Collected Papers on *Snake Venoms*

Contributions from The Pharmacological Institute, National Taiwan University, Taipei, Taiwan, China, 1948-1973

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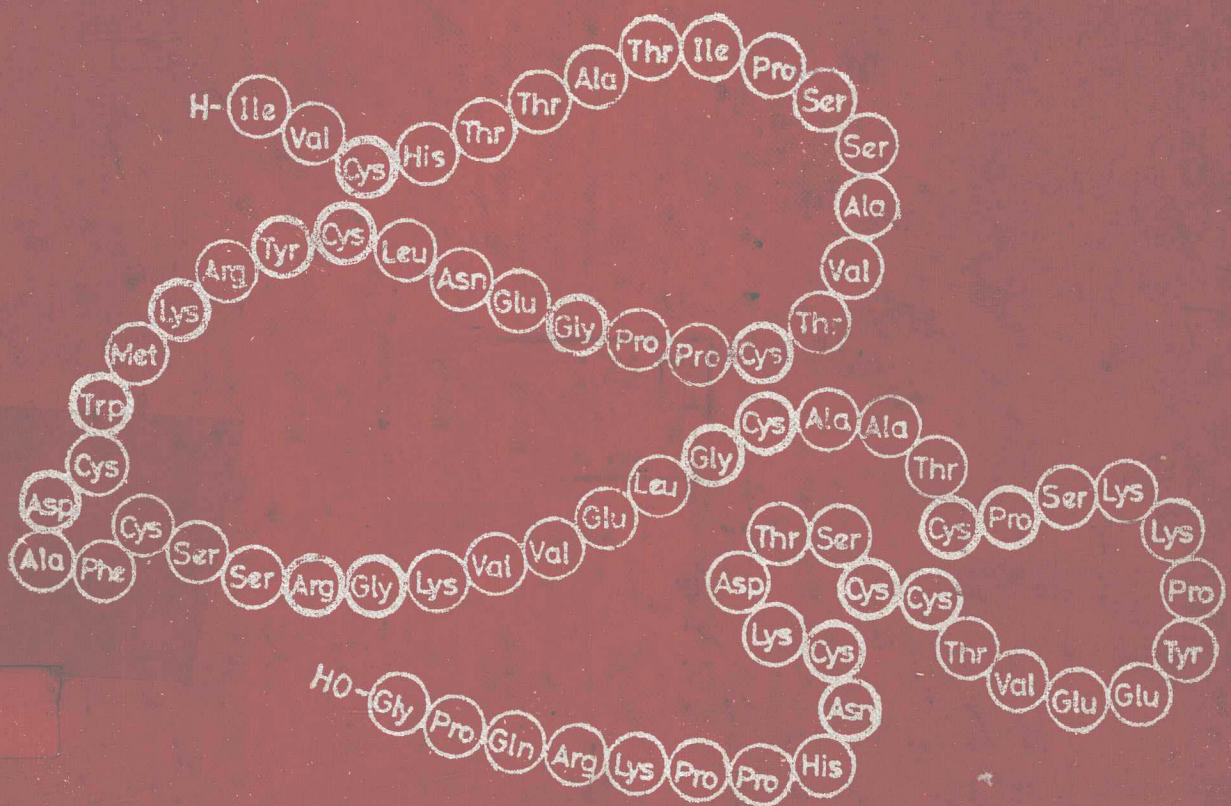

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College of Medicine, National Taiwan University

Dedicated with affection and respect to

Professor Tsungming Tu

in celebration of his eightieth birthday,

who initiated pharmacological studies on

snake venoms in Taiwan.

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Elapid Neurotoxins and Their Mode of Action

C. Y. Lee

Pharmacological Institute
College of Medicine
National Taiwan University
Taipei, Taiwan, China

INTRODUCTION

Venoms of many species of snakes belonging to the family Elapidae (cobras, kraits, corals, mambas, tiger snakes, death adders, black snakes, taipan, etc.) are highly toxic and produce flaccid paralysis and respiratory failure. These effects have been attributed to the so-called "neurotoxins" contained in the venoms. The term "neurotoxin," however, has been ill-defined and used indiscriminately. Russell [1] has stated that neurotoxins can and do have cardiotoxic or hemotoxic activities, or both. Confusion has arisen when the term "neurotoxin" has been applied to a whole venom, for most venoms are complex mixtures of various enzymes and other toxins besides neurotoxins. The purified neurotoxins, however, have been shown to be devoid of any cardiotoxic or hemotoxic activities (cf. [2], also see section "Mode of Action of Elapid Neurotoxins").

Gitter and de Vries [3] have defined neurotoxins as the active components of snake venoms, responsible for the disturbances in the central nervous system and for the impairment of peripheral nerve activity and neuromuscular transmission. This is a rather comprehensive definition, but all the elapid neurotoxins so far studied have been shown to affect selectively the neuromuscular transmission without any appreciable effect on the central nervous system.

CHEMISTRY OF ELAPID NEUROTOXINS

The main cause of death due to elapid venoms has been shown to be peripheral respiratory paralysis caused by their neurotoxins (see section "Mode of Action of Elapid Neurotoxins"). All of the elapid neurotoxins so far isolated are basic polypeptides. The content of neurotoxins in elapid venoms varies from one species to another, and there is ample evidence that more than one kind of neurotoxin is present even in the same venom [4-7].

Isolation and Nomenclature

The early attempts to isolate neurotoxins from snake venoms, especially from elapid venoms, have been reviewed by Slotta [8], Christensen [9], and more recently by Meldrum [10] and Boquet [11]. Recent advances in separation methods based on molecular size and charge have been discussed by Porath [5].

Yang [12] has succeeded in isolating a crystalline neurotoxin from the venom of *Naja naja atra* by ammonium sulfate fractionation followed by repeated chromatography on carboxymethyl cellulose column and subsequent crystallization. The crystalline toxin thus obtained was named "cobrotoxin." Its molecular weight was at first reported to be 11,000 but later calculated to be 6949 from amino acid composition [13].

A neurotoxin, called "toxin α," has been isolated from the venom of *Naja nigricollis* by ion-exchange chromatography on Amberlite IRC-50 [14], and another neurotoxin, also called "toxin α" was recently isolated from the venom of *Naja haje haje* by gradient chromatography on Amberlite CG-50, followed by gel filtration on Sephadex G-50 [15].

These three purified neurotoxins from different cobra venoms have been shown to be homogeneous and free from any known enzyme activities. They are not only similar in their chemical structures but also pharmacologically almost indistinguishable from each other and may all be called "cobra neurotoxin."

Among the three toxic fractions isolated from *Hemachatus haemachatus* venom [5], peaks 3 and 5 represent highly toxic neurotoxins, whereas peak 12 appears to be identical with the direct lytic factor (DLF) isolated from the same venom [16], judging from their amino acid compositions.

Two different types of neurotoxins have been separated from the venom of *Bungarus multicinctus* by means of zone electrophoresis on starch at pH 5.0 [4]. One called "α-bungarotoxin" produces a "nondepolarizing" type of neuromuscular block by acting postsynaptically on the motor

endplate. The two most electropositive fractions, called β- and γ-bungarotoxin, respectively, both produce a neuromuscular block by acting presynaptically on the motor nerve endings (see section "Mode of Action of Elapid Neurotoxins"). Both α- and β-bungarotoxins have recently been further purified by CM-Sephadex chromatography followed by repeated rechromatography on CM-cellulose column and found to be free from any enzyme activities contained in the crude venom [17].

None of these purified neurotoxins has been shown to be glycoprotein as reported by Braganca and Patel [18]. The "low molecular weight" toxins from elapid venoms reported by Fischer and Kabara [19] may be fragments of these larger molecular weight neurotoxins, but so far no evidence has been obtained to support such a possibility.

Amino Acid Composition

In Table 1, the amino acid composition of five neurotoxins isolated so far from different cobra venoms is compared with that of α- and β-bungarotoxins [20] as well as with neurotoxins isolated from sea-snake venoms [21-23]. All of the cobra neurotoxins are composed of 61 to 62 residues of 15 common amino acids but devoid of alanine, methionine, and phenylalanine. They consist of a single peptide chain cross-linked by four disulfide bridges and terminated by leucine and asparagine at their amino- and carboxyl-terminal ends, respectively.

It is interesting to note that the neurotoxins from sea-snake venoms also consist of 61 to 62 amino acids in a single chain cross-linked by four disulfide bonds. The similarity in amino acid composition with cobra neurotoxins is also remarkable; they are all basic polypeptides and devoid of alanine and methionine in their molecules.

From the amino acid analyses and estimation of molecular weight by sedimentation equilibrium, it has been tentatively concluded that α-bungarotoxin consists of 74 amino acids in a single chain cross-linked by five disulfide bridges and terminated by isoleucine at its amino-terminal end, whereas β-bungarotoxin is composed of about 179 residues with ten disulfide bonds [20]. It is noteworthy that some similarities in amino acid composition are to be found between α-bungarotoxin and cobra neurotoxins which have a similar mode of neuromuscular blocking action (see section "Mode of Action of Elapid Neurotoxins"). The molecular weight of β-bungarotoxin has been estimated to be approximately 28,500, but it could be a dimer. Its amino acid composition is quite different from that of other neurotoxins (see Table 1).

Table 1
Amino Acid Composition of Neurotoxins Isolated From Elapid- and Sea-Snake Venoms

Amino acid	*Naja nigricollis* (Toxin α)	*Naja haje haje* (Toxin α)	*Naja naja atra* (Cobrotoxin)	*Hemachatus Haemachatus* (Peak 3)	*Hemachatus Haemachatus* (Peak 5)	*Bungarus multicinctus* (Bungarotoxin) α	*Bungarus multicinctus* (Bungarotoxin) β	*Laticauda semifasciata* (Erabutoxin) a	*Laticauda semifasciata* (Erabutoxin) b	*Laticauda laticaudata* (Laticotoxin a)
Lysine	6	6	3	4	6	6	13	4	4	4
Histidine	2	2	2	2	2	2	5	1	2	2
Arginine	3	4	6	5	4	3	14	3	3	5
Aspartic acid	7	7	8	9	5	4	22	5	4	9
Threonine	8	7	8	7	9	7	12	5	5	4
Serine	2	4	4	3	4	6	6	8	8	5
Glutamic acid	6	7	7	5	8	5	12	8	8	7
Proline	5	4	2	5	4	8	8	4	4	5
Glycine	5	5	7	5	5	4	16	5	5	5
Alanine	0	0	0	0	0	5	11	0	0	0

Half-cystine	8	8	8	8	8	10	20	8	8	8
Valine	2	1	1	1	1	5	4	2	2	1
Methionine	0	0	0	0	0	1	2	0	0	0
Isoleucine	3	3	2	3	1	2	8	4	4	2
Leucine	2	1	1	2	2	2	7	1	1	1
Tyrosine	1	1	2	1	1	2	13	1	1	1
Phenylalanin	0	0	0	0	0	1	6	2	2	1
Tryptophan	1	1	1	1	1	1		1	1	1
Amide NH_3	7	9	9	10	8	4		10	10	
Total	61	61	62	61	61	74	~179	62	62	61
N-terminal	Leucine	Leucine	Leucine			Isoleucine		Arginine	Arginine	Arginine
C-terminal	Asparagine	Asparagine	Asparagine					Asparagine	Asparagine	Asparagine
Molecular weight	6787	6835	6949	6828	6823	7983	~28,500	6837	6857	6880
Reference	[14]	[15]	[13]	[5]		[20]		[21-23]		[22]

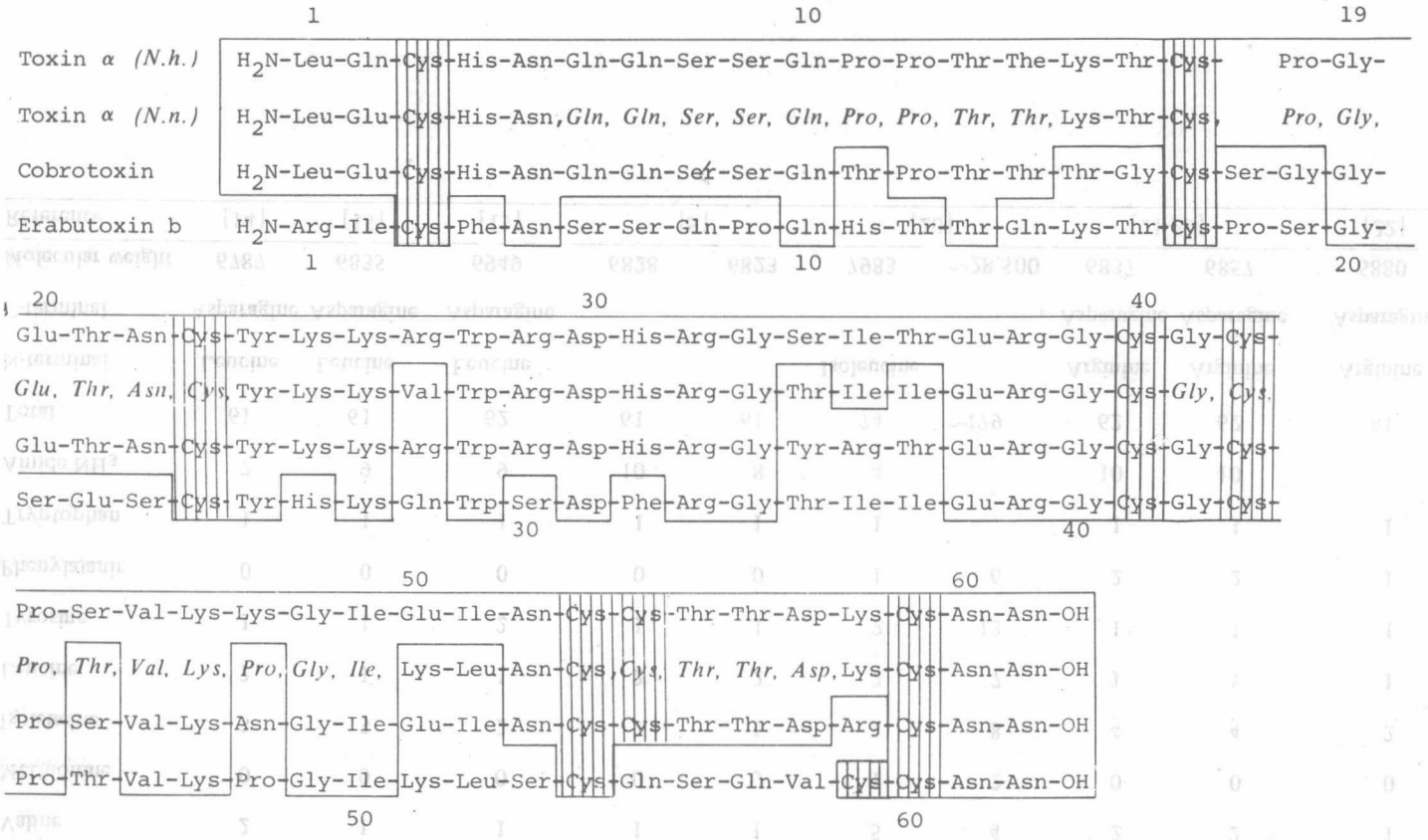

Fig. 1. Comparison of amino acid sequences of toxin α of *N. haje haje (N.h.)* [15], toxin α of *N. nigricollis (N.n.)* [25], cobrotoxin of *N. naja atra* [24] and erabutoxin b of *Laticauda semifasciata* [23]. The parts of the *N. nigricollis* toxin sequence in *italics* were assigned by similarity to the sequences of other two toxins.

Amino Acid Sequence

In Fig. 1, the amino acid sequences of three cobra neurotoxins, cobrotoxin from *Naja naja atra* [24], toxin α from *Naja haje haje* [15], and toxin α from *Naja nigricollis* [25] are compared with that of erabutoxin b from *Laticauda semifasciata* [23]. it is evident that a remarkable degree of similarity exists especially among the three cobra neurotoxins. The two α toxins are identical from the amino terminus to position 26 and also in their carboxyl terminal sequences from positions 52 to 61. In the region from position 27 to 51, only seven amino acid differences are found between the two neurotoxins. There are also only eight amino acid differences between cobrotoxin and toxin α from *Naja nigricollis* if serine at position 18 in cobrotoxin is disregarded. It is noteworthy that half-cystinyl residues in these neurotoxins, which form four disulfide bonds for maintaining the polypeptides in their active conformation, are in the same positions. The similarity in amino acid sequence is found not only among cobra neurotoxins but also between erabutoxin b and cobra neurotoxins. Thus, 28 amino acid residues are found to be common to these neurotoxins and seven out of eight half-cystine residues are in the same positions. Similar amino acids tend to be clustered together in their molecules and the location of all of the half-cystine residues near the ends of the molecules leaves the center sequence from 24-25 to 39-40 free. It has been speculated that this central non-cross-linked sequence containing most of the basic amino acids and all of the aromatic amino acids in close order might be the "active site" of the neurotoxin molecules [25]. This uncross-linked loop, possibly projecting outward from the molecule because of its hydrophilic character, is the only region in the molecule where, potentially, a considerable degree of α-helical structure could be present [15].

Structure-Activity Relationship

It has been repeatedly demonstrated that the integrity of the disulfide bonds in the neurotoxin molecules is essential for their biological activity [26-30]. Reduction breaks the disulfide bridges and results in loss of toxicity. The reduced cobrotoxin regains full toxicity on re-oxidation [30]. An optical rotatory dispersion (ORD) study of cobrotoxin discloses that it contains a left-handed α-helical structure [31], and a subsequent study of its circular dichroism (CD) spectrum indicates the presence of β-structure in its molecule [32]. On reductive cleavage of the disulfide bonds, cobrotoxin becomes a mixture of a large amount of random coil and a small amount of α-helix or β-structure. The re-oxidized cobrotoxin, however,