UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR
SNAKE VENOMS: BIOCHEMICAL EFFECTS – An Overview

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 pray 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 sake 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** and
  - **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 A_2 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 Ca^{++} 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,
    - 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

Reference:

1. WWW.vghtpe.gov.tw (Inappropriate Therapy of Snake Bites)
2. WWW.kingsnake.com/toxinology/
3. WWW.toxinology.com/
4. WWW.mysite.mweb.co.za/
5. WWW.wch.sa.gov.au/paedm/clintox/venoms
6. WWW.coloherp.org/venom.html