Store independent activation and properties of Orai3/STIM1 mediated current

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Recently discovered members of Orai family of proteins, Orai1, Orai2 and Orai3, form store-operated Ca^{2+} channels when expressed with stromal interaction molecule 1 (STIM1), a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor in the endoplasmic reticulum. The functional properties of Orai1 have been well described using overexpression studies, but less is known about the properties of Orai2 and Orai3. The aim of these experiments was to characterise the functional properties of Orai3/STIM1 mediated current in H4IIE liver cells using whole cell patch clamping.

Overexpression of Orai3 and STIM1 generated a large store-operated Ca^{2+} current (I_{SOC}) in H4IIE cells which was activated by depletion of intracellular Ca^{2+} store by 20μM IP_3 included in the patch pipette. In general, Orai3/STIM1 mediated I_{SOC} had properties similar to that of Orai1/STIM1. Similarly to Orai1/STIM1, Orai3/STIM1 mediated I_{SOC} showed high selectivity for Ca^{2+} over monovalent cations and fast Ca^{2+} dependent inactivation at negative potentials. However, there was a significant difference between Orai1 and Orai3 in regard to the effects of 2-aminoethoxydiphenyl borate (2-APB), a known blocker of store-operated Ca^{2+} entry in a variety of cell types.

External application of 50-100μM 2-APB first briefly potentiates and then completely blocks Orai1/STIM1 mediated inward Ca^{2+} current in H4IIE cells. In contrast, we found that in cells expressing Orai3 and STIM1 50μM 2-APB caused strong rapid potentiation of the inward current, which was closely followed by rapid development of a large outward current. This effect was completely reversed by washing out of 2-APB from the bath. Analysis showed that there was a direct correlation between maximum Orai3 current amplitude and maximum 2-APB-induced inward current, indicating that 2-APB did not directly activate another type of channel. Activation of the outward current by 2-APB suggested a significant change in Orai3 selectivity. This was confirmed by cation substitutions in the external solution.

Following 2-APB application, large Orai3-mediated current existed even after removal of Ca^{2+} from the external solution, indicating that a significant part of the current was carried by Na^+. Orai3 activated by store depletion was impermeable to Cs^+ even in the absence of divalent cations. However, in the presence of 2-APB Orai3 did not discriminate between Na^+ and Cs^+, as replacing Na^+ with Cs^+ in the external solution did not affect the amplitude of the current. Oddly, we found that large cations such as $NMDG^+$ or $Tris^+$, at 10mM concentration completely blocked both inward and outward Orai3-mediated current activated by 2-APB, while they had no effect on Orai3-mediated current activated by store depletion. Voltage independence of this block suggested a more complex mechanism than a simple binding inside the pore. To investigate whether 2-APB effects on Orai3 require presence of STIM1 or depletion of the stores, we applied 2-APB to cells not transfected with STIM1 and/or without IP_3 in the patch pipette. Even without depletion of Ca^{2+} stores, no exogenous STIM1, and no development of I_{SOC}, application of 50μM 2-APB to H4IIE cells expressing Orai3 alone still activated large inward and outward currents with properties identical to those observed in cells overexpressing both Orai3 and STIM1. Therefore it appears that 2-APB activates current through Orai3 independently of Ca^{2+} stores and STIM1.

These data suggest that 2-APB increases the size of the pore formed by Orai3 polypeptides, possibly by affecting their assembly in the channel. The exact mechanism, however, remains to be investigated.