

NEDD4-2 Contributes to Neurogenic Hypertension Via ACE2 Internalization and Degradation

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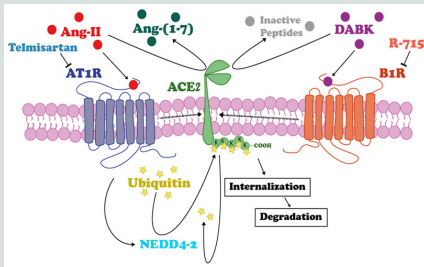


Background

- Nearly 50% of Americans have hypertension, which significantly increases risk for cardiovascular disease.¹
- Activation of bradykinin type 1 receptor (B₁R) and Ang-II type 1 receptor (AT₁R) promotes inflammation-mediated neurogenic hypertension.²
- Angiotensin-converting enzyme 2 (ACE2) is a key compensatory enzyme in the renin-angiotensin system (RAS) pathway which cleaves angiotensin (Ang)-II to Ang-(1-7).³
- ACE2 activity is impaired in hypertension, partially due to ubiquitination.⁴
- NEDD4-2 is a ubiquitinase involved in salt-sensitive hypertension and we recently demonstrated its interaction with ACE2.

Hypothesis

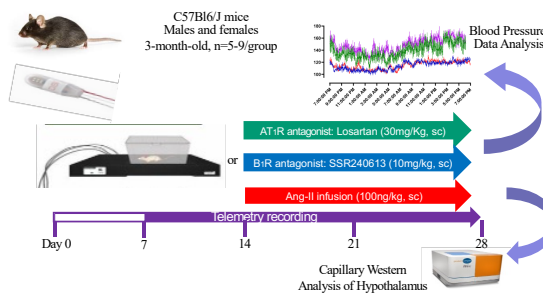
Hypertension leads to NEDD4-2 up-regulation, ACE2 ubiquitination and the loss of compensatory activity



Methods

C57BL6/J mice implanted with blood pressure (BP) probes (telemetry) and after one week of recovery, baseline BP was recorded over a 24-hour period. A subset of both male and female mice were then implanted subcutaneously with an osmotic pump containing either Ang-II (490 ng/Kg/min/4weeks) or saline. BP of all mice was recorded over a 24-hour period weekly. After 2 weeks of Ang-II infusion, the mice were implanted with an additional osmotic pump containing Losartan (30 mg/Kg/min) or SSR240612 (10 mg/Kg/min) for 2 weeks. Average mean arterial pressure (MAP) was calculated for the active and resting phases of the circadian cycle. After 4 weeks, the animals were sacrificed, and the hypothalamus was isolated from all groups. Tissue was homogenized, and protein expression for NEDD4-2, pNEDD4-2, and ACE2 was assessed using capillary Western (Jess ProteinSimple).

Methods



B₁R and AT₁R Blockers blunt Ang-II-Induced Hypertension in Male Mice

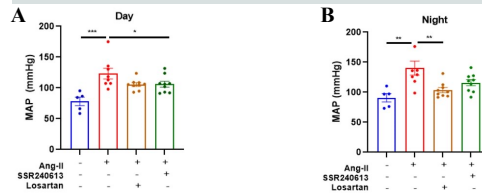


Figure 1: Hypertension, induced by Ang-II (490 ng/kg/min), was blunted in mice co-treated with Losartan (AT₁R antagonist) and SSR240612 (B₁R antagonist) in day (A) and nighttime (B). *p value<0.05, **p value<0.01 ***p value<0.001, two-way ANOVA, n=5-9/group.

Upregulation of NEDD4-2 is Associated with Downregulation of ACE2 in Hypertensive Male Mice

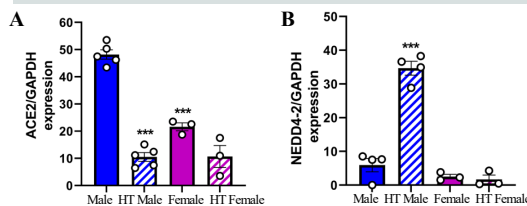


Figure 2. ACE2 is downregulated in hypertensive males and in control female mice (A). Only hypertensive males also exhibit high NEDD4-2 protein levels (B). ***p value<0.001, two-way ANOVA, n=3-4/group.

NEDD4-2 is Inhibited in Hypertensive Females

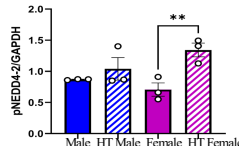


Figure 3. NEDD4-2 is inactivated by phosphorylation. We observed that hypertensive female mice show higher level of pNEDD4-2 compared to their male counterparts. **p value<0.01 two-way ANOVA, n=3/group.

Summary and Conclusion

- Our data show that Ang-II-induced hypertension was prevented by selective inhibition of AT₁R and B₁R.
- In hypertensive mice, ACE2 protein level is downregulated in the hypothalamus.
- ACE2 downregulation is associated with NEDD4-2 upregulation in hypertensive males but not in females.
- Hypertensive females exhibit higher levels of phosphorylated NEDD4-2 (inactive form) possibly contributing to a less severe salt-sensitive hypertension.

Conclusion:

NEDD4-2 plays a critical role in ACE2 ubiquitination in hypertensive male mice.

Future Directions

Further studies will investigate the mechanisms by which NEDD4-2 phosphorylation is preventing ACE2 ubiquitination in hypertension. We will investigate the role of SGK-1 in the phosphorylation of NEDD4-2 and its impact on ACE2 activity.⁵

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