The Role of Notch Signaling on Heart Rate and Atrial Conduction

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Heart disease is the leading cause of death worldwide and can result in arrhythmias, or dysregulation in the electrical activity 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 is activated autonomously in CMs by exposure to activated Delta (Dlx). We hypothesize that Notch activation promotes slowed HR through downregulation of major cardiac voltage-gated sodium channel (Na\textsubscript{s},1.5) and atrial gap junction (Connexin43, Cx40). A "Tale of two"-downstream-activated system using transgenic adult mice was used to activate Notch specifically in CMs. We analyzed various determinants of CM function, including fibrosis, cellular hypertrophy, and Na\textsuperscript{+} and Ca\textsuperscript{2+} channel and gap junction expression. Trichrome stain and histochemistry indicated normal levels of non-conductive fibroblasts. To determine whether Notch activation is associated with pathophysiologic hypertrophy,1 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\textsubscript{s},1.5 or Cx40 expression within the atrial myocardium. However, localization of Na\textsubscript{s},1.5 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 reducing morphologic changes.

**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 non-autonomous effects on the SAN by performing ECGs.

**Aim 2:** Evaluate the effect of Notch on the morphological determinants of conduction velocity.

- Determine changes in ion channel and gap junction expression in the SAN and atrial cardiomyocytes through immunolocalization.
- Determine the amount of fibrosis through trichome staining and hydroxyproline quantification.
- Determine if Notch induces pathophysiologic hypertrophy by quantifying cell area.

Abstract

Heart disease is the leading cause of death worldwide and can result in arrhythmias, or dysregulation in the electrical activity 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 is activated autonomously in CMs by exposure to activated Delta (Dlx). We hypothesize that Notch activation promotes slowed HR through downregulation of major cardiac voltage-gated sodium channel (Na\textsubscript{s},1.5) and atrial gap junction (Connexin43, Cx40). A "Tale of two"-downstream-activated system using transgenic adult mice was used to activate Notch specifically in CMs. We analyzed various determinants of CM function, including fibrosis, cellular hypertrophy, and Na\textsuperscript{+} and Ca\textsuperscript{2+} channel and gap junction expression. Trichrome stain and histochemistry indicated normal levels of non-conductive fibroblasts. To determine whether Notch activation is associated with pathophysiologic hypertrophy,1 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\textsubscript{s},1.5 or Cx40 expression within the atrial myocardium. However, localization of Na\textsubscript{s},1.5 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.

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- Determine if Notch induces pathophysiologic hypertrophy by quantifying cell area.

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

**Figure 2:** Model of Non-autonomous Notch Activation within All Cardiomyocytes. When mice are 8 weeks old, Notch signaling is activated autonomously in CMs and non-autonomously in CMs by exposure to activated Delta (Dlx). A) The genotype of the mice is MHC-creER/+. The genotype utilizes the Tetraclone-on system in which the Notch intracellular domain (NICD) is activated specifically in CMs (driven by the alpha-myosin heavy chain promoter) upon doxycycline induction.

**Figure 3:** Model of Non-autonomous Notch Activation within Cardiomyocytes. When mice are 8 weeks old, Notch signaling is activated autonomously in CMs and non-autonomously in CMs by exposure to activated Delta (Dlx). B) The genotype of the mice is MHC-creER/+. The genotype utilizes the Tetraclone-on system in which the Notch intracellular domain (NICD) is activated specifically in CMs (driven by the alpha-myosin heavy chain promoter) upon doxycycline induction.

**Figure 4:** Model of Autonomous SAN Notch Activation. When mice are 8 weeks old, Notch signaling is activated autonomously in CMs and non-autonomously in CMs by exposure to activated Delta (Dlx). C) The genotype of the mice is MHC-creER/+. The genotype utilizes the HCN4-creER tamoxifen-inducible system in which the Notch intracellular domain (NICD) is activated specifically in CCS cells by exposing the hyperpolarization-activated cyclic nucleotide 4 gene upon tamoxifen gavaging. Cre2-EGFP immunostaining allows easy visualization of conduction system cells for electrophysiological and morphological studies.

**Figure 5:** Model of Autonomous SAN Notch Activation. When mice are 8 weeks old, Notch signaling is activated autonomously in CMs and non-autonomously in CMs by exposure to activated Delta (Dlx). D) The genotype of the mice is MHC-creER/+. The genotype utilizes the HCN4-creER tamoxifen-inducible system in which the Notch intracellular domain (NICD) is activated specifically in CCS cells by exposing the hyperpolarization-activated cyclic nucleotide 4 gene upon tamoxifen gavaging. Cre2-EGFP immunostaining allows easy visualization of conduction system cells for electrophysiological and morphological studies.

**Figure 6:** Model of Autonomous SAN Notch Activation within the Sinusoidal Node. When mice are 8 weeks old, Notch signaling is autonomously activated specifically within the SAN by exposing HCN4-creER tamoxifen-inducible system. The genotype utilizes the HCN4-creER tamoxifen-inducible system in which the Notch intracellular domain (NICD) is driven by the hyperpolarization-activated cyclic nucleotide 4 gene upon tamoxifen gavaging. Cre2-EGFP immunostaining allows easy visualization of conduction system cells for electrophysiological and morphological studies.
**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 over a decade.\(^1\) About 50% of all cardiac-related deaths are due to sudden cardiac death (SCD).\(^2\) Fatal arrhythmias, which result from electrical dysregulation, are often caused by SCD. Despite the major contribution of arrhythmias to mortality rates, there is very little known about the mechanisms of arrhythmogenesis. One of the major risk factors for SCD is myocardial infarction (MI); 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.\(^3,4\) 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.\(^5\) 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 MI frequently experience cardiac arrhythmias. Little is known about the role of Notch in the regulation of ion channels and the role of 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.

**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**

**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)

**References**

3. Zhou, W. et al. Notch activation in atrial myocardium during 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.