Cyclophilin D is a Mitochondrial Sensor of Nano-Pulse Stimulation

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Abstract

Nano-Pulse Stimulation (NPS), a pulsed power-derived technology, stimulates structural and functional changes in plasma membranes and cellular organelles. NPS induces a Ca^{2+} influx and opening of the mitochondrial permeability transition pore (mPTP) that dissipates the mitochondrial membrane potential ($\Delta\Psi_m$) and, when sustained, induces regulated cell death. Here we show that in rat cardiomyoblasts (H9C2) cyclophilin D (CypD) is a mitochondrial sensor for NPS as defined by observations that loss of $\Delta\Psi_m$ is Ca^{2+} and mitochondrial reactive oxygen species (mROS) dependent and cyclosporin A (CsA)-sensitive, which are diagnostic qualities for effects on CypD and the mPTP. Mechanistically, NPS stimulates increases in intracellular Ca^{2+} which enhances mROS in a dose dependent manner. The regulatory role of CypD on mPTP activation, is effectively inhibited at low Ca^{2+} concentrations and/or by CsA. Although NPS-induced dissipation of $\Delta\Psi_m$ is largely Ca^{2+}-dependent, the degree of Ca^{2+} sensitivities vary among cell types. Nevertheless, knockdown of the proapoptotic protein, APAF-1, and overexpression of the antiapoptotic protein, Bcl-xl, in human Jurkat T lymphocytes (E6.1) did not affect NPS-induced dissipation of $\Delta\Psi_m$ or cell death. Taken together, these results indicate NPS induces activation of the mPTP through Ca^{2+}-dependent, mROS-dependent, CsA-sensitive dissipation of the $\Delta\Psi_m$ that is independent of caspase activation and insensitive to protection by Bcl-xl.

Objectives

Determine the effects of the following on the NPS-induced loss of viability and/or dissipation of $\Delta\Psi_m$:

- **dysregulated apoptotic proteins**
- **Ca^{2+}-dependence**
- **mROS-dependence**
- **CsA inhibition**

Conclusions

- **NPS-induced cell death is independent of Bcl-xl protection.**
- **Knockdown of APAF-1 and overexpression of Bcl-xl did not prevent dissipation of ΔΨm.**
- **NPS-induced dissipation of $\Delta\Psi_m$ was dependent on Ca^{2+} and mROS and was sensitive to CsA inhibition.**
- **NPS effects CypD and the mPTP.**
- **CypD is a sensor for NPS.**

Results

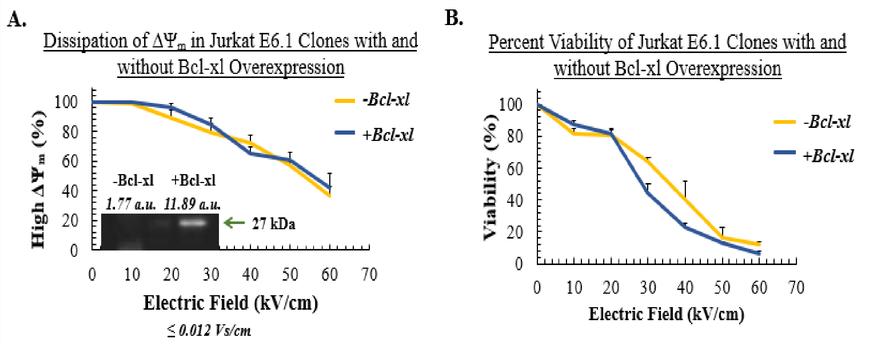

Fig. 1. NPS induced dissipation of $\Delta\Psi_m$ (*A*) and cell death (*B*) in Jurkat E6.1 clones with (*blue*) and without (*gold*) Bcl-xl overexpression in an electric field dependent manner. The cells were treated with 10, 60ns pulses at electric field strengths between 0 and 60 kV/cm. TMRE was used to determine the percentage of cells maintaining high $\Delta\Psi_m$ (*A*) shortly after treatment, and CellTiter-Glo luminescent cell viability assay (*B*) was used to determine the percentage of cell viability 24-hr post-treatment. Data values were normalized to the controls and represent the mean ± SE (n = 3).

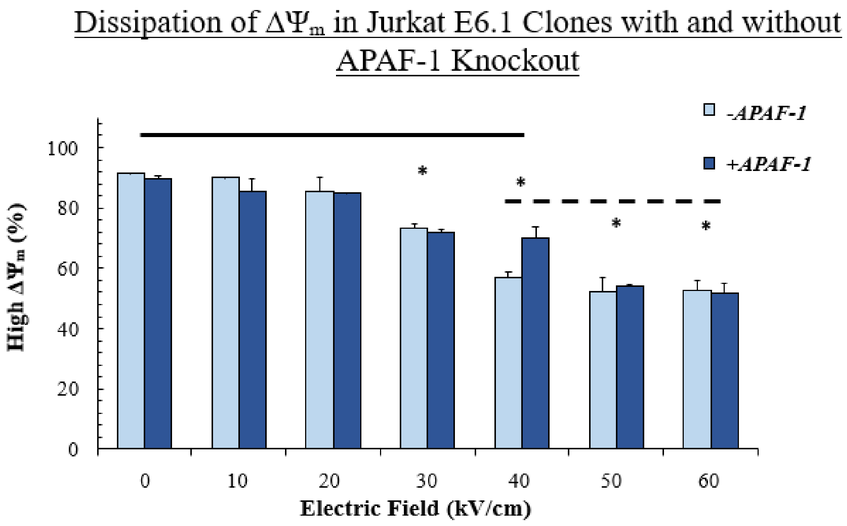

Fig. 2. NPS induced dissipation of the $\Delta\Psi_m$ in Jurkat E6.1 clones without (*light blue*, APAF-1 KO) and with (*dark blue*, normal APAF-1) APAF-1 expression. Cells were treated with 10, 60ns pulses at electric field strengths between 0 and 60 kV/cm. TMRE was used as described to determine the percentage of the cells that maintained high $\Delta\Psi_m$ after treatment with NPS. Data values represent the mean ± SE (n = 3); *p-values < 0.05.

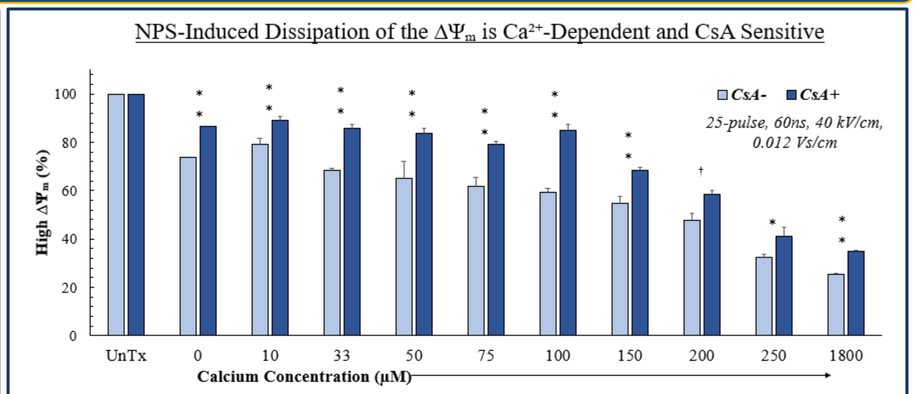

Fig. 3. NPS-induced dissipation of $\Delta\Psi_m$ is calcium dependent and CsA sensitive in H9C2 cells. Cells were treated with 25, 60 ns pulses at 40 kV/cm in increasing concentrations of calcium (0-1800μM Ca^{2+}) with and without CypD inhibition with 5 μM CsA. Data values were normalized to untreated controls and represent the mean ± SE (n=9); **p < 0.0001, *p < 0.05, † p < 0.1.

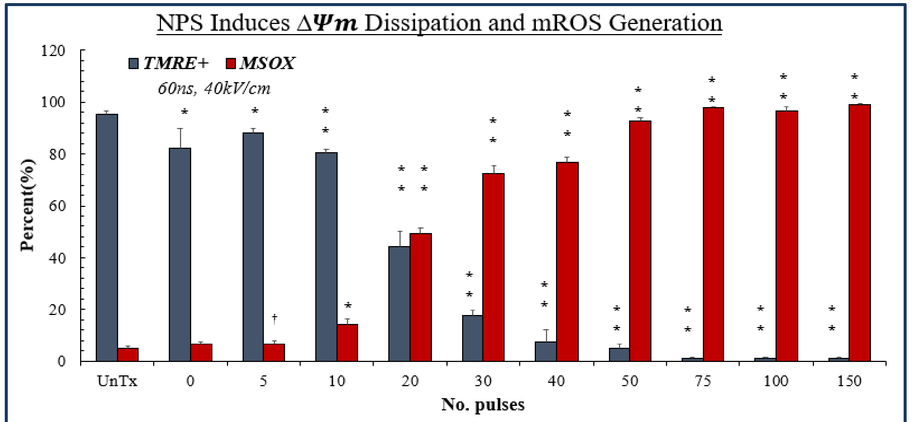

Fig. 4. NPS induced the dissipation of $\Delta\Psi_m$ and mROS generation in H9C2 cells in a dose dependent manner. The $\Delta\Psi_m$ and mROS generation were probed with TMRE and MitoSOX-Red, respectively. Cells were treated with 60 ns pulses at 60 kV/cm by increasing the number of pulses. Since $\Delta\Psi_m$ dissipation and mROS generation are Ca^{2+}-dependent, cells were treated in the presence of calcium. The values from each point represent the mean ± SE (n=3); **p < 0.0001, *p < 0.05, † p < 0.1.

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