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Effects of DIDS on single Ca^{2+} release channel behavior of skeletal muscle

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Evidence has suggested that an anion channel blocker, 4,4'-diisothiocyanatostilbene-2,2' disulfonic acid (DIDS) could trigger Ca release from skeletal sarcoplasmic reticulum (SR) by binding to a 30 kDa SR protein. Since the high molecular weight Ca^{2+} release channel (CRC)/ryanodine receptor (RyR) is the main SR protein that conducts Ca^{2+} efflux in skeletal muscles, the relationship between CRC and the 30 kDa protein remains to be elucidated. The present study examined the effects of DIDS on CRC in planar lipid bilayer. For the bilayer incorporation, both junctional SR (JSR) vesicles and purified CRC were used to distinguish the roles of the 30 kDa protein in the modulation of CRC activities. When JSR vesicles were used, DIDS increased the mean open probability (Po) without affecting unitary conductance. Analysis of lifetime for single CRC showed that DIDS induced a new open time component (3^{rd}), contributing to long-lived open states. DIDS concentration-dependence on P_o showed a hyperbolic saturation curve with EC_{50} = 10.2 ± 5.5 μM. DIDS shifted both ascending and descending phases of the bell-shaped CRC activation and inactivation curve upward with significantly increased Ca^{2+} affinity at the ascending phase (EC_{50}: 11.23 ± 2.43 μM vs. 4.93 ± 1.65 μM). On the other hand, when purified CRC was used for bilayer incorporation, DIDS became much less potent (at least 200 times). To investigate the possibility that the much lower potency of DIDS on purified CRC was due to partial denaturation of CRC protein, we examined the effects of caffeine on the purified CRC. However, both the purified CRC and native CRC showed a similar response to 3 mM caffeine (P_o: 0.21 – 0.32), 10 μM ryanodine (typical subconductance state) and 5 μM ruthenium red (complete blockage), suggesting that the effects of DIDS on CRC could be mediated by the 30 kDa protein in SR