Redox Modifications of Proteins Involved in Cardiac Arrhythmogenesis During Reperfusion

Cecilia Mundiña-Weilenmann

FINANCIAL DISCLOSURE:
No relevant financial relationship exists

Centro de Investigaciones Cardiovasculares,
Facultad de Ciencias Médicas, Universidad Nacional de La Plata
CCT-La Plata CONICET
Argentina
BACKGROUND

Myocardial injury

Ca^{2+}-overload \leftrightarrow \text{Oxidative Stress}

\rightarrow \text{Contractile dysfunction} \quad \rightarrow \text{Apoptosis} \quad \rightarrow \text{Necrosis} \quad \rightarrow \text{Arrhythmias}
Mechanical recovery and arrhythmias during reperfusion

Preischemia

Ischemia

Reperfusion

LVDP

MAP

0.2 sec

DAD

EAD

0.5 sec

0.5 min

1 min

20 min
Triggered arrhythmias: early and late postdepolarizations

BACKGROUND

Triggered arrhythmias: early and late postdepolarizations

- Early Afterdepolarizations (EAD)
- Late Afterdepolarizations (LAD)

Triggered arrhythmias are caused by abnormalities in the cardiac electrical and mechanical properties. These abnormalities can lead to arrhythmias, which are abnormal heart rhythms that can be dangerous and cause the heart to beat too fast, too slow, or irregularly. Arrhythmias can lead to symptoms such as palpitations, shortness of breath, chest pain, or syncope (fainting). Arrhythmias can be caused by a variety of factors, including heart disease, medication side effects, and electrolyte imbalances.
CaMKII-inhibition decreases mechanical recovery and arrhythmias in reversible I/R


BACKGROUND

Myocardial injury

CaMKII

Ca\(^{2+}\)-overload

Oxidative Stress

Contractile dysfunction

Arrhythmias

ISCHEMIA - REPERFUSION
CaMKII substrates
CaMKII and its downstream targets during reperfusion

**BACKGROUND**

- **pCaMKII**
  - Pre-Ish: ND
  - Reperfusion: ND

- **pThr17-PLN**
  - Pre-Ish: ND
  - Reperfusion: ND

- **pSer2814-RyR2**
  - Pre-Ish: ND
  - Reperfusion: ND
CaMKII and its downstream targets during reperfusion

**RESULTS**

- **pCaMKII**
  - Protein levels of pCaMKII under different conditions.
  - Bar graph showing % of control with markers indicating significant changes.
  - Images of Western blots for CQ5 and another protein band at 45 kDa.

- **pSer16-PLN**
  - Protein levels of pSer16-PLN showing reperfusion effects.
  - Bar graph with significant markers.
  - Western blot images for PLN and pSer16-PLN bands at 20 kDa.

- **pSer2808-RyR2**
  - Protein levels of pSer2808-RyR2 during reperfusion.
  - Bar graph and Western blot images showing bands at 205 kDa.
Contribution of SR CaMKII targets to mechanical recovery and arrhythmias of reperfusion

Ser2814 mutated to Ala RyR2-S2814A

Thr17 mutated to Ala PLN-T17A

CaMKII
Role of CaMKII-dependent PLN phosphorylation in ischemia-reperfusion

**PLN-T17A mice**

**WT mice**

**PLN-DM mice**

**BACKGROUND**


Role of CaMKII-dependent RyR2 phosphorylation in ischemia-reperfusion

**RyR2-S2814A mice**

**t$_{1/2}$**

- **Pre-Ischemia**
  - **WT**
  - **RyR2-S2814A**

- **Reperfusion**
  - **WT**
  - **RyR2-S2814A**

**+dP/dt**

- **Control**
- **Ischemia**
- **Reperfusion**

- **WT**
- **S2814A**

**Number of PBs/3 min**

- **Pre-Ischemia**
- **Reperfusion**

**BACKGROUND**


**MAP**

- **0.5mV**
- **0.5 sec**
Role of CaMKII in reversible IR

↑ PThr17–PLN
↓↑ SR Ca$^{2+}$ handling
Preservation of Ca$^{2+}$ transient
↓ Amelioration of depressed contractility of stunned heart
Intracellular Ca^{2+} handling after IR

PLN-DM mice have an altered intracellular Ca$^{2+}$ handling after IR
Role of CaMKII in reversible IR

- Preservation of Ca\(^{2+}\) transient
- Amelioration of depressed contractility of stunned heart

Beneficial

- \(\uparrow\) PThr17-PLN
- \(\uparrow\) SR Ca\(^{2+}\) handling

Detrimental

- \(\uparrow\) PS2814-RyR2
- \(\uparrow\) SR Ca leak

ARRHYTHMIAS
CaMKII increases Ca$^{2+}$ sparks during reperfusion
Role of CaMKII in reversible IR

- **Beneficial**
  - PThr17-PLN
  - SR Ca\(^{2+}\) handling
  - Preservation of Ca\(^{2+}\) transient
  - Amelioration of depressed contractility of stunned heart

- **Detrimental**
  - PS2814-RyR2
  - SR Ca leak
  - ARRHYTHMIAS

Ca\(^{2+}\)-overload vs. Oxidative Stress
Oxidation of CaMKII

**CaMKIIox**
- Pre-Isch: ND
- Reperfusion: *%

**pCaMKII**
- Pre-Isch: ND
- Reperfusion: *

**PT17-PLN**
- Pre-Isch: ND
- Reperfusion: *

**PS2815-RyR2**
- Pre-Isch: ND
- Reperfusion: *%

### RESULTS

- **CaMKIIox**
  - Pre-Isch: ND
  - Reperfusion: *

- **pCaMKII**
  - Pre-Isch: ND
  - Reperfusion: *

- **PT17-PLN**
  - Pre-Isch: ND
  - Reperfusion: *

- **PS2815-RyR2**
  - Pre-Isch: ND
  - Reperfusion: *%
**RESULTS**

**Effects of Tiron on mechanical recovery and reperfusion arrhythmias**

- **LVDP**
  - Graph showing % preischemic values over time (min) for LVDP with Tiron treatment.

- **Reperfusion arrhythmias**
  - Graph showing number of PEs/3 min and % hearts with VT (VT = ventricular tachycardia) with Tiron treatment.

**Graphs**

- **LVDP** graph with data points for Tiron treatment showing increased recovery over time.
- **Reperfusion arrhythmias** graph with bars indicating decreased number of PEs and percentage of hearts with VT for Tiron treatment compared to control.
Redox modifications

- T Tubule
- RyR2
- CQS
- LTCC
- SERCA2a
- PLN
- NCX
- Na ch
- NOX
- eNOS
- Cav3
- NADP+NADPH
- ROS/RNS
- CaMKII
- nNOS
- NOS
- Cav3
Nitroso/redox alterations of RyR2 and reperfusion arrhythmias

RESULTS

**GSH**

\[ \begin{align*}
\text{Pre-isch} & \quad \text{Reperfusion} \\
\text{ND} & \quad \text{APO} \\
\end{align*} \]

**SNO**

\[ \begin{align*}
\text{Pre-isch} & \quad \text{Reperfusion} \\
\text{ND} & \quad \text{APO} \\
\text{L-NAME} & \quad \text{APO} \\
\end{align*} \]

**Reperfusion arrhythmias**

\[ \begin{align*}
\text{Pre-isch} & \quad \text{Reperfusion} \\
\text{WT} & \quad \text{S2814A} \\
\text{WT} & \quad \text{S2814A} \\
\end{align*} \]
Redox modifications of RyR2 (S-glutathionylation and nitrosylation) provide a protective mechanism in the generation of arrhythmias, independently of the CaMKII-dependent phosphorylation of RyR2.

Oxidation of CaMKII is not responsible for the increased activity of CaMKII during reversible ischemia-reperfusion injury.

CaMKII-dependent phosphorylation of RyR2 plays a major role in the generation of reperfusion arrhythmias.

Redox modifications of RyR2 (S-glutathionylation and nitrosylation) provide a protective mechanism in the generation of arrhythmias, independently of the CaMKII-dependent phosphorylation of RyR2.
ACKNOWLEDGEMENTS

ARGENTINA
Bárbara Román
Matilde Said
Romina Becerra
Carlos Valverde
Leticia Vittone
Alicia Mattiazzi

CHILE
Gina Sanchez
Paulina Donoso

USA
Litsa Kranias
John Dedman
M.A. Kaetzel
Xander Wehrens
Ariel Escobar

THE INTERNATIONAL SOCIETY FOR HEART RESEARCH (ISHR)

XXII ISHR World Congress 2016