Ischemia–reperfusion destabilizes rhythmicity in immature atrioventricular pacemakers: A predisposing factor for postoperative arrhythmias in neonate rabbits

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BACKGROUND Postoperative arrhythmias such as junctional ectopic tachycardia and atrioventricular block are serious postoperative complications for children with congenital heart disease. We hypothesize that ischemia–reperfusion (I/R) related changes exacerbate these postoperative arrhythmias in the neonate heart and administration of postoperative inotropes is contributory.

OBJECTIVE The purpose of this study was to study the effects of I/R and postischemic dopamine application on automaticity and rhythmicity in immature and mature pacemaker cells and whole heart preparations.

METHODS Single pacemaker cells and whole heart models of postoperative arrhythmias were generated in a rabbit model encompassing 3 primary risk factors: age, I/R exposure, and dopamine application. Single cells were studied using current clamp and line scan confocal microscopy, whereas whole hearts were studied using optical mapping.

RESULTS Four responses were observed in neonatal atrioventricular nodal cells (AVNCs): slowing of AVNC automaticity (from 62 ± 10 to 36 ± 12 action potentials per minute, $P < .05$); induction of arrhythmicity or increased beat-to-beat variability (0.08 ± 0.04 to 3.83 ± 1.79, $P < .05$); altered automaticity (subthreshold electrical fluctuations); and disruption of calcium transients. In contrast, these responses were not observed in mature AVNCs or neonatal sinoatrial cells. In whole heart experiments, neonatal hearts experienced persistent postischemia arrhythmias of varying severity, whereas mature hearts exhibited no arrhythmias or relatively transient ones.

CONCLUSION Neonatal pacemaker cells and whole hearts demonstrate a susceptibility to I/R insults resulting in alterations in automaticity, which may predispose neonates to postoperative arrhythmias such as junctional ectopic tachycardia and atrioventricular block.

KEYWORDS Automaticity; Atrioventricular node; Neonate heart; Junctional ectopic tachycardia; atrioventricular block

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Introduction

Postoperative arrhythmias occur in 7.5% to 48% of children undergoing surgical procedures for congenital heart disease.[1–5] Cardiopulmonary bypass (CPB) can result in a number of physiologic disturbances, including dysfunction of the conduction system.[6,7] Common and potentially severe forms of postoperative arrhythmias include junctional ectopic tachycardia (JET), sinus node dysfunction, and atrioventricular nodal (AVN) block.[2,8–10] JET is a malignant arrhythmia characterized by a normal QRS complex at rates greater than the upper limits of sinus rhythm and often accompanied by ventriculoatrial (VA) dissociation.[11–13] JET is believed to originate in proximity to the AVN and is seen primarily in the pediatric population.[13,14] Although part of this may reflect the nature of the surgical procedures, a developmental component likely underlies the susceptibility to this arrhythmia, given that it is substantially more common in younger patients.[12–14] The most important AV conduction disturbance is complete heart block, in which there is temporary or permanent disruption of conduction through the AVN.

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Lesser degrees of atrioventricular block (AVB) are also observed.[2,4,5]

Despite the prevailing hypothesis that these postoperative arrhythmias are caused by mechanical insults to the conducting system, the epidemiologic literature suggests that factors other than solely mechanical trauma play a role in their etiology. For this study, we developed both cellular cardiac pacemaker and whole heart models, and examined 3 primary clinical risk factors in order to understand further the basic etiology of such arrhythmias. The prevalent risk factors are (1) age—particularly for JET the highest risk is seen in newborns and infants ≤12 months old;[12,15] (2) CPB duration—longer CPB duration correlates with longer aortic cross-clamp duration;[12,15] and (3) postoperative administration of inotropic agents including dopamine (DA).

Methods

We used both isolated single nodal cells and whole heart optical mapping in 10-day-old (10-d) and 56-day-old (56-d) rabbits. These protocols were approved by the Animal Care Committee of the University of British Columbia and were in accordance with the Canadian Council on Animal Care. All chemicals used were obtained from Sigma (St. Louis, MO) unless otherwise stated.

In brief, single cells were isolated from sinoatrial and AV nodes. The isolation and nodal cell characterization procedures have been previously reported.[16] We investigated spontaneous action potentials (APs) using patch clamp and spontaneous calcium transients using confocal microscopy. Whole hearts were loaded with a potentiometric indicator (RH-237) and optically mapped. Subsequently, spontaneous APs as a result of various ischemia–reperfusion (I/R) protocols were analyzed.

Isolated single pacemaker cell studies

Single cell isolation

Ten 10-d neonate nodal cells (five 10-d atrioventricular nodal cells [AVNCs] and five 10-d sinoatrial cells [SANCs]) and eight 56-d mature AVNCs were used. In brief, hearts isolated from the rabbits were cannulated and retrograde perfused with solution A (see Online Supplemental Table 1). Detailed descriptions of the procedures and solution compositions specific to each age group and to each nodal tissue type are given in the Online Supplementary Material. Enzyme concentrations and perfusion rates were optimized (see Online Supplemental Table 1) to ensure viable Ca^{2+}-tolerant AVNCs and SANCs derived from 10-d or 56-d heart. Integrity, characterization, and nodal specificity of the single cell isolation can be found in our previous publication.[16] Only Ca^{2+}-tolerant nodal cells that contracted spontaneously and synchronously at a regular rhythm for 20 minutes before commencing recording were used for the patch-camp and confocal microscopic studies.

Single cell electrophysiologic studies

Perforated (amphotericin B; 240 μg/mL) whole-cell current-clamp technique was used to record spontaneous APs (MultiClamp 700A, Axon Instruments, Union City, CA). During AP recordings, the cells were sequentially superfused with solutions that mimicked the 3 phases of ischemia reperfusion: preischemia, ischemia, and reperfusion or reperfusion with DA. Detailed description of this protocol and methods can be found in the Online Supplementary Material.

Spontaneous cytosolic Ca^{2+} transients studies

Isolated fluo-4–loaded 10-d AVNCs were superfused with solutions of the I/R protocol. Time lapse images were acquired using a Zeiss (Jena, Germany) LSM Pascal confocal microscope. The average fluorescence intensity of each frame was normalized to the mean intensity of the cell during a quiescent phase to obtain F/F_0.

Whole heart optical mapping study

To study the effect of I/R insults on intact immature vs mature rabbit hearts, we used optical mapping techniques. Details of the excision procedure and optical mapping are described in the Online Supplementary Material.

Optical mapping protocols

To study the effect of young age, increased ischemic duration, and postischemia administration of DA, 3 protocols were used. Figure 1 shows the optical mapping setup (Figures 1A and 1B) and a schematic of these protocols (Figures 1C–1E).

Arrhythmia definitions

During reperfusion, the following distinct electrical patterns were observed (Figure 2):

Sinus rhythm: Defined by a 1:1 atrial-to-ventricular conduction pattern, in which the heart rate was not significantly different from the baseline heart rate during initial perfusion or control conditions.

Atrioventricular block: Defined by an abnormal AV conduction pattern, in which the electrical signals from the right atrium did not conduct in a 1:1 manner to the right ventricle. As a result, the frequency of atrial optical APs exceeded that of the ventricle (A > V). If the atrial rate was similar to baseline, this was an isolated AVB and was categorized the same as in the clinical setting (i.e., first degree, second degree, third degree).

Sinus bradycardia: Defined as a sinus rhythm with heart rate ≤0.75 times that of control (A = V).

Sinus tachycardia: Defined as a sinus rhythm with heart rate ≥1.25 times that of control (A = V).

Tachycardia with VA dissociation: Defined as a ventricular rate that was 1.25 times that of control and/or with clear VA dissociation (A < V).