Hypothesis

A POSSIBLE STRUCTURAL BASIS FOR THE DIFFERENT MODES OF ACTION OF NEUROTOXINS AND CARDIOTOXINS FROM SNAKE VENOMS

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1. Introduction

The short neurotoxins and the cardiotoxins from snake venoms are a large group of homologous proteins [1,2]. Spectroscopic studies indicated that the homology among these toxins includes also the three-dimensional structure and that the conformation type found in single crystals of a particular neurotoxin, erabutoxin b [3,4], prevails quite generally for the proteins in this group [5–8]. On the other hand it appears that in spite of the structural similarities, the mechanism of action of the neurotoxins is quite different from that of the cardiotoxins. Neurotoxins bind to a protein receptor at the postsynaptic level and block acetylcholine transmission [9]. Cardiotoxins cause irreversible depolarization of cell membranes, e.g., desactivating the sodium–potassium adenosine triphosphatase [10]. Evidence was presented that cardiotoxins bind to lipid components of cell membranes [10], and the association of cardiotoxin with negatively charged phospholipids was demonstrated [11,12]. We propose here a structural basis for the different functions of neurotoxins and cardiotoxins based on recent observations by others and in our laboratory.

2. Proposed model for cardiotoxin–membrane interactions

The model is based on the following structural data:

(i) The single crystal structure of one short neurotoxin, erabutoxin b from *Laticauda semifasciata* is known [3,4]. It contains three hairpin loops which are combined into an extended β-sheet comprising five antiparallel segments of the polypeptide chain. This is presented schematically in fig.1A.

(ii) From Raman scattering [5], proton NMR [6] and circular dichroism [8] it was concluded that the solution conformations of short neurotoxins and cardiotoxins contain comparable amounts of β-structure, which correspond to that found in the crystal structure of erabutoxin b [3,4].

Additional ^{1}H NMR data show that certain nearest neighbor relations between amino acid side chains found in erabutoxin b are preserved in the solution conformations of other toxins [7,13]. Since it thus appears that the same structure type prevails for short neurotoxins and cardiotoxins, we have fitted the amino acid sequence of a cardiotoxin, cardiotoxin $V^{II}4$ from *Naja mossambica mossambica* [14], to the backbone conformation of erabutoxin b. To accommodate the deletions of the residues 4, 6 and 7 [14], the first hairpin loop was shortened and rearranged so that the locations of Leu 1, Lys 2 and the disulfide bond Cys 3–Cys 24 were preserved (fig.1B). A second smaller modification was made by the addition of a small bulge between the disulfide bridges Cys 17–Cys 41 and Cys 45–Cys 56 to accommodate the insertion of the two residues Asn 43 and Val 44.

The cardiotoxin structure of fig.1B contains a quite striking feature in that all the hydrophobic segments of the amino acid sequence are found in the three hairpin loops. These are the fragment Leu 9–Ile–Pro–Ile–Ala–Tyr 14 in the first loop, the fragments Met 27–Met–Leu–Ala 30 and Met 34–Val–Pro–Val 37 in the central and longest

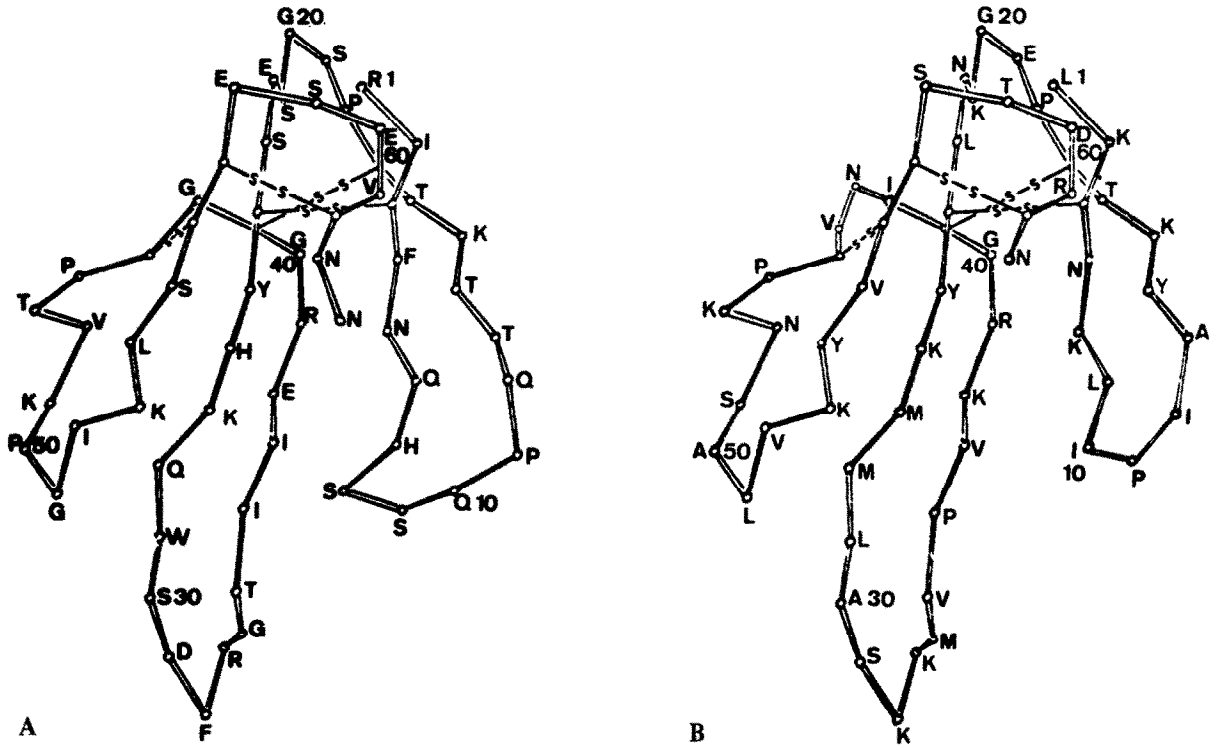

Fig.1. α-Carbon diagrams of (A) the X-ray structure of erabutoxin b from *Laticauda semifasciata* [3]. (B) the proposed homologous conformation of cardiotoxin $V^{II}4$ from *Naja mossambica mossambica* (see text). The amino acids are designed by the IUPAC one-letter code: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; Y, Tyr; V, Val; W, Trp.

loop, and the fragment Ala 50–Leu–Val 52 in the third loop (fig.1B). On both sides of these hydrophobic fragments the amino acid sequence contains hydrophilic residues, mostly lysines (fig.1B). Thus Lys 32 and Lys 33 are located at the tip, and Lys 26 and Lys 38 near the base of the central hairpin loop. In an antiparallel β-sheet structure the distance between the α-carbon atoms of these two pairs of lysine is ~18 Å. Hence if the lysine side chains at the two ends of the loop are oriented in opposite directions, away from each other, the positive charges of the ε-amino groups are at a distance of ~30 Å, which corresponds to the thickness of the hydrophobic core separating the polar head groups in a lipid bilayer [15].

Figure 2 presents a schematic view of the cardiotoxin–membrane complex which we propose from the protein structure of fig.1B. The four hydrophobic peptide fragments in the three hairpin loops of the cardiotoxin are all located in the hydrophobic interior of the membrane, and the central loop penetrates the lipid bilayer. In a membrane containing negatively charged lipids, the positively charged ε-amino groups of eight lysines can form salt bridges in the polar head group regions of both the inside (Lys 32 and 33) and the outside (Lys 8, 15, 26, 38, 47 and 53) layers. The major portion of the protein globule, which contains all disulfide bridges, extends into the aqueous phase. In a cardiotoxin–membrane complex of the type shown in fig.2, the protein structure of fig.1B would readily explain why binding is preferentially to negatively charged phospholipids [11,12] and why cardiotoxin action may lead to perturbations of the lipid bilayer structures in the membranes [16,17].

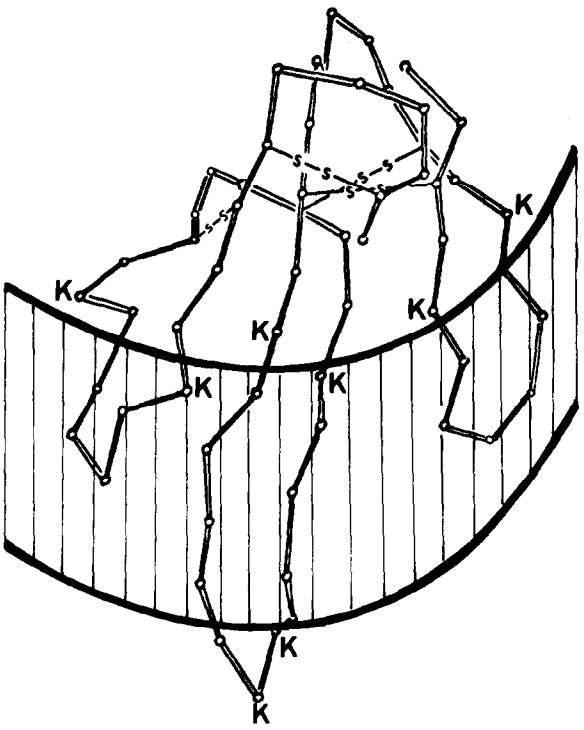

Fig.2. Schematic representation of the structure proposed for the cardiotoxin–membrane complex.

3. Discussion

Since it includes ionic interactions on the membrane surface and penetration of the lipid bilayer by hydrophobic fragments of the protein, the model for cardiotoxin–membrane interaction proposed in fig.2 is quite satisfactory in that it appears to be compatible with the experimental observations on cardiotoxin binding [10–12,16,17]. In addition, as is discussed in the following, it seems to provide an easy explanation for the observed different functional behavior of neurotoxins and cardiotoxins [2,10] and suggests a structural interpretation of the different toxicities found in four different cardiotoxins from the same snake [18].

In neurotoxins, residues with pronounced hydrophilic properties replace the hydrophobic segments in the hairpin loops of the cardiotoxin structure of fig.1B (see, e.g., fig.1A) [2]. In a neurotoxin–membrane complex of the fig.2 type, numerous hydrophilic groups such as Arg, Ser, Thr and Glu would therefore be located in the hydrophobic central region of the lipid bilayer, which would be a very unfavorable situation. Therefore, if one assumes that cardiotoxin–membrane interactions are of the fig.2 type, the protein structures imply that neurotoxins have markedly different functional properties.

Four different cardiotoxins have been isolated from the venom of *Naja mossambica mossambica,* i.e., cardiotoxins $V^{II}1$–$V^{II}4$, and it was reported that the toxicity decreases in the order $V^{II}1 > V^{II}2 > V^{II}3 > V^{II}4$ [18]. If, as a working hypothesis, it is assumed that the toxicity is related to the extent of perturbation of the lipid structure by cardiotoxin binding, the model of fig.2 suggests an intriguing structural interpretation of the variable toxicities. Comparison of the amino acid sequences of the cardiotoxins $V^{II}1$–$V^{II}4$ (fig.3) [14,19] shows that the hydrophobic fragments 9–14 and 50–52 are conserved, as well as five of the six lysines which are located on the external membrane surface in fig.2. Furthermore, the amino acid composition and hence the predominantly hydrophobic character of the central loop is conserved, so that the driving forces for the reactions leading to the structures of the type of fig.2 should be nearly the same in all four proteins. However, the amino acid sequences in the central loop are different (fig.3). Whereas in cardiotoxin $V^{II}4$, Lys 32 and Lys 33 are at the tip of the loop (fig.1B), the hydrophilic groups in $V^{II}3$ and $V^{II}2$ are Arg 30 and Lys 34, and in the central loop of $V^{II}1$ there is only one charged group, i.e., Arg 30. In the structure of fig.2 it is then more difficult, if not impossible, that the extended loop in cardiotoxins $V^{II}1$–$V^{II}3$ can traverse the hydrophobic core of the membrane to the extent that Lys 34 and/or Arg 30 are located near the inside membrane surface. Since the length of the hydrophobic part of the central hairpin loop in $V^{II}1$–$V^{II}3$ is thus too short to match the thickness of the lipid bilayer, formation of salt bridges between the positively charged peripheral groups of Lys 34 and Arg 30 and the polar head groups of the inner lipid bilayer would require much stronger perturbations of the lipid structures than in the case of cardiotoxin $V^{II}4$. Following the above-mentioned working hypothesis this increased perturbation of the lipid bilayer structure would correspond to increased toxicity of the proteins.

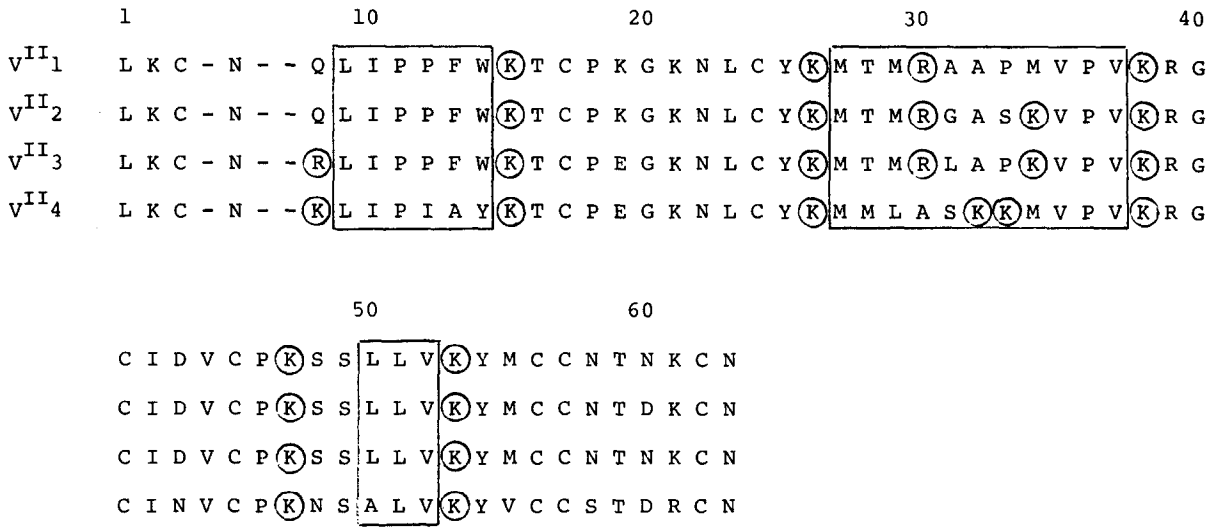

Fig.3. Amino acid sequences of the cardiotoxins $V^{II}1$–$V^{II}4$ from *Naja mossambica mossambica* [14,19]. The numeration of the residues corresponds to that used for homologous arrangements with neurotoxins. The hydrophobic fragments and the positively charged residues discussed in the text (see also fig.2) are shown in boxes and in circles, respectively. For the IUPAC one-letter symbols see fig.1.

Acknowledgements

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