審査の結果の要旨

論文題目

Low dose N-nitro-L-arginine Methyl Ester (L-NAME) causes salt sensitive hypertension via increases in activation of Na^+-Cl^- cotransporter (NCC).

(一酸化窒素の低下はナトリウムークロール共輸送体を活性化し、食塩感受性高血圧を発症させる)

氏名 おう　そうけい

王　　聡慧

（WANG　CONGHUI）

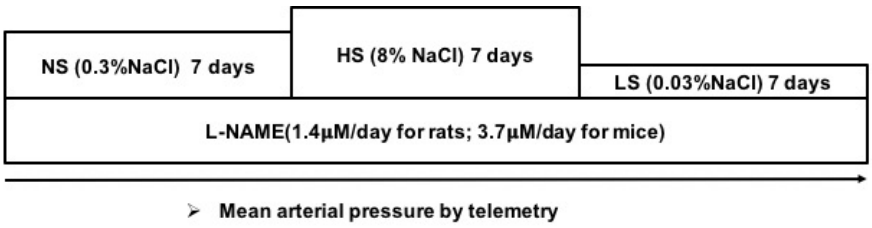
Background:

Hypertensives can be divided as salt-sensitive and salt-nonsensitive according to blood pressure response to salt intake. It has been hypothesized that renal dysfunction in salt excretion is the cause of salt-sensitive hypertension. However, recent clinical studies challenged this hypothesis and it is speculated that vascular dysfunction is the key in induction of salt sensitive hypertension. In studies with normotensives, salt-sensitive hypertension is not necessarily accompanied by an excess circulating volume and is associated with inappropriate elevation of vascular resistance.

Salt-sensitive hypertension has a high prevalence with increasing age or diabetes. It is well known that endothelial function is also impaired with aging and diabetes and nitric oxide (NO) synthesis decline. NO is not only a potent vasodilator but also possibly regulates sodium homeostasis at kidney tubules. High salt diet breaks the balance between NO and superoxide in the kidney. However, the precious mechanism is unclear. I speculated that NO plays a role in salt-sensitive hypertension. I blocked NO synthesis systemically by L-NAME and investigated the pathophysiological alterations both in vivo and in vitro study.

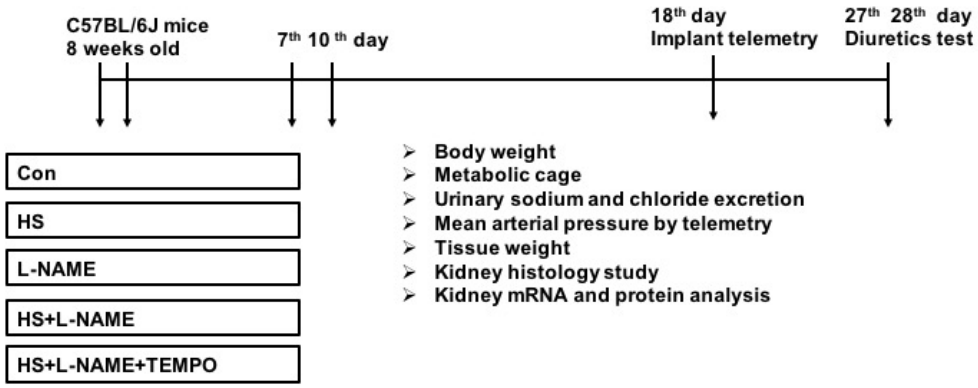
Method: We have three parts in vivo and vitro experiments.

Part 1



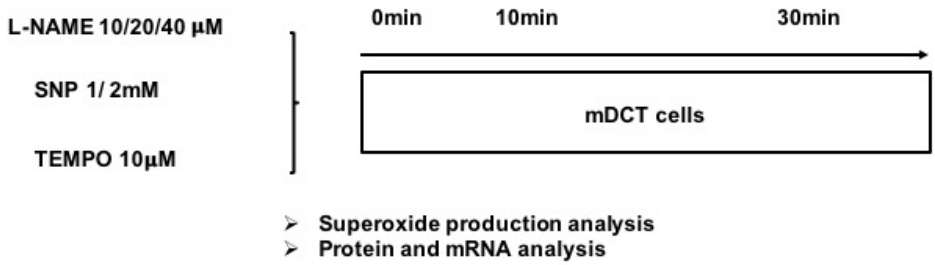
SD male Rats 10 weeks old
C57/B6j male mice 8 weeks old
NCC KO male mice 8 weeks old

Part 2



HS: high salt 8%
L-NAME: 3.7μM/day in drinking water
TEMPO: 20mg/kg/day by mini-subcutaneous-pump

Part 3



Results:

In rats, short-term dietary intervention resulted in salt-sensitive hypertension. Under normal chow, L-NAME treatment did not elevate the BP in rats. Under a high-

salt diet, the MBP was elevated significantly from 99.5 ± 4.5 to 120.5 ± 3.0 mmHg within 1 wk. After implementation of a low-salt diet, BP gradually returned to normal.

Regarding the long-term (4 weeks) effect of salt loading in mice, compatible with acute effect of salt loading. MBP did not change by this dose of L-NAME under normal diet but it significantly increased under a high-salt diet and shifted the pressure natriuresis curve toward the right. No significant morphological changes were observed in the kidney. However, significant changes of sodium and chloride excretion were observed after hydrochlorothiazide treatment but not after amiloride treatment, which was consistent with increases in p-NCC in the kidney. This highly suggested NCC, not ENaC was activated by L-NAME to cause salt-sensitivity.

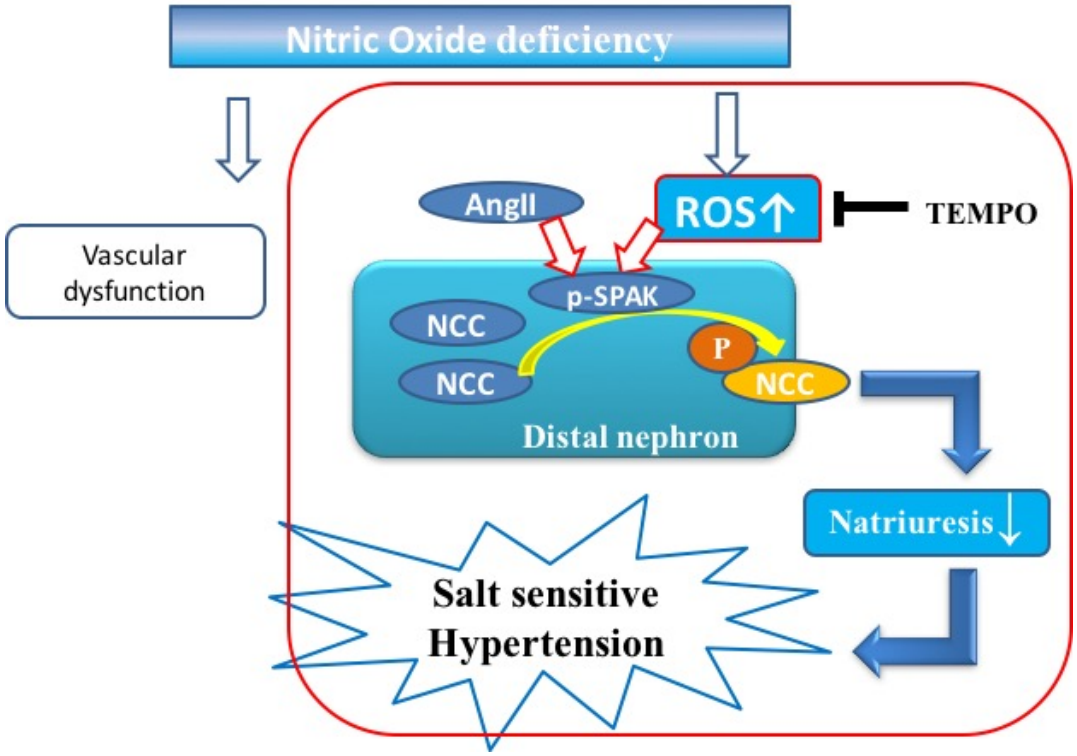
To confirm the importance of NCC, NCC knock out mice was used. L-NAME failed to induce salt-sensitivity in NCC-/- mice.

In order to exclude effect of microcirculation in the kidney in L-NAME treated rodents, I investigated L-NAME effect in vitro. Firstly, I confirmed the existence of eNO synthases mRNA in mouse distal convoluted tubule (mDCT) cells. Secondly, p-NCC expression was increased by L-NAME in mDCT cells in time and dose dependent manner. Consistent with it, NO donor—SNP decrease p-NCC in time and dose dependent manner. Thirdly, L-NAME increased oxidative stress and p-SPAK signaling, which was normalized by TEMPO, an SOD mimetic and p-SPAK inhibitor.

To confirm the role of oxidative stress, I performed the vivo experiment again. After 4 weeks' treatment, TEMPO attenuated the L-NAME-induced increases in superoxide, blood pressure and p-NCC expression in the C57BL/6J mice.

Conclusion:

Low dose L-NAME inappropriately and directly activate NCC via ROS-SPAK pathway , and finally induces salt-sensitive hypertension.



Clinical perspectives

In the present study, systemic inhibition of NO induced salt-sensitive hypertension and those data could be translated into pathophysiology of aged or diabetes who show salt-sensitive hypertension.