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Development of a Coronary-Perfused Interventricular Septal Preparation as a Model for Studying the Role of the Septum in Arrhythmogenesis

A. Glass, S. Sicouri, and C. Antzelevitch

Masonic Medical Research Laboratory Utica, New York

Abstract

Background— Coronary-perfused ventricular wedge preparations have proven valuable in the elucidation of the mechanisms of arrhythmias. This study was undertaken to develop an arterially-perfused model of the interventricular (IV) septum.

Methods— A canine septal preparation was developed via cannulation of the septal artery. Action potentials were recorded from ventricular endocardial surfaces and locations within the septum using floating microelectrodes; a trans-septal electrocardiogram (ECG) was simultaneously recorded. In some experiments, the calcium agonist BayK 8644 was used to enhance trans-septal heterogeneity of action potential duration (APD).

Results— Distinctive ECG waveforms and dissimilar action potential morphologies and durations were observed across the IV septum. The range of APDs observed exceeded that found in the left ventricular wedge. BayK 8644 further accentuated these differences and induced Torsade de Pointes arrhythmias.

Conclusions— The arterially-perfused septal preparation is a sensitive model for the study of arrhythmias that may arise from the IV septum.

Keywords

Electrophysiology; dispersion of repolarization; arrhythmias; pharmacology

Introduction

The development of the arterially-perfused ventricular wedge heralded a new paradigm in cardiac electrophysiology, allowing the measurement of action potentials from transmural regions containing endocardial, epicardial and mid-myocardial (M) cells simultaneously. The characterization of these three electrophysiologically distinct cell-types within the ventricular myocardium led to the understanding that disparities in the times of repolarization of cardiac cells can contribute to the development of arrhythmias.

Electrophysiological heterogeneity, the presence of action potentials (APs) with different durations and morphologies within the same tissue, has been previously identified within the

Corresponding author: Serge Sicouri, M.D., Masonic Medical Research Laboratory, 2150 Bleecker Street Utica, New York 13501-1787, Telephone: (315) 735-2217 ext. 157, Fax: (315) 735-5648, Email: sicouris@mmrl.edu.

There are no conflicts of interest.

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canine interventricular (IV) septum using superfused tissue slices (Sicouri et al., 1994) and dissociated septal cells (Ramakers et al., 2005).

This study was undertaken to develop an arterially-perfused preparation derived from the IV septum in order to confirm the presence of heterogeneity within the intact septum, quantitate the trans-septal dispersion of repolarization (TDR) and delineate its potential role in arrhythmogenesis.

Materials and Methods

In compliance with guidelines established by the Institutional Animal Care and Use Committee, canines with a mass between 20 and 35 kg were anticoagulated with heparin (180 IU/kg) and anesthetized with sodium pentobarbital (35 mg/kg) intravenously. A left-thoracotomy procedure was used to excise the animal's heart, which was immediately immersed in an ice-cold cardioplegic solution ($[K^+]_0 = 8 \text{ mM/L}$).

Tyrode's solution composed of (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, NaHCO_3 20, CaCl_2 1.8, MgSO_4 0.5, and D-glucose 5.5 was employed as a perfusate throughout all experiments. Several different approaches to deliver the perfusate to the septal preparation were attempted before a stable model was developed.

All approaches to a functional septal preparation began with the removal of the right and left ventricular free walls approximately 1 cm from the IV septum and removal of the atria and any tissues superior to the atrioventricular (AV) valves. Portions of the heart not used in the septal preparation were distributed among other investigators at our institution, ensuring the organ's complete utilization for research.

The Double-Cannulated Preparation

Our initial attempt at a septal preparation employed cannulation of the left coronary artery (LCA) and the dorsal interventricular coronary artery (commonly known as posterior interventricular artery, PIVA). To sustain perfusion pressure between 40 and 50 mmHg, the circumflex artery, any severed branches of the ventral interventricular artery (commonly referred as left anterior descending, LAD) and the PIVA were ligated. To evaluate the quality of perfusion in the double-cannulated preparation, Evan's blue dye (Sigma-Aldrich, St. Louis, MO) was dissolved in the perfusate and pumped through both cannulas concurrently for 20 min. Upon sectioning the tissue transversely, scattered regions of dye-colored myocardium were observed to comprise less than half of the preparation. The less than optimal perfusion in the double-cannulated preparation prompted us to explore a different approach.

The LCA-Perfused Preparation

The canine IV septum receives blood primarily from the ventral coronary vessels, with only the dorsal periphery being supplied by the PIVA (Miller, 1964). Therefore, in another attempt to develop a septal preparation, the decision was made to abandon the dorsal portion of the septum. This markedly reduced the number of cut-vessels requiring ligation. In the LCA-perfused preparation, the LCA was cannulated and the circumflex artery and LAD branches tied-off. The septum was dissected frontally from AV valve cusps to apex and the dorsal half discarded. Dye studies showed that the perfusion of the LCA-perfused preparation was only marginally improved over that of the double-cannulated preparation.

The Septal Artery-Perfused Preparation

The majority of the IV septum irrigation is supplied by a single artery originating from, in all but 6% of specimens, either the LAD or LCA (Miller, 1964). For this approach, the septum was dissected at the bifurcation of the LCA into the LAD and circumflex arteries, allowing the visualization and cannulation of the proximal *septal* artery. In this case, dye studies indicated excellent perfusion of most of the IV septal preparation. Because of the marginal apical perfusion present in some preparations, the inferior-most 1 to 1½ cm of the preparation was excised in the final model.

The coronary-perfused interventricular septal preparations were placed in a large bath and allowed to recover for a 2 hour period prior to the start of data collection. During this equilibration period, the preparations were held at a constant temperature of $37\pm0.5^{\circ}\text{C}$ and paced at a basic cycle length (BCL) of 2000 ms using point stimulation delivered to the right ventricular (RV) endocardium.

Following this 2 hour equilibration period, floating glass microelectrodes were used to obtain action potential recordings from the preparation's impalement surface; a frontal plane located at mid-septum. Two Ag/AgCl half cells (2mm diameter x 4 mm) positioned at least 0.5 cm from the RV and left ventricular (LV) endocardial septal surfaces were used to generate a trans-septal electrocardiogram (ECG). Point stimulation was applied to either the RV or LV endocardium at various BCLs.

Results

During stimulation from the LV endocardial surface, a negative R wave and a positive T wave were recorded on the ECG. Conversely, positive R waves and inverted T waves were recorded during stimulation from the RV endocardium (Figure 1).

Figure 2 illustrates the diverse action potential durations (APDs) and morphologies recorded across the ventricular septum. Differences in APDs and spike and dome morphology were observed across the septum. The difference between the longest and briefest action potential was 122 ms. The trans-septal dispersion of repolarization (TDR) found across the ventricular septum exceeded that observed transmurally in LV wedge preparations (Antzelevitch, 2006). TDR was further amplified upon the addition of the calcium agonist BayK 8644, permitting the development of both spontaneous and programmed electrical stimulation-induced arrhythmias, especially Torsade de Pointes.

Discussion

Due to the lack of a functional model, the electrophysiological properties of the intact ventricular septum have been poorly defined. The coronary-perfused septal preparation developed in this study provides for the first time a detailed view of the transmembrane electrophysiology across the septum, demonstrating remarkable electrophysiological heterogeneity in the form of trans-septal dispersion of repolarization, capable of being further amplified by agents with Class III actions, setting the stage for the development of intramural reentrant arrhythmias. Our results indicate that the coronary-perfused interventricular septal preparation is a sensitive model for assessment of the role of the IV septum in the development of cardiac arrhythmias under a wide variety of conditions.

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Figure 1.

Left panel: Mid-frontal cross-section of canine heart, dorsal aspect. The approximate boundaries of the septal preparation are represented by a dashed line. Positions of stimulating and ECG electrodes are represented by “stim” and “e” respectively. **Right panel:** Recordings of ECG morphology during stimulation from LV endocardium. (LV stimulation, top panel) and from RV endocardium (RV stimulation, bottom panel). Basic cycle length = 300 ms. LA, left atrium; RA, right atrium.

Figure 2.

Recordings obtained from a trans-septal preparation. Action potential tracings recorded from an LV endocardial cell, four mid-septal cells and an RV endocardial cell display varied morphologies. Action potential duration at 90% repolarization values are shown associated with each action potential. Recordings were obtained during RV stimulation at a basic cycle length of 2000 ms.