Measuring the Stiffness of Embryonic Avian Myocardium Using Micropipette Aspiration Technique

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Abstract:

Mechanical forces are essential to cardiac formation. The mechanosensitive (MS) ion channel, Piezo1, responds to different mechanical forces and converts them into intracellular signals. Yoda1 is a synthetic drug that serves as a specific agonist for the Piezo1 ion channel by binding to it, causing conformational changes that open the channel. Congenital heart defects (CHD) occur when cardiac morphogenetic processes are disrupted. CHDs affect approximately 2% of infants and are the primary cause of death in children under one year of age. CHD are the largest class of birth defects and account for 25% of all human congenital abnormalities. Chick embryos were used as a research model due to ease of microsurgical accessibility in the egg, and developmental similarities with human embryos, including a four-chamber heart. In this experiment, chick embryos were windowed and injected with Yoda1 to investigate the mechanical properties of the myocardium. We hypothesized that higher concentrations of Yoda1 would stiffen the myocardium. Our findings show that Yoda1 stiffened the myocardium and thus successfully stimulated the Piezo1 ion channels in the cardiomyocytes.

Summary of Research:

Cardiac morphogenesis varies across species but can be broken down into four key steps across vertebrates: heart tube formation, looping, trabeculation, and valve formation/septation. At the beginning stages of cardiogenesis, the cardiac wall is composed of an inner endocardial layer and outer myocardial sheath. Between these two layers is the cardiac jelly. As shown in Figure 1, when the heart tube extends and elongates, looping occurs and will promote growth of the atria and atrial septum. In cardiac trabeculation, endocardial extensions grow toward the myocardial layer, giving rise to trabeculae. Septation of the atrium and ventricles transpires when trabeculae compact and endothelial cells (ECs) fuse.

The myocardium is responsible for contractility of the heart and pumping action. The cardiac muscle must contract with the necessary force so enough blood can supply the metabolic demands of the body. Yoda1 caused an increase in Piezo1 activity which in turn made the myocardium more sensitive to mechanical loads.

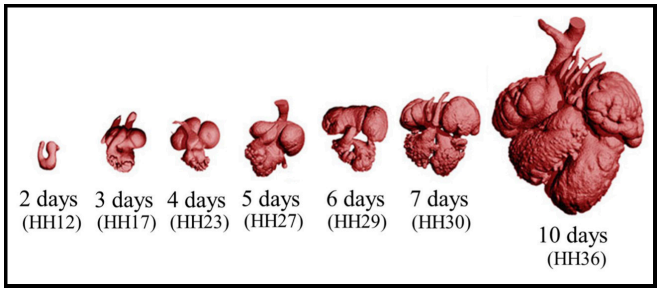

Figure 1: Images of chick embryo heart throughout development.

In the study, chick embryos were incubated until they reach HH 27. The eggs were then windowed in ovo and the eggshell membrane was removed to expose the chorioallantoic membrane (CAM). A pulled glass pipette needle was secured to a micromanipulator. Demonstrated in Figure 2, embryos were injected into the vitelline vein of the vasculature so the solution flowed directly to the heart.

Twelve embryos for three of the four HH stages used in this study (HH 31, HH 36, HH 38) were injected with Yoda1 and Hanks' Balanced Salt Solution (HBSS). For the three HH stages, three embryos were injected with a concentration of 5 μmol of Yoda1, three embryos were injected with a concentration of 10 μmol of Yoda1, three embryos were injected with a concentration of 15 μmol of Yoda1, finally, three embryos were injected with HBSS. All chick embryos were windowed and injected on HH 27. The other HH stages are the days the embryo's hearts were removed and measured. The twelve embryos whose hearts were removed in the fourth group, HH 27, received neither saline nor Yoda1 injections.

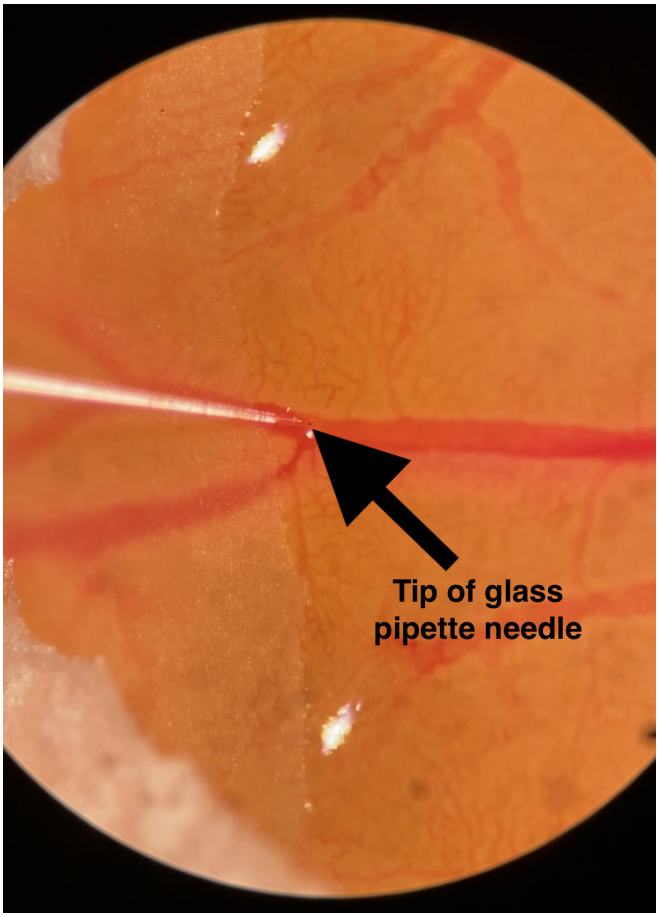

Figure 2: The needle is inserted in a vein, where blood flows toward the heart.

During the chick embryo heart removal, a portion of the left ventricle (LV), right ventricle (RV), and interventricular septum were removed for micropipette aspiration (MPA). MPA applies a controlled vacuum pressure and oversees tissue displacement within the tip of the pipette. MPA was used to determine the stiffness of the myocardium and how saline and different concentrations of Yoda1 influenced the stiffness of the myocardium. The ventricle and septum samples were secured to a polydimethylsiloxane (PDMS) Petri® dish for measurement. The PDMS was placed on the stage of a stereo microscope under a magnification of 150x. Before the tissue is measured it is first "massaged" to relax the cardiac muscle and ensure it is in contact with the pipette tip. Once massaged, a vacuum pressure was applied in increments of 2 μl, 2 μl, 3 μl, 3 μl, 4 μl, 4 μl, 5 μl, 5 μl, 6 μl, 6 μl, 7 μl, 7 μl, 8 μl, 8 μl to the myocardium surface in the interior of the pipette. The aspirated tissue was then imaged and measured in the research application, ImageJ, using a scale bar.

Conclusions and Future Steps:

Results from MPA showed that the sampled heart tissue injected with Yoda1 had a stiffer myocardium than the sampled heart tissue injected with HBSS. Figure 3 shows the formula our experimental values were fitted to.

$$\sigma_{yy} = \alpha C \exp\left[\alpha\left(\lambda^2 + \frac{2}{\lambda} - 3\right)\right]\left(\lambda^2 - \frac{1}{\lambda}\right)$$

Figure 3: Change pressure is represented by σ_{yy} and α and C are the variables that are changed to fit the measured data.

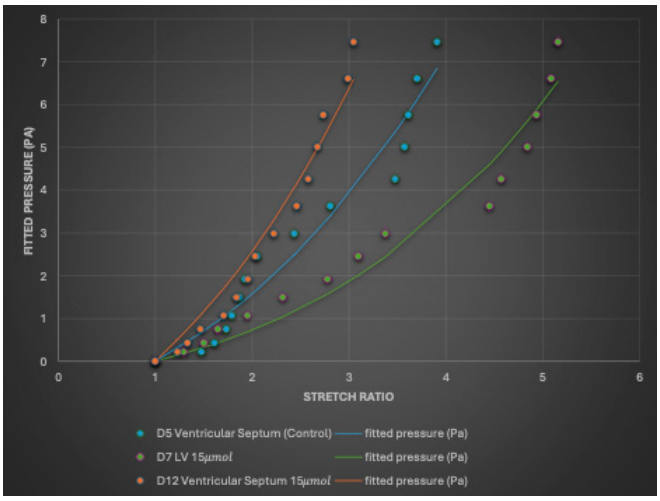

Figure 4: Stretch ratio always begins at "1" and fitted pressure always starts at "0" in this experiment.

In Figure 4, HH 38 had a steeper experimental curve compared to the HH 27 control group. A steeper value indicated a stiffer myocardium. In this experiment, over 60% of chick embryos died in this experiment, including all HH 36 embryos. Survival Rate (SR) of embryos was between 30% and 40%. For future studies, we want to improve the SR of chick embryos. Controlling contamination and ensuring embryos do not bleed out during injections is a possible solution to improving SR.

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