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Action Mechanism Research of Lanthanons to Slow Vacuolar Ion Channels in Raphanus Satirus L. (Xinlimei) Radish by Patch-Clamp

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Abstract: We used whole-vacuolar patch-clamp recording mode to study the action mechanism of La^{3+} to Slow Vacuolar (SV) channels for the first time. We recorded SV channel currents of Xinlimei (Raphanus satirus L.) vacuolars. The minimum activation potentials of voltage-dependent SV channels lied in 25±5 mV. The increase in cytoplasmic Ca^{2+} led to enhancement of SV-type currents. It was found that the threshold potential of activation shifted towards more depolarized values whenever cytoplasmic Ca^{2+} was increased. When 10^{-10} mol/L free La^{3+} was added to the bath, SV-type current was suppressed by 60~75%. These data showed La^{3+} reduced ion permeabilities of Xinlimei root vacuolar membrane.

Keywords: Whole-vacuolar recording, SV-type current, cytoplasmic Ca^{2+}, La^{3+}, Patch-Clamp.

Rare earth fertilizers were applied widely in China. They were employed by 3.3×10^6 hm^2 every year and the production of crops increased up to 10^9 kg. The economic benefit is huge, but the action mechanism of these fertilizers is not clear yet[1]. The distributions of rare earth elements in plant cell were still disputed. Because lanthanons in organism were in minute quantities (10^{-10}~10^{-8} in mass percentage), it was necessary to choose a sensitive and exact analytical method.

In recent years Patch-Clamp technique was used to study membrane ion channels as an effective method. The investigations with Patch-Clamp techniques have shown that ion channels and pumps as pathways for the movement of ions and metabolites[2]. As we know, SV channels are cation selective channels with poor selectivity among monovalent cations (K^+, Na^+ and Cs^+) and divalent cations (Ca^{2+}, Mg^{2+} and Ba^{2+})[3]. Voltage- and time-dependent SV channels are activated by cytosolic Ca^{2+} [4]. Since RE^{3+} and Ca^{2+} have many similar chemical properties, it is very important to study the biological and physical properties of SV ion channels and action mechanism of RE^{3+} to SV channels.

Results and Discussion

XinLiMei vacuoles were isolated according to the literature 2. We first recorded the SV channel currents of XinLiMei vacuoles (n >40). The minimum activation potentials of voltage-dependent SV channels were 25±5 mV. Secondly we studied calcium-

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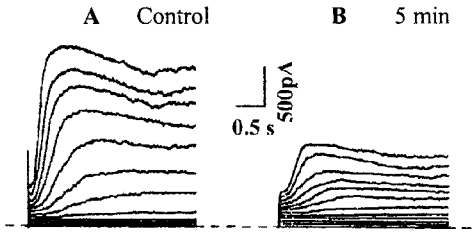
dependent property of SV channels. We found in the absence of cytosolic Mg^{2+}, cytosolic Ca^{2+} at less than 10 μ mol/L did not activate SV channel currents. The increase in free Ca^{2+} from 10^{-5} to 4×10^{-3} mol/L led to the increase of SV-type currents and the decrease of activation potentials. We concluded that a high concentration of cytosolic Ca^{2+} alone can bind to both high-affinity Ca^{2+}binding site and low-affinity binding site on the cytosolic side which can be occupied by either Mg^{2+} or Ca^{2+}.

Figure 1 The inhibitory effect of cytosolic free La^{3+} to SV-type channels



When we added free La^{3+} (10^{-10} mol/L) to the bath, the maximum activation current reduced from 1640 PA in the control to 539 PA after 5 min. The inhibitory effect of cytosolic free La^{3+} to SV-type channels was very obvious and current was suppressed by 67.1% (**Figure 1**). Based on these results, we surmised that free La^{3+} and high-affinity channel proteins on the cytosolic side combined to form a kind of binding-protein which inhibits SV-type channel current. Our findings indicated that $LaCl_3$ decreased ion permeabilities of Xinlimei root vacuolar membrane. Action mechanism researches of other rare earth ions to SV type channels are going on.

Acknowledgments

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