Sympathetic denervation and vascular smooth muscle cell phenotype: implications for vascular therapies

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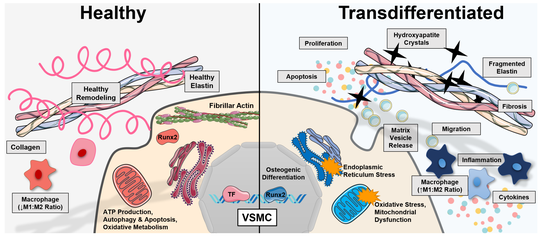
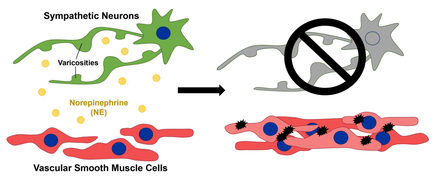
Introduction

- Healthy arteries are innervated by the sympathetic nervous system to not only regulate vascular smooth muscle cell (**VSMC**) contractility and tension, but also to regulate arterial maturation and structure. [1]
- Tissue innervation is a critical component for successful regeneration in any transplanted tissue. [2] However, little is known regarding vascular remodeling due to sympathetic nerve degeneration or injury. One potential consequence of sympathetic denervation is VSMC transdifferentiation.
- VSMCs can switch from a contractile phenotype to a synthetic (proliferative) [3] or osteo-chondrogenic (bone-like) [4] phenotype. Proliferation and migration can thicken the vessel and restrict blood flow, while osteogenic cells can deposit hydroxyapatite crystals into the artery wall.



Goal: Elucidate the relationship between sympathetic innervation and vascular pathogenesis by creating a novel mouse model of arterial denervation and by probing cellular responses to neuronal signals.

HYPOTHESIS: Sympathetic denervation of the femoral artery will lead to transdifferentiation of arterial VSMCs and pathological remodeling of the arterial structure while nerve-like signaling to VSMCs will promote contractile phenotypes



Methods

Animals: BALB/c mice, half female (approved by Northwestern IACUC)

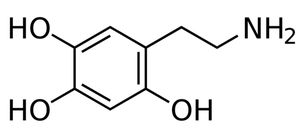
Procedure:
- 6-OHDA subcutaneously injected on a weekly basis near femoral artery for four weeks (n=9) with buffer vehicle solution injection in contralateral limb as control

Analysis:
- Blood flow with Laser Doppler Imaging
- Innervation and extracellular matrix (**ECM**) remodeling studied with histology and immunohistochemistry

Cell Work: Human aortic smooth muscle cells

Treatment:
- Growth media, norepinephrine (**NE**), phenylephrine (**PE**), or differentiation media for 1-7 days

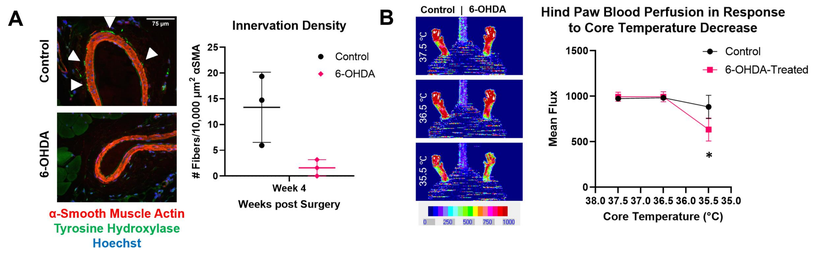
Analysis:
- Viability, morphology, expression of phenotype-specific markers



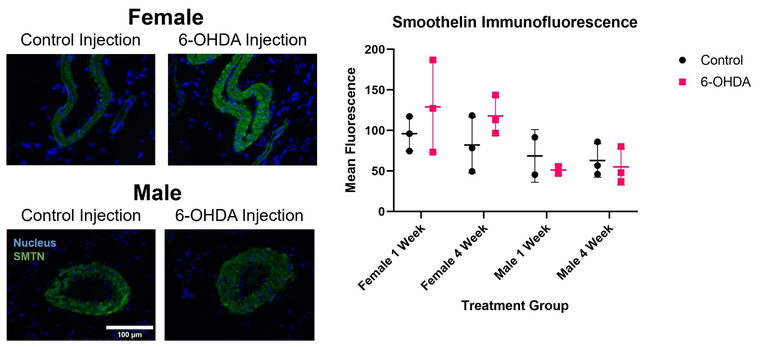
6-hydroxydopamine

Results

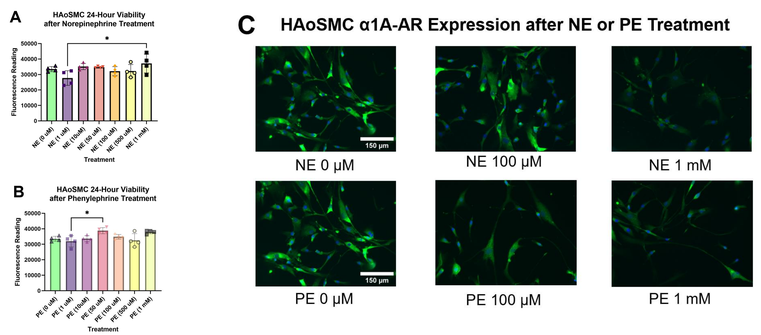
1. Weekly injection of 6-OHDA leads to sustained denervation of the treated limb without affecting the control limb (A). Male 6-OHDA-treated limbs show a change in hemodynamics by lower blood perfusion at low temperature (B).



2. Repeated subcutaneous injection of 6-OHDA for 4 weeks impacts expression of contractile marker smoothelin in the medial layer in a sex-specific manner in pilot studies.

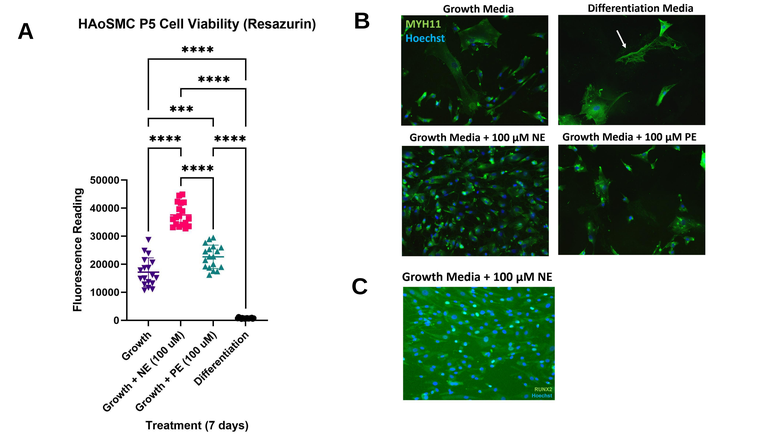


3. Vascular smooth muscle cells remain viable in **NE** and **PE** up to 1 mM (A-B). Cells downregulate α_{1A}-adrenergic receptors in response to high levels of **NE** or **PE** (C).



Results

4. Vascular smooth muscle cells demonstrate increased proliferation in response to high levels of **NE** and **PE** (A). Cells maintain synthetic phenotypes, shown by lack of organized myosin heavy chain (MYH11) staining (B), with **NE** treatment leading to early-stage osteogenic marker (RUNX2) expression (C).



Conclusions

1. **Denervation**: Local, prolonged denervation of the femoral artery is possible by repeated subcutaneous injection with 6-OHDA.
2. **Function**: Arterial denervation causes changes in hemodynamics in response to core temperature changes in male mice.
3. **Remodeling**: Femoral denervation in healthy mice leads to changes in cell phenotype with possible sex-dependence currently under investigation.
4. **Mechanism**: Vascular smooth muscle cell proliferation is increased after high **NE** or **PE** treatment, and cell morphology and protein expression indicate cells do not regain contractile phenotypes.

IMPACT: If sympathetic denervation or hyper-innervation causes VSMC transdifferentiation and pathological remodeling, nerve regeneration or stimulation strategies may be viable targets for therapeutic intervention.

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