What is snake venom?
- It is highly modified saliva produced by modified salivary glands (venom glands) in venomous snakes
- Snake venom is toxic saliva consisting of a complex mixture of potent biochemical compounds

What are the general functions of snake venom?
- Functions of snake venom:
  - Venom serves as a defense system for the snake
  - Venom is used for immobilizing the victim/prey
  - Venom is used to digest food
  - Some venoms can paralyze the prey within a short time, to prevent the prey from moving away and escaping
  - Enzymes in venom can kill the prey and digest it

What is the composition of snake venom?
- Snake venom is a cocktail of several different proteins and enzymes
- Protein constitute 90% of dry weight of venoms
  - Proteins include Lethal Polypeptides and Hydrolytic enzymes (Proteases, Nucleases, Peptidases, Lipases, etc) that can either enhance and/or contribute to the toxic effect of the venom
  - Enzymes cause both local and systemic destruction of tissues and lead to necrosis of skin, muscle and subcutaneous tissue
- About 20 different enzymes have been found in snake venoms
  - Most snakes have between 6 to 12 enzymes in their venom
- Some of these enzymes aid in the digestive process, while other specialize in paralyzing the prey/victim

What are some of the enzymes identified in snake venom?
- **Cholinesterase**:
  - It hydrolyses Acetylcholine in neuromuscular junction, relaxing muscles to the point where the victim has very little control
- **L-amino acid oxidase**:
  - It degrades amino acids,
  - Plays a role in digestion and the triggering of other enzymes
  - It gives the characteristic light yellowish coloration of venoms
- **Hyaluronidase**:
  - Degrades Glycosaminoglycans (GAGS) and causes other enzymes in the venom to be absorbed more rapidly into tissues
- **Protease (Proteinase)**:
  - Digestion of proteins and break down of tissues,
  - Causes extensive tissue damage in human victims
- **Adenosine Triphosphatase (ATPase):**
  - Plays central role in causing shock and immobilizing victims/preys

- **Phosphodiesterase:**
  - May be responsible for the negative cardiac reactions in victims, most notably a rapid drop in blood pressure

**How is snake venom classified clinically?**
- Complexity and diversity of snake venom content makes it difficult to classify
- Clinically convenient to classify snake venoms into two major types based on the main symptoms caused by the snake bite
- Two major types of venom
  - Neurotoxic venom
  - Hemorrhagic venom (also called Hemotoxic venom)

**Neurotoxic Venoms:**
- Affect normal functions of the nervous system
- Attacks Central Nervous System of victims
- Causes heart failure and/or breathing difficulties
- Can cause respiratory paralysis, hypoxia and death

  - Examples of snakes with Neurotoxic Venom include:
    - Cobras, Mambas, Sea snakes, Kraits and Coral Snakes

**Hemotoxic Venoms:**
- Causes abnormal bleeding by interfere with the normal clotting mechanism of blood
- Attacks the circulatory system damages blood platelets and affects normal clotting, causing bleeding signs and symptoms
- Produce local or systemic hemorrhage
- Damages muscle tissue causing excessive Scarring, Gangrene, permanent disuse of muscle, affect motor skill and sometime leads to amputation of affected area

  - Examples of snakes with Hemotoxic Venom include:
    - Rattle snakes, Copperheads, and Cottonmouths
  - Venom of some snakes contains both Neurotoxin and Hemotoxin

**NEUROTOXIC VENOMS (NEUROTOXINS):**

What are the modes / mechanisms of action of the Neurotoxins in venoms?
- Neurotoxic substances interfere with normal processes of Neurotransmission
- Neurotoxins in snake venom disrupt synaptic transmission by either:
  - Inhibit the release of Neurotransmitters from Exocytosis of Synaptic Vesicles via Presynaptic membrane or
  - Bind to the Neurotransmitter Receptors on Postsynaptic membrane

- Two major types of Neurotoxins are:
  - Bungarotoxins (alpha-Bungarotoxin and beta-Bungarotoxin) and
  - Crotoxin
**Alpha-Bungarotoxin:**
- Acts on the **Postsynaptic sites**
  - It irreversibly binds to Nicotinic Acetylcholine Receptor on the Postsynaptic membrane and blocks depolarization in the Postsynaptic site
- Alpha-Bungarotoxin blocks Neuromuscular transmission by interacting with the Motor end-plate Acetylcholine Receptor
- Action of Alpha-Bungarotoxin can be prevented by d-Tubocurarine, a specific but reversible antagonist of Neuromuscular Cholinergic Receptors

**Beta-Bungarotoxin:**
- Acts on the **Presynaptic sites**
  - It disrupts the release of Acetylcholine stored in Synaptic Vesicles, thus blocking Synaptic transmission
- Beta-Bungarotoxin has Phospholipase A₂ activity
- Beta-Bungarotoxin has no Postsynaptic action on:
  - Membrane potential,
  - Action potential, or the
  - Sensitivity to Acetylcholine at the Motor end-plate
- Paralytic action of Beta-Bungarotoxin appears to take place in two processes:
  - First, it binds with the respective target sites, and,
  - Second, it inhibits changes in the target macromolecule of the nerve terminals, leading to failure of transmitter release

**What are the mechanisms of action of Beta-Bungarotoxin?**
- Exact mechanisms of action of Beta-Bungarotoxin are not fully known
- Several mechanisms by which Beta-Bungarotoxin can disrupt Presynaptic Functions have been proposed:

  - **Block Voltage-Gated Na⁺ K⁺ Channels:**
    - If Beta-Bungarotoxin blocks voltage-gated sodium and potassium channels, then Action Potential cannot reach the Axon Terminal, thus synaptic vesicles cannot be released

  - **Block Na⁺ K⁺ ATPase:**
    - Sodium-Potassium ATPase (Sodium Potassium pump) is essential for maintaining Resting Potential of cell membranes
    - If Beta-Bungarotoxin disrupts the function of Sodium-Potassium ATPase, it can disrupt the Resting Potential, and thus disrupt the Conductivity of membrane for Action Potential

  - **Block voltage-gated Ca²⁺ channels:**
    - Beta-Bungarotoxin can also block voltage-gated Calcium channels in the Axon Terminal
    - Action potential induces voltage-gated Calcium channels to open for Calcium ions to enter the cytosol of axon terminal
    - If voltage-gated Calcium channels are blocked, even if action potential exists; Calcium still cannot enter the cytosol, thus synaptic vesicles cannot be released
- **Interact with Ca\(^{++}\) pump of mitochondria:**
  - Mitochondria can uptake excess Calcium ions in the cytosol
  - If Beta-Bungarotoxin increases the activity of Ca\(^{++}\) pump in mitochondria, excessive amount of Calcium ions will enter the mitochondria, reducing the Calcium in cytosol, the effect of calcium ions will be disrupted

- **Inhibit binding of Ca\(^{++}\) with Calmodulin:**
- **Inhibit Protein Kinase C:**
- **Inhibit binding of Ca\(^{++}\) with Synaptophysin (Docking of synaptic vesicle):**
- **Inhibit the function of Synaptotagmin (exocytosis):**

**In summary:**
- Proposed mechanisms by which Beta-Bungarotoxin can inhibition impulse transmission in the Presynaptic membrane involves the action of Phospholipase A\(_2\)
- Because Voltage-gated Na\(^+\) K\(^+\) channels, Na\(^+\) K\(^+\) ATPase, voltage-gated Ca\(^{++}\) channels, Ca\(^{++}\) pump, Synaptophysin, Synaptobrevin, Syntaxin, and Synaptotagmin are all membrane proteins
- If Beta-Bungarotoxin is a Phospholipase A\(_2\), then it can degrade the membrane of the synaptic vesicles and the Presynaptic membrane and therefore affect the structures and functions of membrane proteins
- Resulting effect is inhibition of impulse transmission from Presynaptic sites

**Crotoxin:**
- Crotoxin like Beta-Bungarotoxin has Phospholipase A\(_2\) activity
- Crotoxin can disrupt release of synaptic vesicle at Presynaptic sites, but its mechanism is quite different from Beta-Bungarotoxin
- Crotoxin is a potent neurotoxin consisting of a basic and weakly toxic Phospholipase A\(_2\) subunit (component B) and an acidic non-enzymatic subunit (component A)
- Nontoxic component A enhances the toxicity of the Phospholipase A\(_2\) subunit by preventing its nonspecific adsorption

**What are some of the consequences of Neurotoxic Venoms acting at the Presynaptic Terminal of Neuromuscular Junction?**

**Snake Venoms acting at Presynaptic:**
- Causes disruption of Synaptic Vesicles, Damage to Terminal Axon and Cessation of Release of Acetylcholine (Ach),
- Thus completely blocking neuromuscular transmission
- Ultimate effect is Flaccid Paralysis of affected muscles

On reaching the NMJ the Presynaptic Neurotoxin must:
- Bind to the terminal axon membrane,
- Damage the membrane, and then exert its toxin effects
- Initially this may cause release of Ach, with some muscle twitching, rarely noticed clinically, before destroying vesicles and blocking further Ach release
- Process may take up to an hour in some victims
Clinically, because of the extra time taken for the neurotoxin to be absorbed, reach the circulation, exit again to the extra-vascular compartment, then reach the NMJ, a process that may take from half an hour to several hours, presynaptic paralysis is unlikely to manifest in less than 1-2 hours post bite.

Clinical features of early paralysis are usually first seen in the cranial nerves, with Ptosis (drooping of the upper eyelids) the most obvious first sign.

Presynaptic venoms are poorly responsive to Anti-venom therapy.

Once severe flaccid paralysis is established, with respiratory involvement, anti-venom is unlikely to reverse paralysis.

Crucial to recognize early signs of paralysis and give anti-venom early, before more major and irreversible paralysis occurs.

Presynaptic neurotoxins are found principally in some snake venoms, such as kraits (beta-bungarotoxin), some Australian elapids (Notexin, Taipoxin, Textilotoxin) and a few vipers (Crotoxin).

What are some of the consequences of Neurotoxic Venoms acting at the Postsynaptic terminal of Neuromuscular Junction?

- Postsynaptic Neurotoxic venoms are less potent, but more rapid in action, and are certainly lethal in potential.
- They block binding of Acetylcholine to receptors at the muscle end plate, causing Flaccid Paralysis.
- Clinically the first signs of flaccid paralysis, such as Ptosis, are still rarely seen in less than one hour post bite, and often may be delayed for several hours.
- Postsynaptic neurotoxins are accessible to anti-venom because they remain exposed on the cell surface, in the extracellular compartment.
- Postsynaptic paralysis may be reversible with anti-venom therapy.
- As an alternative, if increased amounts of Ach are released they may overwhelm the postsynaptic neurotoxin, thus overcoming the blockade and re-establishing NMJ transmission.
- One technique is to effectively increase Ach concentration by blocking its removal, for example, by using Anti-cholinesterase agent such as Neostigmine.
- Postsynaptic NMJ neurotoxins are widely distributed in snake venoms, especially elapid venoms, the classic component being alpha-bungarotoxin, from krait venom.

Mode of action of other snake venoms:

- **Dendrotoxins**: (Neurotoxins in the venoms of Mamba elapid snakes)
  - Act Presynaptically at the NMJ
  - Mode of action is different from Beta-Bungarotoxin
    - **Dendrotoxins cause Flaccid Paralysis**
      - Blocks some Potassium channels on the terminal axon membrane, causing over-release of Ach, resulting in initial stimulation, then blockade of release.

- **Fasciculins or "Angusticeps-type" toxins**: (Venom in Mamba snakes)
  - Presynaptic neurotoxins,
    - Potent inhibitors of Cholinesterase, causing a build up of Ach in the NMJ extracellular space.
They act synergistically with Dendrotoxins,
- Fasciculins cause increasing release of Ach while Dendrotoxins prevent its metabolism,
- Greatly increasing the quantity of Ach swamping Ach receptors on muscle end plate

HEMORRHAGIC VENONS (HEMOTOXINS):

- Toxins of the hemorrhagic snakes have very complex actions on the coagulation system
- Major toxicity is to reduce the number of Platelets,
- Inhibit or stimulate the function of Platelets, and to
- Inhibit the functions of other coagulation factors
- Symptoms vary according to the amount of venom present in the body, and thus it is difficult to judge the identity of the snake from the symptoms and signs
- Russell's viper snakes possess both neurotoxins and hemorrhagic toxins
- Main toxic effect is bleeding, because its venom contains Pro-coagulation factors similar to coagulation factors V and factor X
- These can cause a disseminated intravascular coagulation reaction and consume large amounts of normal coagulation factors, reducing their concentrations and leading to systemic bleeding

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