Investigating the effect of doxorubicin and tetracaine in sarcoplasmic reticulum calcium leak

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Summary

The sarcoplasmic reticulum in heart cells acts as a calcium store. Leak from this store during diastole (when the heart is meant to be relaxing) is increased in doxorubicin-induced cardiotoxicity. The leak occurs primarily via RYR2 channels. MG23 is believed to also play a part as a calcium leak channel. When tetracaine was used to inhibit the RYR2 channels, a small leak remained. There is convincing evidence to suggest that MG23 is responsible for the RYR2-independent leak.

Background

Intracellular Calcium Dynamics

- In cardiac muscle, highly controlled release of calcium from sarcoplasmic reticulum (SR) intracellular stores is vital for excitation-contraction coupling, and the contraction of the heart.
- The major intracellular calcium release channel in the SR is the Ryanodine type 2 receptor (RYR2).
- Mitstugumin-23 (MG23) is a voltage-gated, cation-conducting channel, permeable to both K^+ and Ca^{2+}, and expressed in abundance on the SR/ER[1].
- Disturbances to calcium homeostasis, such as leaks from the SR, have been implicated in the pathophysiology of the failing heart[2].

Intracellular Calcium Dyshomeostasis in Heart Failure

- Doxorubicin (DOX) has been shown to upregulate diastolic calcium leak[3].
- The RYR2's are the greatest contributor to the diastolic calcium leak in heart failure; however, it has been proposed that the leak from MG23 also becomes significant in disease states, resulting in depleted SR Ca^{2+} stores, arrythmias, and weaker contractions[4].

Hypothesis and Aims

Hypothesis

The hypothesis of this research project is that MG23 is a target of the anti-cancer drug, doxorubicin, and is responsible for the diastolic RYR2-independent leak associated with cardiotoxicity.

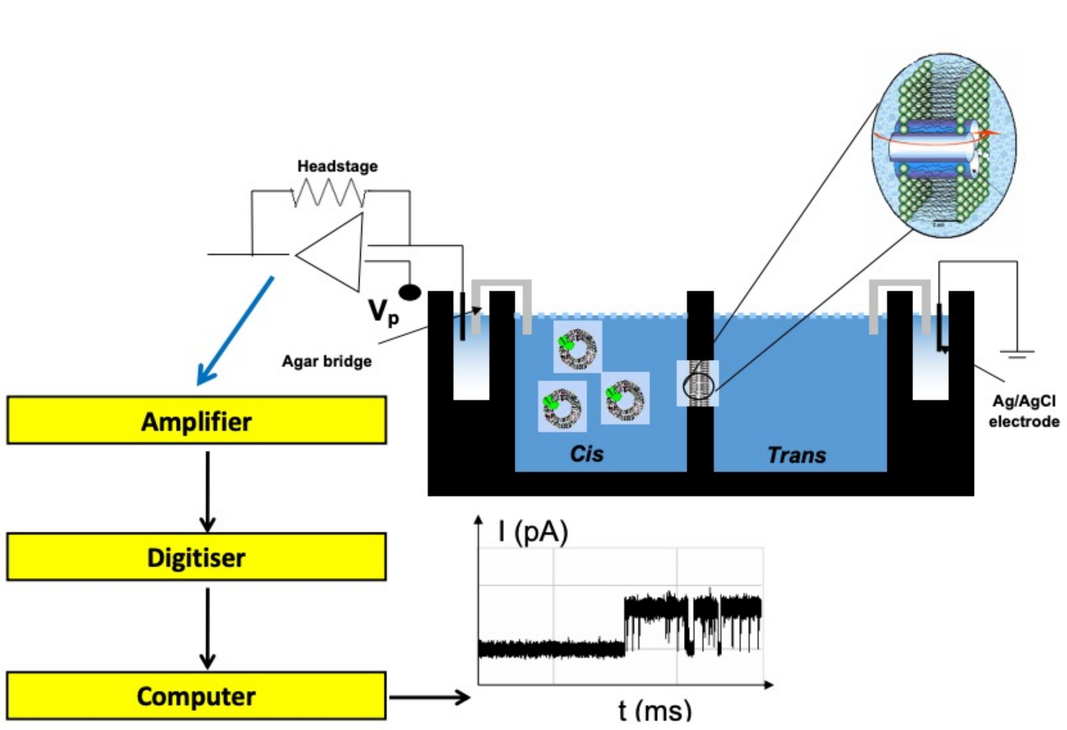
Aims

- Use electrophysiology techniques to investigate the effects of doxorubicin and tetracaine on MG23 isolated from mouse heart

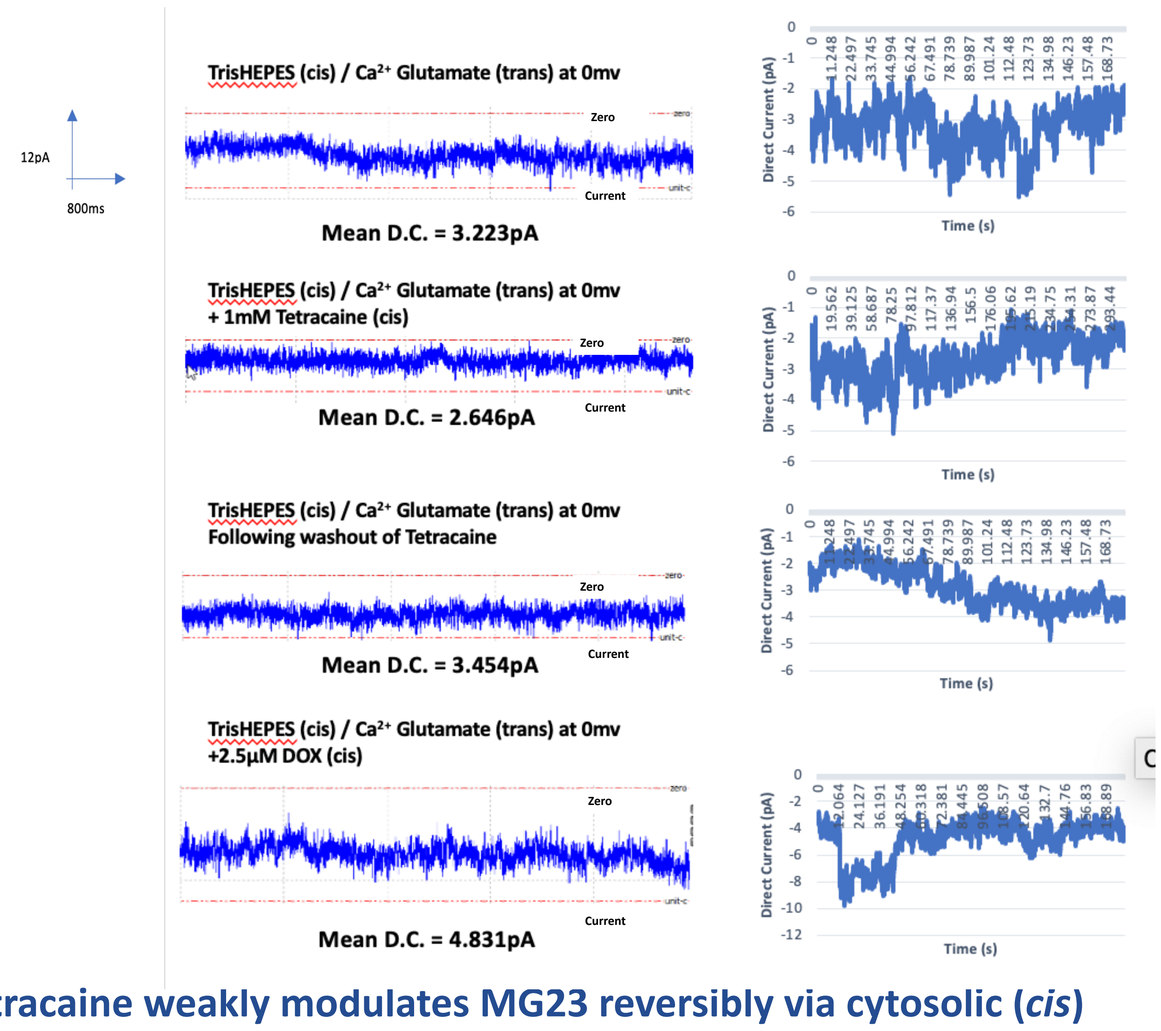
Methods

Planar Phospholipid Bilayer

Native RYR2 and MG23 channels were isolated from mice hearts and incorporated into an artificial bilayer under voltage-clamped conditions following well established protocol[1,5]. Below is a schematic of the apparatus used[6].



Results



Tetracaine weakly modulates MG23 reversibly via cytosolic (*cis*) addition at mM concentrations

Noise analysis using WinEDR 4.0.0 software was done to analyse mean direct current of MG23 channels throughout the recordings. As illustrated above, tetracaine reduced the mean direct current (D.C.) slightly. By washout, this effect was reversed, and mean D.C. returned to control levels.

A clinical dose of doxorubicin activates MG23 via cytosolic (*cis*) addition

As shown in the figure, DOX had an almost immediate effect in increasing current through MG23. This demonstrates how DOX could increase the MG23 responsible leak in disease states.

Conclusion

Our data indicates that tetracaine is a partial antagonist of the calcium leak channel. In the presence of high concentrations of tetracaine (1mM) added to the cytosolic face of the channel, current fluctuations were still observed suggesting that tetracaine could not fully close MG23. Our data also reveals that the anti-cancer drug, doxorubicin, increases MG23 channel activity even after addition of tetracaine. In a whole cell, the concentration of Tetracaine at the single protein channel level is likely to at lower µM concentrations. Going forward, a dose-response curve investigating the effect of Tetracaine on MG23 at µM concentrations would be needed to better understand the pharmacology of this interaction in whole cells. This would provide opportunity to better quantify the 'invisible' calcium leak in whole cells by potentially selectively inhibiting RYR2 but not MG23.

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6 -Schematic of planar lipid bilayer apparatus; unpublished data Pitt lab