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The Role of Notch Signaling on Heart Rate and Atrial Conduction

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Abstract

Heart disease is the leading cause of death worldwide and can result in arrhythmias, or dysregulation in the electrical activation of the heart. Sick Sinus Syndrome (SSS) is characterized by sinus bradycardia (slowed heart rate, HR), slowed conduction through atrial myocardium, and can predispose to the development of atrial fibrillation. A developmental signaling pathway, Notch, regulates cellular identity through differentiation of cardiomyocytes (CMs) into cardiac conduction system-like cells. Previous data show that Notch electrically remodels the right atrium, causing slowed conduction velocity (CV) and hallmarks of SSS including sinus pauses, sinus bradycardia and a predisposition to atrial fibrillation. However, the molecular mechanisms behind these phenotypes are not known. We hypothesized that Notch activation produces slowed CV through downregulation of major cardiac voltage-gated sodium channel ($Na_V1.5$) and atrial gap junction (Connexin40, Cx40). A "Tet-On" doxycycline-activated system using transgenic adult mice was used to activate Notch specifically in CMs. We assayed various determinants of CV, including fibrosis, cellular hypertrophy, and Na^+ channel and gap junction expression. Trichrome stain and hydroxyproline assay indicated normal levels of non-conductive fibroblasts. To determine whether Notch activation is associated with pathophysiological hypertrophy, I quantified cell area using immunohistochemistry and found no difference in Notch activated hearts when compared with controls. Furthermore, immunohistochemistry indicated no gross changes in $Na_V1.5$ or Cx40 expression within the atrial myocardium. However, localization of $Na_V1.5$ and Cx40 within the plasma membranes of CMs, as well as post-translational modifications that may result in slowed conduction velocity are yet to be analyzed. Future studies will determine whether Notch-induced slowed HR is due to autonomous changes within the pace-making sinus node (SAN) region or non-autonomous changes within the atrial myocardium. Notch will be activated specifically in the SAN of the adult mouse heart using an HCN4-creER tamoxifen-inducible system and HR will be evaluated using electrocardiograms.

Hypothesis: Notch induces electrophysiological changes through ion channel and gap junction expression, without inducing morphological changes.

Notch Signaling Pathway

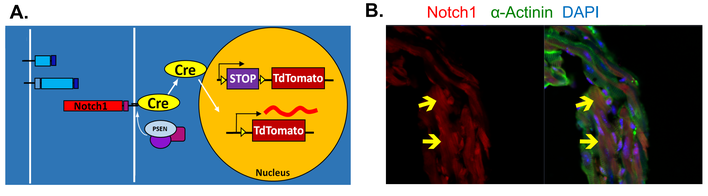

Figure 1: **Notch is activated during development.** A) Genetic model of N1IP::CreHI;R26R^{tdtom} mouse (Liu et al., 2015). After an agonist binds to the Notch1 ligand-gated transmembrane protein, the intracellular domain, Cre, cleaves the stop codon, allowing expression of the red fluorescent protein, tdtomato, during development. B) From Confocal microscopy, overlap of CM-specific alpha-actinin and tdtomato confirms Notch activation within CMs.

Objective and Methods

Studies have shown that cardiac injury to the adult mouse heart electrically remodels the right atrium to induce symptoms resembling SSS.

Aim 1: Determine the effect of Notch signaling on heart rate.
- Determine whether Notch signaling affects heart rate through non-autonomous effects on the right atrium by performing ECGs.
- Determine whether Notch signaling affects heart rate through autonomous effects on the SAN by performing ECGs.

Aim 2: Evaluate the effect of Notch on the morphological determinants of conduction velocity.
- Determine the changes in ion channel and gap junction expression in the SAN and atrial cardiomyocytes through immunostaining.
- Determine the amount of fibrosis through trichrome staining and hydroxyproline quantification.
- Determine if Notch induces pathophysiological hypertrophy by quantifying cell area.

Model of Non-autonomous Cardiomyocyte Notch Activation

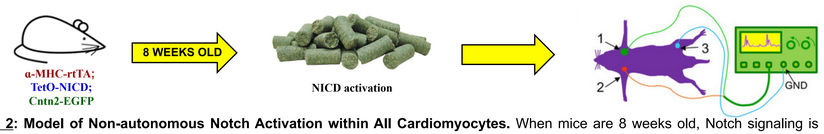

Figure 2: **Model of Non-autonomous Notch Activation within All Cardiomyocytes.** When mice are 8 weeks old, Notch signaling is activated non-autonomously in CMs by exposing mice to doxycycline (Dox) food. The genotype of the mice is a-MHC-rtTA;TetO-NICD. This genotype utilizes the Tetracycline-on system in which the Notch intracellular domain (NICD) in activated specifically in CMs (driven by the alpha-myosin heavy chain promoter) upon doxycycline induction.

Preliminary Data: Heart Rate and Conduction Velocity Slow Upon Non-autonomous Notch Activation

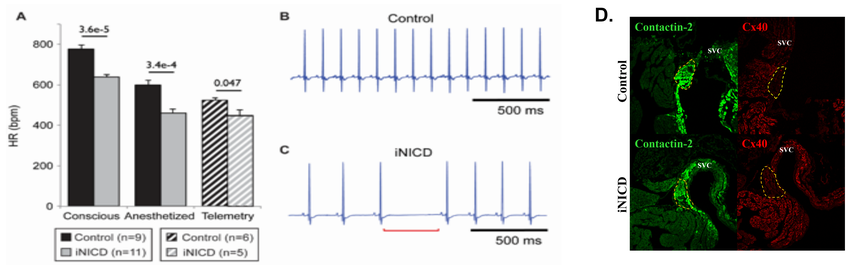

Figure 3: **Non-autonomous Notch activation within cardiomyocytes causes decreased heart rate.** A) 8 week-old mice had Notch activated for 3 weeks and Electrocardiogram (ECG) recordings were collected three different ways. The heart rate significantly decreased in iNICD mice compared to controls in conscious, anesthetized, and Langendorff perfused hearts. B) Representative telemetry recordings from a control (top, B) and iNICD (bottom, C) mouse. iNICD mice exhibit bradycardia along with sinus pause (red line, C). D) *Cx40* and *Cntn2* immunostaining reveals no unusual SAN morphology.

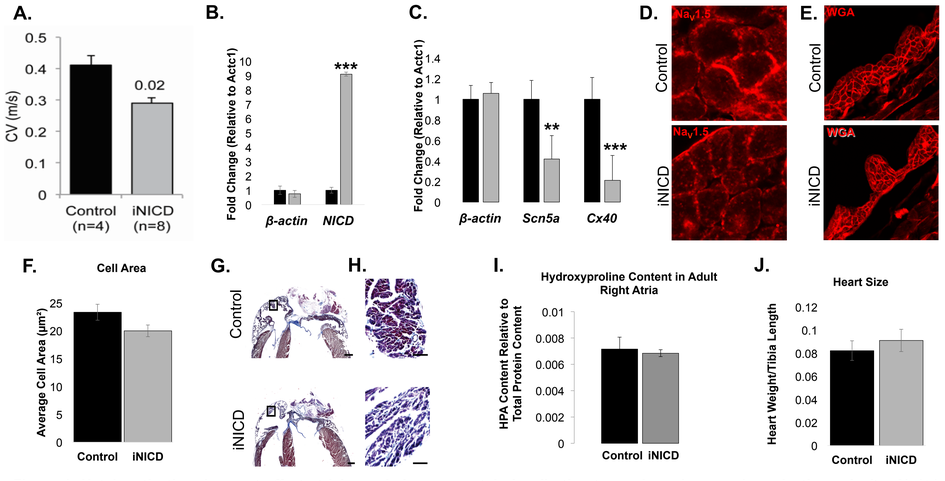

Figure 4: **Notch activation does not affect atrial morphology or protein localization to produce decreased conduction velocity. A)** Atrial conduction velocity is significantly decreased as a result of CM-specific Notch activation at 8 weeks of age. **B-C**) Gene expression in the right atrium of mice exposed to dox chow for 3 weeks. **B**) *NICD* is significantly upregulated in iNICD RA. **C**) Important determinants in conduction velocity, *Scn5a* and *Cx40*, are significantly downregulated in iNICD RA. **D**) Immunostaining shows proper localization of $Na_V1.5$ in plasma membranes of control and iNICD mice. **E**) Wheat-germ agglutinin (WGA) staining was performed and the area of approximately 100 cells perpendicular to the plane of sectioning were outlined and quantified using Axiovision. Only the circumference of circular cardiomyocytes were included in the analysis, and elongated cells were excluded. **F**) Quantification of cell area was performed in 3 distinct regions of the right atria data from all 3 regions were pooled for comparison. There was no statistical difference in CM size. **G, H**) Trichrome stain and hydroxyproline quantification indicates similar levels of fibrosis in controls and iNICD mice. **G**) Trichrome images of control (top) and iNICD (bottom) hearts. There are no gross changes in RA size. **H**) Magnified image of the right atrial myocardium from the boxed region in panel **G**. There is no difference in fibrosis in iNICD vs. control RA. **I**) Hydroxyproline content, an indicator of fibrosis, is not different between iNICD and control mice. **J**) Total heart size is no different in iNICD mice vs. controls.

Model of Autonomous SAN Notch Activation

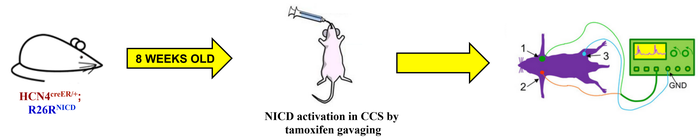

Figure 5: **Model of Autonomous Notch Activation in SAN.** When mice are 8 weeks old, Notch signaling is activated autonomously by exposing mice to tamoxifen. The genotype of the mice is HCN4$^{creEr/+;}$ R26R$^{NICD/+}$. This genotype utilizes the HCN4-creER tamoxifen inducible system in which the Notch intracellular domain (NICD) in activated specifically in CCS cells by expressing the hyperpolarization-activated cyclic nucleotide 4 gene upon tamoxifen gavaging. Cntn2-EGFP immunostaining allows easy visualization of conduction system cells for electrophysiological and morphological studies.

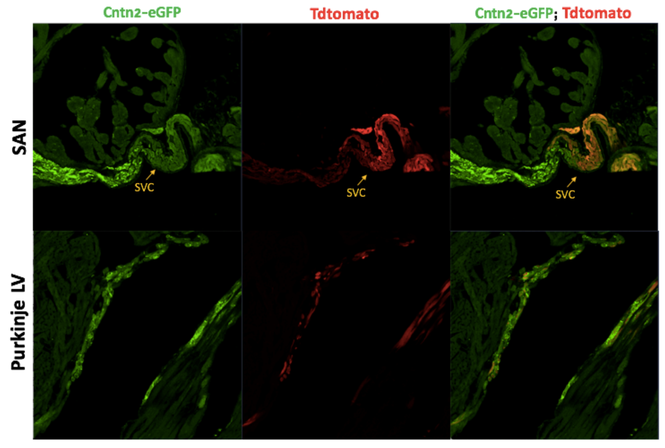

Figure 6: **Model of Autonomous Notch Activation within the Sinoatrial Node.** When mice are 8 weeks old, Notch signaling is conditionally activated specifically within the SAN by exposing HCN4$^{creEr/+;}$ R26R$^{tdtomato/+}$ mice to tamoxifen. This genotype utilizes a tamoxifen inducible system in which the Notch intracellular domain (NICD) is driven by the hyperpolarization-activated cyclic nucleotide 4 (Hcn4) promoter, and therefore specifically activates in cells which express Hcn4, including the SAN. Cntn2-EGFP immunostaining allows easy visualization of conduction system cells for electrophysiological and morphological studies.

Ongoing Plans & Future Directions

1. Investigate how different durations of Notch signaling activation may differentially affect electrical remodeling of the right atrium.
2. Investigate how autonomous Notch activation in CCS may decrease HR.
3. Investigate how different types of cardiac injury may differentially induce Notch activation in cardiomyocytes (MI, Transverse Aortic Constriction, Ischemia Reperfusion)
4. Investigate whether Notch signaling is working through other signaling pathways (such as Wnt signaling) to promote arrhythmogenesis.

Acknowledgements

I would like to thank the Developmental Biology Histology & Microscopy Core at Washington University in St. Louis for help with slicing hearts for histology experiments. I would also like to thank Ornitz and Skeath labs for lending equipment for slicing, and allowing use of the confocal.

Elucidating the Role of Notch Signaling Activation in Atrial Arrhythmogenesis

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Abstract

Background: Heart disease is the leading and most costly cause of death in the United States as well as the world and this statistic has not changed in over a decade.[1-3] About 50% of all cardiac-related deaths are due to sudden cardiac death (SCD).[4] Fatal arrhythmias, which result from electrical dysregulation, are often the cause of SCD. Despite the major contribution of arrhythmias to mortality rates, there is very little known about the mechanism(s) of arrhythmogenesis. One of the major risk factors for SCD is myocardial infarction (MI).[5] Notch signaling, a developmental signaling pathway important in cell processes including proliferation and differentiation, is upregulated following cardiac injury such as myocardial infarction (MI) in the cardiomyocytes (CMs) of the adult mouse and adult zebrafish heart.[1,6–8] Notch signaling has the capability of converting a ventricular myocyte to a Purkinje-like phenotype by altering the electrical program of the cell when overexpressed during development.[9] Therefore, it is also possible that Notch activation after cardiac injury in the adult is an important contributor to the development of cardiac arrhythmias through electrical remodeling of CMs. This may explain why individuals who undergo cardiac injury are subsequently more likely to experience cardiac arrhythmias. Little is known about the role of Notch in the regulation of ion channels in atrial myocytes. Atrial myocytes are an often overlooked, yet important cell type to investigate because one of the most common types of arrhythmias is atrial fibrillation.

Hypothesis: Notch activation regulates electrical remodeling of adult right atrial cardiomyocytes and this remodeling may be involved in the progression of arrhythmias after cardiac injury.

Preliminary Data

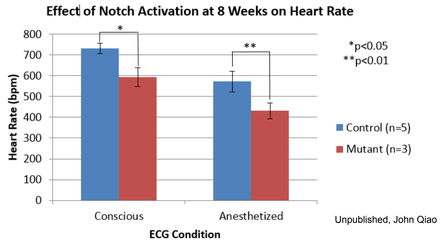


Figure 1: Notch activation causes sinus bradycardia. 8 week-old mice had Notch activated and Electrocardiogram (ECG) recordings were collected two different ways 3 weeks later. The heart rate significantly decreased in mutant mice compared to controls in both conscious and anesthetized ECGs.

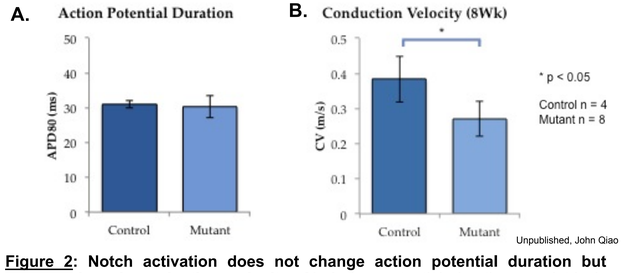


Figure 2: Notch activation does not change action potential duration but causes slowed conduction velocity. A) Action potential duration, an intrinsic myocyte property, is not significantly increased as a result of CM-specific Notch activation at 8 weeks. **B)** Atrial conduction velocity is significantly decreased as a result of CM-specific Notch activation at 8 weeks.

Electrophysiology of Cardiac Cells

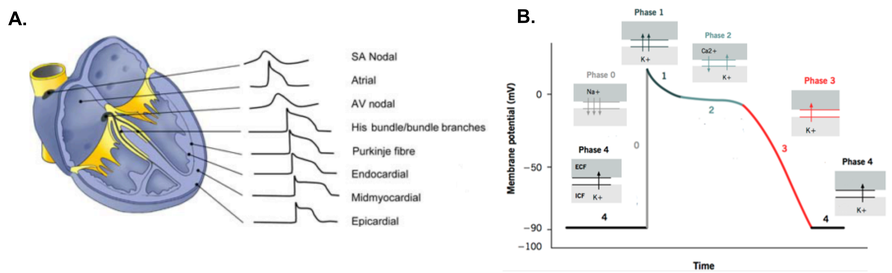


Figure 3: A.) Action potential waveforms vary based on location of cell in the heart.[11] B.) Action potential of a typical ventricular cardiomyocyte. Numbers on the action potential waveform represent the phase of the action potential. Action potential phases are characterized by a unique combination open and closed ion channels, and therefore a difference currents.

Aims & Methods

Specific Aim 1: To investigate the mechanism for Notch-induced sinus bradycardia and electrical remodeling of atrial cardiomyocytes

Subaim 1.1: Determine whether Notch activation is causing morphological changes to the sinoatrial node

- Histological Staining
  - SAN: Cntn2+;Cx40-

Subaim 1.2: Determine whether Notch activation is acting cell autonomously on sinoatrial nodal cells, or indirectly through effects on right atrial cardiomyocytes, to cause sinus bradycardia

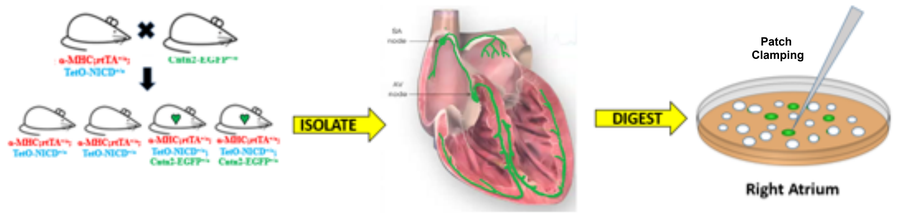
- Single cell electrophysiology-current clamp
  - Resting membrane potentials
  - Action potential waveforms

Subaim 1.3: Determine which ionic currents are regulated by Notch in atrial cardiomyocytes

- Single cell electrophysiology-voltage clamp
  - Ion channels (i.e. Voltage-gated Na+, voltage-gated K+)

Subaim 1.4: Investigate the transcription effects of Notch activation in atrial myocardium using qPCR

- Ion channels (i.e. Scn5a)
- Gap junctions (i.e. Cx40)



Model of Arrhythmogenesis

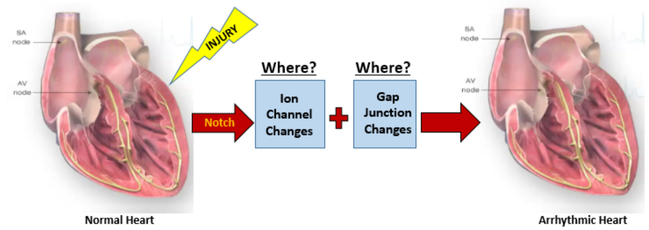


Figure 4: **Our current model of arrhythmogenesis**. Notch activation after cardiac injury alters expression of ion channels and gap junctions in cardiomyocytes, promoting the onset of arrhythmias. The specific tissue sites of Notch activation after different cardiac injuries are yet to be determined.

Ongoing Plans & Future Directions

1. Investigate the duration of Notch activation necessary to induce arrhythmogenesis and electrical remodeling of the right atrium (ongoing)
2. Investigate whether “rescuing” the effect of Notch activation at 8 weeks of age by using Notch inhibitors prevents arrhythmogenesis and electrical remodeling of the right atrium
3. Investigate how different types of cardiac injury induce Notch activation in cardiomyocytes (MI, Transverse Aortic Constriction, Ischemia Reperfusion)

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References

1. Russell, J. L. et al. A dynamic notch injury response activates epicardium and contributes to fibrosis repair. Circ. Res. **108,** 51–59 (2011).
2. Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T. & Murray, C. J. L. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet **367,** 1747–57 (2006).
3. https://www.nhlbi.nih.gov/about/documents/factbook/2012/chapter4.htm
4. Zipes, D. P. & Wellens, H. J. J. Sudden Cardiac Death. Circulation **98,** 2334–2351 (1998).
5. Virmani, R., Burke, A. P. & Farb, A. Sudden cardiac death. Cardiovasc. Pathol. **10,** 275–82 (2001).
6. Gude, N. a et al. Activation of Notch-mediated protective signaling in the myocardium. Circ. Res. **102,** 1025–35 (2008).
7. Raya, A. et al. Activation of Notch signaling pathway precedes heart regeneration in zebrafish. Proc. Natl. Acad. Sci. U. S. A. **100 Suppl 1,** 11889–95 (2003).
8. Zhang, R. et al. In vivo cardiac reprogramming contributes to zebrafish heart regeneration. Nature **498,** 497–501 (2013).
9. Rentschler S. R. *et al.* Myocardial Notch signaling reprograms cardiomyocytes to a conduciton-like phenotype. *Circulation* **126**, 1058-1066 (2012).
10. Wyndham, C. R. C. Atrial fibrillation: the most common arrhythmia. *Tex. Heart Inst. J.* **27,** 257-267 (2000).
11. Postma, A. V, Christoffels, V. M. & Bezzina, C. R. Developmental aspects of cardiac arrhythmogenesis. *Cardiovasc. Res.* **91,** 243–51 (2011).