Effect of 5-HTR2a Inhibition on Embryonic Valve Biomechanical Remodeling

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I. Compaction

Compaction is an important process cells utilize to remodel their microenvironment. Stimulated cells apply contractile forces on the surrounding matrix which alters the overall tissue density and may serve as a mechanism for modifying material properties.

- Treated HH25 AV cushions with 10, 100, & 1000nM MDL100,907
- Cushion cross-sectional area measured initially and after 24 hrs.
- Compaction defined as the ratio , A/f = Area Final / Area Initial

II. Micro-Mechanical Testing

The material behavior of the embryonic cushions is regulated by numerous factors including genetic, biochemical, and mechanical stimuli. To identify the effect of TGFβ3 and 5-HT on cushion mechanics we performed micro-mechanical testing via the pipette aspiration technique.

- Vacuum pressure applied to the cushion draws tissue into micropipette (diameter ~70μm)
- Deformation is measured as the aspirated length, L.
- Stress-Strain data is analyzed to calculate the Effective Modulus, or stiffness, of the cushions following 24 hr. treatment.

III. RT-PCR Analysis

Cells routinely generate mRNA instructions for protein production to maintain homeostasis, but also in response to stimuli. RT-PCR is a technique that quantifies mRNA content of particular gene of interest. The mechanically relevant genes that have been investigated in the broader study are listed in Fig 3D. The focus of this analysis was to determine possible cross-talk mechanisms between the TGFβ3 and 5-HT pathways. We designed primers for the 2a & 2c 5-HT to see if our treatment conditions enhanced or diminished these avenues for 5-HT consumption. Primer design was facilitated by NCBI online resources, such as Primer3, Blast, and ensemble.

- Inhibition of 5-HTR2a does not effect cushion compaction
- MDL100,907 does not alter low compaction behavior of TGF-β3 treated cushions

Motivation

- TGFβ2 KO mice have hyperplastic AV valves1 (See below)
- Cardiac defects occur in 5-HTR2b(serotonin receptor 2b) KO2
- Serotonin(5-HT) up-regulates TGFβ1 in porcine AV/ICs3
- TGFβ & serotonin both increase 4-hydroxyproline uptake
- Aortic valve mechanical properties are sensitive to 5-HT administration5

References


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Curriculum Plans

- Adopt a Disease Project: Students study a specific disease over the course of the year
- Ex-ovo Chick Culture Project
- Bioethics Seminar

I. Introduction

Appropriately controlled transforming growth factor-beta (TGF-β) signaling is critically important for normal valvular formation and remodeling in the heart. Recent studies have shown that serotonin (5-HT) up-regulates TGF-β and collagen production in cultured aortic valve cells suggesting a possible interaction effect between them. Of particular interest is the 5-HT2 family of serotonin receptors which are considered the more mechanosensitive of the 5-HT receptors. Here we investigate the effect of 5-HT2a inhibition on HH25 AV cushion mechanics, tissue compaction, and mRNA production of HH25 heart valve cushions from developing chick embryos.

Defective heart valves manifest aberrant geometry as well as a perturbation from normal tissue mechanics. The interplay of tissue stiffness, mechanical loading, and the biochemical environment is a complex system of stimulation and feedback which directs parts of valvulogenesis. Though not well understood, studies such as the present one provide important pieces to the puzzle, namely the relationship between TGFβ3 and 5-HT in altering mechanics.

Therapeutic opportunities resulting from this research include potentiating TGFβ3 signaling with specific 5-HT receptor inhibitors to correct mechanical properties of defective tissue during development, or in certain disease states with a similar TGFβ3/5-HT interaction phenomenon.