Elapidae Snake Bites—Biophysical Aspects of the Neuroparalytic Envenomation

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Abstract: Snakebite is classified by the WHO (World Health Organization) as a neglected tropical disease, envenoming in a significant public health problem in tropical and subtropical regions. Neurotoxicity is a key feature of different types of envenomations and there are many unanswered questions regarding this manifestation. Acute neuromuscular weakness with respiratory involvement is the most clinically important neurotoxic effect. We present how the neuroparalytic poison affects the human body and what actually happens when the poison is injected into the human body. Neuroparalytic poisons are one of the most lethal, because they cause paralysis of the eye, throat and chest muscles. A well-known member of the Elapidae family is the cobra. Bungarotoxins are a group of closely related neurotoxin proteins. The α -bungarotoxin inhibits the binding of acetylcholine (ACh) to nicotinic acetylcholine receptors; β -bungarotoxin and γ -bungarotoxins act presynaptically causing excessive acetylcholine release and subsequent depletion.

Key words: Elapidae, action potential, neuromuscular synapse, poison, bungarotoxin, paralysis.

1. Introduction

Neuroparalytic envenomation, caused by Elapid snake bites, is one of the most lethal, leading to paralysis of the eye, throat and chest muscles [1]. A member of Elapidae family is the well-known cobra (the biggest specie which reaches up to 5.6 m (170 ft). The snakes live in the tropical and subtropical regions of Asia, Australia, Africa, North and South America—that is the reason why most of the clinical cases are from India, Taiwan, China (Fig. 1). Professor Chuan-Chiung Chang [2] from The National Taiwan University was the one who isolated in 1950 the neurotoxic protein in the snake venom bungarotoxin. Thirteen years later, the different types were distinguished: α -bungarotoxin, β -bungarotoxin and γ -bungarotoxin.

The bungarotoxin consists of proteins and lipids and its neuroparalytic effect is caused by the curare-like action of the toxin at the presynaptic and postsynaptic levels of the neuron, followed by neuromuscular

blockade—a failure of impulse transmission. The mechanism of action of any of the three types bungarotoxins is different and to be understand easily; let us briefly review the current knowledge on how the neuromuscular impulse travels through the neuromuscular junction.

The process consists of the following steps:

- Step 1: Action potential travels through the axon to the terminal part of the axon (Fig. 2);
- Step 2: The generated voltage opens Ca^{2+} channels and Ca^{2+} ions enter the terminal part of the axon;
- Step 3: Diffusion of Ca^{2+} ions causes exocytosis and release of acetylcholine from the vesicles;
- Step 4: Acetylcholine enters the presynaptic cleft and connects with the acetylcholine receptors, which consist of five protein subunits. Then, the conformation of the subunits changes and Na^+ channels open (Fig. 3);
- Step 5: Na^+ ions enter the muscle fiber, while K^+ ions enter the synaptic cleft—this leads to depolarization of the membrane. When a certain level of depolarization is reached, action potential travels along the sarcolemma and muscle contraction occurs;

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Fig. 1 Regions where Elapidae snakes can be found [3].

Fig. 2 The states of sodium and potassium channels during the change of action potential [2].

Fig. 3 MEPPs (miniature endplate potentials).

Source: www.mybrainfacts.com.

- Step 6: The final step includes removal of the remaining acetylcholine from the synaptic cleft and its dissociation to acetic acid and choline by enzyme called acetylcholinesterase. Then choline is transferred to the terminal part of the axon to take part in resynthesis of acetylcholine.

There are biophysical methods for recording such miniature potentials using special voltmeters.

The neurotoxins of the snake venom block this process at different levels. The different sites of action of neurotoxins in general on neuromuscular junction are shown in Figs. 4 and 5:

(1) Synaptic vesicular proteins: Snake toxins: β -bungarotoxin (*Bungarus spp.*), taipoxin (*O. scutellatus*); Other toxins: botulinum toxin, tetanus neurotoxin;

(2) Voltage-gated calcium channel: Snake toxins: calciseptine (*Dendroaspis spp.*), β -bungarotoxin (*Bungarus spp.*);

(3) Pre-synaptic membrane: Snake toxins: phospholipase A2 toxins;

(4) Pre-synaptic ACh receptor: Snake toxins: candoxin (*Bungarus candidus*); Other toxins: curare;

Pharmacological substances: atracurium;

(5) Voltage-gated potassium channels: Snake toxins: dendrotoxins (*Dendroaspis spp.*);

(6) Acetylcholine: Lysis by exogenous acetylcholinesterase in snake venom: cobra venom (*Naja spp.*);

(7) Acetylcholinesterase: Inhibitors of endogenous AChE in snake venom: fasciculins (*Dendroaspis spp.*);

(8) Post-synaptic ACh receptors: Snake toxins: α -bungarotoxin (*Bungarus spp.*), Disease states: myasthenia gravis;

(9) Voltage-gated sodium channels: Snake toxins: crotamine (*Crotalus spp.*); Other toxins: pompilidotoxin (wasps), δ -conotoxin (*Conus spp.*), tetrodotoxin (pufferfish).

2. Methodology

Our goal was to find information about the mechanism and biophysical aspects of the poison of the cobra, since there is not much about this topic. We have gathered the information through dedicated search in Internet. We have used different keywords,

Fig. 4 Minimum threshold must be reached in order action potential to be generated.

Source: www.studyinukraine.eu.

Fig. 5 Sites of action of snake neurotoxins and other substances on the neuromuscular junction. Schematic representation of the neuromuscular junction showing different sites of action of snake neurotoxins, other toxins, and pharmacological substances, and sites of involvement in disease states (examples indicated where relevant) [4].

such as “cobra envenomation”, “cobra venom”, “bungarotoxin” and “neuromuscular junction”. Altogether we have put the most relevant information into our paper.

3. Bungarotoxins α and β

The β -BGT (β -bungarotoxin) is more lethal. He affects the terminal part of the axon and blocks the release of acetylcholine, but does not affect the sensitivity of the acetylcholine receptors to the acetylcholine. The mechanism of the β -BGT is complex. His effect is similar to phospholipase A₂, which hydrolyses phosphatidylcholine, but it also connects and blocks K⁺ channels. The level of neuromuscular blockade depends on the temperature—it lowers with the decrease of

temperature. The victims of β -BGT do not respond to antidotes. Drugs, such as Anticholinesterases, have a very little or no effect. Harris and colleagues demonstrate, that an hour after the bite, a lot of presynaptic terminal structures show signs of physical destruction (destruction of the mitochondria, Schwann cells enter the synaptic cleft) and are deprived of synaptic vesicles and, after 24 h, 70% of the muscle fibers are de-innervated. That is why envenomations caused by β -neurotoxins, are often followed by heavy prolonged paralysis, which are very difficult to be treated. The recovery period is long while the muscle terminal of the axon regenerates and new neuromuscular junction is formed. Re-innervation occurs after three days and ends after seven days. That is why long assisted ventilation is required, due to the

fact that the respiratory muscles are paralyzed (Fig. 6). Recovery of the breathing is hard, sometimes not complete and the lethality is high [5].

The α -BGT (α -Bungarotoxin) binds competitively to the nicotine acetylcholine receptor, which not only blocks the binding of acetylcholine, but also the postsynaptic depolarization of the membrane (Steps IV, V and VI—curare-like effect). Although the irreversible binding, clinicians report positive effect from the antidote. It is considered that the antidote causes dissociation of the toxin-receptor complex and recovery of the contractions of respiratory muscles.

It is clear that the blockage of action potential with α -Bungarotoxin is post-synaptic, while β -Bungarotoxin (β -BGT) is pre-synaptic but there are many challenges to the study of all the details of neurotoxicity of cobra venom. There is considerable variation between individual patients in symptom

evolution and recovery patterns of weakness, respiratory involvement and response to antivenom and anticholinesterases and it seems to depend on snake species and geographical location (Fig. 7).

Fig. 6 Patient during assisted ventilation [4].

Fig. 7 Curare effect on neuromuscular synapses, which is similar to α -bungarotoxin action.

Source: www.studentconsult.com.

4. Conclusions (Interesting Fact)

In diagnostic aspect, α -Bungarotoxin is used in

series of studies in order to define the number and density of acetylcholine receptors in patients with

myasthenia gravis and to evaluate the quantity of the antibodies that have targeted these receptors and the activity of the disease.

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