Online Supplement for
CaMKIIδ_C slows $[Ca]_i$ decline in Cardiac Myocytes by promoting Ca sparks

by T. Guo, T. Zhang, K.S. Ginsburg, S. Mishra, J.H. Brown & D.M. Bers

Ca sparks during the declining phase of twitch $[Ca]_i$ decline in KO/TG

Data in Figures 7 and 8 of the paper suggest that SR Ca release events (including Ca sparks) occur much more frequently in mice overexpressing CaMKII, and that these may contribute to slowing twitch $[Ca]_i$ decline as observed in these mice (with unaltered SERCA expression and where both are PLB-KO mice). Fig S1A shows a typical line scan image from a KO/TG mouse, where numerous Ca sparks are apparent throughout all phases of the Ca transients and diastole. Individual lines are shown at right. In location 1, three sparks are apparent (asterisk), one early and two late in $[Ca]_i$ decline. Location 2 shows one early and one late Ca spark and location 3 shows no readily identifiable Ca sparks. One can readily see at least ten other release events at other loci during this beat (white marks). Notably, in typical KO/TG myocyte pre-treated with the CaMKII inhibitor peptide AIP (Fig S1B), the image looks very different with a much more spatially uniform $[Ca]_i$ decline and no Ca sparks apparent on the declining phase. The difference is striking. It should also be noted that detectable release events during the earlier phases of $[Ca]_i$ decline would be strongly disfavored because of the lower luminal $[Ca]_{SR}$ (due to both a lower [Ca] driving force and luminal Ca effect on gating), refractoriness of RyR and the higher ambient local $[Ca]_i$ against which it must be detected. However, there can be robust SR Ca leak that is not detectable as Ca sparks[1,2] and conditions (like CaMKII phosphorylation of RyR) that favor Ca sparks would also favor SR Ca leak that is smaller in amplitude than Ca sparks. Thus, even without identifiable Ca sparks at a given locus, SR Ca leak is likely increased. It is then easy to visualize how increasing SR Ca leak (only part of which is visible as Ca sparks)[1,2] may contribute to the different global $[Ca]_i$ decline seen in KO/TG myocyte with and without CaMKII activity (Fig S1C and Figs 1E, 7C and 8).

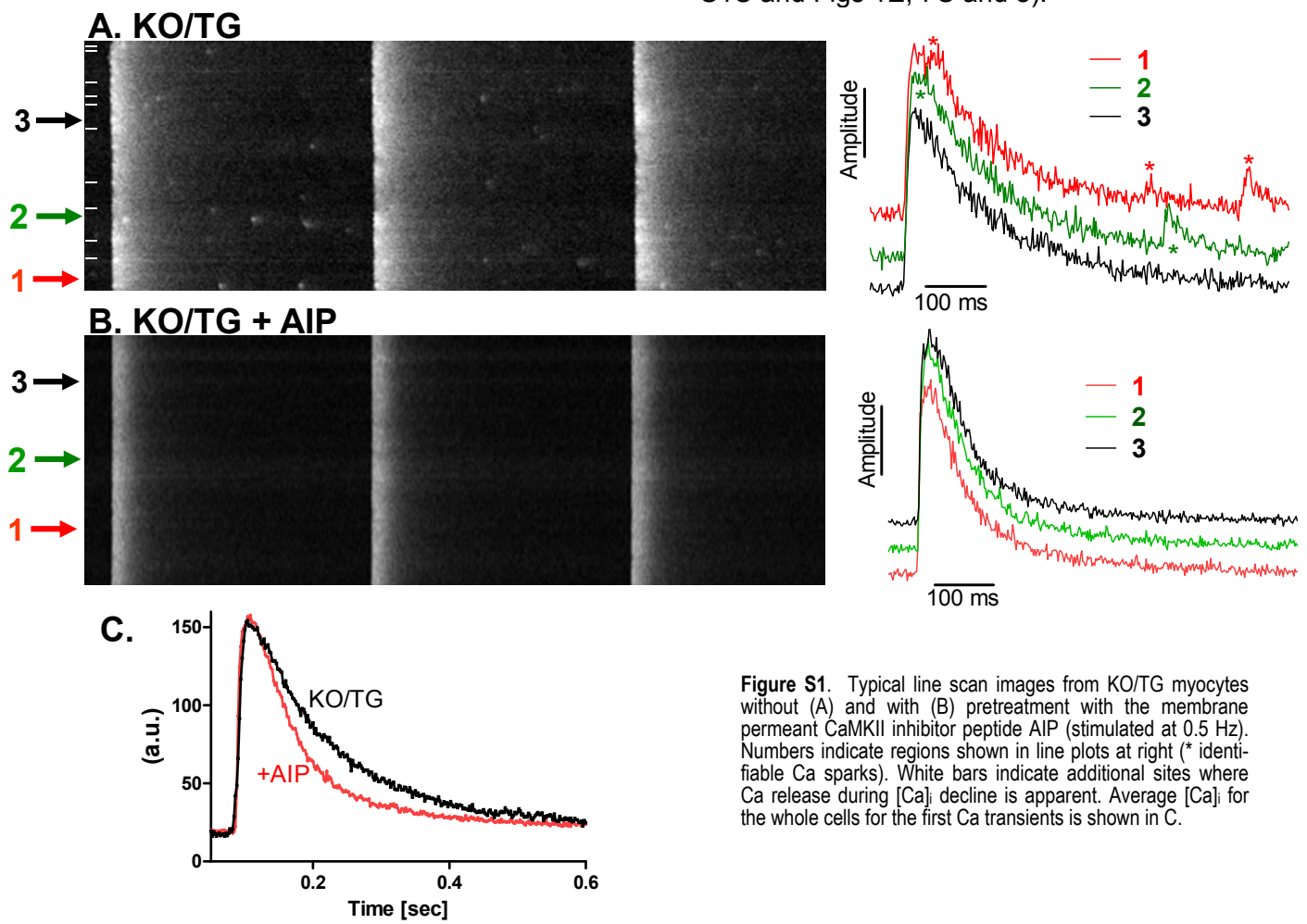

Figure S1. Typical line scan images from KO/TG myocytes without (A) and with (B) pretreatment with the membrane permeant CaMKII inhibitor peptide AIP (stimulated at 0.5 Hz). Numbers indicate regions shown in line plots at right (* identifiable Ca sparks). White bars indicate additional sites where Ca release during $[Ca]_i$ decline is apparent. Average $[Ca]_i$ for the whole cells for the first Ca transients is shown in C.

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Calculation of the impact of SR Ca leak on the rate of twitch $[Ca]_i$ decline

To test the theoretical influence of SR Ca leak on the τ_{twitch} during $[Ca]_i$ decline we developed a simple Ca flux model during $[Ca]_i$ decline, like that developed by Bassani *et al.*[3] and previously used in PLN-KO mice by Li *et al.*[4]. As described in more detail previously[3], we first converted free $[Ca]_i$ to total cytosolic [Ca] ($[Ca]_T$) accounting for Ca buffering ($[Ca]_T = [Ca]_i + 215/(1+673\ nM/[Ca]_i)$ in μmol/l cytosol). Then the rate of $[Ca]_T$ decline ($d[Ca]_T/dt$) was fit by a transport function of the form

$$d[Ca]_T/dt = V_{max}/(1 + \{K_m/[Ca]_i\}^h) + V_{min} \quad (1)$$

where V_{max} is the maximal transport rate, K_m is the $[Ca]_i$ for half maximal activation, h is a Hill coefficient and V_{min} allows net flux to approach zero at reasonable diastolic $[Ca]_i$ in this simplified model. The $[Ca]_i$ decline during twitch and caffeine exposure are analyzed this way to infer the parameters in Eq 1 above (see Fig S2) for both twitch and caffeine-induced Ca transients, where the latter reflects NCX function (plus the Slow systems; plasma membrane Ca-ATPase and mitochondrial Ca uptake). Subtracting the "NCX" flux curve (J_{NCX}) from the twitch curve (J_{twitch}) we obtain an SR Ca-ATPase curve (J_{SR}), which can then be fit to Eq 1 to establish parameters. All three curves are shown in Fig S2 and reflect the functional data in Fig 1 and 2 for PLN-KO mice.

Having established these $d[Ca]_T/dt$ functions for SERCA (J_{SR}) and NCX (plus slow; J_{NCX}) we can simulate $[Ca]_i$ decline during a twitch when all systems are functioning

$$d[Ca]_T/dt = J_{SR} + J_{NCX} - J_{Leak} \quad (2)$$

where we can vary the leak flux (J_{Leak}). Normal SR Ca leak at normal SR Ca load is typically 4-6 μmol/l cytosol/s (refs 1,5), and we varied the value to include up to a 10-fold increase in leak from the normal value (based on the 7-fold increase in Ca spark-mediated leak assessed in this study). Figure S3 shows the calculated $[Ca]_i$ decline for leak values of 0, 6 and 60 μmol/l cytosol/s, and the leak dependence of twitch τ for $[Ca]_i$ decline is shown as Fig 8B in the paper.

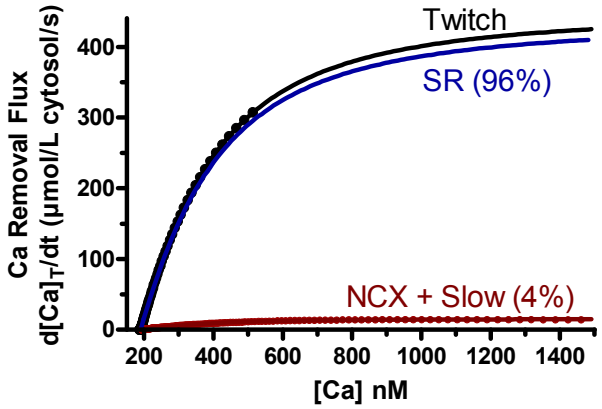

Figure S2. Ca transport rates observed experimentally during twitch and caffeine-induced Ca transients (symbols) and fits using Eq. 1 (curves). The blue curve for SR is the difference of black and red curves fit with Eq 1. The % values refer to fraction of flux by SR and NCX at 560 nM $[Ca]_i$.

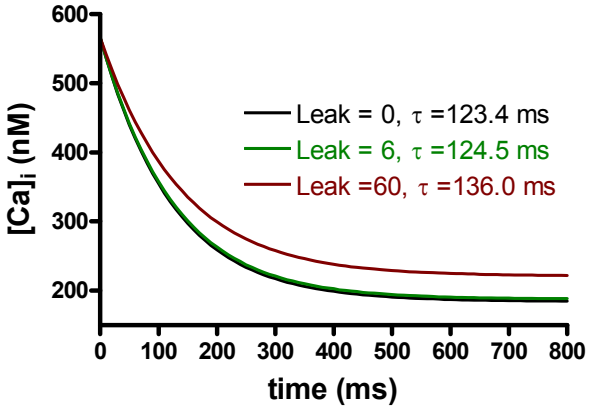

Figure S3. $[Ca]_i$ decline calculated using Eq. 2. Initial $[Ca]_i$ was fixed at 566 nM and fluxes and buffering were as indicated in the text. Leak values are in μmol/l cytosol/s and time constants (τ) were from single exponential fits.

References

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