High Salt-Induced Changes in Mitochondrial Bioenergetics in the Kidney Proximal Tubules During the Development of Hypertension – Role of mTORC1

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Background and Rationale

- Kidney proximal tubules (PTs) reabsorb nearly 65% of Na^+ from the glomerular filtrate in the cortex. Under high salt (HS) feeding, this imposes a significant metabolic stress upon the PT mitochondria to reabsorb greater Na^+ which requires enhanced ATP production and O_2 consumption to meet the increased energy demand.
- **Goal:** In this study, we hypothesized that mitochondrial bioenergetics in the kidney PTs is progressively impaired while meeting the energy demand, via activation of mTORC1 signaling during the development of salt-sensitive (SS) hypertension

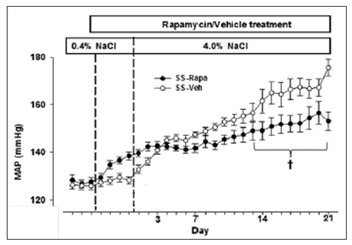

Fig. 1. Mean Arterial Pressure (MAP) time course: The MAP of SS rats fed a HS diet (4.0% NaCl) is presented over 21 days for vehicle-treated and Rapamycin-treated SS rats [1]. Rapamycin is a potent inhibitor of mTORC1 signaling.

Materials and Methods

1. PT isolation

- Kidney cortical tissues from SS rats were digested with collagenase at 37° C, yielding a digested mixture (DM) containing ~ 60% proximal tubules (PT). Sieving of the DM produced a suspension mixture (SM) enriched to ~85% PT, based on morphometric analysis of ~ 500 segments.
- The isolated PT were mechanically permeabilized via shear stress (PT_p), which were used for mitochondrial respiration studies (**Fig. 2**).

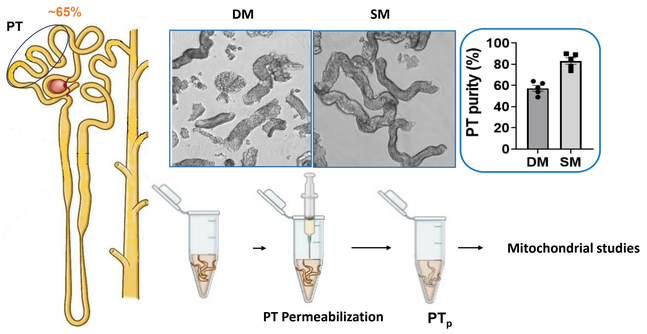

Fig. 2. **PT isolation and permeabilization for mitochondrial studies:** PT were isolated by collagenase digestion and sieving, and permeabilized via shear stress through a needle/syringe to assess mitochondrial respiration. DM: Digestion mixture, SM: Suspension mixture, PT_p: Permeabilized PT.

2. Measurement of mitochondrial respiration

- Tubular respiration was studied at low salt (LS) and at days 7,14 and 21 of the high salt (HS) diet.
- Mitochondrial O_2 consumption rates (OCR or JO_2) of the PT_p were determined at states 2, 3, & 5 when energized with substrates for complex I (pyruvate + malate (PM)) or complex II (succinate). The PT_p were stimulated in two ways: 1) a saturated dose of ADP and 2) sequentially increasing doses of ADP (**Fig. 3**). RCI and ADP/O ratio were calculated to determine the overall efficiency and coupling of mitochondrial oxidative phosphorylation (OxPhos). Data shown as mean ± SEM. *p<0.05 vs LS.
- To assess mTORC1's role in HS-induced mitochondrial dysfunction, PT respiration was compared between HS14 SS rats treated with rapamycin (1.5 mg/kg/day, i.v.) and vehicle controls.

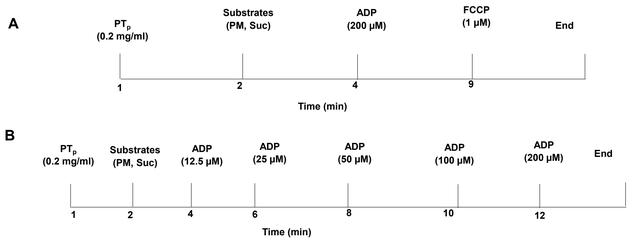

Fig. 3. Timeline experimental protocols for measuring mitochondrial JO_2 with ADP of: (A) single dose, and (B) sequentially incremental doses.

Results

1. Effect of high salt on PT_p mitochondrial bioenergetics.

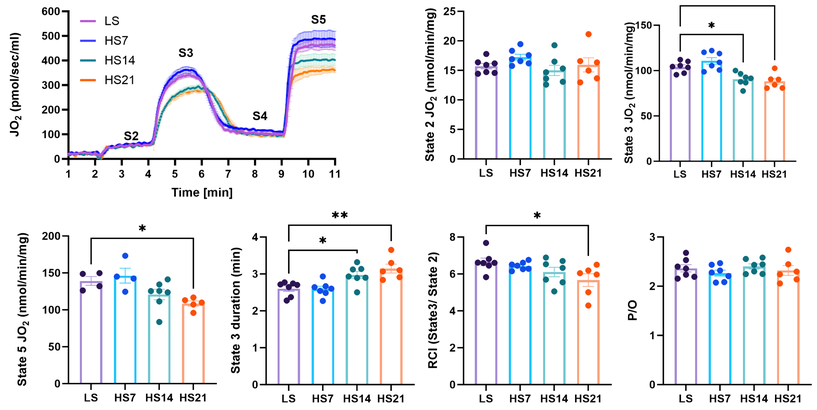

Fig. 4. JO_2 traces and states of PT_p from HS-fed SS rats, when energized with PM. Mitochondrial respiration was compromised in HS14 and HS21 (late phases of HT).

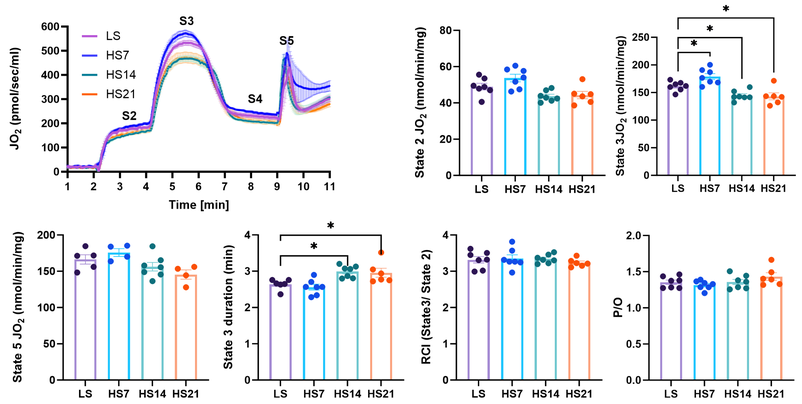

Fig. 5. JO_2 traces and states PT_p from HS-fed SS rats, when energized with Suc. Mitochondrial respiration was compromised in HS14 and HS21 (late phases of HT).

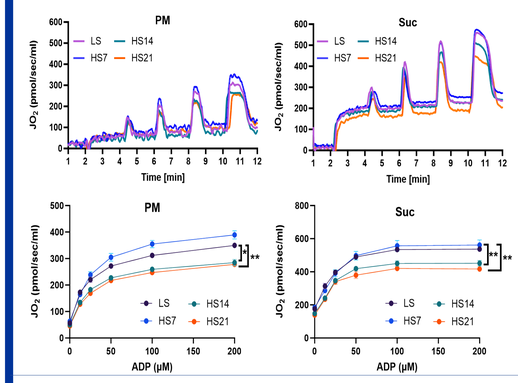

Fig. 6. Upper panel: Representative time courses of JO_2 for PT_p upon HS treatment during sequential additions of incremental ADP in the presence of PM and succinate**.** **Lower panel**: ADP-stimulated state 3 respiration for different doses of ADP in HS groups. A significant decline in S3 respiration in HS14 and 21 days is observed.

2. Role of mTORC1 on PT bioenergetics in HS fed (14 days) SS rats.

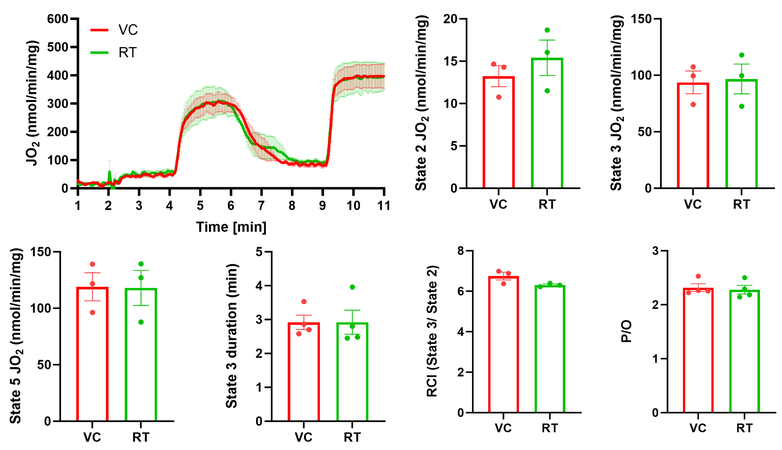

Fig. 7. OCR of PT_p from HS14 SS rats showed a slight increase in basal respiration with rapamycin (RT) vs. vehicle (VC) when using PM as substrate.

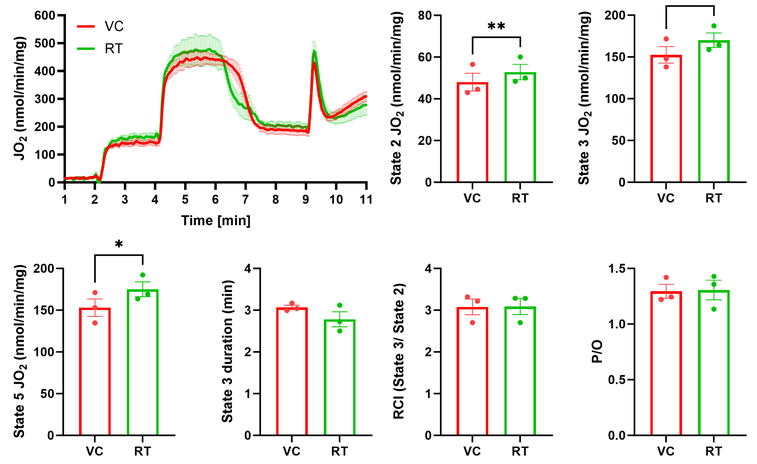

Fig. 8. OCR of PT_p from HS14 SS rats were significantly increased by rapamycin (VC) treated vs. vehicle (VC) when using succinate as substrate (*p<0.05). Both basal and maximal mitochondrial respiration were improved.

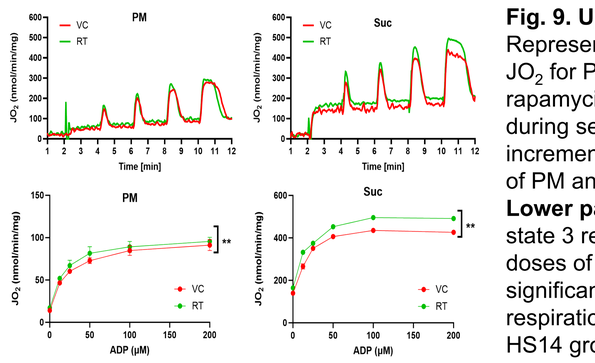

Fig. 9. Upper panel: Representative time courses of JO_2 for PT_p upon vehicle/ rapamycin treatment in HS14 during sequential additions of incremental ADP in the presence of PM and succinate**.** **Lower panel**: ADP-stimulated state 3 respiration for different doses of ADP. *p<0.05 vs VC. A significant improvement in respiration in rapamycin treated HS14 group is observed.

Summary

PT_p from HS14 and HS21 fed SS rats showed reduced ADP-stimulated OCR and prolonged S3 duration with PM and succinate, indicating impaired CI/II activity and OxPhos inefficiency during chronic HS feeding. In HS14 rapamycin treated SS rats, basal (S2) and ADP-stimulated (S3) OCRs were improved compared to vehicle treated rats, particularly with succinate, suggesting enhanced mitochondrial content and CI/CII activity due to rapamycin.

Conclusion

In HS fed SS rats, PTs maintain OxPhos efficiency for about 10 days, followed by a marked decline during late-stage of SS hypertension. Rapamycin improved PT OCR, highlighting mTORC1's key role in mediating mitochondrial dysfunction during hypertension development.

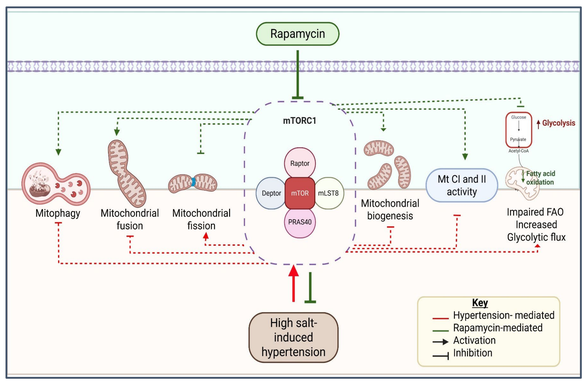

Fig. 10. We hypothesize that mTORC1 inhibition by rapamycin promotes mitochondrial biogenesis and mitophagy [2],thereby increasing healthy mitochondria, enhancing respiration, and reducing blood pressure.

References

[1] Kumar V *et al*. Hypertension. 2017;70(4):813-821. PMID: 28827472.
[2] Melser S *et al*. Biochimica et biophysica acta. 2015; *1853*(10 Pt B):2812–2821.

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