was greater (70% of the maximum twitch tension generated) and lasted twice as long as that observed with crude venom at double the concentration. In both cases, contracture was followed by irreversible blockade of the twitch-tension evoked by indirect stimulation (50% paralysis in 35 ± 3 min for P.o. venom and 18 ± 3 min for Peak I). P.o. venom and Peak I were also able to block the contractile response to acetylcholine and potassium. CK release was observed with the crude venom but not with Peak I. Peak I evoked minor morphological alterations when compared with the crude venom. The principal effect observed in both cases was hypercontractility of the muscle fibres. Based on the above results, we conclude that Peak I contains all neurotoxic activity of crude P.o. venom.

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Double role of the non-toxic and non-enzymatic subunit of crotoxin in the toxin mechanism of action. C. Bon, V. Choumet, E. Délot, G. Faure, A. Robbe-Vincent and B. Saliou (Unité des Venins, Institut Pasteur, Paris, France).

Crotoxin, the main toxin from the venom of rattlesnake, is a phospholipase A_2 (PLA_2) β-neurotoxin which consists of the non-covalent association of two subunits: a basic and weakly toxic PLA_2 subunit (CB) and a non-toxic, non-enzymatic subunit (CA) that enhances the toxicity of CB. Crotoxin complex (CACB) dissociates upon interaction with membranes: CB binds while CA does not. Multiple variants of each subunit give different crotoxin complexes characterized by different lethal potency and different enzymatic activity. (1) The more toxic isoforms block neuromuscular transmission more efficiently than weakly toxic isoforms; (2) the less toxic complexes have the same K_m and V_{max} as CB alone, while the more toxic isoforms are enzymatically less active than CB; (3) the differences correlate with the stability of the complexes, the less toxic isoforms are less stable (K_d 25 nM) and dissociate rapidly (half-life 1 min), whereas the more toxic isoforms are more stable (K_d 4 nM) and dissociate more slowly (half-life 15 min); (4) the rate of interaction of crotoxin complexes with vesicles of negatively charged phospholipids parallels the rate of dissociation of the complexes in the absence of vesicles. These observations indicate that CA enhances the lethal potency of CB by preventing its non-specific adsorption, the stability of the complex playing a major role in the synergistic action of crotoxin subunits. Binding studies were performed with ^{125}I-labelled crotoxin on *Torpedo* presynaptic membranes. (1) In the range of concentrations that are effective on synaptosomes, crotoxin binds to a single class of sites without cooperativity; (2) the binding of crotoxin was inhibited by related PLA_2 neurotoxins but not by CB; (3) although CA alone does not bind, it competes efficiently with crotoxin. An analysis of the kinetics of crotoxin binding to *Torpedo* synaptosomal membranes further indicated that CA participates with CB to the formation of a ternary complex with the crotoxin acceptor site, preceding the release of CA.

β-Bungarotoxin does not affect binding of synaptic vesicles to immobilized synaptic plasma membranes. P. Rosenberg and R. Chapell (The University of Connecticut, School of Pharmacy, Department of Pharmaceutical Sciences, Storrs, CT 06269, U.S.A.).

Synaptic vesicles (SV) are tethered in a highly organized system to the synaptic plasma membrane (SPM) of the presynaptic terminal in close proximity to the Ca^{2+} channel, forming a prefusion complex to facilitate membrane fusion and neurotransmitter release upon receipt of the Ca^{2+} signal. It was therefore of interest to determine whether β-bungarotoxin (β-BuTX), a potent and specific presynaptic acting toxin which decreases acetylcholine release, interferes with the binding of SV to the SPM. Purified SV were isolated from porcine brain and radiolabeled with ^{125}I. SPM were isolated from the same source and immobilized by binding to nitrocellulose sheets. The binding of SV to the SPM was then measured by gamma counting. Binding was specific, protein dependent and could be modulated by treatment of the SV and/or SPM by various agents. *N*-Ethylmaleimide (NEM), kinase II fragment (inhibitor of calmodulin) and staurosporine (inhibitor of protein kinases) all inhibited binding. A phorbol ester which stimulates protein kinase C (PKC) increased binding, which was antagonized by calphostin C (a PKC inhibitor) and NEM. β-BuTX and *Naja naja atra* PLA_2 at 15 nM concentrations had no effect on binding while a higher concentration (100 nM) of *N. n. atra* PLA_2 (but not β-BuTX) increased binding, probably due to the release of arachidonic acid which is known to activate PKC. Our results suggest that the inhibitory effect of β-BuTX on neurotransmitter release occurs at a stage prior to the binding of SV to SPM. This conclusion is in agreement with our previous suggestion that β-BuTX may decrease the number of free SV in the presynaptic terminal available for binding by inhibiting phosphorylation of the cytoskeletal protein synapsin I.

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