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IMMUNOLOCALIZATION OF PURIFIED CROTOXIN FROM *Crotalus durissus terrificus* VENOM AT MOTOR END PLATE OF STRIATED MUSCLE IN CBA/J MICE.

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Crotoxin, a potent neurotoxic fraction of the venom from *C.d.terrificus*, has a potent pre- and post-synaptic action as detected by electrophysiological assays. In order to verify tissue localization of purified crotoxin, we performed immunohistochemical detection of this toxin, using a single step immunoperoxidase assay.
Crotoxin was purified from crude venom by gel filtration and isoeletric pH precipitation, with a LD_{50} in mice of 80 ug/kg. Purification was assessed by SDS-PAGE. CBA/J mice(18-22g) were injected in retroorbital plexus with 5 ug of purified crotoxin in sterile PBS, resulting in death of all animals in 3hs. Mice were killed at 15, 30 and 60 minutes after injection and organs carefully removed and fixed by immersion in phosphate buffered formalin. The immunohistochemical assays were made with a peroxidase conjugated antivenin, using periodate as coupling agent, with a 1/400 titer in an ELISA assay. Tissue sections of Paraplast-plus^{R} embedded striated muscle, in glue coated slides, were incubated with the conjugate at 1/50 dilution and revealed by DAB-H_2O_2. Controls muscle and also muscle from animals after 15 min injection showed no staining. After 30 min of the injection, we found clearly stained neuromuscular motor end plate, including thin terminations, without any morphological alteration. After 60 min of the injection, we failed to observe intact motor end plate, only roughly formed stained areas, without clear identification of their structure.
This study shows crotoxin specific binding to neural motor end plate at striated muscle, with probably time dependent toxin induced degeneration of this structure.

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