Cardiac functional recovery post ischemia/reperfusion is impaired by cardiomyocyte mineralocorticoid receptor activation in male and female hearts

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Mineralocorticoid receptor (MR) activation in the heart is involved in the development of cardiovascular disease and heart failure. Selective deletion of MR in cardiomyocytes reduced mineralocorticoid/salt induced cardiac fibrosis and inflammation in male mice (Rickard *et al.*, 2012). Clinical observations suggest MR-mediated disease may be sex-specific, therefore identifying both cell and sex differences in MR signaling may provide evidence for the development of cardiac-and/or gender-specific MR therapies. The aim of this study was to characterize the role of cardiomyocyte MR signaling in post-ischemic cardiac functional recovery and to identify sex-specific MR regulation of cardiac function.

8 week old, male and female, wild-type (WT) and cardiomyocyte MR knockout mice (myo-MRKO) underwent uninephrectomy (anaesthetic: 80 mg/kg *i.p.* ketamine, 10mg/kg *i.p.* xylazil, analgesic: 5 mg/kg *i.p.* carprofen, 20 ug.kg *i.p.* buprenorphine) and were (i) maintained on high salt diet (vehicle, 0.9% NaCl, 0.4% KCl) or (ii) maintained on high salt diet plus a subcutaneous deoxycorticosterone pellet (DOC:0.3 mg/day, 0.9% NaCl). After 8 weeks of treatment hearts excised (anaesthetic: 100mg/kg i.p. sodium pentobarbitone) were Langendorff perfused. *Ex vivo* hearts underwent 30 minutes of basal equilibration, 20 minutes global ischemia and 45 minutes reperfusion (n = 7-9 per group).

Basal cardiac function was equivalent between all groups. Post-ischemia/reperfusion, recovery of left ventricular developed pressure (LVDevP) was greater in myo-MRKO *vs* WT hearts regardless of sex and mineralocorticoid excess (% basal LVDevP: male WT; Veh: 73 ± 5 mmHg, DOC: 78 ± 2 mmHg *vs*, myo-MRKO; Veh: 88 ± 6 mmHg, DOC: 90 ± 3 mmHg, female WT; Veh: 66 ± 9 mmHg, DOC: 69 ± 7 mmHg *vs* myo-MRKO; Veh: 86 ± 5 mmHg, DOC: 85 ± 5 mmHg, $p < 0.05$). Mineralocorticoid excess reduced time to onset of ischemic contracture in male hearts only (male WT; Veh: 15 ± 2 min, myo- MRKO; Veh: 14 ± 1 min *vs* WT DOC: 12 ± 1 min, myo-MRKO DOC: 12 ± 1 min, $p < 0.05$). Time to onset of ischemic contracture occurred earlier in male hearts compared to female hearts regardless of genotype (male WT Veh: 15 ± 2 min, myo-MRKO Veh: 14 ± 1 min, WT DOC: 12 ± 1 min, myo-MRKO DOC: 12 ± 1 min *vs* female WT Veh: 16 ± 1 min, myo-MRKO Veh: 16 ± 1 min, WT DOC: 16 ± 0.5 min, myo-MRKO DOC: 15 ± 1 min, $p < 0.05$).

This study demonstrates that under basal conditions, loss of cardiomyocyte mineralocorticoid receptor signaling and mineralocorticoid status does not alter cardiac function regardless of sex or genotype. After acute ischemia/reperfusion, activation of cardiomyocyte mineralocorticoid receptor reduces cardiac functional recovery in both sexes. Male WT and myo-MRKO hearts treated with mineralocorticoid excess had reduced time to onset of ischemic contracture suggesting worse injury compared to vehicle treatment. Time to onset of ischemic contracture was significantly longer in female hearts compared to males hearts suggesting female hearts sustained less injury during the ischemic period. Mineralocorticoid excess did not alter female time to onset of ischemic contracture indicating that mineralocorticoid status may be more important in males for modulating ischemic injury. Interestingly, mineralocorticoid excess did not alter functional recovery regardless of sex or genotype. These data show that cardiomyocyte mineralocorticoid receptor signaling is critical for cardiac functional recovery post-acute ischemia/reperfusion. On-going studies are investigating sex- and cardiomyocyte mineralocorticoid receptor-dependent modulation of ischemia/reperfusion-activated pathways.

Rickard AJ, Morgan J, Bienvenu LA, Fletcher EK, Cranston GA, Shen JZ, Reichelt MR, Delbridge LMD & Young MJ. (2012) *Hypertension*, **60**, 1443-50.