Mem. Inst. Butantan
51(4):177-193, 1989.

BIOCHEMICAL STUDIES OF THE SEA SNAKE NEUROTOXINS

Anthony T. TU
Roger A. MILLER

ABSTRACT: Sea snakes belonging to the family Hydrophiidae are marine-adapted, serpents found widespread in tropical and subtropical costal waters of the Indian and Pacific Oceans. Sea snake venom is a mixture of different proteins and contains potent neurotoxins. The LD_{50} for sea snake venom can be as low as 0.01 mg/Kg. The purified type I postsynaptic neurotoxins consist of 60-62 amino acid residues with four disulfide bonds. They are basic proteins with isoelectric points of 9 to 10 and range in molecular weight from 6,000 to 8,000. Sea snake neurotoxin work in this laboratory has centered on the postsynaptic neurotoxin of *Laticauda semifasciata, Lapemis hardwickii, Pelamis platurus* and *Acalyptophis peronii*. Sea snake neurotoxins show considerable homogeneity in their amino acid sequences with many invariant residues. Raman studies indicate the neurotoxins are a mixture of beta sheet and beta turns with no alpha helical secondary structure. The origin of lethality comes from the fact the sea snake neurotoxin strongly binds to the acetylcholine receptor at the neuromuscular junction which leads to muscle paralysis and respiratory arrest. Chemical modification of the conserved tryptophan residue has lead to the loss of the specific binding of the acetylcholine receptor and the loss of toxicity, but the modified toxin retained the ability to bind to neurotoxin antibodies. This suggested that neurotoxins can be converted into toxoids. A single tyrosine residue, some arginine and lysine residues are also essential to neurotoxicity. In addition to specific residues, some regions of polypeptide backbone are also important for toxicity.
KEY WORDS: Neurotoxins, Venoms, Acetylcholine Receptor.

INTRODUCTION

The scope of this review and discussion is restricted mainly to the work in this laboratory related to sea snake neurotoxins and interaction with acetylcholine receptors (AChR) in order to restrict the length of the review. There

Department of Biochemistry Colorado State University Fort Collins, CO 80523.
Dedicated to Dr. Saul Schenberg's 70th birthday
Received 04/7/1989; accepted 02/8/1989.

are many review articles on this subject, and readers are encouraged to read these for an overall view of neurotoxins.[1-4]

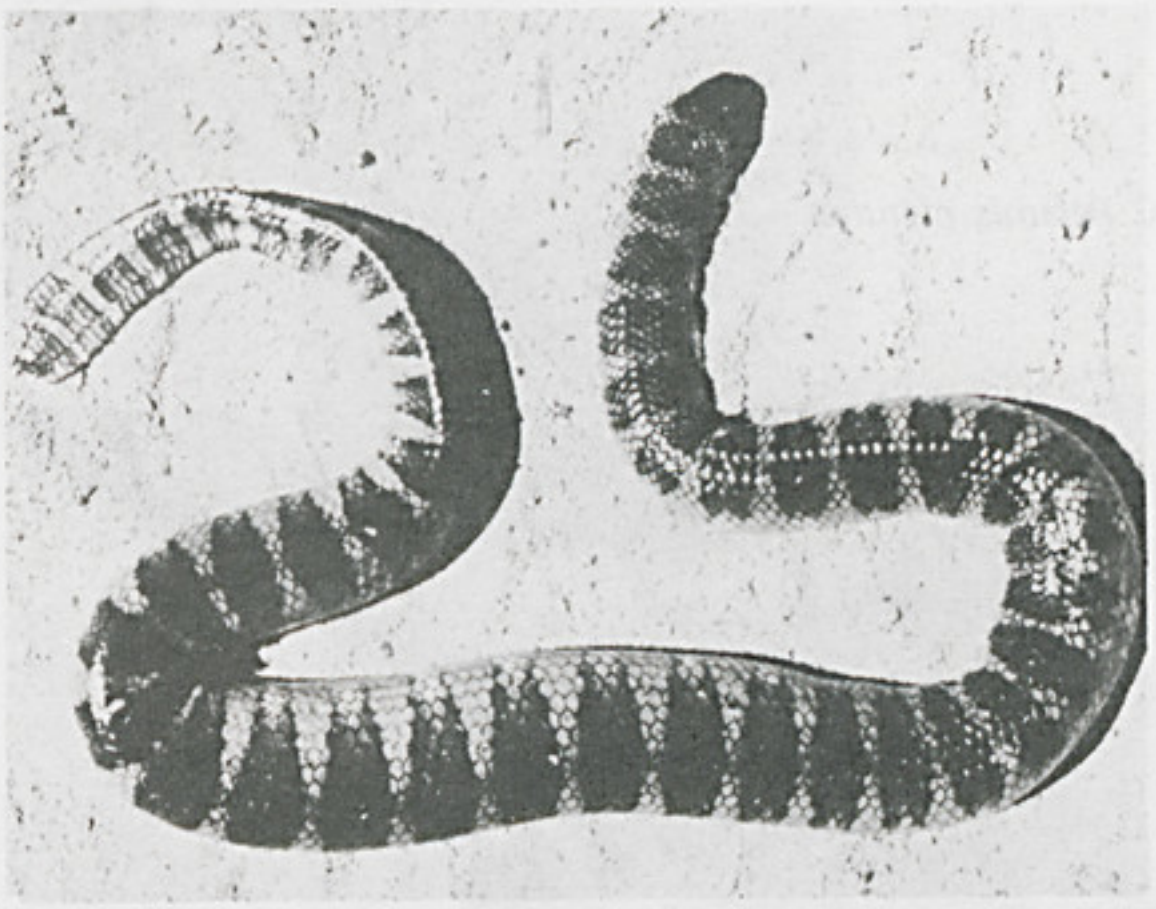
The sea snake is a marine-adapted serpent belonging to the family of Hydrophiidae and they are found widespread in tropical and subtropical coastal waters of he Indian and Pacific Oceans, however they are not found in the Atlantic Ocean. (Fig. 1) There are many varieties of sea snakes with different colors, shapes, and sizes. They are well adapted for the marine environment and have a flat tail and a salt gland. There are two subfamilies within the family Hydrophiidae. They are Hydrophiinae and Laticaudinae. The two types of sea snakes have distinct differences in their ventral scales. The former usually does not have ventral scales distinguishable from the surrounding scales, whereas the latter has wide ventral scales. These differences in ventral scale patterns eventually dictate their habitats. The reason a snake can crawl is due to the movement of its ventral scales. Since Hydrophiinae do not have ventral scales, they spend their entire lives in the sea. On the other hand, the Laticaudinae can swin in the sea and crawl on the beach and rocks.

Fig. 1. Distribution of sea snakes

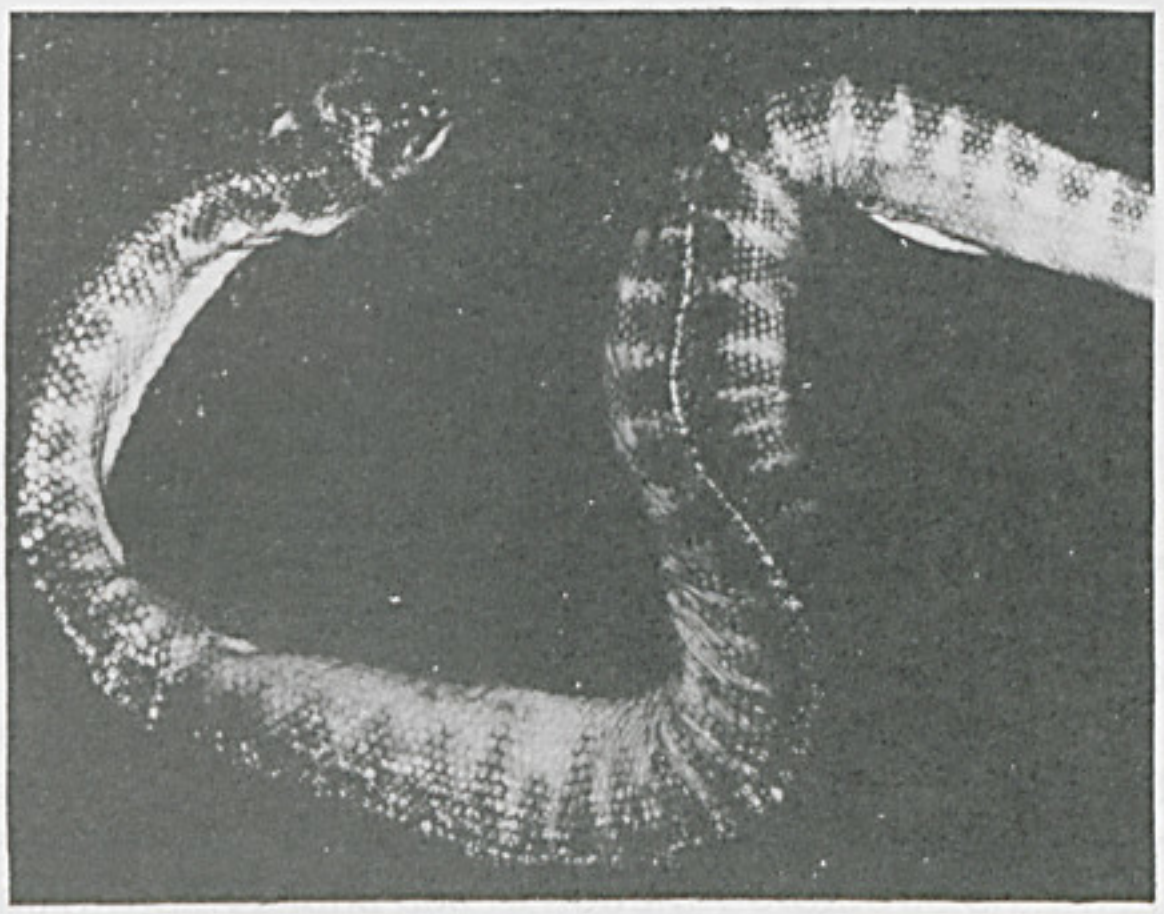
Sea snake venom is a mixture of different proteins, of which some are toxic and some components are relatively nontoxic. Because a venom contains highly toxic postsynaptic neurotoxins venom as a whole exhibits high toxicity. The potent neurotoxins in their venoms can cause muscle paralysis and respiratory failure of a victim which may lead to morbidity or death.

Most of the sea snake work in this laboratory has centered on the venom from four species of sea snakes: *Laticauda semifasciata* captured in the waters near the Philippines (Fig. 2A), *Lapemis hardwickii* captured in the Gulf of Thailand (Fig.2B), *Pelamis platurus* captured in the Pacific coastal waters of Costa Rica (Fig. 2C) and *Acalyptophis peronii* captured in the Gulf of Thailand (Fig. 2D).

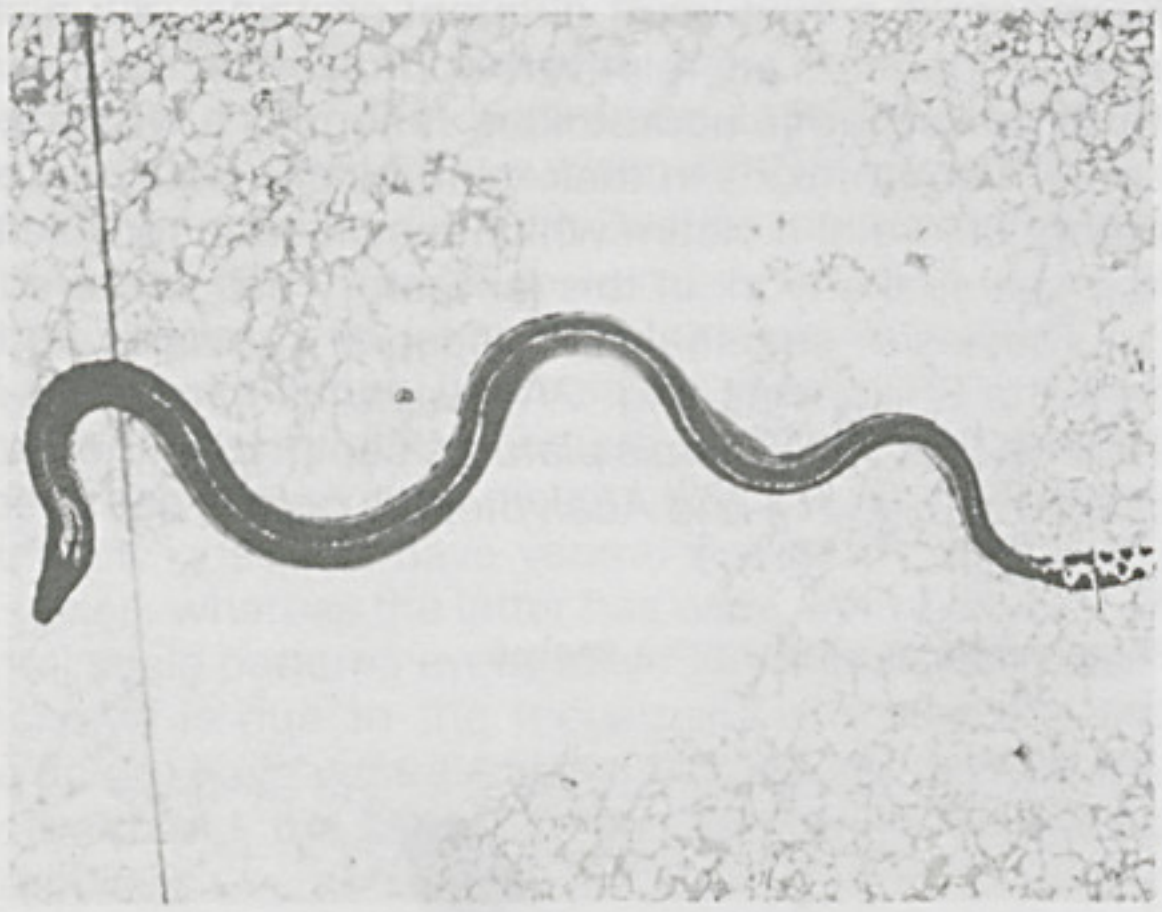
Fig. 2. (A-D) Photographs of selected sea snakes



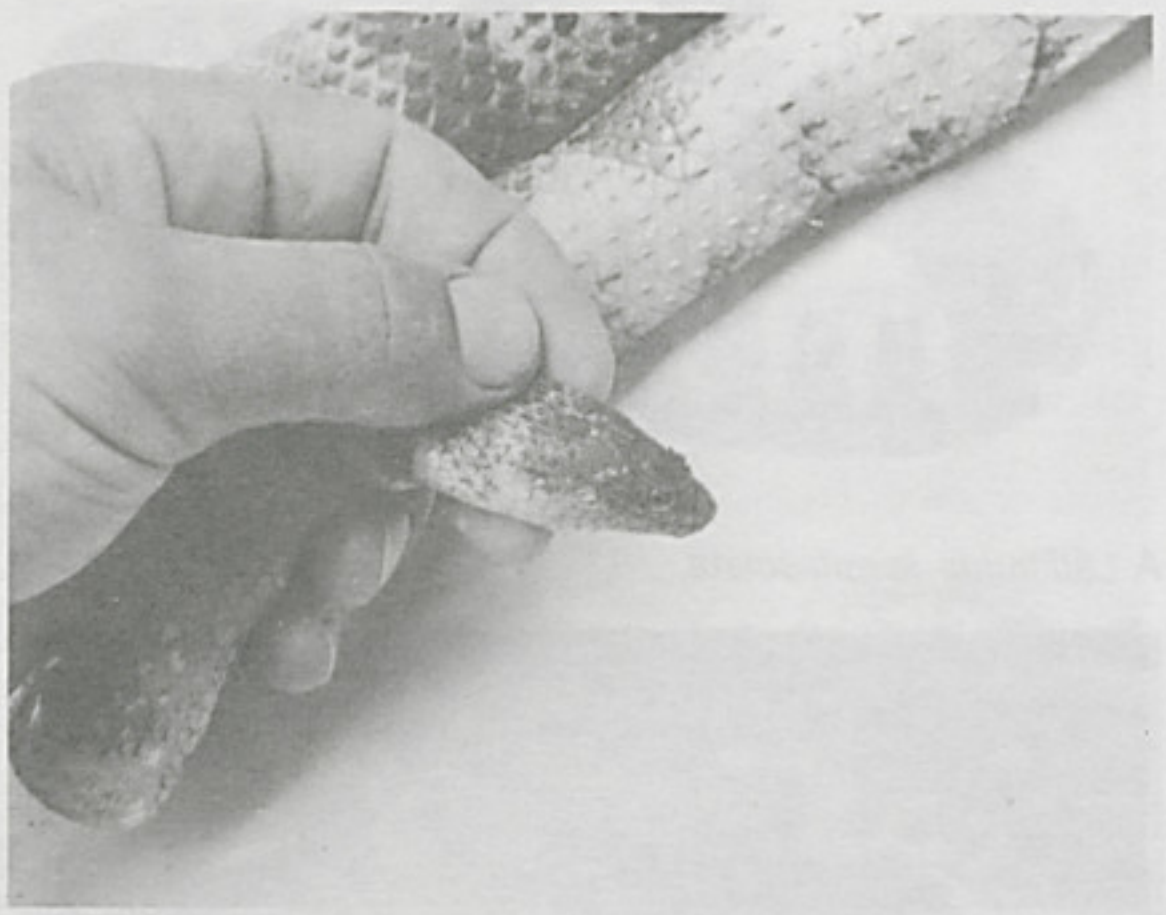
A *Laticauda semifasciata*



B *Lapemis hardwickii*



C *Pelamis platurus*



D *Acalyptophis peronii*

The yellow-bellied sea snake, *Pelamis platurus*, which is considered to be the most widespread sea snake in the world, is found in most of the coastal waters shown in Figure 1.[5] *Acalyptophis peronii* is the third most common sea snake in the Gulf of Thailand. The appearance, color pattern, and other morphological characteristics of this snake are quite different from other sea snakes. This sea snake is readily recognizable by its horn-like lifted-up scale near the eye.[1,6,7]

The remainder of this review will summarize the work in this laboratory in understanding the structure-function of sea snake neurotoxins and interaction studies with the AChR.

STRUCTURE FUNCTION RELATIONSHIPS

All sea snakes are poisonous and their venoms are extremely toxic. The LD_{50} for crude sea snake venom can be as low as 0.01 mg/Kg mouse body weight. For purified neurotoxin the LD_{50} is even lower, suggesting the high toxicity of sea snake venoms.[1]

Although the toxicity of sea snake venom is high, the yield of venom from a sea snake is very small with yields of 0.6 to 19.0 mg per snake, depending on the species.[8,9]

When various sea snake venoms were examined using immunodiffusion methods different venoms immunologically crossinteracted with each other (Fig. 3) indicating the close similarity in the composition of the venoms among different sea snakes.[10,11]

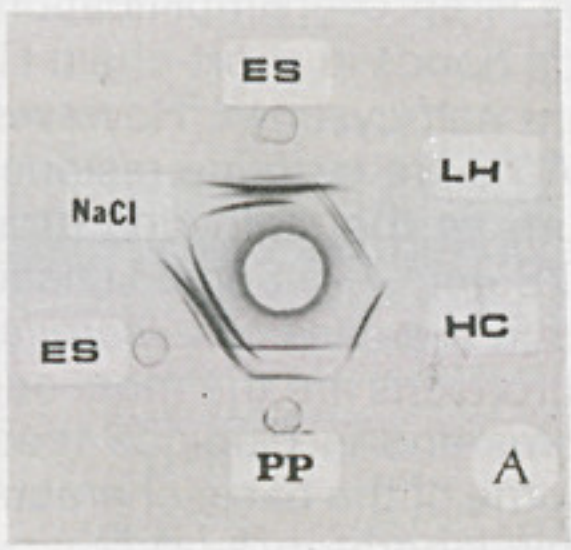


Fig. 3 Immunodiffusion study of sea snakes venoms
Immunologic cross-reaction of different sea snake venoms.
Antibody used was from anti-*E. schistosa* venom manufactured by Commonwealth Serum Laboratory, Melbourne, Australia. ES, *E. shistosa;* LH, *L. hardwickii;* HC, *H. cyanocinctus;* PP, *P. platurus.*

Sea snake venom seems to lack cytolytic activity. When venoms of various sea snakes *(E. schistosa, L. semifasciata, L. hardwickii, and P. platurus)* were added to the cell cultures of KB cells, Yoshida sarcoma cells, and normal peritoneal cells, no lysis was observed.[12]

Most sea snake neurotoxins consist of 60-62 amino acid residues with four disulfide bonds. These are the type I or short-chain neurotoxins. However, several type II or long-chain toxins were also isolated. Both types I and II are postsynaptic toxins, but type II toxins have five disulfide bonds. Some neurotoxins have structures between type I and II; they contain four disulfide bonds but have many other features similar to type II neurotoxins.[1]

Most sea snake venoms seem to contain only the postsynaptic neurotoxin. Only in *Enhydrina schistosa* venom, which also possesses a postsynaptic toxin, was a presynaptic type found and identified as phospholipase A. Therefore, to classify neurotoxins solely on the basis of their disulfide bonds or amino acid sequences is an oversimplification.[1]

There are four disulfide bonds in short-chain (Type I) neurotoxins which means that there are eight half-cystines. However, all Hydrophiinae toxins have nine half-cystines. An extra cysteine residue can be readily detected from the Raman spectrum as the sulfhydryl group shows a distinct S-H stretching vibration at 2578 cm^{-1}.[13,14] Some Laticaudinae toxins do not have a free cysteine residue as in the case of *L. semifasciata* toxins. These two types of postsynaptic neurotoxins are the most commonly found neurotoxins in Hydrophiidae venoms and in Elapidae (cobras and kraits) venoms.

Table 1 summarizes some of the basic characteristics of selected toxins purified and studied in this laboratory.[6,7,14-16]

TABLE 1

BIOCHEMICAL CHARACTERISTICS OF SELECTED POSTSYNAPTIC NEUROTOXINS DONE IN THIS LABORATORY

Neurotoxin	MW	pI	LD_{50}	#res/molec
Laticauda semifasciata				
Toxin **b**	6,800	> 9.5	0.05 mg/Kg	62
Lapemis hardwickii				
Lapemis Toxin	6,800	9.6	0.01 mg/Kg	60
Pelamis platurus				
Toxin **b**	6,800	8.7	0.185 mg/Kg	60
Acalyptophis peronii				
Major Toxin	6,600	> 9.5	0.125 mg/Kg	60
Acalyptophis peronii				
Minor Toxin	6,600	> 9.5	0.10 mg /Kg	60

*(i.v. mice)

The amino acid sequences of many sea snake neurotoxins have been determined. The complete amino acid sequence of the neurotoxins sequenced in this laboratory are listed in Table 2.[6,7,16-18] There are two toxic fractions in the *Acalyptophis peronii* venom, the most toxic and abundant fraction was isolated and termed major toxin. The *A. peronii* minor toxin was identified and compared to that of the major toxin. The only difference between the major and the minor toxins is in the 43rd residue. The major toxin at this position contains glutamine, while the minor toxin contains glutamic acid.[6,7]

TABLE 2

AMINO ACID SEQUENCES OF SEA SNAKE POSTSYNAPTIC NEUROTOXINS COMPLETED IN THIS LABORATORY

Laticauda semifasciata
Toxin b
RICFNQHSSQPQTTKTCPSGQSSCYHKQWSDFRGTIIERGCGCPTVKPGIKLSCCESERCNN
Lapemis hardwickii
Lapemis Toxin
MTCCNQQSSQPKTTNCAESSCYKKTWSDHRGTRIERGCGCPQVKPGIKLECCHTNECNN
Pelamis platurus
Toxin b
MTCCNQQSSEPKTTTNCAESSCYKKTWSDHRGTRIERGCGCPQVKSGIKLECCHTNECNN
Acalyptophis peronii
Major Toxin
MTCCNQQSSQPKTTTNCAGNSCYKKTWSDHRGTIIERGCGCPQVKSGIKLECCHTNECNN
Acalyptophis peronii
Minor Toxin
MTCCNQQSSQPKTTTNCAGNSCYKKTWSDHRGTIIERGCGCPEVKSGIKLECCHTNECNN

In order to understand the exact mechanism of the neurotoxic action, it is important to know the secondary structure of the neurotoxins as well. It is now known that postsynaptic neurotoxins attach to the a-subunits of acetylcholine receptor (AChR).[1] The conformation of sea snake neurotoxins has been extensively studied. The results of these studies are summarized in Table 3.[5,16,18,19]

Raman spectroscopic examination of pelamis toxin b indicates that the toxin contains a considerable amount of antiparallel β-structure, β-turn, and random coil without α-helix as the amide I band appears at 1673cm^{-1} and the amide III band at 1246 cm^{-1}. Circular dichroic studies also indicate a typical β sheet structure. The pelamis toxins b is a typical postsynaptic neurotoxin as it binds to the AChR competitively with a well known toxin, α-bungarotoxin.[16]

TABLE 3

CONFORMATION OF SEA SNAKE NEUROTOXINS AS DETERMINED BY RAMAN SPECTROSCOPY IN THIS LABORATORY

Venom	Toxin	Conformation
Enhydrina schistosa	Major toxin	Mixture of β turn and β sheet and no α helix
Lapemis hardwickii	Lapemis toxin	Mixture of β turn and β sheet and no α helix
Pelamis platurus	Pelamis toxin **a**	Mixture of β turn and β sheet and no α helix
Pelamis platurus	Pelamis toxin **b**	Mixture of β turn and β sheet and no α helix

TABLE 4

CHEMICAL MODIFICATION OF SEA SNAKE NEUROTOXINS IN THIS LABORATORY

Residue	Toxin: Conclusion
Arginine	
	L. semifasciata
	toxin a: No loss of toxicity when 1 of 3 residues modified
	toxin b: No loss of toxicity when 1 of 2 residues modified
Lysine	
	L. semifasciata
	toxin a: No loss of toxicity when 3 of 4 residues modified
	toxin b: No loss of toxicity when 4 of 5 residues modified
Tryptophan	
	E. schistosa
	Major toxin: Loss of toxicity
	L. hardwickii
	Lapemis toxin: Loss of toxicitv
	L. semifasciata
	Toxins a & b: Loss of toxicity
Tyrosine	
	L. hardwickii
	Lapemis toxin: Loss of toxicity
Sulfhydryl group	
	P. platurus
	Pelamis toxin: Less toxic but retains toxicity, still bind to AChR
Disulfide bond	
	P. platurus
	Pelamis toxin: Loss of toxicity

The amide I band and III band for *Enhydrina schistosa* toxin were at 1672 cm^{-1} and 1242 cm^{-1}, respectively. These wave numbers are characteristic for anti-parallel β sheet structure. The presence of β-sheet structure found by Raman spectroscopic study was later confirmed by X-ray diffraction study on *Laticauda semifasciata* toxin b.[19]

Sea snake short-chain toxins have a molecular weight of only 6,800. The small size with four disulfide bonds makes these toxins very compact and stable molecules. Therefore, when the *Pelamis platurus* toxin is subjected to heat treatment at 100 °C and subsequent cooling, it does not change its conformation substantially. Amide I and III bands and S-S stretching vibration did not change by heat treatment.[5]

The four disulfide bonds of the neurotoxin are clustered in one area and there is a protruding loop. It is suspected that this loop is the one that plays an important role in binding to the AChR. The four disulfide bonds are believed to be important for maintaining the specific conformation and have been studied extensively. The conformation of the disulfide bond in C-C-S-S-C-C network is *gauche-gauche-gauche* conformation at the S-S stretching vibration appearing at 510-512 cm^{-1} of Raman spectrum.[1,21]

Figure 4 shows the two-dimensional structure and tertiary structure of *Lapemis hardwickii* lapemis toxin adapted from the more well studied toxin b of *Laticauda semifasciata* venom.[22] Note the three major structural loops of the tertiary structure termed loop A, B, and C from the amino terminal to the carboxyl terminal respectively.

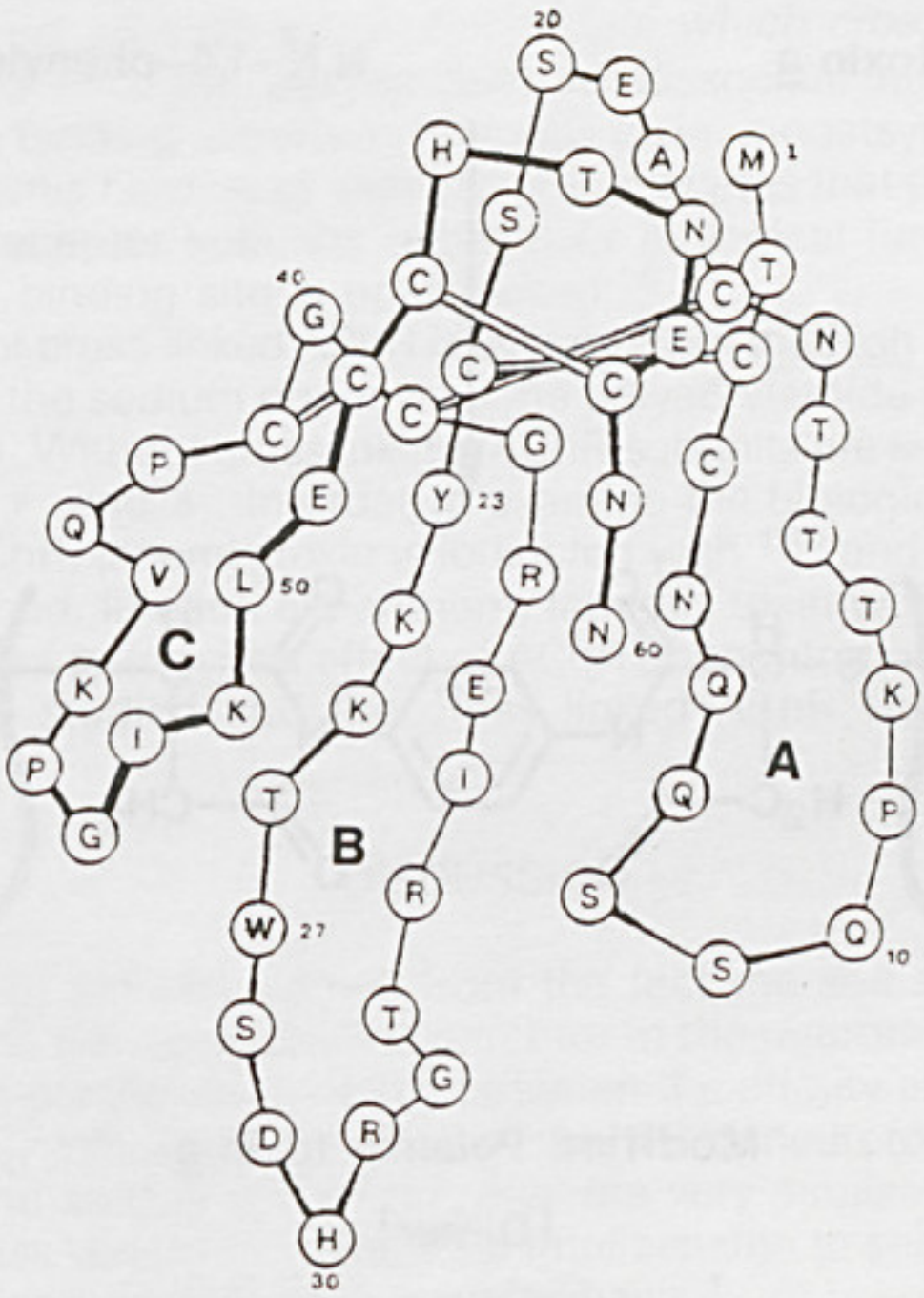


Fig. 4. Tertiary view of *Lapemis hardwickii* Lapemis toxin

In order to elucidate structure and function relationships, some amino acid residues were chemically modified and the effects of this modification on toxicity or acetylcholine receptor binding ability of the postsynaptic neurotoxins were investigated. Chemical modification of sea snake neurotoxins is summarized in Table 4.[14,19-23]

The amino acid residues in neurotoxins which are important for neurotoxic action are still not entirely clarified. Some neurotoxins contain one free SH group, while others do not. From this fact, it would be logical to assume the sulfhydryl group is not essential. This was actually proven to be the case.[20]

When N,N'-1,4-phenylenedimaleimide was used for modifying the sulfhydryl group in *pelamis* toxin, 2 mole of toxins combined with 1 mole of the reagent. With the sulfhydryl group modified, the S-H stretching vibrational band at 2578 cm^{-1} disappeared. The modification of the single sulfhydryl group did not alter the binding ability to the AChR or Toxicity (Fig. 5).[20]

Fig. 5. Chemical modification study example on neurotoxin

Disulfide bonds, however, are important in maintaining the particular toxin structure and have been shown to be essential for toxicity. When all four *disulfide bonds are reduced and alkylated, the neurotoxin loses its toxicity.*[24]

The one residue most extensively studied is tryptophan. Since it was *easily modified, it indicated that the tryptophan residue is exposed. Raman* spectroscopic analysis of a sea snake neurotoxin indicated that a single tryptophan residue is indeed exposed. The tryptophan residue lies in the im*portant loop consisting of segment 4. Modification of the tryptophan* residue induces the loss of AChR binding ability as well as the loss of toxicity.[14,19,20,22]

There is only one tyrosine residue in some sea snake neurotoxins. This residue is usually quite difficult to modify, but once it is modified, the toxicity is lost.[23]

Arginine and lysine are believed to be important, but results are not clear because sea snake neurotoxins contain several residues of these amino acids.[14]

The acetylcholine receptor connects the nerve impulse from the axon to the muscle by receiving a nerve transmitter, acetylcholine. The receptor consists of five subunits of which two are identical; it is expressed as $\alpha_2\beta\gamma\delta$. It is known that the subunit is the site for the acetylcholine binding and also for its antagonist, snake postsynaptic neurotoxin. It is generally recognized that the subunits β, γ, and δ are also essential to maintain the integrity of the acetylcholine receptor. In order to further understand the role of the subunits in the acetylcholine receptor function, the subunits *were cross-linked with dimethylsuberimidate which cross-links NH_2. The* cross-linked acetylcholine receptor does not dissociate into its components and retains the binding activity to Lapemis toxin, a postsynaptic toxin from sea snake *Lapemis hardwickii* venom. This indicates that convalently linked acetylcholine receptor subunits retain their biological function as long as the neurotoxin binding site is not blocked.[25]

Formation of cross-linked AChR is evident from the high molecular weight band shown in the sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Without crosslinking, AChR subunits are separated into four subunits, α, β, γ, and δ. In order to examine the biological activity of the cross-linked AChR, lapemis toxin is iodinated with ^{125}I and the mutual binding was examined. In each experiment, lapemis toxin without crosslinking was used as the control. The effect of AChR concentration on lapemis toxin binding was studied and the cross linked AChR showed very weak binding.[26]

DISCUSSION

The origin of lethality comes from the fact the sea snake neurotoxin strongly binds to the acetylcholine receptor at the neuromuscular junction. Thus, there is a parallel relationship between the affinity and toxicity as expressed by LD_{50}. When the structure of sea snake neurotoxins is compared with that of land snakes (Elapidae), they are very similar. Fortunately, sea snake injects less venom in a bite; thus fatalities due to sea snake are fewer than those of land snakes on a per bite basis.[1]

The binding mechanism of neurotoxins to acetylcholine receptors is still

unclear. Neurotoxins are fairly small polypeptides (6800-8000 Daltons), and many of their primary structures are already known. However, owing to the large size of the multi-subunit AChR, detailed binding sites of the receptor are still less well understood.[1]

Sea snake neurotoxins show considerable homogeneity in their amino acid sequences. There are many invariant residues. That there is greater similarity in sequences within a subfamily and more differences between the two subfamilies of sea snakes is very interesting since these chemical data agree well with morphological differences between the two subfamilies.[1]

Similarity in the structures of sea snake neurotoxins are also reflected in immunologic similarities. Usually the antibody (antiserum or antivenin) produced against sea snake venom neutralizes the toxicities of other sea snake venoms quite well. Sea snake neurotoxins are not only similar among themselves but also closely resemble neurotoxins from some land snake venoms, such as those of the Elapidae (cobras and kraits). Therefore, it is advantageous to discuss the neurotoxins of Elapidae and Hydrophiidae together for a better understanding of their structure and function. The higher toxicity of Hydrophiidae venoms compared with that of Elapidae venoms is due to the higher concentration of neurotoxins in Hydrophiidae venoms.[1]

Chemical modification of each residue in the sea snake neurotoxin indicated the tryptophan-modified neurotoxin abolished the lethality and neuromuscular blocking activity, thereby indicating the essential role of tryptophan residue. It is of interest to note that both tryptophan and tyrosine residues are conserved and located at exactly the same position in the amino acid sequence regardless of the species of sea snake. When the tryptophan residue was modified with N-bromosuccinamide, the toxicity of the sea snake neurotoxins disappeared; however, they still retained the ability to bind to neurotoxin antibody. This suggested that neurotoxins can be converted into toxoids.[19]

Sea snake venoms contain potent neurotoxins that bind almost irreversibly to the postsynaptic acetylcholine receptors. Neurotoxins are the most extensively studied proteins of all the components present in sea snake venoms. Sea snake toxins are basic proteins with isoelectric points of 8.7-10. They range in molecular weight from 6,000 to 8,000. The neurotoxin is the main protein fraction although other proteins with molecular weights up to 29,500 can be found in *Pelamis platurus*. The AChR is composed of five subunitis, $\alpha_2 \beta \gamma \delta$. A neurotoxin attaches to the α subunit. Since there are 2 moles of the α subunits, 2 moles of neurotoxins attach to 1 mole of AChR. A neurotransmitter, acetylcholine (ACh), also attaches to the α subunit. When the ACh attaches to the AChR, the AChR changes conformation, opening up the transmembrane pore so that cations (Na+,K+) can pass through. By this mechanism the depolarization wave from a nerve is now conveyed to the muscle. The difference between neurotoxin and ACh is that the former's attachment does not open the transmembrane pore. As a consequence, the nerve impulse from a nerve cannot be transmitted through the postsynaptic site (Fig. 6).

Since neurotoxins are basic proteins and the AChR is acidic, it would be logical to assume that the binding of these two proteins is due to acidic and basic protein interactions. Yet, simple acidic and basic protein binding

cannot explain the extremely high affinity of these two proteins based solely on the ionic state. Therefore, there must be some other factor involved in the binding. One such factor is likely to be complementary topography of the two molecules, which allows the two proteins to lock firmly into each other.

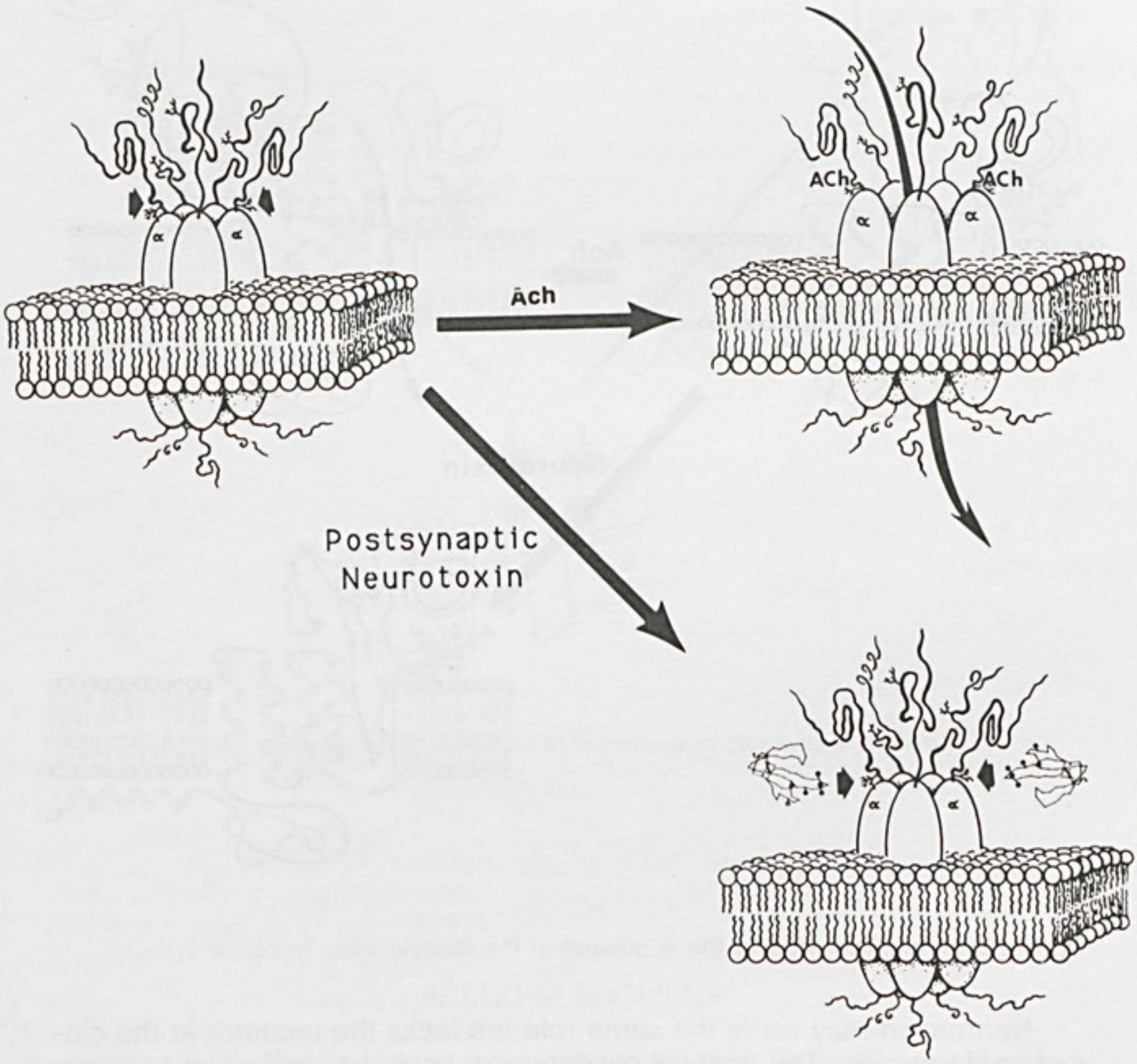


Fig. 6 Diagrammatical structure of Acetylcholine Receptor ACh (acetycholine)

The binding is very tight as stated and suggests the following working hypotheses by these authors. As mentioned the ionic state and topography of the two molecules (neurotoxin, α -AChR) play a major role in the recognition and binding. The importance of the invariant tryptophan residue of the neurotoxin may play a role in receptor recognition. The neurotoxin molecule would then lock the receptor in the closed conformation effectively blocking the receptor function to transmit the electrochemical neuronal impulse to the musculature leading to paralysis. Figure 7 diagrammatically depicts the interaction of neurotoxin with the α subunit of the AChR which is imbedded in the lipid bilayer of the muscle cell. The branched structure represents the carbohydrate moiety on the exterior surface of the muscle cell. These authors believe that there may occur a disulfide bond exchange when acetylcholine binds and the opening of the ionic channel occurs due to the conformational change in the two α subunits of the AChR.

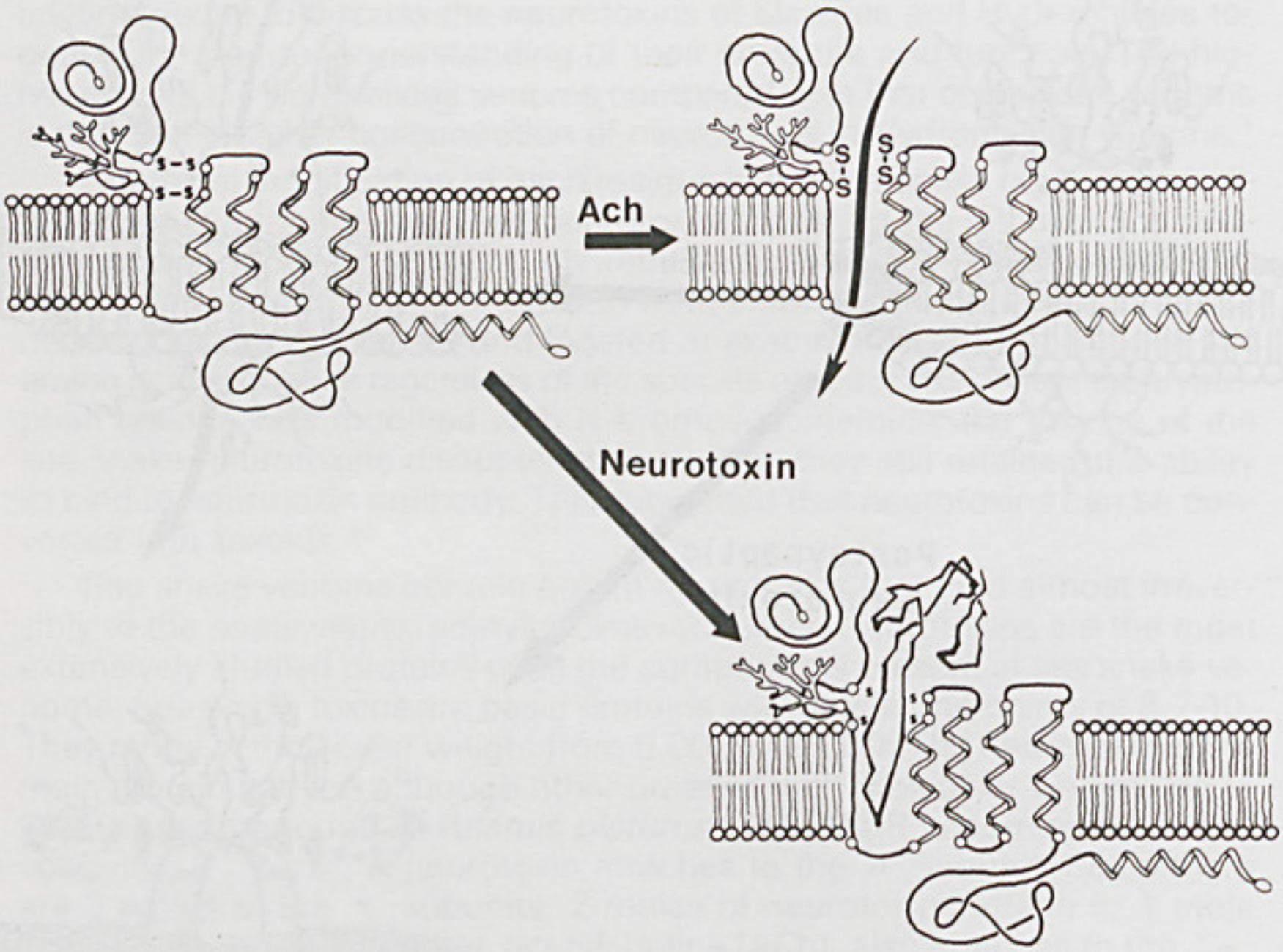


Fig. 7 Diagrammatical view of the α subunit of the Acetylcholine Receptor

Neurotoxin may serve the same role but locks the receptor in the closed conformation. The acetylcholinesterase would be ineffective to remove the neurotoxin as it does acetylcholine. This would therefore be an irreversible blockage of the receptors. The specificity and irreversibility of this action by neurotoxin probably explains the high toxicity of these molecules.

An alternative hypothesis, which is very similar to the above but may account better for the very tight binding known to occur between the AChR and neurotoxin, is that the disulfide bonds under local reducing environment actually exchange between receptor and neurotoxin molecule giving a covalent bonded receptor toxin interaction (Fig. 8). The invariant tryptophan may play a role in providing the localized reducing environment of the AChR critical disulfide bonds and the neurotoxin. Experiments are currently being designed to probe these hypothesis

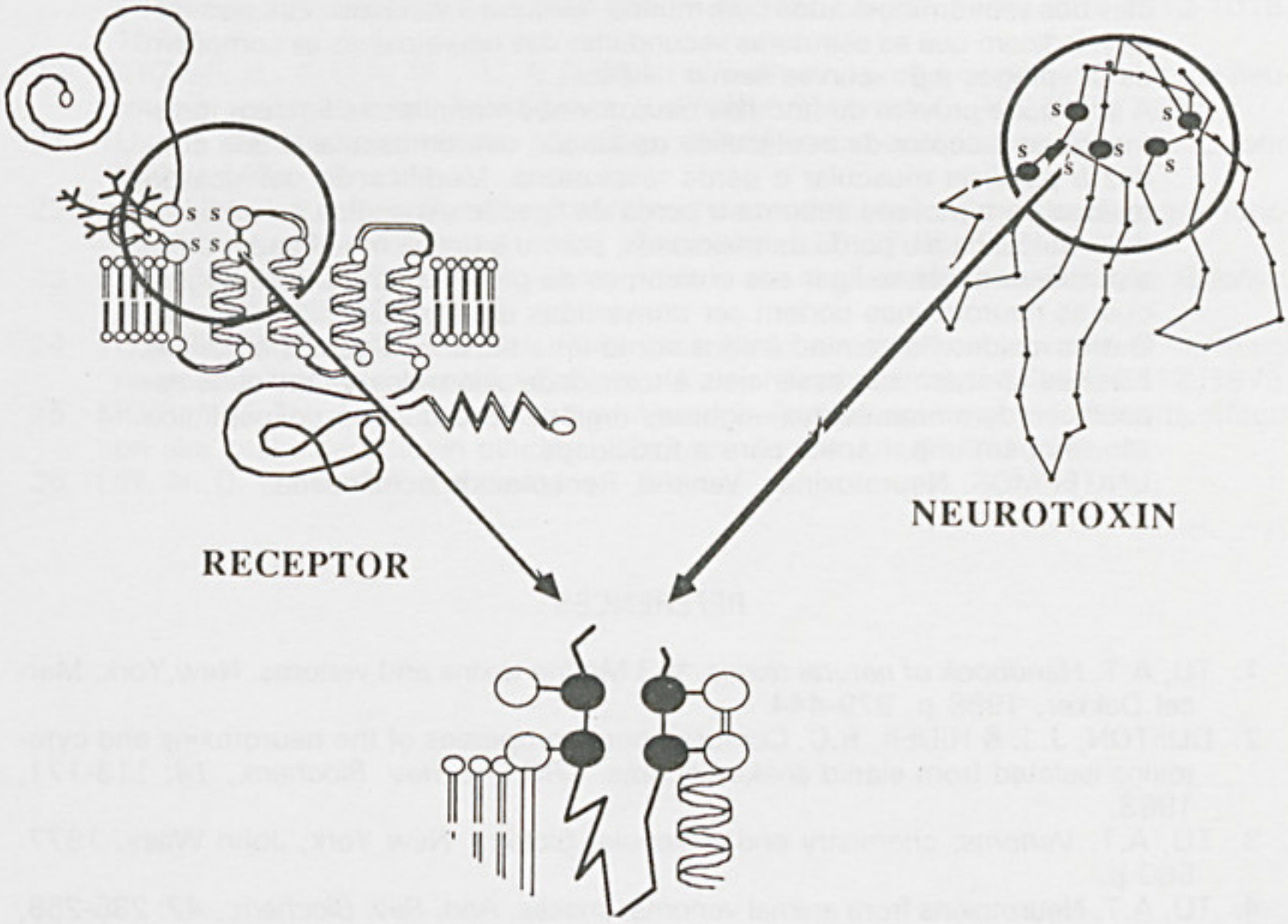


Fig. 8 Expanded view of the Disulfide (S-S) Exchange of Neurotoxin and AChR

ACKNOWLEDGMENT

This work was supported by U.S. Army Medical Research and Development Contract DAMD17-89-Z-9019. The views, opinions, and/or findings contained in this review are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

TU, A.T.; MILLER, R.A.; Biochemical studies of the sea snake neurotoxins. **Mem. Inst. Butantan, 51**(4)177 - 193, 1989.

RESUMO: Serpentes marinhas pertencentes à família Hidrofidae são serpentes adaptadas à vida aquática e são encontradas dispersas nas águas costeiras das zonas tropical e subtropical dos oceanos Indico e Pacífico.
O veneno da serpente marinha é uma mistura de várias proteínas e contém neurotoxinas potentes. A DL_{50} para o veneno da serpente marinha pode chegar até o baixo valor de 0,01 mg/kg.
As neurotoxinas pós-sinápticas do tipo I purificadas consistem de 60-62 resíduos de amino-ácidos com quatro pontes de disulfeto. São proteínas básicas com pontos isoelétricos de 9 a 10 e de pesos moleculares variando de 6.000 a 8.000 daltons. Pesquisas deste laboratório foram focalizadas sobre neurotoxinas pós-sinápticas dos venenos de *Laticauda semifasciata, Lapemis hardwickii, Pelamis platurus e Acalyptophis peronii.*
As neurotoxinas marinhas mostram grande homogeneidade das seqüências dos seus amino-ácidos com muitos resíduos invariáveis. Estudos Raman indicam que as estruturas secundárias das neurotoxinas se compõem de β -pregas e β -curvas sem α -hélices.
A letalidade provém do fato das neurotoxinas marinhas se ligarem fortemente ao receptor de acetilcolina da junção neuromuscular o que conduz à paralisia muscular e perda respiratória. Modificação química do resíduo de triptofano acarreta a perda da ligação específica ao receptor da acetilcolina e a perda da toxicidade, porém a toxina modificada retém a propriedade de se ligar aos anticorpos da proteína nativa. Isso sugere que as neurotoxinas podem ser convertidas em toxóides.
Outros resíduos de amino ácidos como uma tirosina, algumas argininas e lisinas também são essenciais à toxicidade. Além desses resíduos específicos de amino-ácidos, algumas regiões do esqueleto polipeptídico são também importantes para a toxicidade.
UNITERMOS: Neurotoxinas, Veneno, Receptor de acetilcolina.

REFERENCES

1. TU, A.T. *Handbook of natural toxins*. V.3 Marine toxins and venoms. New York, Marcel Dekker, 1988 p. 279-444.
2. DUFTON, J.J. & HIDER, R.C. Conformational properties of the neurotoxins and cytotoxins isolated from elapid snake venoms. *CRC Crit. Rev. Biochem., 14:* 113-171, 1983.
3. TU, A.T. *Venoms*; chemistry and molecular biology. New York, John Wiley, 1977. 560 p.
4. TU, A.T. Neurotoxins from animal venoms; snakes. *Ann. Rev. Biochem., 42:* 235-258, 1973.
5. TU, T.; TU, A.T.; LIN, T. Some pharmacological properties of the venom, venom fractions, and pure toxin of Yellow-bellied sea snake, *Pelamis platurus. J. Pharm. Pharmacol., 28:* 139-45, 1976.
6. MORI, N. & TU, A.T. Isolation and primary structure of the major toxin from sea snake *Acalyptophis peronii*, venom. *Arch. Biochem. Biophys., 260:* 10-17, 1988.
7. MORI, N. & TU, A.T. Amino-acid sequence of the minor neurotoxin from *Acalyptophis peronii* venom. *Biol. Chem. Hoppe-Seyler, 369:* 521-525, 1988.
8. TU, A.T. Sea snake investigation in the Gulf of Thailand. *J. Hepertol., 8:* 201-210, 1974.
9. TU, A.T. Sea snake venoms and neurotoxins. *J. Agr. Food Chem., 22*: 36-43, 1974.
10. TU, A.T. & SALAFRANCA, E.S. Immunological properties and neutralization of sea snake venoms (II). *Am. J. trop. Med. Hyg., 23:* 135-138, 1974.
11. TU, A.T. & GANTHAVORN, S. Immunological properties and neutralization of sea-snake venoms from Southeast Asia. *Am. J. trop. Med. Hyg., 18:* 151-154, 1969.
12. TU, A.T. & GILTNER, J.B. Cytotoxic effects of snake venoms on KB and Yoshida sarcoma cells. *Res. Commun. Chem. Pathol. Pharmacol., 9:* 783-786, 1974.
13. YU, N.T.; LIN, T.S.; TU, A.T. Laser Raman scattering of neurotoxins isolated from the venoms of sea snake *Lapemis hardwickii* and *Enhydrina schistosa. J. Biol. Chem., 250*: 1782-1785, 1975.
14. TU, A.T.; HONG, B.S.; SOLIE, T.N. Characterization and chemical modifications of toxins

isolated from the venoms of the sea snake, *Laticauda semifasciata*, from Philippines. *Biochemistry, 10*: 1295-1304, 1971.

15. TU, A.T. & HONG, B.S. Purification and chemical studies of a toxin from the venom of *Lapemis hardwickii* (Hardwick's sea snake). *J. Biol. Chem., 246:* 2772-2779, 1971.
16. MORI, N.; ISHIZAKI, H.; TU, A.T. Isolation and characterization of *Pelamis platurus* (Yellow-bellied Sea Snake) Postsynaptic Isoneurotoxin. *J. Pharm. Pharmacol., 41*: 331-334, 1989.
17. TSERNOGLOU, D.; PETSKO, G.A.; TU, A.T. Protein sequencing by computer graphics. *Biochim. Biophys. Acta, 491:* 605-608, 1977.
18. FOX, J.W.; ELZINGA, M.; TU, A.T. Amino acid sequence of a snake neurotoxin from the venom of *Lapemis hardwickii* and the detection of a sulphydryl group by laser Raman spectroscopy. *FEBS Lett., 80:* 217-220, 1977.
19. TU, A.T. & HONG, B.S. Purification and chemical studies of the toxic component of *Enhydrina schistosa* (common sea snake) venom. *J. Biol. Chem., 246:* 1012-1016, 1971.
20. ISHIZAKI, H.; ALLEN, M.; TU, A.T. Effect of sulfhydryl group modification on the neurotoxic action of a sea snake toxin. *J. Pharm. Pharmacol., 36*:36-41, 1984.
21. TU, A.T. *Raman Spectroscopy in biology;* principles and applications. New York, John Wiley. 448p.
22. ALLEN, M. & TU, A.T. The effect of tryptophan modification on the structure and function of sea snake neurotoxin. *Mol. Pharmacol., 27:* 79-85, 1985.
23. RAYMOND, M.L. & TU, A.T. Role of tyrosine in sea-snake neurotoxin. *Biochim. Biophys. Acta, 285:* 498-502, 1972.
24. TU, A.T.; LIN, T.S.; BIEBER, A.L. Purification and chemical characterization of the major neurotoxin from the venom of *Pelamis platurus. Biochemistry, 14:* 3408-3413, 1975.
25. MORI, N. & TU, A.T. Cross-linking of Acetylcholine Receptor subunits and its effects on sea snake neurotoxin binding. *Biochem. Archiv., 4*: 85-89, 1988.
26. LIN, N. Q. & TU, A.T. (in press)