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CONOTOXINS ACTING ON THE ACETYLCHOLINE RECEPTOR: A REVIEW

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Introduction

Conus species are marine snails which are greatly admired for the beautiful patterns on their cone-shaped shell. At the same time, these species are notorious for their poisonous stings. Although cones usually react to disturbance by retracting into their shell, accidental stinging of humans (1, 2, 3, 4) have resulted from careless and prolonged handling of the live animal. In fact, several human fatalities have been reported over the years. Most dangerous are the fish eating or piscivorous species, particularly the larger ones such as *Conus geographus.*

Cones are basically hunters which use a well-developed venom apparatus for catching prey such as worms, other molluscs and fish (5). Two types of strategies have been observed among piscivorous *Conus* species. *C. geographus,* upon sensing a fish in the vicinity, opens up its flexible funnel-shaped rostrum or mouth like a blooming flower. As soon as the unsuspecting fish swims into the funnel, the snail stings and envelopes the fish with its rostrum. In contrast, *C. magus* and other closely related species (e.g., *C. purpurascens, C. achatinus*) bury themselves under the sand and entice prey by extending a brightly colored proboscis. When a fish comes to feed on the worm-like proboscis of a snail, it stings and hooks in the paralyzed fish as it emerges from the sand to engulf the fish with its distensible mouth.

The venom apparatus of cones (6) consists of a muscular venom bulb (probably acting as a pump), a long duct filled with venom, and a radula sac containing numerous hollow radula-tooth at different stages of formation. As the cone gets ready to strike, it positions one of the harpoon-shaped tooth at the end of the extensible proboscis. The highly modified tooth first acts as a hypodermic needle through which venom is injected then as a hook for pulling in the catch.

Symptoms of *Conus* Stinging

Various symptoms have been described in stinging cases of humans. One well documented case (7) is a 28-year old man who died within 4½ hours after being pierced in the hand by a *Conus*. He complained of numbness of the hand in 5 to 6 minutes after stinging. The sensation extended upwards to the lips and mouth in a few minutes, and there was blurring of vision. Within an hour, the victim was unable to speak. He was completely paralyzed before death.

In mice, intraperitoneal injection of crude venom produce symptoms similar to those seen with many snake venoms. Death from asphyxiation occurs from a few minutes to about half an hour after injection due to paralysis of the respiratory muscles (8).

Types of Conotoxins

The venom of *Conus* species is a complex mixture of digestive enzymes (9), quaternary ammonium compounds (6), fast-acting toxic peptides, and other components. The generic name "conotoxins" was suggested (10) for all toxic peptides isolated from *Conus* venoms, with a capital letter to indicate the species and a Roman numeral to denote the particular variant. Small Greek letters preceding the name of toxins has also been suggested as a means to indicate the physiological action.

Toxins isolated from *Conus* venom have differing physiological action with some acting on the neuromuscular system and others on the central nervous system. *Conus* species have evolved a series of neuromuscular toxins to ensure the effectiveness of venom injected to the prey. One group, the ω-conotoxins irreversibly block nerve stimulus evoked release of transmitter at the frog neuromuscular junction (11). Another group (α-conotoxins) inhibits the post-synaptic terminus of vertebrate neuromuscular junction (12, 13). A third group (μ-conotoxins) rapidly blocks muscle action potentials in frog and mouse (14, 15). The cones are thus equipped with a set of toxins acting at three stages of impulse transmission. Although this may seem to be an "overkill", it ensures survival of the species.

The α-Conotoxins

The most well-characterized of the conotoxins are of the α-type. The group consists of a homologous set of small basic peptides. The amino acid sequences (12, 16) of those isolated so far are given in Fig. 1. The peptides designated by G all come from *C. geographus* and the M peptide from *C. magus*. All of them have a blocked carboxyl terminus. Conotoxins GI and GIA are essentially identical except that GIA has additional Gly. Lys at the carboxyl end. GII differs from GI only in the conservative replacement of Asn4 by histidine, Arg9 by lysine and Tyr11 by phenylalanine. MI differs from GI not only in the conservative replacement of Asn4 by His and His11 by Asn but also in the radical substitution of Glu1 (an acidic amino acid) by Arg (a basic amino acid). Obviously, there is considerable flexibility

	0	5	10	15
Conotoxin GI	Glu.Cys.Cys.Asn.Pro.Ala.Cys.Gly.Arg.His.Tyr.Ser.Cys.NH_2			
Conotoxin GIA	Glu.Cys.Cys.Asn.Pro.Ala.Cys.Gly.Arg.His.Tyr.Ser.Cys.Gly.Lys.NH_2			
Conotoxin GII	Glu.Cys.Cys.His.Pro.Ala.Cys.Gly.Lys.His.Phe.Ser.Cys.NH_2			
Conotoxin MI	Gly.Arg.Cys.Cys.His.Pro.Ala.Cys.Gly.Lys.Asn.Tyr.Ser.Cys.NH_2			
Ancestral Toxin	Gly. Arg. Glu.	Cys.Cys.His.Pro.Ala.Cys.Gly.Lys.	Asn. His.	Tyr.Ser.Cys.NH_2

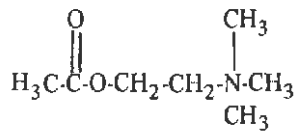
Figure 1. Amino acid sequences of α-conotoxins and the proposed ancestral toxin (16).

at the amino terminus of the conotoxins. The disulphide bondings are Cys2 to Cys7, and Cys3 to Cys13 (17). Chemically synthesized conotoxins GI and MI have been demonstrated to be identical, both biochemically and pharmacologically, to the native toxins (17, 18).

Presumably, conotoxin GI and conotoxin GII arose by gene duplication and divergence within *C. geographus.* There is reason to believe that GIA arises during processing of GI from a larger precursor. Assuming that species divergence between *C. magus* and *C. geographus* occurred first before gene duplication in *C. geographus,* possibilities for the sequence of the ancestral toxin may be as shown in Fig. 1.

Physiological data (12, 13) indicate that conotoxins act at the muscle end plate region. No inhibition of either nerve or muscle action potential has been detected. McManus *et al.* (13) showed that conotoxins GI and GII compete with α-bungarotoxin for binding to the acetylcholine receptor. Although MI has not been used in physiological experiments, the mode of action is presumed to be the same as the G series because of the close structural homology.

Many potent toxins similarly act by competing with acetylcholine for its receptor, without causing depolarization of the muscle membrane. So far two major classes of nicotinic Ach receptor inhibitors have been available: low molecular weight alkaloids (such as curare) and small proteins from snake venoms (e.g., α-bungarotoxin, cobratoxin, and erabutoxin). The α-conotoxins comprise a third class which is intermediate in size between the small alkaloids and the snake α-neurotoxins which contain 60 to 74 amino acid residues. All these toxins mimic acetylcholine which has the formula:



The onium head with its positve charge spread over the methyl cluster in an important structural feature for both muscarinic and nicotinic Ach action (19).

Correlation of activity with amino acids sequences coupled with model building suggest a conformation for α-conotoxins which is analogous to the "active tip" of the short α-neurotoxins of snakes (20). Both *Conus* and snake neurotoxins can undergo a conformational flip with one of the conformations being functionally equivalent to the calabash alkaloids and curare. All these α-neurotoxins have a characteristic cationic pair.

It is remarkable that conotoxin MI is the most active peptide yet discovered, being about 25 times more potent than d-tubocurarine and ten times more so than α-bungarotoxin on a molar basis. It is thus comparable to C-alkaloid E, whose rigid structure is assumed to be a close complimentary fit to the active site of the acetylcholine receptor protein (20).

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Clara Y. Lim-Sylianco, Discussant

There is not really much to say after that very excellent presentation of Dr. Cruz. Everything was very clear. It is a very good example as to what collaboration over these years can do. It shows that one can go deep until the molecular level to investigate problems regarding our Philippine conus species. I am very much impressed with what has been accomplished until the analysis, determination of the sequence of the amino acids and some structural studies that would really give us an idea how deep one can go into the study of some of those observations that we have in our own Philippine waters. And lastly, I would like to say that Dr. Cruz must be congratulated for her continuing interest in all these aspects of conotoxin inspite of the fact that she has always been saddled with administrative and teaching responsibilities. Thank you.

Edgardo Gomez, Discussant

I thought that I was going to be the most brief of the reactors but Dr. Sylianco pre-empted me from that role. You see, I am a little bit out of kilter here in the sense that this is a biochemistry paper and I am a marine biologist. The only thing we really have in common here is that I am interested in the animals that they are studying. I was going to leave all biochemistry to her to sort out. But she indicated that Dr. Cruz has presented a very clear paper so that perhaps there isn't very much need to dwell further, dig deeper into the biochemistry of this conotoxin.

What I thought I would do is just maybe make a few general remarks or observations and encourage more research along this area. Again, as a marine biologist I can't look into the biochemistry of it except perhaps to mention that in the marine environment, we have a wide variety of toxic and venomous marine animals, many of which are of interest to medicine. It is unfortunate that so few researchers in this country are looking at these substances.

I might very briefly mention a few as examples. Dr. Cruz mentioned the seasnakes of which we have plenty in the Philippines. Among the other vertebrates, we have a number of fish including the lion fish and the scorpion fish, both of which have toxins or venoms. I am not quite sure how you differentiate between a venom and a toxin and a poison. I tend to associae toxins with the things that you ingest and venoms with those used by animals for stinging. It is very difficult sometimes to draw the line on why you say this thing is poisonous as against being venomous. I use the terms interchangeably.

Among the invertebrates, the molluscs which we are studying have been mentioned. Then you have a whole range of coelenterates such as jellyfish and corals,

all of which have some type of toxin or venom. A number of worms also are known to have some toxins. Some of you have heard of the octopus. There is a small octopus that is famous in Australia that apparently kills people every year. And many of these animals are found locally, in our own waters. Perhaps when Dr. Cruz branches out we can give her a whole array of marine organisms that she can start to look at.

Now I would just like to mention very briefly a little bit about some of the types of toxins in the marine environment. She is working on conotoxin. You also have in the puffer fish tetraodotoxins. And then you have the saxotoxins, some of which are found in bivalve molluscs and it is not very clear whether these are manufactured by the bivalves or they are concentrated by them. One of the interesting things is that some of these toxins such as the tetraodotoxins have also been found in amphibians. The question that sometimes comes to mind is how is it that two very different groups of animals have come up with the same toxins? Is it some kind of convergent evolution or do they somehow follow genetically. Are they animals related ancestrally or what? Anyway there are a lot of areas for research in this field and I think the medical applications can be very exciting.