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Supporting Material

RYANODINE RECEPTOR LUMINAL Ca2+ REGULATION: SWAPPING CALSEQUESTRIN AND CHANNEL ISOFORMS

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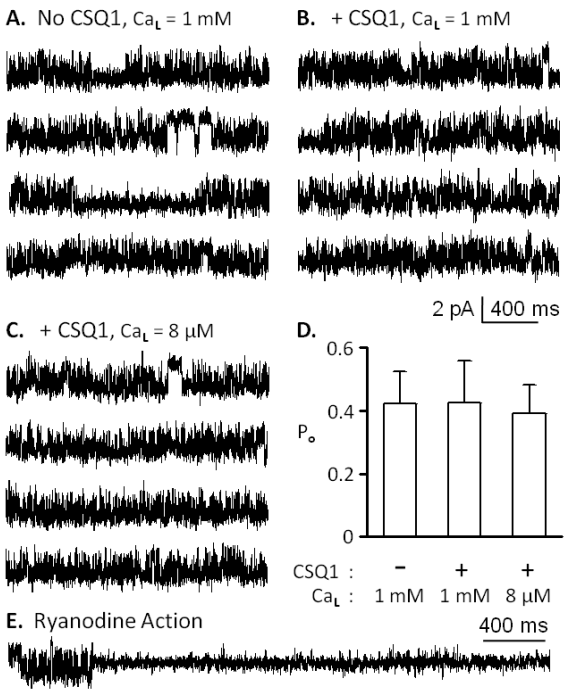
Supplemental Figure:



Absence of a detectable action of CSQ1 on single RyR1 channel Po in qausi-physiological salt solutions. In this experiment, the cytosolic solution contained 5 mM ATP, 1 mM Mg^{2+} (free) and 20 µM Ca^{2+} (free). The luminal solution contained 100 mM Cs-methanesulfonate, 1 mM Mg^{2+} and 1 mM (or 8 µM) Ca^{2+}. Unit current at 0 mV was small and difficult to resolve. Thus, all recordings here were done at a holding potential of 20 mV. Open events are shown as upward deflections. All recordings shown are from the same channel (except that in part E). **A.** Example recordings when no CSQ1 was present and the luminal free Ca^{2+} concentration (Ca_L) was 1 mM. **B.** Example recordings after 5 µg/ml CSQ1 was added to the luminal solution (Ca_L = 1 mM). **C.** Example recordings with 5 µg/ml CSQ1 present but now with a Ca_L of 8 µM. **D.** The summary Po presented in this plot was obtained from 4 minute recordings on 3-4 different RyR1 channels. There was no significant Po difference detected when no CSQ1 was present with 1 mM Ca_L, when CSQ1 was present with 1 mM Ca_L or when CSQ1 was present with 8 µM Ca_L. The average (±SEM) Po's were 0.42±0.10, 0.43±0.13 & 0.39±0.09, respectively. **E.** Example recordings taken after 10 µM ryanodine was added to the cytosolic solution. The luminal solution contained 5 µg/ml CSQ1 and a Ca_L of 8 µM. Ryanodine modified the channel into the characteristic long-lived sub-conductance state confirming the channels examined here were indeed RyR channels.

Supplemental Methods:

Chemicals and Drugs. BAPTA (1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid), 5,5´-Dibromo-BAPTA (1,2-Bis(2-amino-5-bromophenoxy)ethane-N,N,N',N'-tetraacetic acid), $Ca(OH)_2$, CsCl, HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid) were obtained from Fluka (Milwaukee, WI). $CaCl_2$ standard for calibration was from World Precision Instruments Inc (Sarasota, FL). Phospholipids were obtained from Avanti Polar Lipids (Alabaster, Alabama). Decane and all other chemicals were obtained from Aldrich Chemical (Milwaukee,WI).

Statistics. Summary unpaired single channel results are presented as mean ± SEM of *n* determinations. Statistical comparisons between means were performed using a Student's *T* test with significance defined at the $p<0.05$ level. To statistically define bursts, the classic PS method was applied (39). The PS method finds bursts by scanning the recording for closely spaced events. It then adds or subtracts neighboring events if they occur within ≤ half but not ≥ twice the mean inter-event interval. This method essentially measures the "surprise" of an event occurring when it does assuming that gating is random. Generally, PS values >10 are considered to be highly significant.