SUPPLEMENTAL DATA

Control of local calcium release activation by DHPR calcium channel openings

Eva Poláková, Alexandra Zahradníková, jr., Jana Pavelková, Ivan Zahradník, and Alexandra Zahradníková, Institute of Molecular Physiology and Genetics, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Slovakia

Latency distribution of Ca^{2+} spikes

Derivation of the latency distribution of calcium spikes. Distribution of single DHPR channel open times can be considered monoexponential (Rose *et al.*, 1992; Sham *et al.*, 1998) with the open time constant τ_O (but see Josephson *et al.*, 2002). Then, according to Colquhoun & Hawkes (1995), the probability density of DHPR channel open times (pdf_{Open}) can be described as

$$pdf_{Open}(t) = \frac{e^{-\frac{t}{\tau_O}}}{\tau_O}, \qquad \text{(Eq. S1)}$$

where t is time.

The time course of RyR activation by a step increase of Ca^{2+} concentration follows higher order kinetics (Zahradnikova *et al.*, 1999; Zahradnikova *et al.*, 2003; Zahradnikova *et al.*, 2007), therefore the probability of RyR activation during a step Ca^{2+} elevation can be described by the function

$$P_{Act}(t) = \left(1 - e^{-\frac{t}{\tau_{act}}}\right)^n \qquad \text{(Eq. S2)}$$

where τ_{act} is the RyR activation time constant at the given Ca^{2+} concentration, and n is the exponent of RyR activation.

The probability density of RyR activation relative to the onset of a step calcium elevation is the time derivative of Eq. S2:

$$pdf_{Act}(t) = \frac{n e^{-\frac{t}{\tau_{act}}}\left(1 - e^{-\frac{t}{\tau_{act}}}\right)^{n-1}}{\tau_{act}}. \qquad \text{(Eq. S3)}$$

Upon stepping the voltage from a potential near the reversal potential V_r of calcium current, at which DHPRs are activated but no calcium enters the cell, to a negative potential, an inward (tail) calcium current starts to flow instantaneously through DHPRs that were open at the time of the voltage change. If the tail potential V_{tail} is sufficiently negative, the DHPR channels which once closed will not reopen again. In other words, at very negative V_{tail}, each open DHPR channel will contribute to RyR activation by a single opening only, and for the duration of this opening. Therefore the probability density of calcium release activation in response to a DHPR opening starting at $t = 0$, pdf_{Cpl}, will be proportional to both, $pdf_{Act}(t)$ and to the integral of the probability density of open times at the interval from t to infinity:

$$pdf_{Cpl}(t) = pdf_{Act}(t)\int_t^\infty pdf_{Open}(t)dt = \frac{n e^{-\frac{t}{\tau_O}-\frac{t}{\tau_{act}}}\left(1 - e^{-\frac{t}{\tau act}}\right)^{n-1}}{\tau_{act}} \qquad \text{(Eq. S4)}$$

The resulting cumulative distribution function for the probability of calcium release activation is then

$$cdf_{Cpl}(t) = \int_0^t pdf_{Cpl}(t)dt \cdot \qquad \text{(Eq. S5a)}$$

Eq. S5a can be solved for any integer exponent of RyR activation, n. We have solved Eq. S5a for n = 1 – 4, 6 and 8. As shown below, the best approximation of the experimental data was obtained for n = 4, for which the solution of Eq. S5a is

$$cdf_{Cpl}(t) = 4\tau_O\left(\frac{1}{\tau_O + \tau_{act}} - \frac{3}{2\tau_O + \tau_{act}} + \frac{3}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right) - 4\tau_O e^{-\frac{t(4\tau_O + \tau_{act})}{\tau_O \tau_{act}}}\left(\frac{e^{\frac{3t}{\tau_{act}}}}{\tau_O + \tau_{act}} - \frac{3e^{\frac{2t}{\tau_{act}}}}{2\tau_O + \tau_{act}} + \frac{3e^{\frac{t}{\tau_{act}}}}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right) \qquad \text{(Eq. S5b).}$$

The probability that a single calcium channel opening will eventually activate a calcium spike, i.e., the coupling fidelity, is then

$$P_{Cpl} = \left[cdf_{Cpl}(t)\right]_{t\to\infty}, \qquad \text{(Eq. S6a)}$$

which for the exponent of RyR activation n = 4 gives

$$P_{Cpl} = 4\tau_O\left(\frac{1}{\tau_O + \tau_{act}} - \frac{3}{2\tau_O + \tau_{act}} + \frac{3}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right). \qquad \text{(Eq. S6b)}$$

Because individual calcium release sites contain n_{DHPR} DHPRs, calcium spike activation is equivalent to activation of calcium release by at least one calcium channel opening, and in analogy to the formula given by Inoue and Bridge (2005), the cumulative distribution of the probability of calcium spike activation will be

$$cdf_{Spike}(t) = 1 - \left(1 - P_O cdf_{Cpl}(t)\right)^{n_{DHPR}}, \quad \text{(Eq. S7)}$$

where P_O is the open probability of calcium channel at t = 0, and $cdf_{Cpl}(t)$ follows Eq. S5. The probability density of calcium spike activation is then

$$pdf_{Spike}(t) = \frac{d}{dt} cdf_{Spike}(t) = \frac{d}{dt}\left(1 - \left(1 - P_O cdf_{Cpl}(t)\right)^{n_{DHPR}}\right), \quad \text{(Eq. S8a)}$$

which for the exponent of RyR activation n = 4 gives Eq. S8b, shown in Box 1. The probability of spike activation during an interval centred at t with duration Δt is

$$P_{Spike}(t, \Delta t) = \int_{t-\frac{\Delta t}{2}}^{t+\frac{\Delta t}{2}} pdf_{Spike}(t)dt = cdf_{Spike}\left(t + \frac{\Delta t}{2}\right) - cdf_{Spike}\left(t - \frac{\Delta t}{2}\right)$$

(Eq. S9a)

and for the exponent of RyR activation n = 4 we obtain Eq. S9b, shown in Box 1. This equation was used for fitting the experimentally obtained distributions of calcium spike latencies.

Fitting of the latency distribution of calcium spikes. Distribution of calcium spike latencies (Figure 3 of the main text) was fitted with Eq. S9a solved for the exponents of RyR activation fixed to n = 1, 2, 3, 4, 6 and 8. Separate fitting sessions were performed for different fixed values of the open probability P_O from the interval 0.025 – 0.6. Four data sets, i.e., latencies of calcium spikes at the tail potential of -120 mV in response to 1.5-ms and 5-ms prepulses in control as well as in the presence of BayK 8644 were simultaneously approximated by the appropriate model. The optimal values of

- n_{DHPR} (equal for all four data sets),
- τ_{act} (separate values for data in control and in the presence of BayK 8644), and
- τ_O (only for the data in control, since the value of τ_O in the presence of BayK 8644 was fixed to the time constant of tail calcium current at the tail potential of -120 mV)

were determined for each combination P_O and n. It was found that for any exponent n, the quality of fit did not depend on P_O. The use of the exponent n = 1 and 2 provided unacceptable results (not shown). For n = 1 the theoretical latency probability density has a maximum at t = 0, while the experimentally observed maximum was at t > 0. For n = 2 clusters of >1000 DHPRs were required to obtain a satisfactory fit. The results for n = 1 and 2 were therefore not analyzed further.

For $n \geq 3$, the values of τ_O and τ_{act} did not significantly depend on P_O (not shown). The value of τ_O did not depend on the exponent of RyR activation, while nP_O and τ_{act} decreased with n almost in parallel, and increasing the exponent of activation increased the estimated value of coupling fidelity only within a certain range, as illustrated in Figure S1A - C, respectively, for P_O = 0.2. The quality of fit was not significantly different for n = 3 and n = 4, while it was

$$pdf_{Spike}(t) = \frac{d}{dt} cdf_{Spike}(t) = \frac{4 n_{DHPR} P_O \tau_O}{\tau_{act}} e^{-t\left(\frac{1}{\tau_O} + \frac{4}{\tau_{act}}\right)} \left(e^{\frac{t}{\tau_{act}}} - 1\right)^3$$

$$\left(1 - 4P_O\tau_O\left(\frac{6{\tau_O}^3}{(\tau_O + \tau_{act})(2\tau_O + \tau_{act})(3\tau_O + \tau_{act})(4\tau_O + \tau_{act})} - e^{-\frac{t(4\tau_O + \tau_{act})}{\tau_O \tau_{act}}}\left(\frac{e^{\frac{3t}{\tau_{act}}}}{\tau_O + \tau_{act}} - \frac{3e^{\frac{2t}{\tau_{act}}}}{2\tau_O + \tau_{act}} + \frac{3e^{\frac{t}{\tau_{act}}}}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right)\right)\right)^{n_{DHPR}-1}$$

(Eq. S8b)

$$P_{Spike}(t, \Delta t) =$$

$$\left(1 - 4P_O\tau_O\left(\frac{6{\tau_O}^3}{(\tau_O + \tau_{act})(2\tau_O + \tau_{act})(3\tau_O + \tau_{act})(4\tau_O + \tau_{act})} - e^{-\frac{(2t-\Delta t)(4\tau_O + \tau_{act})}{2\tau_O \tau_{act}}}\left(\frac{e^{3\frac{2t-\Delta t}{2\tau_{act}}}}{\tau_O + \tau_{act}} - \frac{3e^{\frac{2t-\Delta t}{\tau_{act}}}}{2\tau_O + \tau_{act}} + \frac{3e^{\frac{2t-\Delta t}{2\tau_{act}}}}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right)\right)\right)^{n_{DHPR}} -$$

$$\left(1 - 4P_O\tau_O\left(\frac{6{\tau_O}^3}{(\tau_O + \tau_{act})(2\tau_O + \tau_{act})(3\tau_O + \tau_{act})(4\tau_O + \tau_{act})} - e^{-\frac{(2t+\Delta t)(4\tau_O + \tau_A)}{2\tau_O \tau_A}}\left(\frac{e^{3\frac{2t+\Delta t}{2\tau_{act}}}}{\tau_O + \tau_{act}} - \frac{3e^{\frac{2t+\Delta t}{\tau_{act}}}}{2\tau_O + \tau_{act}} + \frac{3e^{\frac{2t+\Delta t}{2\tau_{act}}}}{3\tau_O + \tau_{act}} - \frac{1}{4\tau_O + \tau_{act}}\right)\right)\right)^{n_{DHPR}}$$

(Eq. S9b)

Box 1. Probability distributions of calcium spike latencies for DHPR activation exponent n = 4.

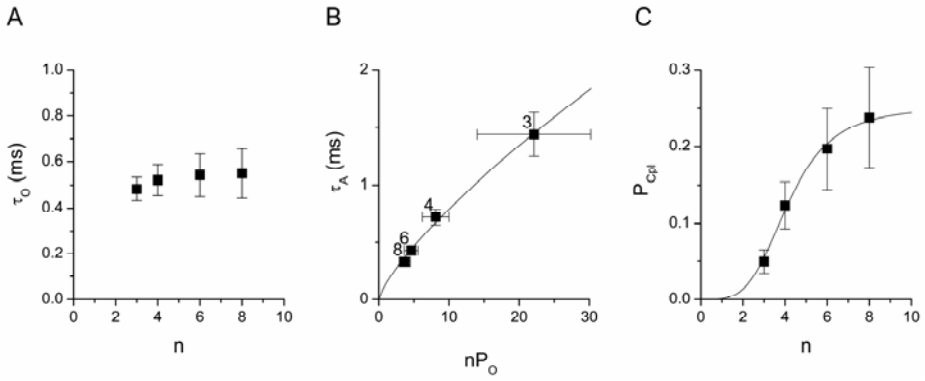

Figure S1. The dependence of kinetic parameters related to calcium release activation on the exponent of RyR activation. **A** – Open time of DHPR channels in the absence of Bay K 8644. **B** – The relationship between the RyR activation time constant (τ_{act}) and nP_O of the DHPR calcium channel cluster. Labels denote the exponent of activation n. The line shows the allometric relationship $\tau_{act} = (0.14 \pm 0.02)\ nP_O^{0.76 \pm 0.04}$. **C** – Coupling fidelity in the absence of BayK 8644. The line denotes the relationship $P_{Cpl} = P_{max}\frac{K^{n_H}}{K^{n_H} + n^{n_H}}$ with $K = 4.15$, $n_H = 4$, $P_{max} = 0.25$.

significantly worse for $n > 4$ ($p < 0.05$) and unacceptable for n = 1 and 2 ($p < 0.001$). The exponent $n = 3$ provided large errors of estimates with a large fitted value of nP_O (Figure S1 B). This value of nP_O was considered unacceptable, because it would require over 100 DHPRs per release site. Therefore only the model with $n = 4$ was considered further. It should be mentioned that the expression for cdf_{Spike} in Eq. S9a provides analytical functions suitable for fitting only for integer values of n.

The estimated values of the parameters τ_O and τ_{act} did not depend on the value of DHPR open probability P_O. As shown in Figure S2 A, B, there was no significant difference between the values of any of these parameters in the P_O range of 0.025 – 0.6. The estimated value of n_{DHPR} was inversely proportional to P_O (Figure S2 C), so that the estimated value of nP_O (Figure S2 D) was independent of P_O. As a result, the values of P_{Cpl} and P_{Spike} (Eq. S6B and Eq. 4 of the main text) were independent of P_O as well (Figure S2 E, F).

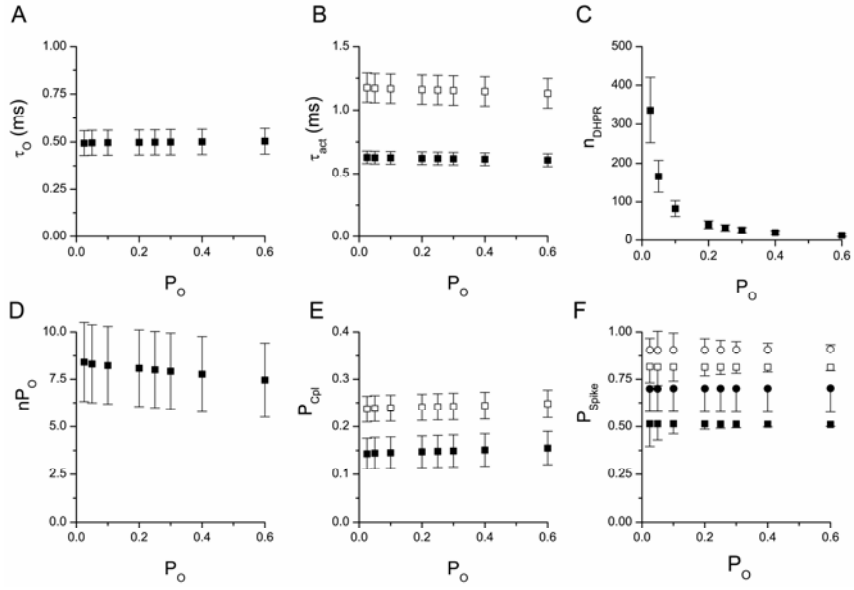

Figure S2. Fitting results at different preset values of DHPR open probability. **A** – DHPR open time in the absence of BayK 8644. **B** – RyR activation time constant under different experimental conditions. **C** - The dependence of n_{DHPR} on the value of preset DHPR open probability. **D** - nP_O of fully activated DHPRs in the absence of BayK 8644. **E** – Coupling fidelity at -120 mV tail potential under different experimental conditions. **F** – Spike probability at -120 mV tail potential under different experimental conditions. In panels B, E and F, filled and open symbols stand for the absence and presence of BayK 8644; in panel F, squares and circles denote 1.5- and 5-ms prepulses, respectively.

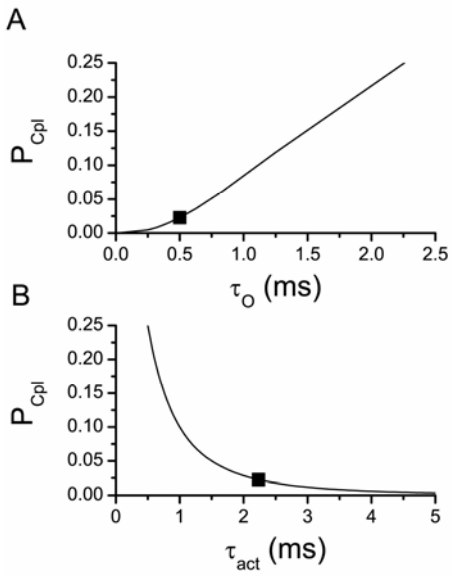

Figure S3. Coupling fidelity at +10 mV as a function of τ_O **(A)** and τ_{act} **(B)**. Solid symbols stay for the respective values derived for control conditions from Table 1 and Eq. 6 of the main text.

Sensitivity of coupling fidelity to changes in coupling parameters

Sensitivity of coupling fidelity to coupling parameters was assessed using Eq. S6B that was solved for various τ_O and τ_{act}. The value of coupling fidelity at a membrane potential of +10 mV, at which the whole-cell calcium current is maximal and which is close to the action potential plateau, was used as the reference. The value of τ_{act} at +10 mV was calculated using Eq. 6 from the value of τ_{act} at -120 mV (Table 1 of the main text), assuming V_r = +60 mV, and is plotted as full symbol in Figure S3B. Then coupling fidelity was calculated with τ_{act} kept constant and τ_O varied (Figure S3A), or with τ_O kept constant and τ_{act} varied (Figure S3B). It can be seen that coupling fidelity is approximately proportional to τ_O, while it is inversely proportional to τ_{act}. Thus, coupling fidelity is sensitive to the expected physiological and pathophysiological variations of DHPR open time and RyR activation time constant.

Simulations of calcium release gain

The experiments of Altamirano and Bers (2007) were simulated by calculating the spike probability using Eq. 4 of the main text, assuming that at V_m = 0 mV, each DHPR channel displays a constant number of openings (n_O) before it inactivates. The value of P_{Cpl} was calculated from Eq. S6b. We chose n_O = 20, so that for P_O = 0.2 and τ_O = 0.5 ms, the duration of calcium currents was ~50 ms. (Qualitatively similar results were obtained for n_O = 10). For standard conditions (holding potential V_h = -60 mV, test potential V_p = 0 mV, $[Ca]_o$ = 1 mM) we set τ_O = 0.5 ms, τ_{act} = 1 ms, P_O = 0.2, n_{DHPR} = 40. The extent of steady-state inactivation at different holding potentials was determined from the Boltzmann equation with midpoint of -40 mV and slope parameter of 5 mV and used to calculate the average number of active DHPR channels per release site. The dependence of i_{Ca} on the external calcium concentration was calculated from the hyperbolic binding curve, assuming EC_{50} = 0.5 mM. The dependence of i_{Ca} on membrane potential was assumed to be exponential ($A\ e^{-kV}$) with k = 0.04 mV^{-1}, as was assumed by Altamirano & Bers (2007). The value of τ_O was kept constant throughout the simulation. The relation between τ_{act} and i_{Ca} was defined by Eq. 6 from the main text.

Normalized I_{Ca} was calculated from the formula

$$I_{Ca}\left(V_p,V_h,[Ca]_O\right)=\frac{i_{Ca}\left(V_p,[Ca]_O\right)n_{DHPR}\left(V_h\right)}{i_{Ca}\left(0,1\right)n_{DHPR}\left(-60\right)}, \text{ (Eq. S10)}$$

derived using the relationship $I_{Ca} = n_{DHPR}\ P_O\ i_{Ca,}$ since P_O of active channels was independent of the conditions of the simulation. Normalized gain was calculated as

$$\left(\frac{P_{spike}}{I_{Ca}}\right)_{rel}\left(V_p,V_h,[Ca]_O\right)=\frac{P_{spike}\left(V_p,V_h,[Ca]_O\right)}{I_{Ca}\left(V_p,V_h,[Ca]_O\right)}\frac{I_{Ca}\left(0,-60,1\right)}{P_{spike}\left(0,-60,1\right)}$$

(Eq. S11).

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