Atrial Remodeling is Attenuated by Dual Cannabinoid Receptor Agonist Via AMPK Activation and AERP Reduction

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Background:

- **Atrial fibrillation (AF)**
  - is the most common sustained cardiac arrhythmia[1]
  - arrhythmic episodes triggered by electrical activity
  - self-perpetuating; greater risk of persistence and recurrence of arrhythmia over time
- **Atrial remodelling**
  - rate-dependent atrial changes
  - molecular mechanisms poorly understood[2]
  - decreased atrial effective refractory period (AERP) and decreased conduction velocity
- Important risk factors and comorbidities for AF development[4] include:
  - cardiovascular disease, age, diabetes mellitus, genetic predisposition
- Therapeutic strategies to treat AF remain suboptimal and can be extremely invasive (e.g. ablation therapy)

- **Cannabinoids**
  - phytocannabinoids; e.g. cannabidiol
  - synthetic cannabinoids; e.g. CB-13
  - endocannabinoids; e.g. anandamide
- *endocannabinoid system (ECS)* is comprised of cannabinoid receptor agonists and the proteins that bind, transport and metabolize these lipids[5]
  - 2 G-couple protein receptors
    - CB1 receptors (CB1R) expressed in brain and heart
    - CB2 receptors (CB2R) expressed in heart
- **CB13** is a synthetic, peripherally restricted dual CB1R/CB2R agonist with limited brain penetration[6]
- CB13 demonstrated to be cardioprotective

Objective:

- Hypothesis: cannabinoid treatment during atrial tachypacing will alter AF substrate development through novel signaling mechanisms
- Aim: to assess the effect of CB13 on atrial electrophysiology in an *ex vivo* Langendorff hanging heart set-up (Fig. 1)
- Significance:
  - identify effect of CB13 on atrial tachypacing-related electrical/metabolic changes utilizing a novel *ex vivo* technique
  - identify role of cannabinoid signaling in AF
    - establish atrial CB receptors as a novel drug target

Methods:

- electrophysiology parameters:
  - mini-hook platinum quadrupole electrode
    - placed on right atrium; stimulates pacing and records atrial signals, including ***atrial effective refractory period (AERP)***

Tachypacing *ex vivo* protocol:

- control SD hearts (n=8), CB13-treated SD hearts (n=8)

Step 1: Hang heart and recovery period
↓
Step 2: 10 Burst paces (cycle length 20ms, duration 20sec)
↓
Step 3: Atrial effective refractory period (AERP) measurement S1S2 protocol

Step 7: Tissue collection (freeze)
↑
Step 6: 10 Burst paces (cycle length 20ms, duration 20sec)
↑
Step 5: Atrial effective refractory period (AERP) measurement S1S2 protocol

Step 4: Rapid pacing (5ms above failed 1:1 atrial capture)

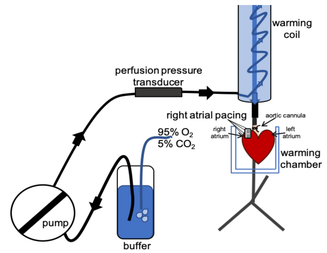

Figure 1. Langendorff hanging heart apparatus.

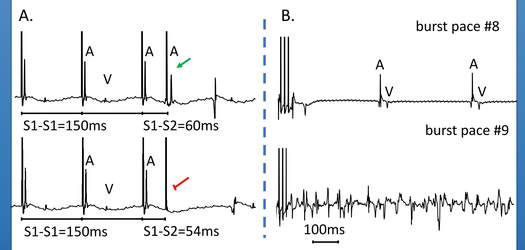

Figure 2. Effects of CB13 on atrial refractoriness and AF substrate were assessed by high resolution electrogram recordings. **(A) S1S2 protocol.** Right atrial (CB-13-treated) recording of AERP capture. 60ms S2 cycle length demonstrates 1:1 atrial signal *(top)*. 54ms S2 cycle length demonstrates failed 1:1 atrial capture *(bottom)*. S1 cycle length at 150ms. **(B) Burst pacing.** 10 burst paces of 20 sec with cycle length (CL) of 20 ms. A, atria; V, ventricle.

Results:

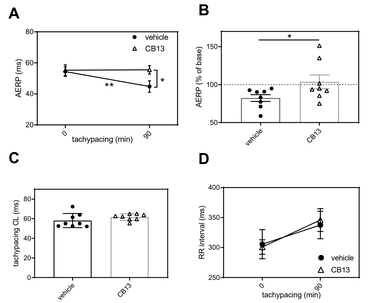

Figure 3. CB13-treatment inhibited tachypacing-induced atrial electrical remodeling.

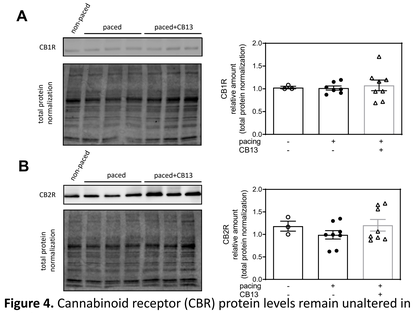

Figure 4. Cannabinoid receptor (CBR) protein levels remain unaltered in atrial tissue.

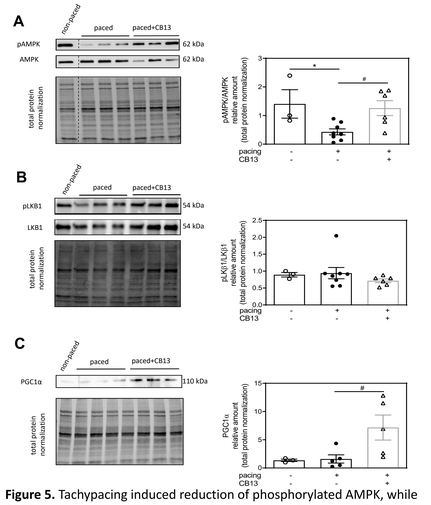

Figure 5. Tachypacing induced reduction of phosphorylated AMPK, while CB13 treatment abrogated tachypacing effects and increased PGC1α signaling.

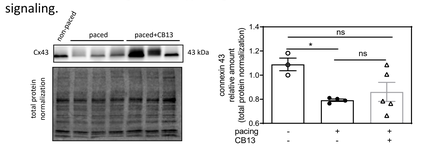

Figure 6. Tachypacing induced reduction of connexin 43 is prevented by CB13. Cx43 is downregulated in tachypaced atrium compared to non-paced atrium.

Summary:

1. Hemodynamic effects:

- CB13 treatment has no effect on left ventricular pressure recordings
  - absence of chronotropic, dromotropic or hemodynamic effects of CB13 in the non-paced rat heart

2. Electrical modifications:

- CB13 treated SD rat hearts prevented the reduction of AERP after rapid pacing in an *ex vivo* Langendorff hanging heart set-up
  - tachypaced control SD rat hearts have a significant reduction in AERP

3. Molecular modifications:

- Signaling mediators:
  - CBR protein levels remain unaltered in atrial tissue
  - tachypacing induced reduction of phosphorylated AMPK
    - CB13 treatment prevented tachypacing effects and increased PGC1α signaling
  - tachypacing-induced reduction of connexin 43 is prevented by CB13

Conclusion:

- CB13 attenuates pathophysiological changes that cause AERP decreases over time, likely through an AMPK mediated response
- CB13, and the subsequent activation of CBRs in the heart, may be a viable treatment strategy for AF

Limitations:

- the exact link between AERP and AMPK activation remains to be elucidated
- detection of changes in CBRs may require longer time periods compared to AMPK activation

Future directions:

- determine the effects of CB receptor activation *in vitro,* using neonatal rat atrial cardiomyocytes
  - investigate CB receptor-dependent mitigation of electrical and/or structural remodeling mechanisms that lead to increased AF substrate
- determine atrial electrophysiology using an *in vivo* tachypacing rodent model
  - in collaboration with the Etzion lab at Ben Gurion University

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