Summary

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Study of skeletal type calcium release channel (RyR1)

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Maurocalcine (MCa), a 33 amino acid peptide toxin obtained from scorpion venom, has been shown to interact with the isolated skeletal-type ryanodine receptor (RyR1) and to strongly modify its gating. In this study, we explored the effects of MCa and its mutants (Lys8Ala, Lys19Ala, Lys20Ala, Lys22Ala, Lys23Ala and Lys24Ala) on single channel currents through RyR1 in artificial lipid bilayer and we studied the effects of toxins on SR Ca^{2+}-pump using coupled enzyme assay technique. The effect of gadolinium ions on purified calcium channel (RyR1) and heavy SR vesicles (HSR) were studied using single channel experiments, $^{45}Ca^{2+}$ flux measurements and [^{3}H] ryanodine binding assay.

Individual LLSSs last even for several seconds and the average length of these events and the frequency of their occurrence are altered in different mutations, according to the spatial distance of the mutated amino acid from the critical residue, ^{24}Arg. If the mutated residue is close to this critical residue, the length and the frequency of these LLSSs lessen resulting in lower LLSS ratio too. The effect is strongly dependent on the direction of the channel current (the polarity). If the direction of the cation current is the same as in case of calcium release, the toxin is about 8-10 fold less effective compared to the opposite current direction. The toxin and its mutants induce similar effect at 50 µM and at 240 nM free calcium concentration. Here we show, that the effect of the toxin is governed by the large charged surface formed by the residues ^{20}Lys, ^{22}Lys, ^{23}Arg, ^{24}Arg, ^{8}Lys as shown by the effect of the 19 mutant, which exhibits almost identical effect with the wild type. Our results suggest that MCa and its mutants do not have any effect on activity of SR Ca^{2+}-pump

In single channel experiments the gadolinium inhibited the activity of calcium channel concentration dependent manner on both (*cis* and *trans*) side. It inhibited the calcium release from SR vesicles and [^{3}H] ryanodine binding. The inhibition of channel did not have any voltage-dependence. Our results suggest that there are sites on *cis* and *trans* side of the RyR1 that are able to bind Gd^{3+} causing inhibition of channel with similar parameters (Hill coefficient, IC_{50}).

Keywords Skeletal muscle · Ryanodine receptor · Maurocalcine · Gadolinium