Acute ischemia of canine interventricular septum produces asymmetric suppression of conduction

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BACKGROUND Acute ischemia depresses tissue excitability more rapidly in the epicardium than in the endocardium of the canine left ventricular (LV) free wall. However, the effects of acute ischemia on conduction in the interventricular septum (IVS), which is composed of right ventricular (RV) and LV endocardium and midmyocardium without epicardium, are less known.

OBJECTIVE The purpose of this study was to evaluate the hypothesis that the IVS exhibits transseptal differences in local tissue response to acute ischemia.

METHODS Isolated canine IVS preparations were perfused through the septal branch of the anterior descending coronary artery, and conduction on the cut-exposed transseptal surfaces was optically mapped before and after two sequential episodes of 8 minutes of global ischemia (separated by >60 minutes of reperfusion). The preparations were paced alternately between the RV endocardium and LV endocardium at cycle lengths of 250, 300, and 1,500 ms.

RESULTS Prior to ischemia, transseptal conduction was radial and symmetric during either RV endocardial or LV endocardial pacing at all cycle lengths. Eight minutes of ischemia depressed conduction velocity more in the RV half than in the LV half of the IVS and caused local conduction block in the sub-RV endocardium, especially during rapid pacing. The K_{ATP} channel blocker glibenclamide (10 μmol/L) prevented development of this transseptal asymmetry and conduction block during ischemia.

CONCLUSION Acute global ischemia increased transseptal heterogeneity and induced sub-RV endocardial block of conduction via activation of the ATP-sensitive potassium current. Such changes could contribute to initiation of arrhythmia in patients with septal infarction.

KEYWORDS Arrhythmia; Interventricular septum; Optical mapping; Ischemia; Conduction

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Introduction

Heterogeneity in the density of ion channels, in action potential (AP) configuration, in electrophysiologic dynamics, and in tissue responses to acute ischemia have been recognized as major contributors to arrhythmogenesis.[1] Acute ischemia depresses excitability and conduction velocity more rapidly in the epicardium than in the endocardium of the canine left ventricular (LV) free wall. Such transmural dispersion of tissue excitability and refractoriness can provide a substrate for transmural reentry and ventricular tachyarrhythmias.[2–6]

Compared to the LV free wall, less is known about the electrophysiologic properties of the interventricular septum (IVS), although substantial differences in cellular composition and function exist. In contrast to the LV free wall, the IVS is composed of right ventricular (RV) and LV endocardium. We have not found M-cell expression in the canine IVS,[7] but other investigators have.[8] Similar to the LV free wall, transseptal gradients exist in the expression and density of membrane ion channels[9] and of AP.[7] However, how these differences affect the response of the IVS to acute ischemia is unknown.

To evaluate the hypothesis that the canine IVS exhibits transseptal differences in local tissue response to ischemia, we optically mapped transseptal conduction in isolated and arterially perfused canine IVS preparations before and after two sequential 8-minute episodes of reversible ischemia (separated by >60 minutes of perfusion) and tested the effects of glibenclamide, an ATP-sensitive potassium current (I_{KATP}) blocker.

Methods

Surgical and tissue preparation

The investigation adheres to the principles on the *Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Transseptal wedges of canine IVS were prepared using procedures similar to those reported in our previous study.[7] In brief, hearts from 14 adult mongrel dogs (weight 25–30 kg) were harvested after intravenous injection of heparin sodium (5,000 units) and pentobarbital sodium (30 mg/kg body weight) and quickly Langendorff-perfused with ice-cold hyperkalemia cardioplegic solution (Tyrode's solution [see below] with 15 mmol/L KCl). The cardioplegic

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perfusion washed out the blood and pentobarbital sodium and protected the hearts during the subsequent period of tissue isolation. The septal branch of the left anterior descending coronary artery was cannulated and perfused, and angiography was performed using an iodine-containing contrast media to identify the branches of the arterial tree and the regions of perfusion. A well-perfused transseptal section of the IVS (30–40 mm long by 8–10 mm wide on the endocardium and 13–17 mm across the septum) containing the cannulated septal artery (diameter ≥1 mm) along its length, about 10–30 mm from the tricuspid valve, was isolated, contrast media was washed out with sufficient perfusion, a second cannula was inserted into the septal branch for monitoring perfusion pressure, and underperfused tissue was trimmed off. Major arterial leaks were ligated with silk sutures. The isolated preparations were mounted in a temperature-controlled (37°C ± 0.5°C) tissue chamber; perfused with 37°C ± 0.5°C Tyrode's solution of the following composition (in mmol/L): 128.0 NaCl, 4.0 KCl, 22.0 $NaHCO_3$, 0.65 NaH_2PO_4, 0.50 $MgCl_2$, 11.1 dextrose, and 2.0 $CaCl_2$, gassed with 95% O_2/5%CO_2 at an arterial pressure of 40–50 mmHg; and immersed in the perfusion efflux.

To reduce intertissue differences in the effects of inevitable ischemic stress during tissue isolation, the preparations were subjected to 6 minutes of preconditioning ischemia (no irreversible damage[10,11]) after being prepared and mounted in a tissue chamber and perfused for approximately 30 minutes. After the preconditioning, the preparations received >60 minutes of additional recovery perfusion, and their physiologic condition and stability were evaluated according to our published criteria.[12,13]

Transseptal mapping of conduction of APs

Each tissue was stained with di-4-ANEPPS [4-(β-[2-(di-n-butylamino)-6-naphthyl]vinyl)pyridinium, ~4 mmol/L in perfusate, Biotium, Hayward, CA, USA], a membrane potential–sensitive fluorescent dye used widely in optical mapping studies, after >60 minutes of tissue recovery and equilibration during continued Tyrode's perfusion.

The tissues were paced (2-ms duration, twice diastolic current threshold, bipolar electrode) from either RV endocardium or LV endocardium. Two silver electrodes were placed in the chamber, one (negative) at the LV side and the other (positive) at the RV side of the preparation, to record the transseptal ECG. An optical mapping system[12,13] with a 256-element (16 × 16) photodiode camera (C4675, Hamamatsu, Japan) and a long-pass (>610-nm) optical filter collected the fluorescence (excited by 520 ± 45 nm light) from an area 19.5 × 19.5 mm^2 on the cut-exposed transseptal surface of the preparation and converted it into 256 channels of electrical signals. Each channel of electrical signal corresponded to a surface area of 1.1 × 1.1 mm^2. A custom data acquisition system recorded the optical mapping signals and transseptal ECG at a rate of 1,000 samples per channel per second after >10 pacing stimuli at the same cycle length. Baseline control data were recorded at each of the sequentially decreasing pacing cycle lengths (PCLs) of 1,500, 300, and 250 ms after the preparations were fully recovered, stabilized, and verified as we previously reported.[6,7,12,13]

Canine transseptal tissue models of ischemia

After stability evaluation and baseline data recording, 8 minutes of global ischemia was induced by stopping perfusion and lowering the bath level below the top of the tissue as we previously reported,[6,13] followed by >60 minutes reperfusion and a second episode of 8 minutes of ischemia. In six preparations, 10 μmol/L glibenclamide, a K_{ATP} channel antagonist, was added to the perfusate after 30 minutes of reperfusion following the first episode of ischemia and the rest of the protocol continued. Data (256 channels of AP and ECG) were recorded before (baseline and second control) and during both episodes of ischemia. Four glibenclamide-free control preparations received the same two episodes of ischemia as the glibenclamide-treated preparations. Another four preparations received only one episode of 8 minutes of ischemia and thus were included in the statistics of baseline data.

Data processing and statistical analysis

All data were processed and analyzed using custom software developed for the optical mapping system. Conduction time was measured from the interval between the pacing spike and the maximum rate of depolarization. All measurements were visually inspected and manually corrected when necessary, as we reported previously.[6,7,13] Local conduction velocity was derived from the conduction times between the pacing site and a site 3.3 mm away transseptally. Distributions (maps) of conduction times were measured at all recording sites on the cut-exposed transseptal surface. All measurements were from data recorded following >10 regularly paced, ectopic-free activation cycles. Statistical analysis was performed using the Student's t-test for paired data. $P < .05$ was considered significant.

Results

Baseline conduction and tissue stability in control experiments

Baseline recordings both before the first episode of ischemia (in all 14 preparations) and before the second episode of ischemia (in the four glibenclamide-free preparations) had similar patterns of radial conduction from the site of stimulation on the transseptal mapping surface (Figures 1A and 1E). These baseline transseptal conductions were symmetric between RV endocardial and LV endocardial stimulations. QRS durations were similar between RV endocardial and LV endocardial stimulations ($P > .05$), similar before both episodes of ischemia (RV endocardial pacing: 63.8 ± 6.6 ms vs 65 ± 7.2 ms, $P > .05$; LV endocardial pacing: 62.8 ± 5.3 ms vs 64 ± 6.4 ms, $P > .05$; first vs second preischemia recording, PCL = 300 ms, four glibenclamide-free preparations), and without prolongation with reduction of PCL from 1,500 to 300 ms (Table 1). Baseline recordings also showed similar local conduction velocities during RV endocardial and LV endocardial stimulation ($P > .05$; Table 1