The Pharmacology of Plant Toxins

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What is Pharmacology?

• **Defined as:** The study of drugs, their sources, preparations, and therapeutic uses.

• **Pharmacodynamics:** The study of mechanisms underlying drug action in the body.

• **Pharmacokinetics:** The study of drug disposition in the body.

• **Pharmacotherapeutics:** The identification and development of clinical applications for drugs to palliate, prevent, or cure disease.
Drug-Receptor Interactions

• The interaction of a chemical (ligand) with specific protein sites (receptors) either in the cell or on its surface.
  – Main classification of drugs.
    • Agonist, a drug that produces a biological effect.
    • Antagonist, a drug that opposes the actions of an agonist.
Classes of Receptors

(A) ION-CHANNEL-LINKED RECEPTOR

(B) G-PROTEIN-LINKED RECEPTOR

(C) ENZYME-LINKED RECEPTOR
Pharmacological Definition of Receptors

The receptor must allow for the recognition and binding of a drug and must satisfy the following criteria:

• **Saturability** – receptors exists in finite numbers.
• **Reversibility** – binding must occur by weak intermolecular forces (H-bonding, van der Waal forces).
• **Stereoselectivity** – receptors should recognize only one of the naturally occurring optical isomers (+ or -, d or l, or S or R).
• **Agonist specificity** – related drugs should bind well, while physically dissimilar compounds should bind poorly.
• **Tissue specificity** – Drug concentrations should be physiologically relevant and binding should occur in tissues known to be sensitive to the endogenous ligand.
Concentration-Effect Relationships of Agonists

- Agonist activity is correlated with its concentration at the receptor.
- This relationship can be plotted as a concentration-effect curve.
- Drug concentration is plotted on the x-axis against graded changes in the magnitude of drug effect on the y-axis.
Affinity

- **Affinity**: The tenacity by which a drug binds to its receptor.

- **$K_D$ (in moles/liter)** expresses the affinity of a drug for a receptor.

  \[ K_D = \text{[ligand]} \text{ which occupies half the receptors.} \]

- Determined by the use of radiolabelled ($^3$H, $^{125}$I)-ligand.

- Is often lower than the concentration required to elicit a half-maximal biological response (EC$_{50}$).

Levy, 2003
Efficacy

- Efficacy: also known as "intrinsic activity". The ability of a drug to produce a maximal biological response relative to other drugs. Expressed as $E_{\text{max}}$.

- Note, true antagonists have no intrinsic efficacy.
Potency

- Potency: ability of a drug to cause a measured functional change relative to other drugs.
- In isolated tissue or cell assays potency must be expressed as the 50% effective concentration (EC50).
Dose

- The quantity of drug usually represented as milligram of drug per kilogram of body weight (mg/kg).
- The dose of a drug that cause a half maximal response in an organism is known as the 50% effective dose (ED$_{50}$).
## Affinities versus Potency of Selected Agonists From Plants

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Plant</th>
<th>Affinity, $K_i$ (nM) $\alpha_7$-nAChR</th>
<th>Potency ($\mu$M) $\alpha_7$-nAChR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>Common Tobacco <em>Nicotiana tabacum</em></td>
<td>400</td>
<td>18</td>
</tr>
<tr>
<td>Anabasine</td>
<td>Tree Tobacco <em>Nicotiana glauca</em></td>
<td>58</td>
<td>16.8</td>
</tr>
<tr>
<td>Anabaseine</td>
<td>Tree Tobacco <em>Nicotiana glauca</em></td>
<td>58-759</td>
<td>6.7</td>
</tr>
<tr>
<td>Lobeline</td>
<td>Indian Tobacco <em>Lobelia inflata</em></td>
<td>11000</td>
<td>No Effect</td>
</tr>
</tbody>
</table>

Drug Potencies Vary by Receptor Type

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Potency (µM) α7-nAChR</th>
<th>Potency (µM) α4β2-nAChR</th>
<th>Potency (µM) α3β4-nAChR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>18</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>Anabasine</td>
<td>16.8</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Anabaseine</td>
<td>6.7</td>
<td>4.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>Lobeline</td>
<td>No Activation</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Cell-Based Assays

• TE-671 cells
  – Express fetal human muscle-type nAChR ($\alpha_1\beta_1\gamma\delta$).

• SH-SY5Y cells
  – Express autonomic type nAChRs containing $\alpha_3$ and $\beta_4$ subunits.
Teratogenic Activity Profile

- Piperidine alkaloids
- Carbon side chain of at least three carbons or larger attached to the carbon alpha to the piperidine nitrogen increased teratogenic activity.
- A methyl group attached to the nitrogen reduced teratogenic activity.
- A double bond at the nitrogen or carbon side chain increases teratogenic activity
Anabasine

- Piperidine Alkaloid
  - Isolated from *N. glauca* (tree tobacco)
  - Agonist
  - Present in the plant as a racemate.
Anabaseine

- Carnivorous marine worm toxin
- Potent at neuromuscular receptors.
- Double (imine) bond between positions 1 and 2 of the piperidine ring.
- Isolated and identified from *Paranemertes peregrina* (purple ribbon worm).
Nicotine

- Pyridine Alkaloid
- *N. tabacum*
- Not very teratogenic when compared to the piperidine alkaloids.
Ammodendrine

- Piperdine alkaloid
- Teratogenic in cattle

*L. sulphureus*, Pendleton, Umatilla, OR
Relationship between potency, percent maximum response, and affinity of the teratogenic alkaloids.

- Each figure presents the potency, and percent maximal response and the affinity (estimated, negative logarithm of the affinity value (pK$_a$), of the alkaloids in TE-671 cells (A) and SH-SY5Y cells (B).

- Suggest the actions of these alkaloids are efficacy dependent.

- High concentrations of affinity-dependent agonists are required to produce a near maximum biological response.

- Lower concentrations of efficacy-dependent agonists can produce a near maximum biological responses. Efficacy-driven agonists would have a greater potential to produce fetal defects at low concentrations relative to their pKa value.
Competitive Antagonists

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

MLA

- Isolated from *Delphinium* Spp. (Larkspur)
- Classified as an antagonist
  - Lacks intrinsic efficacy.
- Acts at nAChR.