Allosteric Activation of Na^+-Ca^{2+} Exchange by L-Type Ca^{2+} Current Augments the Trigger Flux for SR Ca^{2+} Release in Ventricular Myocytes

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ABSTRACT The possible contribution of Na^+-Ca^{2+} exchange to the triggering of Ca^{2+} release from the sarcoplasmic reticulum in ventricular cells remains unresolved. To gain insight into this issue, we measured the "trigger flux" of Ca^{2+} crossing the cell membrane in rabbit ventricular myocytes with Ca^{2+} release disabled pharmacologically. Under conditions that promote Ca^{2+} entry via Na^+-Ca^{2+} exchange, internal [Na^+] (10 mM), and positive membrane potential, the Ca^{2+} trigger flux (measured using a fluorescent Ca^{2+} indicator) was much greater than the Ca^{2+} flux through the L-type Ca^{2+} channel, indicating a significant contribution from Na^+-Ca^{2+} exchange to the trigger flux. The difference between total trigger flux and flux through L-type Ca^{2+} channels was assessed by whole-cell patch-clamp recordings of Ca^{2+} current and complementary experiments in which internal [Na^+] was reduced. However, Ca^{2+} entry via Na^+-Ca^{2+} exchange measured in the absence of L-type Ca^{2+} current was considerably smaller than the amount inferred from the trigger flux measurements. From these results, we surmise that openings of L-type Ca^{2+} channels increase [Ca^{2+}] near Na^+-Ca^{2+} exchanger molecules and activate this protein. These results help to resolve seemingly contradictory results obtained previously and have implications for our understanding of the triggering of Ca^{2+} release in heart cells under various conditions.

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Contraction in cardiac muscle cells results from an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) from a diastolic level of roughly 100 nM to a peak of ~1 μM. The source of most of this Ca^{2+} is release from the sarcoplasmic reticulum (SR), which occurs as a collection of microscopic release events known as Ca^{2+} sparks (1). Ca^{2+} sparks have been shown to be triggered primarily by Ca^{2+} ions crossing the cell membrane through L-type Ca^{2+} channels (2).

As the heart cell relaxes, the electrogenic Na^+-Ca^{2+} exchanger (NCX) removes Ca^{2+} from the cell. This protein imports three Na^+ ions for every Ca^{2+} ion that exits (3). Depending on cellular transmembrane potential (V_m) and the relative concentrations of Na^+ and Ca^{2+} inside and outside the cell, NCX can also work in ''reverse mode,'' in which Ca^{2+} is imported and Na^+ is extruded. The issue of whether Ca^{2+} entry via this pathway can contribute to the trigger for Ca^{2+} release has remained controversial because of apparently contradictory data from different groups. On the one hand, blockade Ca^{2+} entry due to other pathways has led to the conclusion that Ca^{2+} entry via NCX is ineffective at triggering release (4–6). On the other hand, some investigators have demonstrated dramatically increased Ca^{2+} release at positive potentials when internal Na^+ is present compared with when it is absent (7–9). Because NCX is presumably working in reverse mode under these conditions, this extra release has been ascribed to the actions of the exchanger.

One possible confounding factor in these experiments is the SR Ca^{2+} load, which strongly influences the quantity of Ca^{2+} released (10). Because changes in intracellular [Na^+] affect the balance of Ca^{2+} across the cell membrane, measurements of SR Ca^{2+} release with and without reverse-mode NCX might be performed at different SR Ca^{2+} loads. To avoid this potential complication, we measured the V_m dependence of Ca^{2+} flux crossing the cell membrane with SR release disabled. Our results, which indicate that reverse mode NCX can augment the transmembrane Ca^{2+} flux substantially, suggest a resolution to the apparently contradictory results obtained in previous studies.

Experiments were performed on isolated rabbit ventricular myocytes incubated with ryanodine and thapsigargin (1 μM each) to prevent SR Ca^{2+} release. Cells were voltage-clamped in whole-cell mode and loaded with fluo-3. The pipette solution contained 120 mM Cs^+ to block most outward K^+ currents and either 10 mM or 0 mM Na^+. Cells were held at −80 mV, a slow ramp (500 ms) to −50 mV was applied to inactivate Na^+ current, and then cells were depolarized to a range of test potentials. Confocal line scan recordings of fluorescence (F) were made concurrently. This protocol therefore allowed simultaneous measurement of L-type Ca^{2+} current (I_{Ca}) and the total transmembrane flux of Ca^{2+}, assessed by increases in fluorescence ($\Delta F/F_0$).

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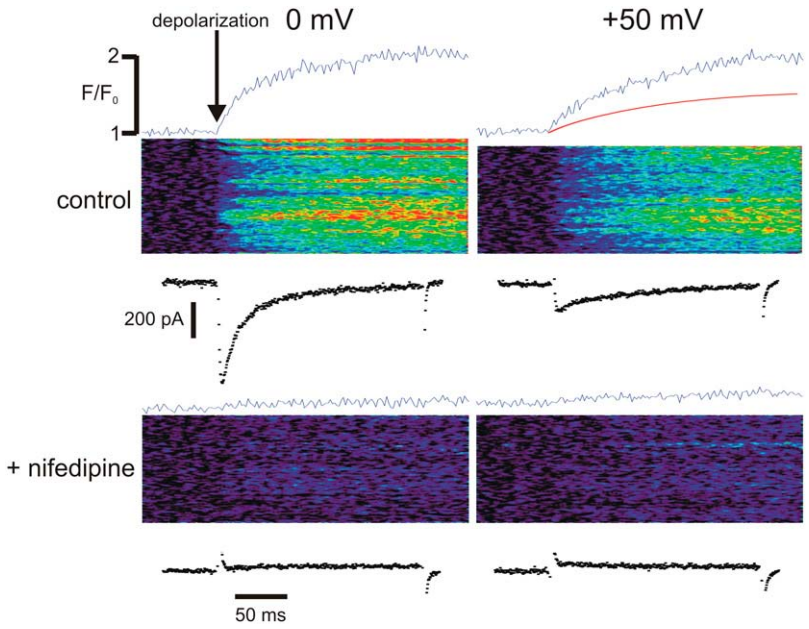

FIGURE 1 Average $\Delta F/F_0$, spatiotemporal changes in F, and I_{Ca} measured at different membrane potentials, before and after adding 40 μM nifedipine. SR Ca^{2+} release was disabled and pipette $[Na^+]$ was 10 mM in all cases.

Examples of patch-clamp and line-scan recordings are shown in Fig. 1. A depolarization to 0 mV (*top left*) induced both a large inward I_{Ca} and a sizable $\Delta F/F_0$. A depolarization to +50 mV (*top right*) caused a much smaller current, but still a substantial $\Delta F/F_0$. This probably occurred because Ca^{2+} was also entering via reverse mode NCX at this potential.

We used two methods to assess the relative contributions of these pathways. First, we added nifedipine (40 μM) to block L-type Ca^{2+} current. $\Delta F/F_0$ was essentially zero at 0 mV (*bottom left*), a potential below the NCX reversal potential under these conditions (+27 mV). At +50 mV, an increase in F was observed (*bottom right*); however, this was much less than that seen with both inward NCX and I_{Ca} present. We also integrated the I_{Ca} recorded at 0 mV and scaled the result to approximate the $\Delta F/F_0$ time course at that V_m. When I_{Ca} at +50 mV was integrated, and the same scaling factor was applied, the estimated $\Delta F/F_0$ due to I_{Ca} was only 51% of the total increase observed (*top right*; *red trace*). Thus, the Ca^{2+} trigger flux at +50 mV under control conditions is much greater than a simple sum of fluxes due to I_{Ca} and reverse-mode NCX measured in the absence of I_{Ca}.

Fig. 2 shows summary data from experiments such as that shown in Fig. 1. Peak I_{Ca} and trigger flux, approximated as the maximal rate of rise of F (dF/dt_{max}; see Supplementary Material, Fig. S1), before and after adding nifedipine, are plotted together on a normalized scale for comparison. Peak I_{Ca} and dF/dt_{max} match one another below +20 mV, but the latter is greater at more positive V_m, consistent with Ca^{2+} entry via reverse mode NCX at these potentials. We considered whether imperfect selectivity of the L-type Ca^{2+} channel, by underestimating Ca^{2+} entry via I_{Ca} at potentials such as +50 mV, could cause the divergence between the two curves. Even after correcting for this, however, the trigger flux at +50 mV assessed by dF/dt_{max} is still ~2.5 times the calculated Ca^{2+} influx through I_{Ca} (see Supplementary Material Fig. S2).

To verify that NCX contributed to the surprisingly large trigger flux observed at positive V_m, we performed additional experiments with 0 mM Na^+ in the patch pipette. Fig. 3 compares dF/dt_{max} measured with 0 mM $[Na^+]$ (*black squares*) and 10 mM $[Na^+]$ (*red circles*). Plots are normalized for comparison. At V_m >+20 mV, dF/dt_{max} is relatively much larger with $[Na^+]$ in the pipette, confirming that reverse-mode

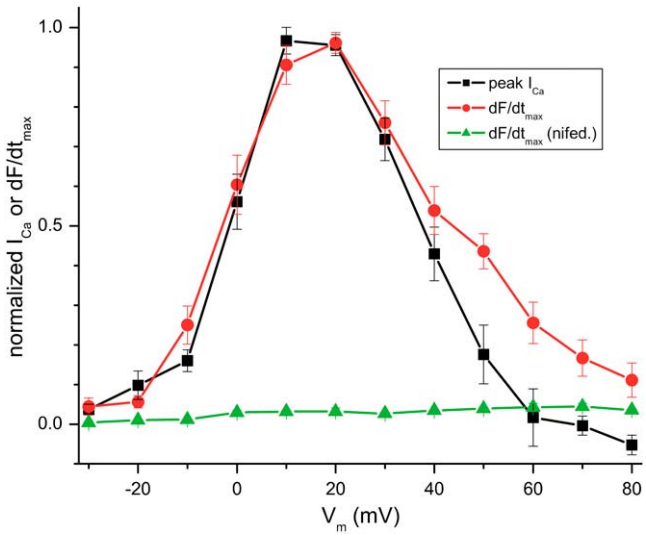

FIGURE 2 Summary data from experiments such as that shown in Fig. 1. Estimated Ca^{2+} trigger flux (dF/dt_{max}; *red circles*) is greater than peak I_{Ca} (*black squares*) only at V_m above +30 mV. dF/dt_{max} measured after blocking I_{Ca} (*green triangles*) is too small to account for this difference.

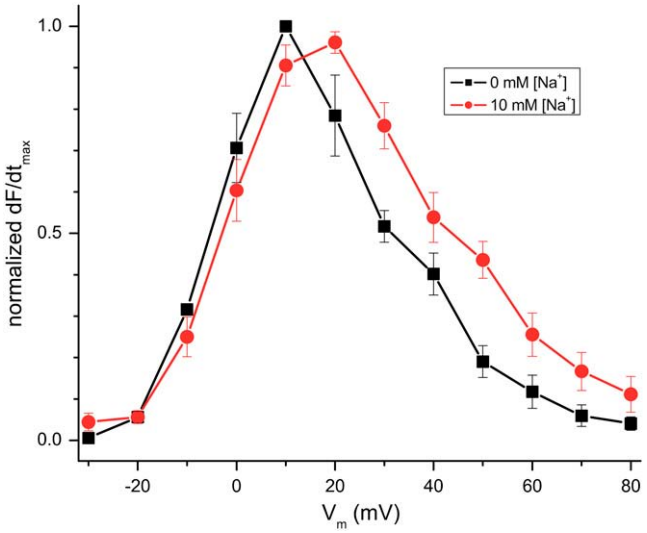

FIGURE 3 ***dF/dt*$_{max}$ as a function of V_m, with (*red circles*; $n = 7$) and without (*black squares*; $n = 3$) 10 mM [Na^+] in the pipette.**

NCX augments the trigger flux at these voltages. It is striking that the flux of Ca^{2+} at +50 mV in the presence of internal [Na^+] is more than twice that measured in its absence.

Overall, these results suggest that the Ca^{2+} ''trigger flux'' at positive V_m is much greater than that estimated from a simple summation of the fluxes through I_{Ca} and reverse mode NCX measured in the absence of I_{Ca}. The most likely explanation for this finding is that Ca^{2+} entering through L-type channels activates the catalytic Ca^{2+}-binding site on NCX (11,12), thereby causing an increase of Ca^{2+} influx on the exchanger. Indeed, early studies on the function of cardiac NCX suggested that this could be the case (13). Reverse-mode NCX after L-type channel block is less than in control conditions because this catalysis does not occur, and, consistent with this idea, large differences in Ca^{2+} fluxes tend to shrink at very positive V_m (e.g., +80 mV), probably because smaller I_{Ca} leads to a reduction in NCX catalysis. This hypothesis therefore provides an explanation for the contradictory results mentioned earlier. More important, the data presented suggest that NCX may contribute substantially to Ca^{2+} entry at V_m corresponding to the early action potential plateau.

These results are similar to observations recently made by Viatchenko-Karpinski et al. in rat myocytes (14). These authors, however, only observed nonlinear summation of I_{Ca} and reverse mode NCX after β-adrenergic stimulation. The fact that we observe such an augmentation of the trigger flux under control conditions is consistent with the greater NCX currents recorded in larger mammals and suggests that NCX may have a larger role in triggering Ca^{2+} release in such species. This species difference also suggests that experiments such as those performed here may provide insight into spatial relationships between L-type Ca^{2+} channels and Na^+-Ca^{2+} exchangers under a range of conditions.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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