Altered regulation of cardiac calcium handling proteins in an *in vivo* rat model of Angiotensin II-induced hypertension

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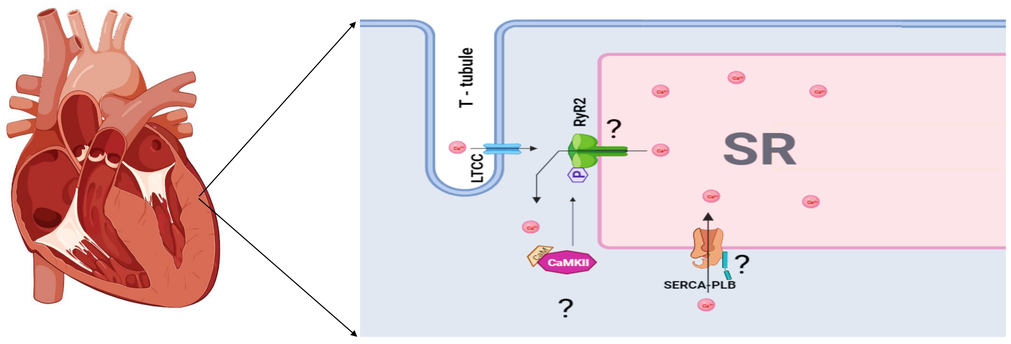
SCAN ME

1. BACKGROUND

Hypertension is a major comorbidity in patients with heart failure with preserved ejection fraction (HFpEF), which remains an increasing global challenge. Cardiac remodelling and dysfunction as well as altered calcium (Ca^{2+}) homeostasis are all characteristics of this disease[1,2]. However, existing models and evidence on the mechanisms driving this form of cardiomyopathy remain contradictory.

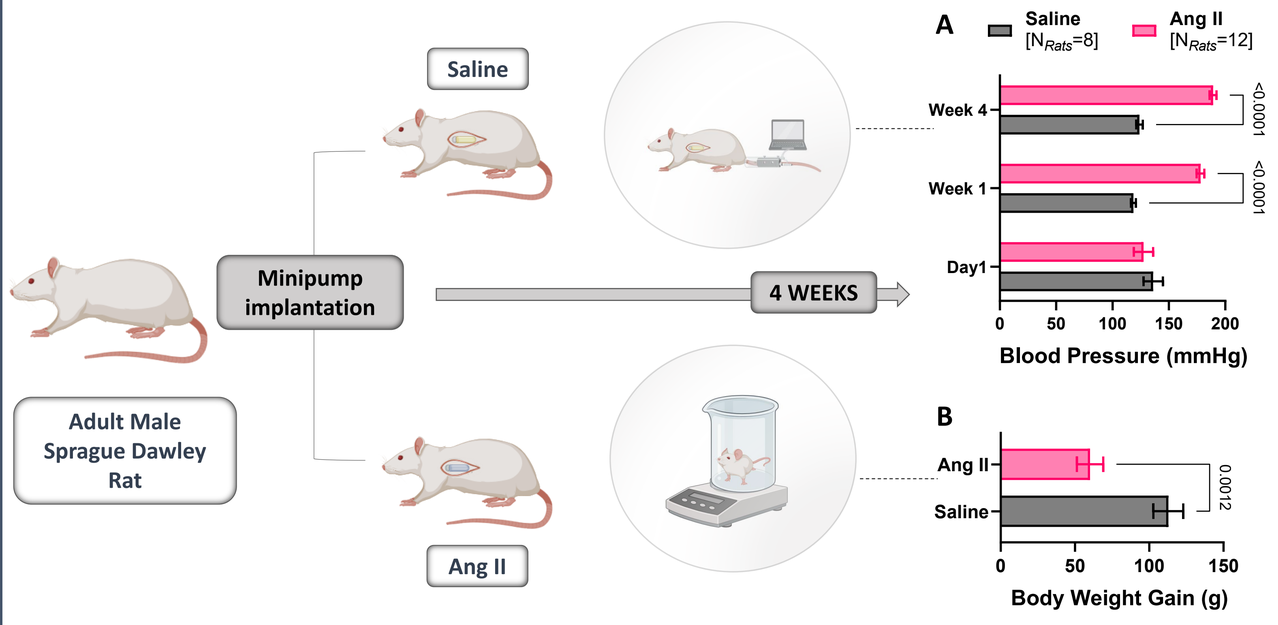


Cardiac Ca^{2+} homeostasis. Extracellular Ca^{2+} influx via ion channels lead to the activation of proximal Ryanodine receptors (RyR2). These facilitate a greater release of Ca^{2+} from the sarcoplasmic reticulum (SR) into the intracellular space, for contraction. Free cytosolic Ca^{2+} are recycled into the SR via Phospholamban (PLB)-regulated SERCA during relaxation. Ca^{2+}/Calmodulin-dependent protein Kinase (CaMKII) regulates the phosphorylation of these proteins.

Aims of study:

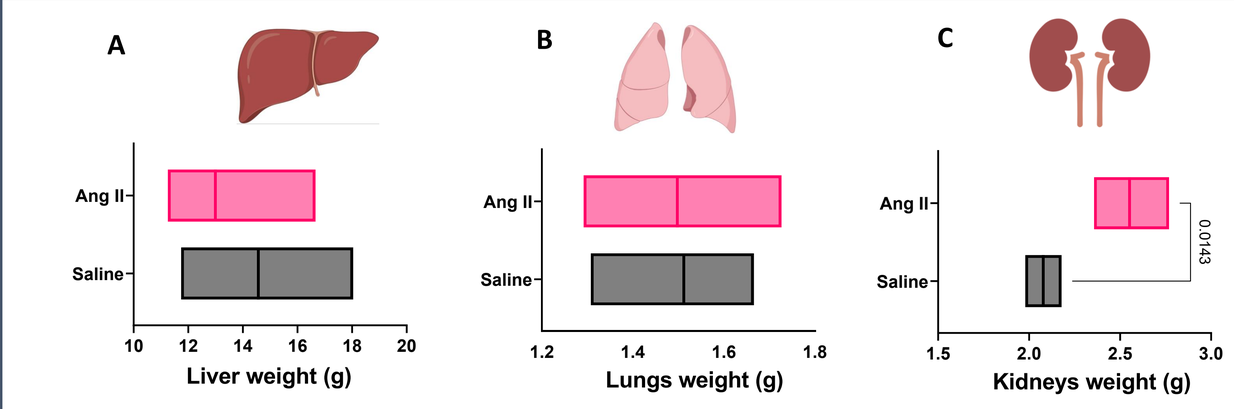
- To establish a relevant *in vivo* hypertensive model, and assess its sufficiency to generate HFpEF.
- To assess alterations in the expression and activation of calcium handling proteins.
- To assess the acute Ca^{2+} signalling alterations in an *in vitro* replica of the *in vivo* model.

2. *IN VIVO* HYPERTENSIVE MODEL GENERATION



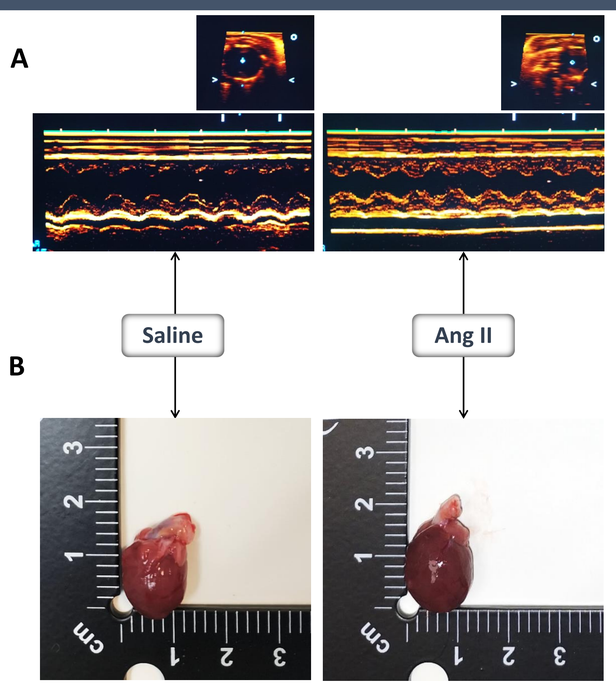
Adult rats were surgically implanted with osmotic mini pumps, containing either Saline as a vehicle control or Angiotensin II (Ang II), infused at 0.576mg/kg/day, for a total duration of 4 weeks. Body weight and blood pressure were recorded before and after surgery. Ang II **(A)** increased blood pressure post-surgery, and **(B)** reduced body weight gain. Data presented as mean±SEM.

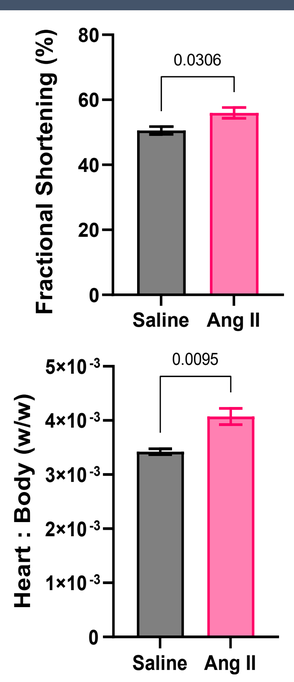
3. NO SYSTEMIC ORGAN DYSFUNCTION OBSERVED



Following treatment with either Saline or Ang II, the liver, lungs and kidneys of rats were weighed. **(A-C)** Floating bar box plots show minimum to maximum range with line at mean.

4. CHRONIC HYPERTENSION → ↑ INOTROPY and ↑ HYPERTROPHY

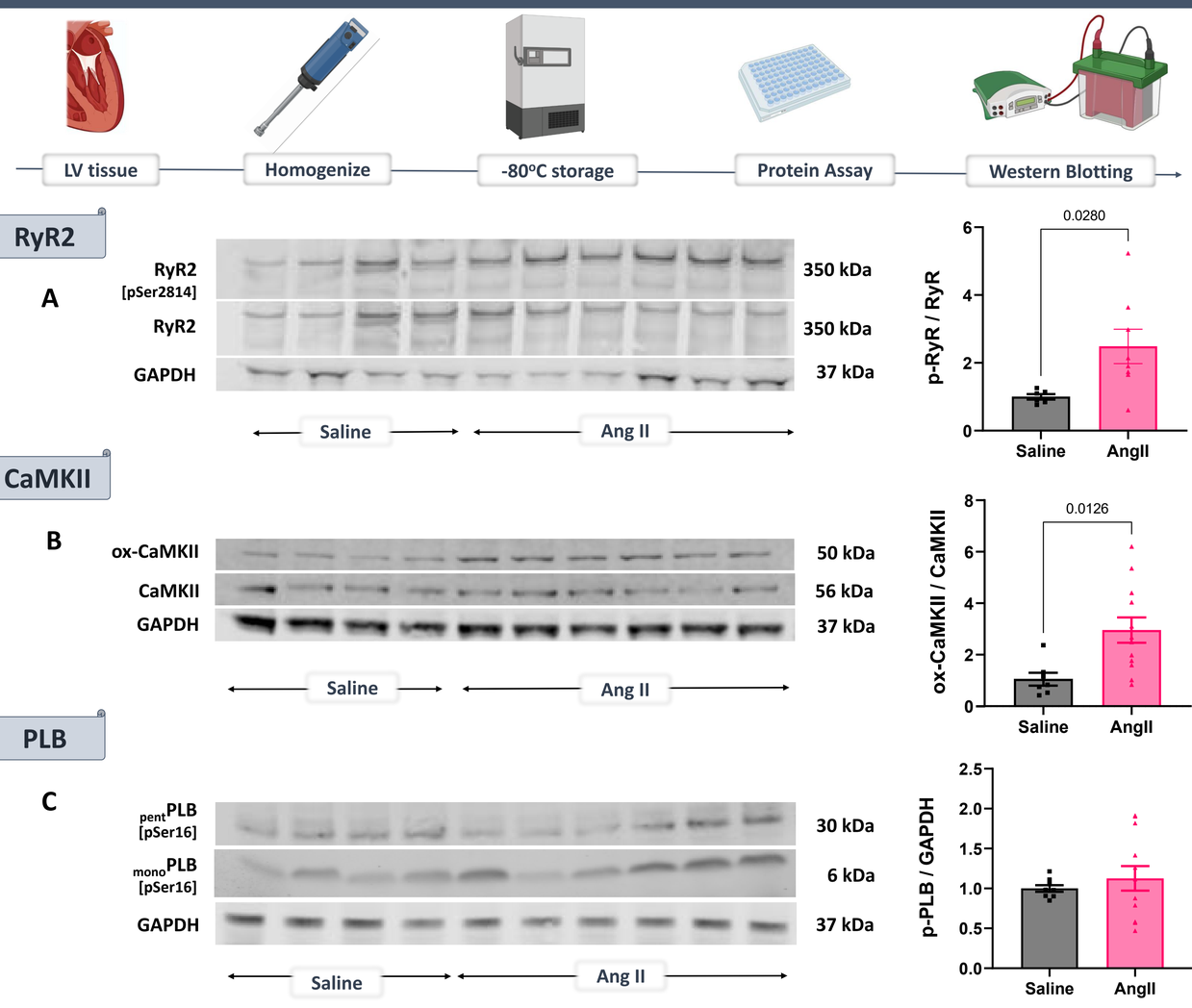




(A) Echocardiographic measurements revealed a slight increase in the fractional shortening of the left ventricular walls, in chronic hypertensive rats.

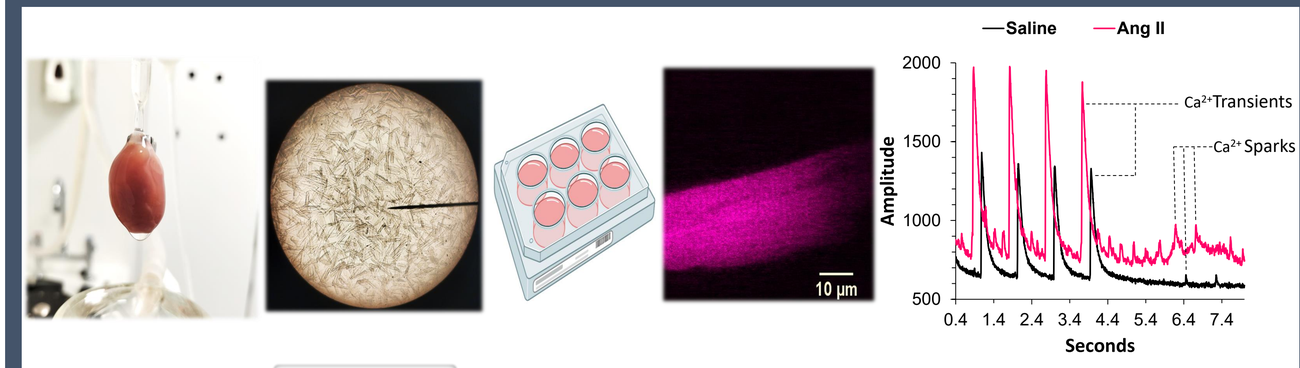
(B) The ratio of heart weight to body weight was significantly increased in chronic hypertensive rats.

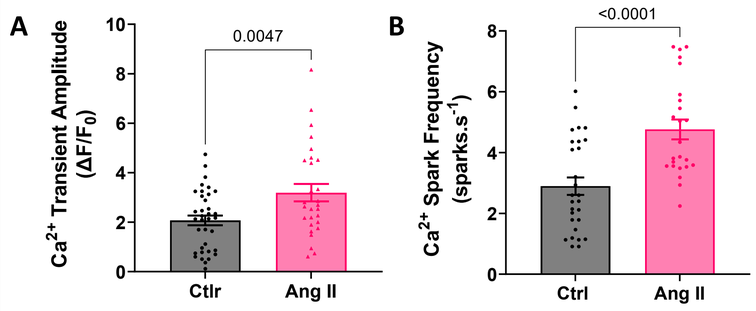
5. CHRONIC ALTERATIONS IN Ca^{2+} HANDLING PROTEINS



Immunoblots of left ventricular cardiac tissue show that chronic Ang II treatment enhanced **(A)** the phosphorylation of RyR2 [Ser2814] at the CaMKII site, and **(B)** the oxidation of CaMKII proteins. **(C)** No change was observed in combined monomeric and pentameric PLB [pSer16], phosphorylated at the PKA site.

6. ACUTE ANG II → ↑ Ca^{2+} TRANSIENT AMPLITUDE and SPARK FREQUENCY





Ventricular cardiac myocytes were isolated from 4 naïve adult male rats by Langendorff perfusion, cultured with or without 200nM Ang II for 24h, and then stained with Cal520 AM calcium indicator. Following electrical stimulation at 1Hz, Ca^{2+} transients and sparks were recorded by live single cell confocal microscopy.

7. SUMMARY and FUTURE WORK

Findings show that:

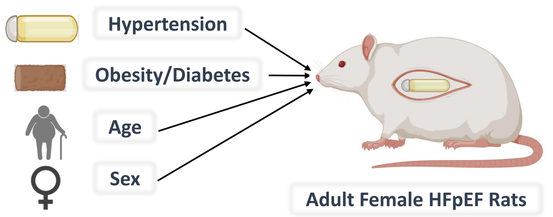
- Chronic Ang II treatment over 4 weeks resulted in cardiac hypertrophy and hypertension with no reduction in fractional shortening.
- Chronic Ang II treatment over 4 weeks resulted in increased pRyR-Ser2814 and increased oxidation of CaMKII.
- Acute Ang II treatment of isolated cardiac myocytes resulted in increased Ca^{2+} transient amplitude and Ca^{2+} spark frequency.
- The enhanced function of RyR2 following acute Ang II treatment may be mediated via CaMKII oxidation and activation.

Therefore:

- Ang II treatment alone is likely to be insufficient to mimic the complex nature of HFpEF.

FUTURE STUDIES

- How do other comorbidities of HFpEF contribute to this cardiomyopathy, in addition to hypertension?
- What are the roles of other cell types in both *in vitro* and *in vivo* models of HFpEF?



8. REFERENCE

1. Frisk M, Le C, Shen X, Røe ÅT, Hou Y, Manfra O, Silva GJ, van Hout I, Norden ES, Aronsen JM, Laasmaa M. Etiology-dependent impairment of diastolic cardiomyocyte calcium homeostasis in heart failure with preserved ejection fraction. Journal of the American College of Cardiology. 2021 Feb 2;77(4):405-19.

2. Kilfoil PJ, Lotteau S, Zhang R, Yue X, Aynaszyan S, Solymani RE, Cingolani E, Marbán E, Goldhaber JI. Distinct features of calcium handling and β-adrenergic sensitivity in heart failure with preserved versus reduced ejection fraction. The Journal of physiology. 2020 Nov;598(22):5091-108.