Angiotensin II Deregulates Mitochondrial Quality Control And Prevents Autophagosome Formation In Skeletal Muscle.

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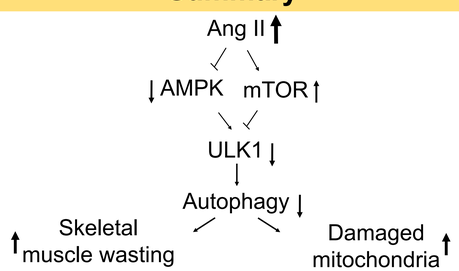
Abstract

Introduction: Mitochondria are the powerhouse of the cells and play a critical role in muscle metabolism. When damaged, mitochondria are selectively degraded by autophagy (i.e. Mitophagy). Since angiotensin II (Ang II) induces a catabolic condition and disrupts energy balance, we aimed to investigate the effects of Ang II in mitochondria quality control and autophagy. **Methods:** FVB mice (10 weeks-old) were infused with Ang II (1.0 µg/kg/day) for 12h, 1, 4 and 7 days; pair-fed group was infused with saline. In skeletal muscle we performed western blot, RT-qPCR, and Transmission Electronic Microscopy (TEM). **Results:** Ang II infusion reduced mouse body weight and caused muscle loss of TA. TEM analyses in EDL showed swollen (abnormal) mitochondria with disorganized cristae at 7d of Ang II. This was associated with disrupted mitochondrial dynamics: Ang II decreased markers of mitochondrial fusion (Mitofusin 2 and OPA1) and fission (Fis1). Furthermore, PINK1 expression was increased in Ang II, suggesting an accumulation of damaged mitochondria. These results indicates disruption of mitophagy. In Ang II, we found decreased conversion of LC3-II and increased p62/SQSTM1, indicating an inhibition of autophagy flux. In contrast, the autolysosome function (lysosomal cathepsin B and L activities) was not altered by Ang II. Additionally, Ang II increased mTOR and impaired AMPK activation, and inhibited ULK1-ATG14 pathway leading to decreased autophagosome formation. **Conclusions:** Our data show that 1: Ang II impairs mitochondrial dynamics and 2: blocks autophagosome formation, likely via modulation of mTOR/AMPK axis, which are detrimental to muscle and may trigger muscle atrophy. Thus, targeting renin-angiotensin system could be an attractive approach to prevent muscle loss.

Summary



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Disclosures: KASS, PD, and TY: **NONE**

Results

1 – Ang II decreases BW and increases SBP

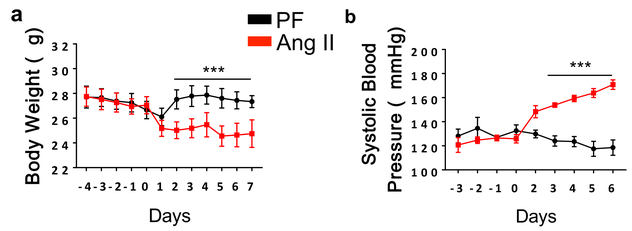

Fig 1: Ang II reduces body weight and increases systolic blood pressure. a) Body weight of FVB mice infused with Ang II or saline (PF) for 7 days. b) Systolic blood pressure is increased in Ang II group compared to PF. N=6 mice/group, ***p=0.0001 vs. PF.

2 – Ang II induces skeletal muscle loss

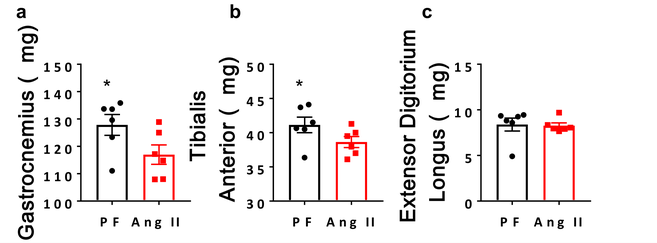

Fig 2: Ang II induces loss of muscle mass after 7 days of Ang II infusion. a) Gastrocnemius weight in FVB mice infused with Ang II or saline (PF) for 7 days. b) Tibialis anterior muscle is decreased in Ang II group compared to PF. c) No difference in EDL muscle between PF and Ang II. N=6 mice/group, p<0.05 vs. Ang II.

3 – Ang II accumulates damaged mitochondria and disrupts mitochondrial quality control

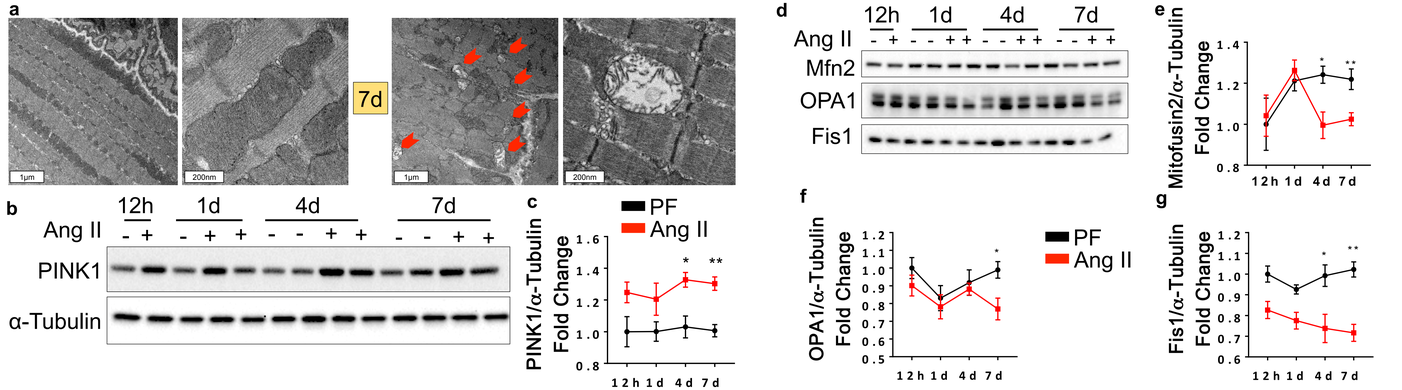

Fig 3: Ang II accumulates damaged mitochondria after 7 days of Ang II infusion. a) Damaged mitochondria in Ang II after 7 days of infusion. b) Representative western blot of PINK1 expression. c) Quantification of PINK1 protein expression. e) Ang II decreases Mitofusin2, OPA1 and Fis1 protein expression at 4 and 7 days of Ang II infusion. e), f), and g) Quantification of Mitofusin2, OPA1 and Fis1 western blot, respectively. Red arrows indicate damaged mitochondria. N=3 to 6 mice/group, *p<0.01 and **p<0.05 vs. PF.

4 – Ang II disrupts signaling pathway that activates autophagy in skeletal muscle

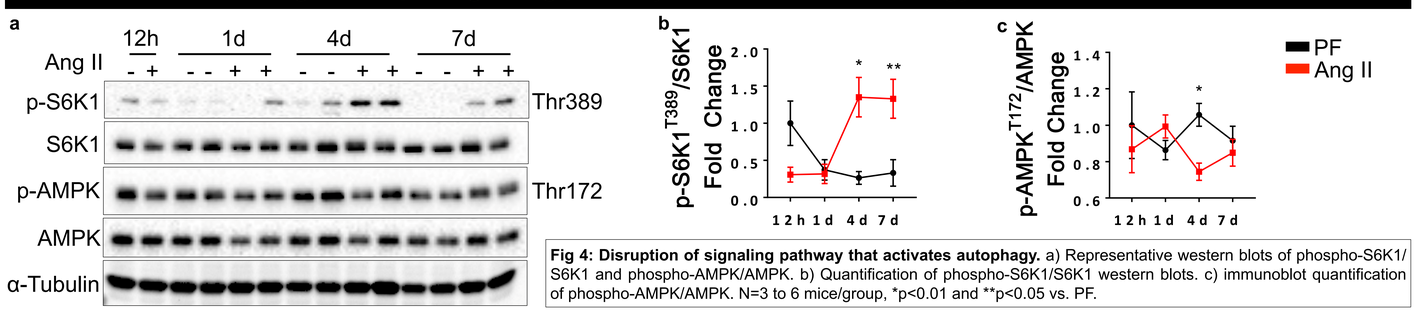

Fig 4: Disruption of signaling pathway that activates autophagy. a) Representative western blots of phospho-S6K1/S6K1 and phospho-AMPK/AMPK. b) Quantification of phospho-S6K1/S6K1 western blots. c) immunoblot quantification of phospho-AMPK/AMPK. N=3 to 6 mice/group, *p<0.01 and **p<0.05 vs. PF.

Results

5 – Ang II blunts ULK1 activation

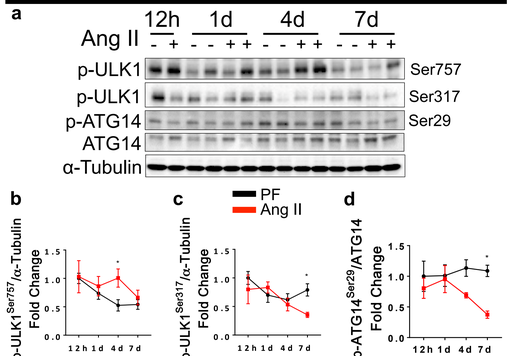

Fig 5: Activation of ULK1 is reduced after Ang II infusion. a) Representative western blots of ULK1 at Ser757 (mTOR site), Ser317 (AMPK site), and ATG14 Ser29. b) Quantification of phospho-ULK1-Ser757/α-Tubulin western blots. c) Immunoblot quantification of phospho-ULK1-Ser317/ α-Tubulin. d) Quantification of ATG14-Ser29/ ATG14. N=3 to 6/mice/group/time course, *p<0.05.

6 – Autophagy is impaired in Ang II

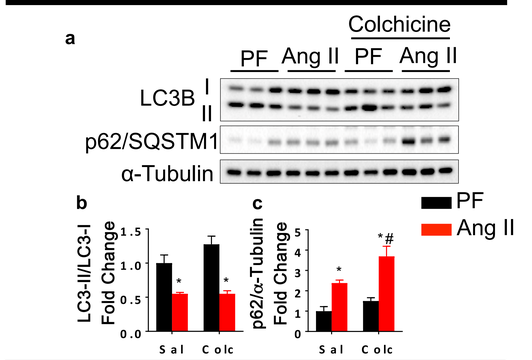

Fig 6: Ang II reduces autophagy flux by decreasing autophagosome formation. a) Representative western blot of LC3B-II conversion. b) Quantification of LC3B-II/I conversion. c) Quantification of p62/SQSTQM1 expression. Mice were treated with colchicine (0.4 mg/kg) for two days before sacrifice. N=6 mice/group, *p<0.05 vs. respective control and #p<0.05 vs. Ang II – Saline.

Conclusion

Ang II suppresses autophagy by impairing autophagosome formation, which leads to accumulation of damaged mitochondria and ubiquitinated proteins. Our data strongly suggest that autophagy plays a critical role in Ang II-induced energy imbalance and skeletal muscle wasting, and that it could be a new therapeutic target in wasting disorders such as HF.