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Effects of Recombinant Imperatoxin A (IpTx_a) mutants on Ca²⁺ Release Channel/Ryanodine Receptor in Rabbit Skeletal Sarcoplasmic Reticulum

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Imperatoxin A (IpTx_a), a 3.7 kDa peptide from the African scorpion *Pandinus imperator*, has been known as an agonist of skeletal ryanodine receptor (RyR). In order to study the structure and function of the toxins on RyR, the IpTx_a cDNA was PCR-amplified using 3 pairs of primers and the toxin was expressed in *E. coli* expression system. The toxin was further purified by various chromatographic methods. Various point-mutants in which the basic amino acid residues were substituted with alanine were prepared by site-directed mutagenesis. The studies of single channel properties by planar lipid bilayer method were carried out to examine the effects of the wild type and mutant recombinant IpTx_a on rabbit skeletal Ca²⁺ release channel/RyR. The results show that the recombinant toxin peptide has the similar effect on RyR to the native toxin. Mutations in specific basic amino acids (Lys¹⁹, Arg²³, or Arg³¹) dramatically reduced the capacity of the peptide to activate RyRs. The appearance of subconductance state was predominant when Lys⁸ was substituted with alanine. These results suggested that some specific basic amino acid residues in IpTx_a are important for activation of RyR and the residue Lys⁸ could be important for the regulation of the gating mode of RyR.