Larkspur Alkaloid Structure and Function
Toxic larkspur (*Delphinium*) species have been responsible for large losses to the cattle industry in Western North America since the beginning of the twentieth century (Marsh et al., 1916; Marsh et al., 1934).
Curariform Activity of Larkspur Alkaloids

- Toxic Larkspur alkaloids are competitive antagonists of nicotinic acetylcholine receptors (nAChR) in voluntary, striated muscle.
Competitive Antagonists

- Competitive antagonists have no efficacy.
- These drugs compete with agonists for the binding of the same receptor site to reduce the potency of agonists.
- The effect of a competitive antagonist can be overcome by excess agonist.

A = agonist alone
B = antagonist (one concentration)
A+B = agonist + antagonist

Levy, 2003
Larkspur Alkaloid Structure

- *N*- (methylsuccinimido) anthranoyllycocotonine type (MSAL-type).
  - MSAL Alkaloids are a predictor of plant toxicity.
  - Methyllycaconitine (MLA) most studied of the MSAL-type.
- 7,8-methylenedioxylycoconine type (MDL-type) norditerpenoid alkaloids.
  - Not as toxic individually, but thought to contribute to overall plant toxicity.
  - Deltaline is a typical MDL-type alkaloid.

![Deltaline (MH+ = 508) - Methyllycaconitine (MH+ = 683)](image-url)
**Affinity**

The tenacity by which a drug binds to its receptor.

**KD** (in moles/liter) represents the affinity of a drug for a receptor.

- **KD** = [ligand] which occupies half the receptors.

The ligand concentration at which half of the receptors are occupied (Kd) is often lower than the concentration required to elicit a half-maximal biological response (ED$_{50}$)

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**Diagram:**

- **Saturation Binding Curve (linear scale)**
- **Legend:**
  - **Drug A**
  - **Drug B**

**Equation:**

$$k_{on} \quad [\text{ligand}] + [\text{receptor}] \leftrightarrow [\text{ligand} \bullet \text{receptor}] \quad k_{off}$$

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Levy, 2003
# Affinities of Selected Antagonists From Plants.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Plant</th>
<th>Affinity, $K_i$ (nM) $\alpha_7$-nAChR</th>
<th>Affinity, $K_i$ (nM) $\alpha_4\beta_2$-nAChR</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-tubocurarine</td>
<td><em>Chondodendron tomenosum</em></td>
<td>25000</td>
<td>13.9</td>
</tr>
<tr>
<td>Methyllycaconitine (MLA)</td>
<td><em>Delphinium spp.</em></td>
<td>0.69</td>
<td>3700</td>
</tr>
<tr>
<td>Erysodine</td>
<td><em>Erythrina spp.</em></td>
<td>4000</td>
<td>5</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td><em>Erythrina spp.</em></td>
<td>9000</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Mechanism of Toxin Action in Larkspur Poisoned Cattle

- Attributed to diterpenoid alkaloids produced by the plant and found in high concentration in plant tissues.
- Toxic alkaloid content can represent 3% of plant dry weight
  - mixture of 10-15 alkaloids
    - Diterpenoid alkaloids
    - Norditerpenoid alkaloids
Methyllycaconitine (MLA)

- MLA has been described as possessing curariform activity.
- Potent competitive antagonist of nicotinic acetylcholine receptors in autonomic neurons and voluntary, striated muscle.
  - Selective for $\alpha_7$-nicotinic acetylcholine receptors.
- Affinity values
  - Nanomolar range at $\alpha_7$-nicotinic acetylcholine receptors.
  - Micromolar range at muscle-type, $\alpha_4\beta_2$ and $\alpha_3\beta_4$-nicotinic acetylcholine receptors.
Other Alkaloids

- Nudicaline has nanomolar affinity at $\alpha_7$-nicotinic acetylcholine receptors.

- Nudicaline and 14-deacetylnudicauline
  - $IC_{50}$ values in the micromolar range in the lizard sciatic nerve extensor digitorum longus preparation (Hardick et al., 1996; Dobelis et al., 1999).
Dose-response relationships of norditerpenoid alkaloids in cattle

- Cattle were orally dosed and monitored for heart rate (beats/minute) and EMG response (percent of control) at time = 0 and 24 h after dosing.
  - Heart rate ED$_{50}$ = 1.74 mg/kg.
  - EMG response ED$_{50}$ = 47.57 mg/kg.

![Figure 2](image-url)
Clinical Signs of Larkspur Poisoning in Cattle
Initial Symptoms of Poisoning in Cattle

- Lack of appetite
- General uneasiness
- Nausea
- Rapid pulse and respiration
- Stiff staggering gait


Signs of Poisoning Progress To:

- Bloating
- Respiratory depression
- Tremors and weakness in locomotor muscles leading to more generalized tremors (i.e. curariform-like activity).
- Failure of voluntary muscle coordination
- Collapse to sternal or lateral recumbency

Clinical observations of animals dosed with 14.54 mg/kg MSAL-type alkaloids

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Predominate Breed</th>
<th>Neostigmine Dose (mg/kg i.m.)</th>
<th>Time</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Angus</td>
<td>0.02</td>
<td>0900</td>
<td>Dosed with larkspur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td>1530</td>
<td>Sternal recumbency, labored breathing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1540</td>
<td>Standing, periodic collapse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1730</td>
<td>Sternal recumbency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1408</td>
<td>Standing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1413</td>
<td>Standing, Periodic collapse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1650</td>
<td>Sternal recumbency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td>1000</td>
<td>Very weak, sternal recumbency, labored breathing</td>
</tr>
</tbody>
</table>
Anticholinesterase Agents

These agents prolong the existence of acetylcholine by the inhibition of acetylcholinesterase at neuromuscular junctions and cholinergic synapses.
Structure of Acetylcholinesterase

Anionic Site

Esteratic Site

Ser
Physostigmine

- Reversible cholinesterase inhibitor.
- Crosses the blood-brain barrier.
- Also known as eserine.
- In humans physostigmine has been used to treat glaucoma and reverse the CNS effects of atropine.
- Physostigmine is not widely used in Veterinary Medicine.
Neostigmine

- Reversible cholinesterase inhibitor.
- Does not effectively cross the blood-brain barrier.
- Sold as Prostigmin®
- In humans has been used as a treatment for myasthenia gravis and for the reversal of non-depolarizing muscle relaxants used in anesthesia.
- In Veterinary Medicine neostigmine has been used as a rumen motility agent in cattle and for the reversal of non-depolarizing muscle relaxants used in anesthesia.

Neostigmine does not effectively cross the blood-brain barrier due to the presence of a charged quaternary amine group.
The effects of anticholinesterase agents on heart rate in larkspur poisoned cattle.

- Animals were given the equivalent dose of 10.4 mg/kg MSAL-type alkaloids in the form of dried ground larkspur and monitored at time = 0 (baseline) and 24 h.
- At 24 h, some animals received 0.04 mg/kg of either neostigmine (A), or physostigmine (B) given by intravenous injection.
  - Bars represent the mean ± S.E. of responses from 3 animals.
  - *P < 0.05 vs. baseline mean
  - **P < 0.01 neostigmine mean vs. 24 hours mean
  - Tukey-Kramer test, repeated measures ANOVA
The effects of anticholinesterase agents on the EMG response in cattle.

- Animals were given the equivalent dose of 10.4 mg/kg MSAL-type alkaloids in the form of dried ground larkspur and monitored at time = 0 (baseline) and 24 h.
- At 24 h, some animals received 0.04 mg/kg of either neostigmine, or physostigmine given by intravenous injection.
  - Bars represent the mean ± S.E. of normalized responses from 9 animals at 24 hours and 3 animals for neostigmine, physostigmine and saline, respectively.
  - *P < 0.05 vs. 100 percent, one sample t test.
  - **P < 0.01 physostigmine mean vs. 24 hours mean
  - A,BP < 0.01 physostigmine mean vs. neostigmine and saline means respectively
  - Tukey-Kramer test, P = 0.005, one-way ANOVA.
Nicotinic Receptor Structure

- Consists of 5 receptor subunits.
  - Seventeen different subunits have been proposed based on genetic sequence.
  - Twelve functional neuronal receptor subunits have been identified $\alpha_{2-10}$ and $\beta_{2-4}$ from a variety of species.
  - Muscle form $(\alpha_1)_2\beta_1\epsilon\delta$
  - Neuronal forms can be a homopentamer or combination of subunits
    - $\alpha_7$
    - $(\alpha_4)_2(\beta_2)_3$

November 2005 Molecule of the Month
www.pdb.org
Bovine Alpha 7 nAChR

CHRNA7 cholinergic receptor, nicotinic, alpha 7 [ Bos taurus ]
GeneID: 282178 updated 01-Feb-2008

See CHRNA7 in MapViewer

www.ncbi.nlm.gov
Structure of Cattle nAChR Genes

Alpha 5

Beta 4

Bos taurus Chromosome 21

www.ncbi.nlm.gov