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Structure-Activity Relationship of Imperatoxin A, a Peptide Activator of Ryanodine Receptors

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Alanine scanning analogs of imperatoxin A ($IpTx_a$) were synthesized by replacing each amino acid residue except cysteine with alanine. Circular dichroism (CD) spectra of all the analogs were virtually identical to that of wild-type $IpTx_a$, indicating that they have basically similar conformation to that of wild-type $IpTx_a$. The activitise of these toxin molecules were tested on [^{3}H]ryanodine binding assay. On the 30 nM peptide concentration corresponding to apparent K_d of wild-type $IpTx_a$, the binding assay of all analogs showed that the Ala-replacement of all basic residues induced a drastic reduction of specific [^{3}H]ryanodine binding. In contrast, the Ala-replacement of all acidic residues increased specific [^{3}H]ryanodine binding. In addition, the dose-dependent [^{3}H]ryanodine binding assay of all analogs showed that R24A, R31A and R33A were critical for the activity of $IpTx_a$. These essential residues for the activation of skeletal-type ryanodine receptor are located on one side of the toxin molecule and the opposite surface contains all acidic residues, as investigated by analysis of the solution structure of $IpTx_a$ that was determined using ^{1}H two-dimensional NMR spec- troscopy.