The role of calmodulin-dependent protein kinase II (CaMKII) in ischemia-reperfusion injury

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Introduction & Hypothesis

In recent years, there has been much interest in the role of calmodulin-dependent protein kinase II (CaMKII) in neuronal injury. It is hypothesized that CaMKII is involved in the regulation of neuronal excitability and synaptic plasticity. This study aimed to investigate the role of CaMKII in ischemia-reperfusion injury.

Materials & Methods

Pharmacological intervention with CaMKII inhibitors was used to evaluate its role in ischemia-reperfusion injury. The effects of CaMKII inhibitors on neuronal survival and function were assessed.

Results - Effect of Inhibition

CaMKII inhibitors were found to significantly reduce neuronal death and improve functional outcomes in ischemia-reperfusion injury.

Conclusions & Future Directions

CaMKII plays a crucial role in ischemia-reperfusion injury, and targeting CaMKII with pharmacological intervention may offer a novel therapeutic strategy.

Acknowledgments & References

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References

INTRODUCTION & HYPOTHESIS

In recent years, there has been much debate as to the role of calmodulin-dependent protein kinase II (CaMKII) in acute ischemia-reperfusion (IR) injury. Previous studies have suggested that CaMKII is an important, druggable target worth studying in the hopes of developing novel therapies to treat IR injury [1]. Other post-translational modifications of this enzyme, such as phosphorylation & oxidation, have been studied in the past, but nitrosylation has yet to be investigated [2]. This study will look at nitrosylation of 2 specific sites on CaMKII, C273 and C290. Previous projects have shown that nitrosylation of the C290 site causes autonomous activation of CaMKII, while nitrosylation at C273, inhibits enzyme activity [3].
Hypothesis:

Nitrosylation at the C290 site is detrimental to recovery from acute IR injury, while nitrosylation at the C273 site improves recovery from IR injury.

We will examine the role of CaMKII nitrosylation in IR injury by assessing the phosphorylation state of CaMKII and one of its prominent targets, phospholamban (PLB) in both wild-type and C290A knock-in mice that are resistant to nitrosylation at the C290 site.
MATERIALS & METHODS

Phase I: Data Collection

- Utilized both wild type and novel C290A knock-in mice resistant to nitrosylation at the C290 site.
- Hearts excised from mice, aorta cannulated for Langendorff perfusion. Each heart was subjected to one of the 6 following experimental protocols:

<table>
<thead>
<tr>
<th>Group 1</th>
<th>WT Control</th>
<th>Hearts hung &amp; perfused on rig for 90 mins - NO ISCHEMIC EVENT</th>
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<tbody>
<tr>
<td>Group 2</td>
<td>WT Isoproterenol + Ischemic Event</td>
<td>Hearts hung &amp; stabilized on rig for 20 mins</td>
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<tr>
<td></td>
<td></td>
<td>Add 10 nM Isoproterenol 10 min prior to global ischemia</td>
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<tr>
<td></td>
<td></td>
<td>Initiate 20 mins of global ischemia</td>
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<tr>
<td></td>
<td></td>
<td>Reperfuse for 40 minutes</td>
</tr>
</tbody>
</table>

| Group 3 | C290A Control | Hearts hung & perfused on rig for 90 mins - NO ISCHEMIC EVENT |

| Group 4 | C290A Isoproterenol + Ischemic Event | Hearts hung & stabilized on rig for 20 mins |
|         | Add 10 nM Isoproterenol 10 min prior to global ischemia |
|         | Initiate 20 mins of global ischemia |
|         | Reperfuse for 40 minutes |

| Group 5 | WT Isoproterenol + GSNO + EGTA + Ischemic Event | Hearts hung & stabilized on rig for 20 mins |
|         | Add 100-150 μM GSNO + 25 μM EGTA 17.5 minutes prior to global ischemia |
|         | Add 10 nM Isoproterenol 10 min prior to global ischemia |
|         | Initiate 20 mins of global ischemia |
|         | Reperfuse for 40 minutes |

| Group 6 | WT Isoproterenol + L-NAME + Ischemic Event | Hearts hung & stabilized on rig for 20 mins |
|         | Add 1 nM L-NAME 17.5 minutes prior to global ischemia |
|         | Add 10 nM Isoproterenol 10 min prior to global ischemia |
|         | Initiate 20 mins of global ischemia |
|         | Reperfuse for 40 minutes |

Phase II: Data Analysis

- Following perfusion protocol hearts were flash-frozen and stored at -80°C
- Hearts were homogenized as previously described (ref https://pubmed.ncbi.nlm.nih.gov/33926209/)
- After protein quantification with BCA assay, samples were run on criterion TGX gels 4-20% before transfer to 0.2 μm nitrocellulose
- Blots were probed with one of the following antibody solutions
  - Anti-pT17 and anti-PLB (Badrilla)
  - Anti-pS16 and anti-PLB (Badrilla)
  - Anti-CaMKII T286 (Badrilla)
  - Anti-CaMKII delta (custom antibody)
• Followed by anti-rabbit IRDye800 and anti-mouse IR Dye680LT before scanning with the Sapphire Biomolecular Imager (Azure Biosystems).
• Blots were subsequently analyzed with Image J
RESULTS - EFFECT OF NITROSYLATION (GSNO + EGTA V. L-NAME)

**Effect of Nitrosylation**

a). PT17

b). PS16 PLB

c). T286 CaMKII

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L-NAME (an NO scavenger) did not reduce phosphorylation of T17. GSNO pretreatment (triggering nitrosylation of CaMKII at C273) did not have a significant effect on PLB T17 phosphorylation.

b). pS16

No significant changes in pS16 phosphorylation following IR, or LNAME or GSNO pretreatment were observed.

c). T286

pT286 antibody was not sensitive enough to display differences between CTL and IR, pretreatments or C290A and littermates.
RESULTS - WT V. C290A

WT vs. C290A

\[ d). \text{pT17 PLB} \]

\[ e). \text{pS16 PLB} \]

\[ f). \text{T286 CaMKII} \]

\[ \text{WT CTL, WT IR, C280A CTL, C280A IR} \]

\[ d). \text{pT17} \]
While phosphorylation of the T17 residue on phospholamban increased with IR injury in the WT mice, it appeared to decrease in the C290A mice. This is consistent with previous studies that found activation of CaMKII in IR injury and increased phosphorylation of its target PLB affecting SR Ca handling.

e). pS16

No significant differences between CTL and IR conditions in WT or C290A mice.

f). T286

pT286 antibody was not sensitive enough to display differences between CTL and IR, pretreatments or C290A and littermates.
CONCLUSIONS & FUTURE DIRECTIONS

Experiments are ongoing to increase n in these biochemical experiments and to assess functional effects such as LVP, dP/dt, and infarct size.

Our initial conclusions are that our data is consistent with activation of CaMKII in acute IR injury (increase in PLB phosphorylation at the CaMKII site (pT17)). However, we were unable to confirm that NO signaling through protein nitrosylation and CaMKII in particular plays a significant role in recovery from IR injury. Overall our data hint at a role for CaMKII in IR injury but additional experiments are required to elucidate the role of CaMKII nitrosylation.
ACKNOWLEDGMENTS & REFERENCES

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References


AUTHOR INFORMATION

Brandon Weiss is a third year DVM candidate at the UC Davis School of Veterinary Medicine. He was born in Los Angeles, CA and attended UC Berkeley for his undergraduate education. There, he completed a Bachelor of Arts degree in Ecology, Evolution, & Organismal Biology and developed a fervent passion for California wildlife, especially birds. Brandon is deciding between many of his passions in veterinary medicine and will most likely either specialize in an area of small animal medicine or practice as an emergency doctor.
REFERENCES

