Neuropilin-1 Regulates Vascular Smooth Muscle Cell Contractility and Blood Pressure

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Introduction: Neuropilin-1 (NRP1) is a transmembrane receptor present in vascular smooth muscle cells (VSMC) that binds to the Class 3 Semaphorin (SEMA) ligand SEMA3A. NRP1 mediates the inhibition of RhoA signaling, however, its role in VSMCs and blood pressure regulation is unknown. **Hypothesis:** We hypothesize that loss of NRP1 in VSMC mitigates SEMA3A-induced RhoA inhibition, thereby increasing vascular tone and blood pressure in vivo. **Methods:** To study the role of NRP1 in SMCs, we generated mice with inducible, smooth muscle cell-specific deletion of NRP1 (SM22a-Cre^{ERT2} X Nrp1$^{flox/flox}$). Following recombination using 4-hydroxy tamoxifen (SM-NRP1 KO) in male and female adult mice (8-12 weeks), systolic blood pressure (SBP) was measured using a tail cuff and compared to age- and sex-matched mice that did not receive tamoxifen (control). Aortic vascular reactivity to contractile agonists and expression of key proteins in the RhoA signaling cascade were measured using ex vivo tension myography and western blotting, respectively. **Results:** SBP was significantly increased in SM-NRP1 KO mice following recombination compared to control mice (SBP: 136.5 ±10.9 vs 112.9 ±5.6 mmHg; p=0.0006). Aortas of SM-NRP1 KO mice displayed significantly enhanced contractile response to phenylephrine, KCl, and the thromboxane agonist U44619. In support of the increased contractility, expression of total myosin light chain and LIMK-2 proteins were increased in SM-NRP1 KO compared to control aortas. In vitro, treatment of murine primary VSMC expressing NRP1 with SEMA3A decreased angiotensin II-induced Rho-GTP activation. Additionally, control and SM-NRP1 KO mice (starting at 2 weeks post-recombination) were administered angiotensin II (490 ng/kg/day) for 4 weeks. While there was no significant difference in SBP at weeks 1 and 2, SM-NRP1 KO mice had significantly lower SBP at weeks 3 and 4 following angiotensin II infusion compared to controls (Week 4 SBP: 150 ±1.4 vs 130.5 ±2.5 mmHg; p=0.02), suggesting a low ejection fraction and cardiac dysfunction in these mice. In support of this observation, gene expression of atrial natriuretic peptide was increased (p=0.06) in hearts of angiotensin II-infused SM-NRP1 KO mice. **Conclusion:** Together, our data point to the role of NRP1 as a novel regulator of basal vascular tone and blood pressure, and the loss of NRP1 promotes the onset of hypertension and exacerbates cardiac dysfunction.

Topic: Cardiovascular disease