preparation. The reduction in heart rate by neostigmine is attributable to the direct inhibition of acetylcholinesterase in intracardiac ganglia.[30] Conversely, physostigmine was more effective at reversing the alkaloids' effect on the external anal sphincter. Physostigmine is a more potent inhibitor of acetylcholinesterase than neostigmine, and this is likely why it reversed the effects of the larkspur alkaloids on the external anal sphincter–evoked EMG response more effectively than did neostigmine.[31] The lack of physostigmine effect on heart rate may have been the result of the drug crossing the blood-brain barrier and subsequent inhibition of acetylcholinesterase in the medullary vasomotor and cardiac centers of the brain, which

would lead to stimulation of these centers and counter the anticholinesterase effects at the heart.[32]

To the authors' knowledge, this is the first report of quantitative assessment of the actions of neostigmine in larkspur-poisoned cattle. At 24 hours after administration of a dose of larkspur that was equivalent to 10 mg of MSAL-type alkaloids/kg, IV administration of neostigmine at a dose of 0.04 mg/kg significantly reduced the observed tachycardia in the study heifers. Furthermore, results of our study indicated that neostigmine given IM at a dose as low as 0.02 mg/kg can be used as a rescue treatment for intoxicated cattle in recumbency. A lower dose of anticholinesterase agent was used as the rescue treatment because those cattle were not restrained and, in light of adverse cholinergic effects, were not safe to approach for treatment.

Larkspur-associated death in cattle is thought to be the result of recumbency caused by the curare-like effects of norditerpenoid alkaloids and the inhibition of eructation.[2] Reversal of recumbency (most importantly, lateral recumbency) by the use of neostigmine may provide a means with which the medical outcome of larkspur poisoning in cattle can be altered without the complication of CNS effects associated with the use of physostigmine. Further research is needed to investigate the usefulness of neostigmine alone and in combination with physostigmine in laboratory and field applications and to investigate the reversal of MSAL-type alkaloid toxicosis in cattle that have ingested lethal amounts of plant material.

The results of the present study suggest that MSAL-type larkspur alkaloids alter neurotransmission at autonomic ganglia and neuromuscular junctions in cattle. Neostigmine reversed the cardiac effects of toxic larkspur alkaloids, whereas physostigmine reversed larkspur-induced decreases in evoked EMG response. Whether these anticholinesterase agents provide a treatment option for the clinical effects of larkspur poisoning in cattle warrants investigation.

a. Gehl Mix-All model 55, Gehl Co, West Bend, Wis.
b. Baxter Healthcare Corp, Deerfield, Ill.
c. Sigma-Aldrich Inc, St Louis, Mo.
d. Hospira Inc, Lake Forest, Ill.
e. Powerlab and Octal Bioamp, ADInstruments Inc, Colorado Springs, Colo.
f. 3M Red Dot model 2670 repositionable monitoring electrodes, 3M Corp, Saint Paul, Minn.
g. Henkel Consumer Adhesive Inc, Avon, Ohio.
h. ADInstrument Chart software package, version 5.4.2, ADInstruments Inc, Colorado Springs, Colo.
i. SRS Medical, Redmond, Wash.
j. Graham-Field Health Products, Atlanta, Ga.
k. Vedco Inc, St Joseph, Mo.
l. GraphPad Prism, version 4.03 for Windows, GraphPad Software, San Diego, Calif.

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