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Role of Mitofusin-2 and Mitochondria-Associated Membrane in Arrhythmogenesis

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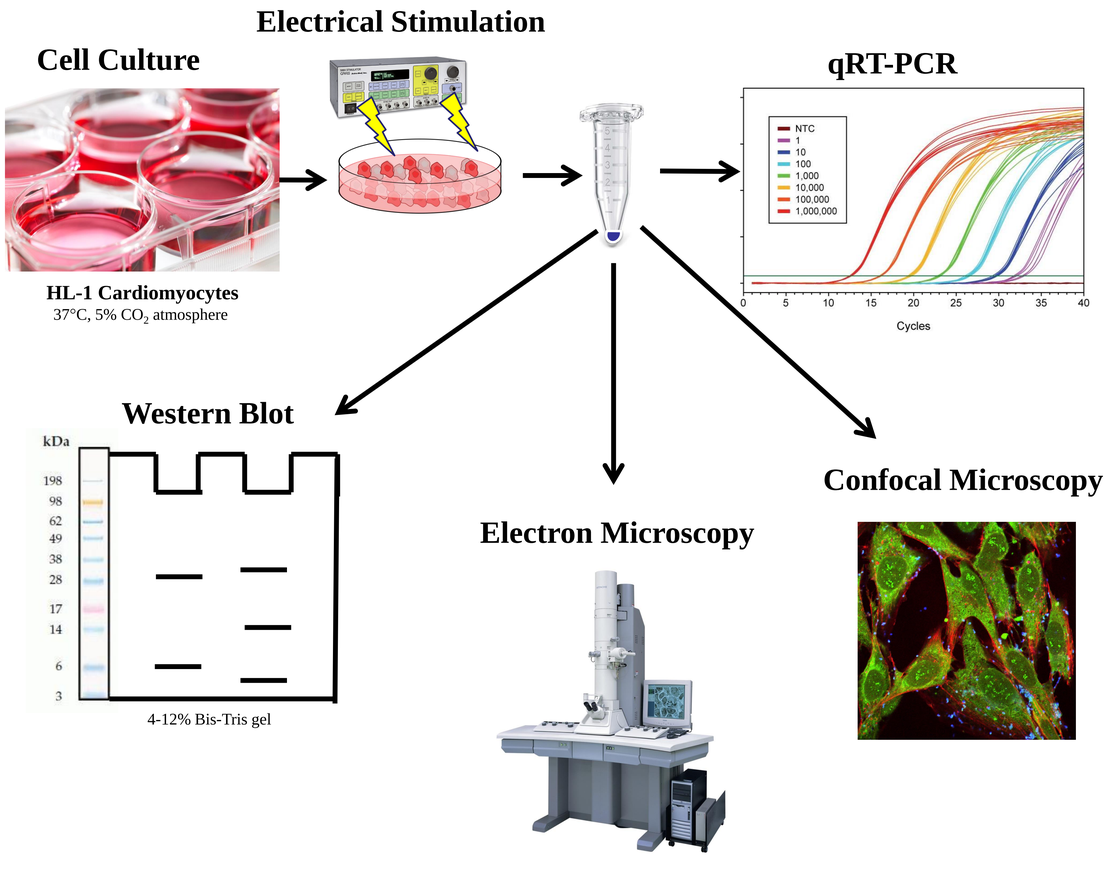
BACKGROUND

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia[1]. The high frequency electrical activity in the fibrillating human atrium *in vivo* is associated *in vitro* with electrophysiological remodeling, calcium overload, and apoptotic cell death[2,3]. Despite intensive investigation of these maladaptive processes, the mechanistic links between arrhythmogenesis and progression to permanent AF remain largely unexplained. The mitochondria-associated membrane (**MAM**) has been recently identified as the physical association of juxtaposed sarcoplasmic reticulum (SR) and mitochondrial membranes facilitating inter-organelle calcium signaling in cardiac myocytes[4]. We have recently established that sustained "fibrillatory-stress" induces mitochondrial hyperfusion, increases MAM formation, and synchronizes the mitochondrial potential with calcium release events[5]. Since the tethering of mitochondria to SR is orchestrated by the mitochondrial fusion protein mitofusin-2 (Mfn-2)[6,7], we hypothesize that sustained high frequency activation, such as occurs during AF, results in Mfn-2 mediated MAM formation, thereby altering calcium handling and promoting mitochondrial hyperfusion and arrhythmogenesis.

METHODS



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RESULTS

Fibrillatory Stress results in mitochondrial hyperfusion and MAM formation

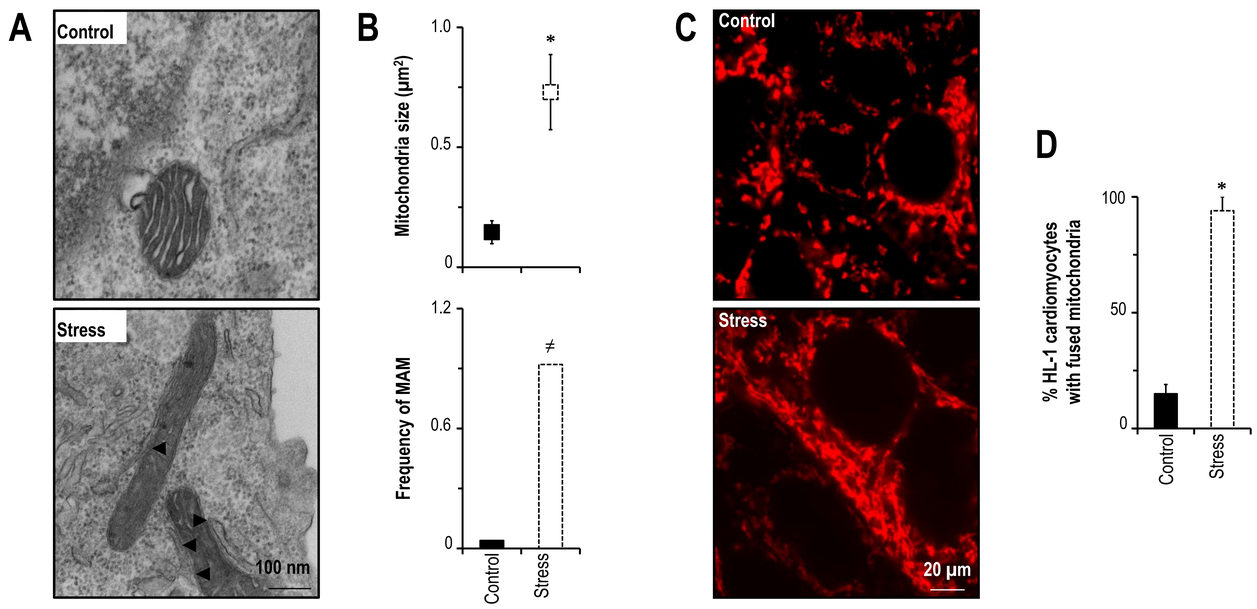

Figure 1. HL-1 cardiomyocytes were preconditioned at 1Hz (control) *cf.* 5 Hz (fibrillatory stress) for 24h in a humid atmosphere of 5% CO_2 – 95% air at 37°C.
Panel A: Electron micrographs of HL-1 cardiomyocytes preconditioned under control or stress conditions. Filled arrowheads indicate MAM. Cells were fixed and thin sections were cut and counterstained for electron microscopy. Digital images were captured using JEOL 1230 TEM and AMT software.
Panel B: Objective mean quantitative data for mitochondrial 2D area and for the occurrence of visible mitochondria: SR contacts (MAM) on 2D electron microscopy. * denotes $p<0.001$, Student's t-test, n= 50; ≠ denotes $p<0.001$, Fisher's exact test and one-way ANOVA, n= 50.
Panel C: Confocal micrographs of HL-1 cardiomyocytes preconditioned under control or stress conditions, and then loaded with TMRE to assess mitochondrial morphology.
Panel D: Quantitative analysis of mitochondria shape is illustrated. 50 images were acquired per condition. Data are mean ± SE.* denotes $p < 0.001$, ANOVA, n= 50.

Fibrillatory Stress results in Mfn-2 recruitment to Mitochondria: SR (MAM)

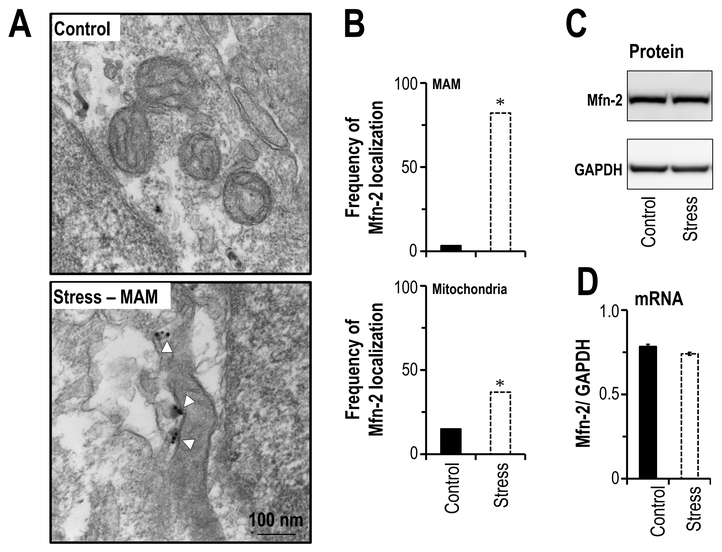

Figure 2. HL-1 cardiomyocytes were preconditioned at 1Hz (control) *cf.* 5 Hz (fibrillatory stress) for 24h in a humid atmosphere of 5% CO_2 – 95% air at 37°C.
Panel A: Immunogold **e**lectron micrographs of HL-1 cardiomyocytes depicting Mfn-2 localization (open arrowheads) at the MAM under stress conditions, compared to control conditions.
Panel B: Objective mean data for the frequency of Mfn-2 localization at the MAM and mitochondria fusion sites, determined by immunogold electron microscopy. * denotes $p< 0.001$, Fisher's exact test and one-way ANOVA, n=50.
Panel C: Immunoblots revealing no noticeable changes in Mfn-2 protein expression in HL-1 cardiomyocytes following 24h of fibrillatory stress, compared to control.
Panel E: Objective mean data obtained using RT-qPCR to determine relative expression levels of Mfn-2 following 24h of fibrillatory stress, compared to control. Kruskal- Wallis test p= 0.39.

CONCLUSIONS

Mfn-2 plays a role in stress-induced mitochondrial remodeling, although the exact mechanism that regulates its activity remains unknown. It is likely that through interactions with other proteins and possibly through post-translational modifications, Mfn-2 is concentrated in the mitochondria and mediates mitochondrial remodeling.

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