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

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Introduction

- CaMKIId is an important regulator of cardiac function and dysfunction in pathological states. It critically modulates ion channels, Ca handling proteins (phospholamban/SERCA, RyR), myofilaments, nuclear transcription and energetics/metabolism. (Fig1) [1]
- CaMKII holoenzyme becomes activated by binding of Ca-CaM, resulting in autophosphorylation of neighboring subunits at T286 and an autonomously active kinase. Other CaMKII targets include T17 on phospholamban and S2814 on RyR (Fig2) [1]
- in addition to autophosphorylation, other post-translational modifications were recently identified that also promote autonomous CaMKII activation: oxidation (M281/282), O-GlcNAcylation (S280) and S-nitrosylation (C290). Interestingly CaMKIId has a second regulatory nitrosylation site (C273) that inhibits instead of activates the kinase. [2]
- PTMs that promote autonomous activity are implicated in cardiac pathology but little is known about the interplay between these PTMs. Oxidized CaMKII contributes to apoptosis post-MI and atrial fibrillation and O-GlcNAcylation contributes to hyperglycemia-induces SR Ca leak and arrhythmia. The role of CaMKII S-nitrosylation has yet to be investigated.
- Previous studies have suggested a key role for CaMKII and nitrosylation in IR injury [3]
- This study will look at the role of nitrosylation at both regulatory sites on CaMKII: C290 and C273S.

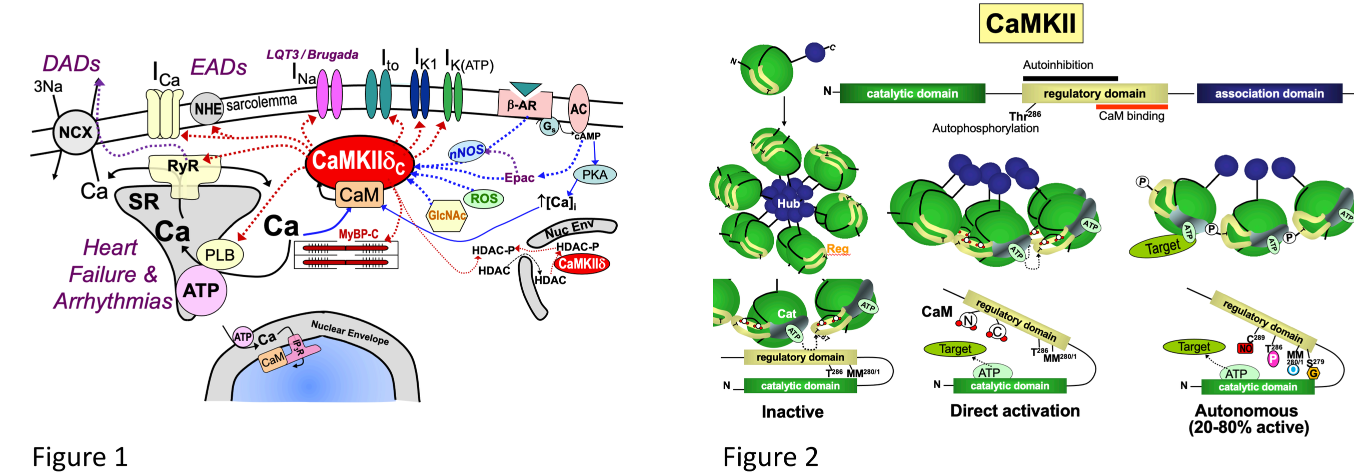


Figure 1 Figure 2

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.

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:

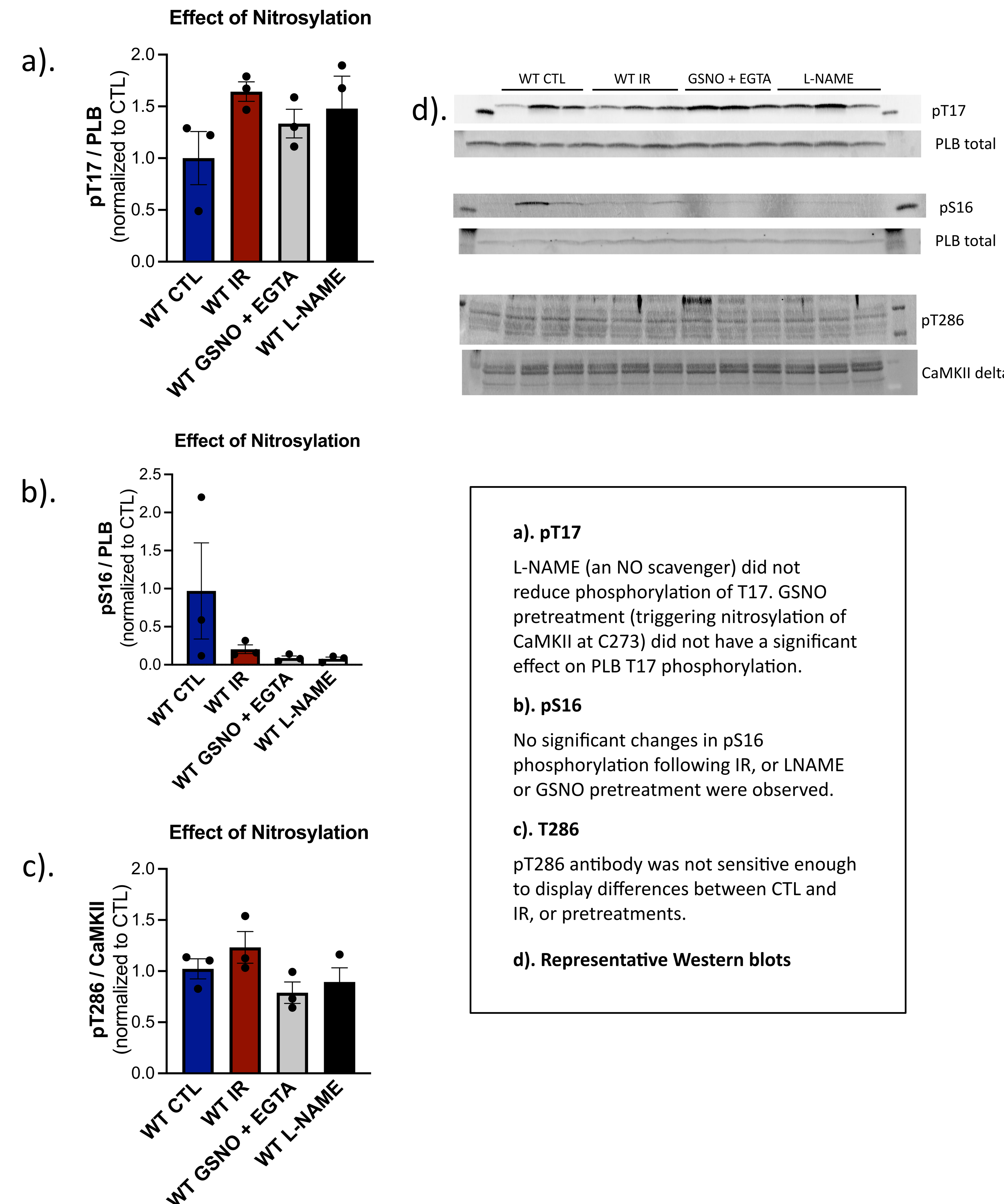
Group	Experimental Protocol
WT Control	Hearts hung on rig and perfused for 90 mins **NO ISCHEMIC EVENT**
WT IR	Hearts hung & stabilized on rig for 20 mins → Add 10 nM isoproterenol 10 min prior to global ischemia → initiate 20 minutes of global ischemia → Reperfuse for 40 minutes
C290A Control	Hearts hung on rig and perfused for 90 mins **NO ISCHEMIC EVENT**
C290A IR	Hearts hung & stabilized on rig for 20 mins → Add 10 nM isoproterenol 10 min prior to global ischemia →Initiate 20 minutes of global ischemia → Reperfuse for 40 minutes
WT GSNO + EGTA	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 minutes of global ischemia → Reperfuse for 40 minutes
WT L-NAME	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 minutes of global ischemia → Reperfuse for 40 minutes

Materials & Methods (cont.)

Phase II: Data Analysis

- Following perfusion protocol, hearts were flash-frozen and stored at -80C
- 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 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



a). pT17

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

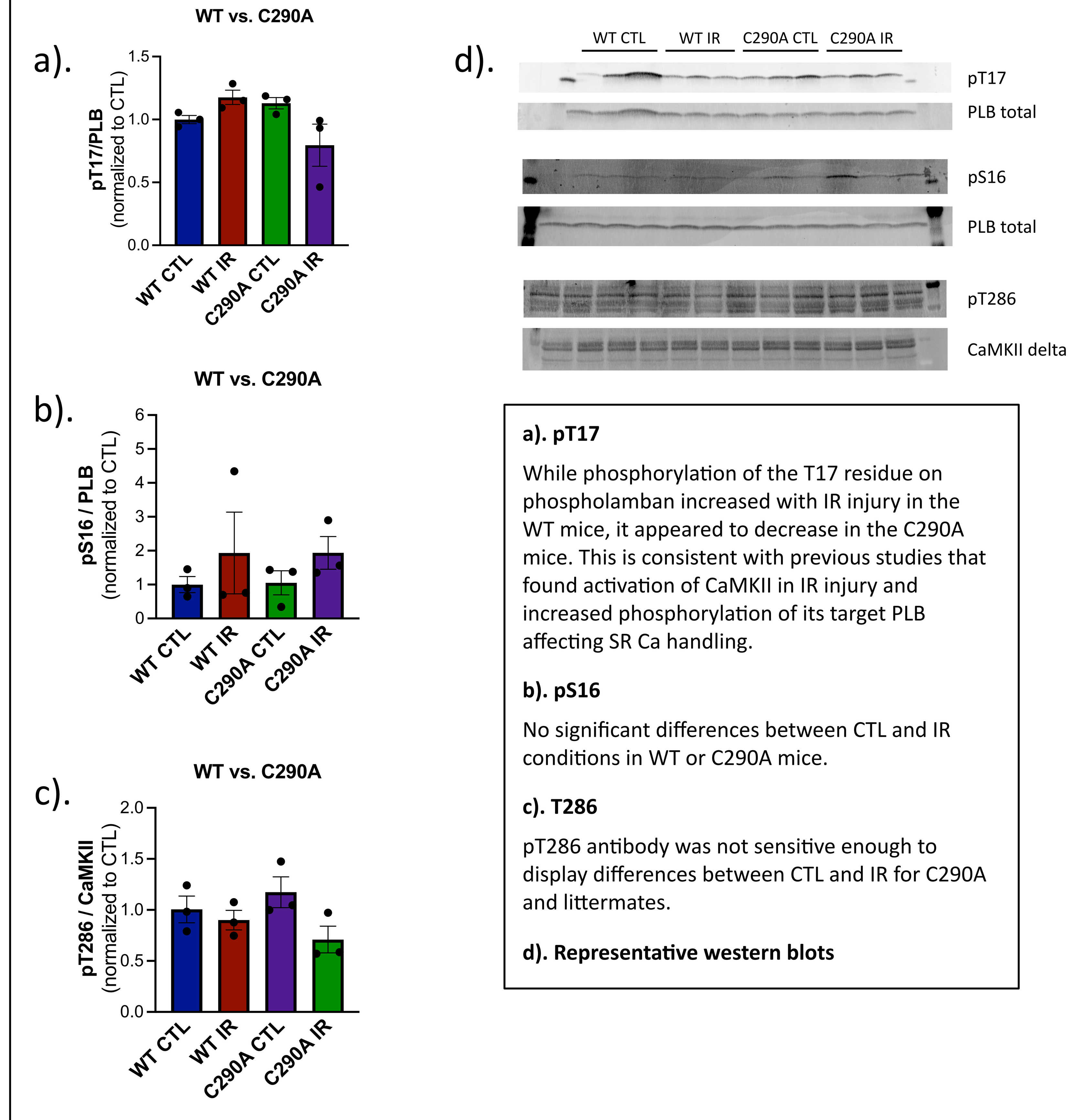
No significant changes in pS16 phosphorylation following IR, or LNAME or GSNO pretreatment were observed.

c). T286

pT286 antibody was not sensitive enough to display differences between CTL and IR, or pretreatments.

d). Representative Western blots

Results WT v. C290A



a). 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.

b). pS16

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

c). T286

pT286 antibody was not sensitive enough to display differences between CTL and IR for C290A and littermates.

d). Representative western blots

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

Acknowledgments

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