# Epigenetic modulation of renal arterioles induced by DOCA-salt loading in mice and the remission of medial hypertrophy and hypertension by inhibitors of histone acetylation.

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## Introduction and aims

- The relationship between salt intake and hypertension is known well. However, the mechanism for the onset has not been revealed enough.
- Previously, we reported that medial hypertrophy of renal arterioles induced by transient salt loading caused lasting renin elevation resulting in elevation of blood pressure in spontaneous hypertensive rat (SHR) [Oguchi et al. Hypertension 2014] (Fig1).
- Activation of matrix metalloproteinase-2 (MMP-2) in the vasculature is known to cause the medial hypertrophy, but the regulatory mechanism of MMP-2 expression has not been examined in detail.
- Last year in ERA-EDTA 52nd Congress, we reported that histone H3 and H4 acetylation in the promoter region of MMP-2 gene was enhanced during salt loading by using Deoxycorticosterone acetate (DOCA)-salt hypertension model mice (Fig2, 3).
- The present study investigated the significance of epigenetic modulation of renal arterioles in the induction of medial hypertrophy and hypertension after transient salt loading by using inhibitors of histone acetylation.

**Fig1. Medial hypertrophy of renal arterioles induced by transient salt loading**

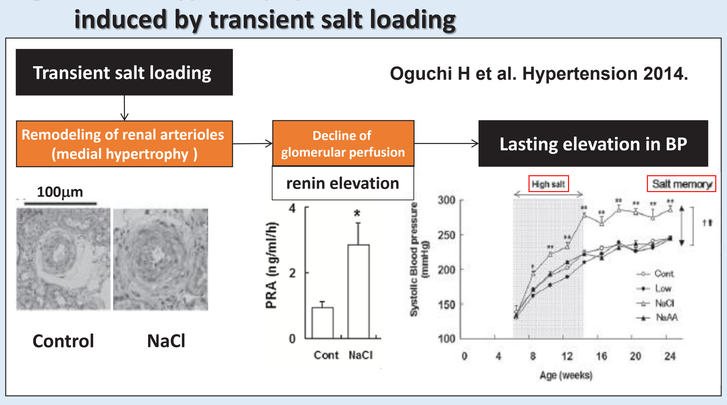



**Fig2. Methods: DOCA-salt hypertension model mice**

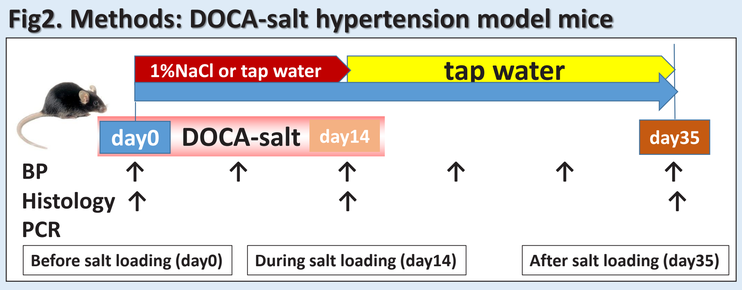
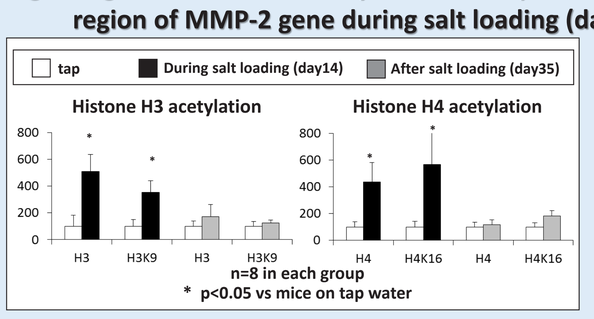



**Fig3. Augmented histone acetylation in the promoter region of MMP-2 gene during salt loading (day14)**




## Methods

- Male 8 week old C57bl6 mice were implanted DOCA pellets and given drinking water containing 1% NaCl for 2 weeks to introduce salt-induced hypertension.
- Blood pressure was measured by tail-cuff method during and after transient salt loading. Histological examinations of the kidney were performed. Gene expressions in the whole kidney and the renal arterioles were quantified using real time PCR and laser capture microdissection (LCM) (Fig2, 4).
- To investigate the significance of histone acetyltransferases (HATs) and histone deacetylases (HDACs) such as Sirts in the induction of hypertension, DOCA-salt mice were treated with the inhibitors of histone acetylation: curcumin or nicotinamide mononucleotide (NMN) during salt loading. The former is a HAT inhibitor and the latter is a Sirts activator (Fig5).

**Fig4. LCM**

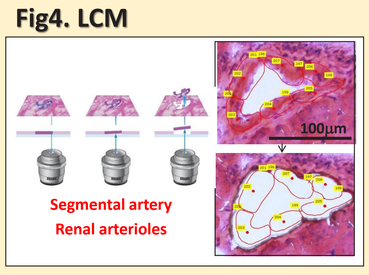
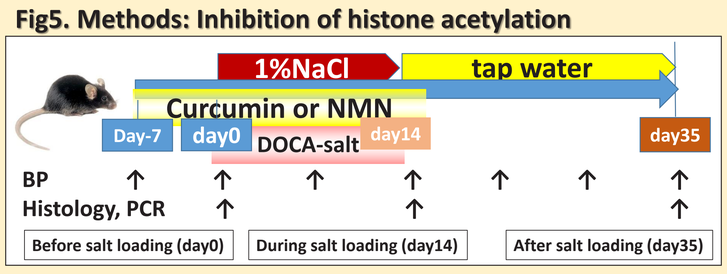



**Fig5. Methods: Inhibition of histone acetylation**




## Results

- Transient salt loading caused medial hypertrophy of renal arterioles and elevation in blood pressure during the loading period, and they remained even after stopping salt loading (Fig6).
- Immunostaining revealed that H3 and H4 acetylation in the renal tubules and arterioles were enhanced during salt loading strongly, and hyperacetylation in the renal arterioles remained even after stopping salt loading (Fig7, 8).
- Real time PCR in the whole kidney revealed that the gene expressions of CGN5, CBP and p300, which are HATs, were elevated; and conversely, those of Sirt1, Sirt3, HDAC1 and HDAC5, which are HDACs, were decreased during salt loading (Fig9).
- The expression of MMP-2 in the renal arterioles was kept elevated even after salt loading had quitted (Fig10).
- Curcumin and NMN reduced the medial hypertrophy of renal arterioles and elevation in blood pressure partially (Fig11, 12).

**Fig6. Results: DOCA-salt hypertension model mice**

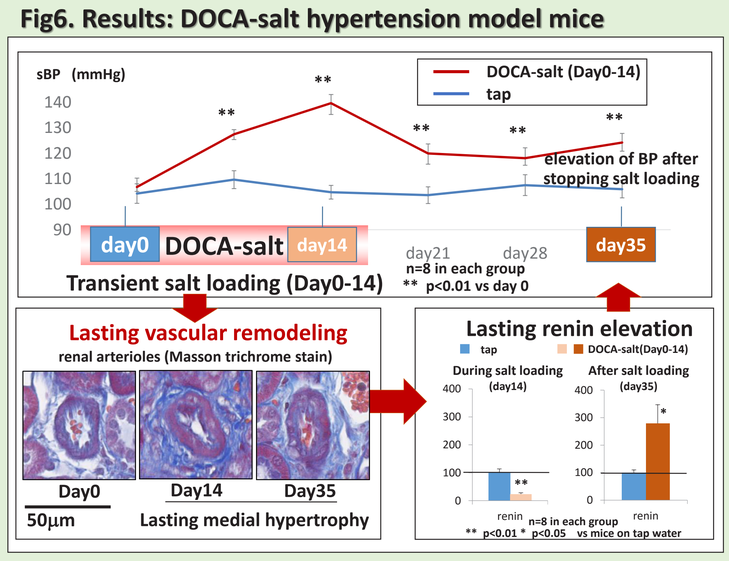



**Fig7. Immunostaining of histone H3 acetylation**

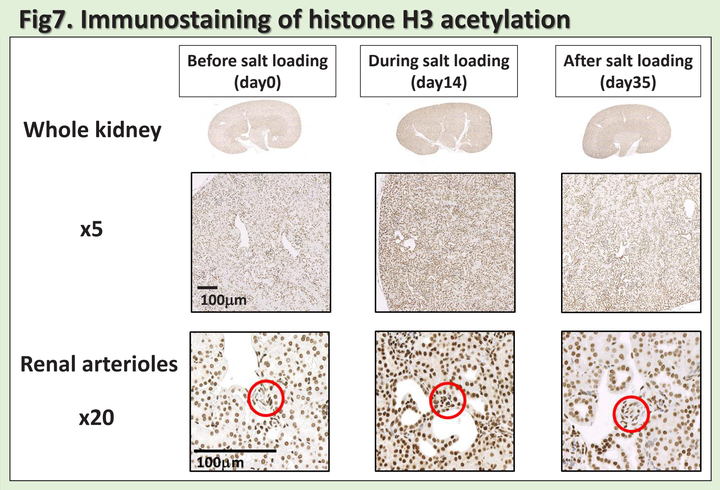



**Fig8. H3 and H4 acetylation in the tubules and arterioles**

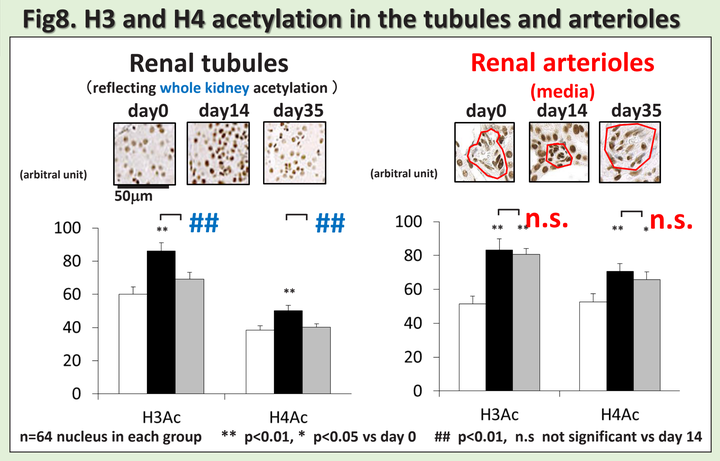



**Fig9. Gene expression in the whole kidney (real time PCR)**

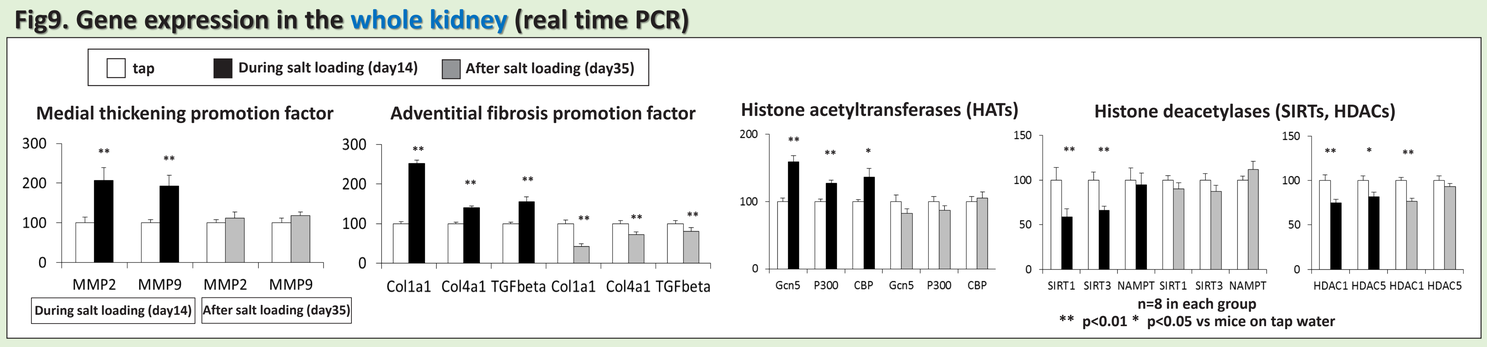



**Fig10. Gene expression in the renal arterioles (LCM)**

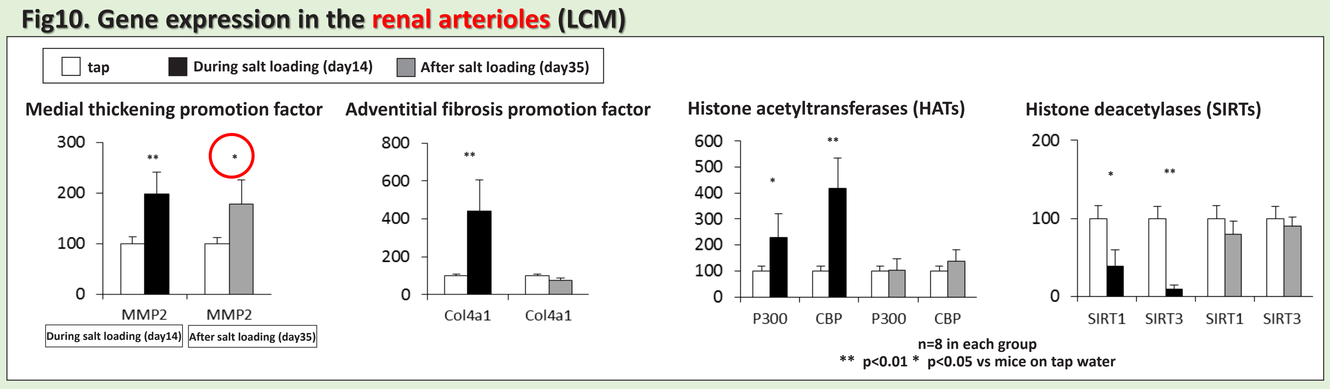



**Fig11. DOCA-salt mice treated with curcumin**

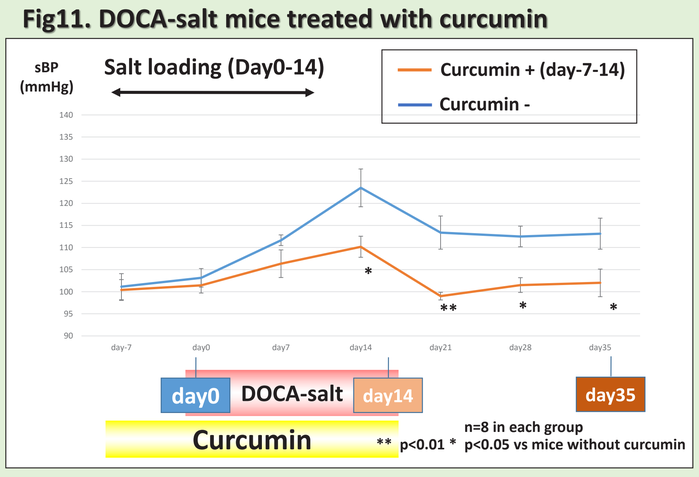
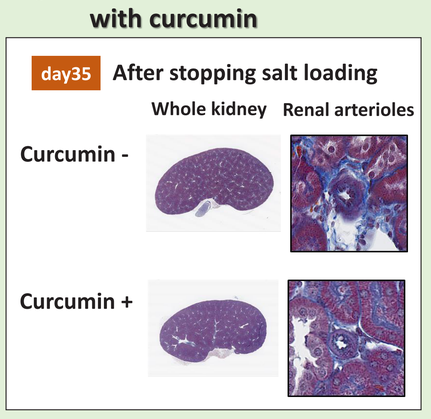



**Fig12. DOCA-salt mice treated with curcumin**




## Conclusions

- Transient salt loading caused the elevation of histone acetylation and MMP-2 gene expression during salt loading not only in the whole kidney but also in the renal arterioles. After stopping salt loading, these changes regressed in the whole kidney, but they remained in the renal arterioles.
- After stopping salt loading, the medial hypertrophy of renal arterioles and partial elevation in blood pressure remained. The lasting medial hypertrophy and renin elevation after transient salt loading along with the increase in histone acetylation and MMP-2 expression in renal arterioles were suggested to cause persistent elevation in blood pressure.
- Increased expressions of HATs and decreased expressions of HDACs during salt loading would be involved in the enhancement of histone acetylation.

**Fig13. Conclusions**

| Whole kidney | During salt (Day14) | After salt (Day35) |
|---|---|---|
| HATs | ↑↑ | → |
| Sirts, HDACs | ↓↓ | → |
| Immunostaining H3Ac,H4Ac | ↑↑ | → |
| Medial thickening promotion factor MMP2,9 | ↑↑↑ | → |
| Adventitial fibrosis promotion factor Col1a1, Col4a1 | ↑↑↑ | ↓↓ |
| H3 and H4 acetylation in the promoter region of MMP-2 | ↑ | → |
| Renin | ↓↓↓ | ↑ |

| Renal arterioles | During salt (Day14) | After salt (Day35) |
|---|---|---|
| HATs | ↑↑ | → |
| Sirts | ↓↓ | → |
| Immunostaining H3Ac,H4Ac | ↑↑ | ↑↑ |
| Medial thickening promotion factor MMP2 | ↑↑ | ↑ |
| Adventitial fibrosis promotion factor Col4a1 | ↑↑↑ | → |
| medial hypertrophy | ↑↑ | ↑↑ |
| sBP | ↑↑ | ↑ |

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