Relationship between Extracellular Potassium Accumulation and Local TQ-Segment Potential During Graded Coronary Flow Reduction in a Porcine Myocardial Ischemia Model

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Background: Changes in resting membrane potential due to extracellular potassium ([K+]e) accumulation are thought to be responsible for TQ segment depression in ischemia. However, the nature of the [K+]e-TQ relationship remains to be fully elucidated.

Methods: We created a carotid-coronary shunt in 21 pigs, and recorded [K+]e and TQ-segment potentials simultaneously during graded left anterior descending artery (LAD) flow reduction via 4–6 K+-sensitive electrodes placed in the LAD. Only data from K+ electrodes with calibration slopes of 55–65 mV/decade change in K+ were used.

Results: While the correlation between the changes in potassium equilibrium potential (E_K) and the TQ shift was linear, the regression slopes initially increased and then decreased during graded flow reduction (S = −0.338, Q = 30 mL/minute, S = −3.253, Q = 10 mL/minute, S = −0.312, Q = 0 mL/minute), i.e., TQ depression at all E_K values became larger, then smaller as the flow was decreased in a stepwise manner. The inhomogeneity of changes in [K+]e and TQ potential changes and their relationship also decreased initially then increased during graded flow reduction (R = −0.237, LAD flow = 30 mL/minute; R = −0.819, LAD flow = 15 mL/minute; R = −0.115, LAD flow = 0 mL/minute).

Conclusions: Although [K+]e and the TQ shift are related linearly, there is large variability in their relationship in the setting of graded coronary flow reduction. Therefore, local TQ-segment potentials cannot be used as indices of the severity of ischemic changes.

Key words: myocardial ischemia, extracellular K+, TQ potential, low-flow ischemia, potassium equilibrium potential

Introduction

Fatal arrhythmias are not uncommon in the early stage of myocardial infarction. Harris et al. first reported extracellular potassium ([K+]e) accumulation as a major determinant of these arrhythmias on the basis of concentrations measured in blood samples drawn from coronary veins draining the ischemic region.1 Direct measurement of [K+]e was later made possible with the introduction of potassium-sensitive electrodes.2 The time course of extracellular potassium accumulation characterizing myocardial ischemia has been described as biphasic, with the first phase comprising a fast rise in [K+]e followed by a plateau during which a transient decrease in [K+]e often occurs, and the second phase comprising an increase in [K+]e that heralds the onset of irreversible injury.3-5 Apart from these time-dependent changes in [K+]e, regional differences in [K+]e have also been reported.3,6 The regional dispersion of [K+]e is inhomogeneous and resembles that seen in other variables during regional myocardial ischemia, such as the refractory period, resting membrane potential, excitability, conduction time, and TQ segment changes.7-10 Inhomogeneity of electrophysiologic variables has been shown to contribute to the occurrence of reentrant arrhythmias.11-13 Previously reported studies described the relation between [K+]e and TQ potential and its link to the occurrence of ventricular fibrillation during acute no-flow regional ischemia.14,15 However, the relation between [K+]e and TQ potential during low-flow myocardial ischemia is unclear. Thus, we undertook a study in in-situ pig hearts to quantify the relation between [K+]e and TQ potential during graded low-flow ischemia.

Material and Methods

1.1. Experimental preparation: Care for all pigs used in this study conformed to the Position of the American Heart Association on Research Animal
Use and was done in accordance with accepted guidelines for the care and treatment of experimental animals at the University of North Carolina (IRB approval number: #179, April 1995). The experimental preparation was similar to the preparation that we reported previously (Fig. 1).\textsuperscript{16,17} Twenty-one domestic swine of either sex and weighing 30–50 kg were anesthetized with sodium pentobarbital (25 mg/kg), and this was followed by $\alpha$-chloralose, as needed. Mechanical ventilation and supplemental oxygen were provided via an endotracheal tube and a Harvard respirator. Arterial blood gases were monitored, and appropriate ventilator adjustments were made to maintain an arterial PO$_2$ of >80 mmHg and a pH of 7.35–7.45. Catheters were placed in the femoral artery for blood pressure monitoring and blood sampling and in the femoral vein for blood sampling and administration of fluids and drugs. Core temperature was continuously monitored with a temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH, USA). A heating blanket was used to maintain the animal’s body temperature at 36–37°C.

The heart was exposed via median sternotomy and suspended in a pericardial cradle. A site midway along the length of the left anterior descending coronary artery (LAD) and free of branches was selected for cannulation and dissected from surrounding tissue. After brief occlusion of the LAD at this site, the epicardial margin between ischemic and non-ischemic tissues was identified, and 4–6 pairs of ion-selective/unipolar and bipolar electrodes were placed at various locations in the center of the ischemic zone, defined as the region >10 mm inside the visible cyanotic border, and in the normal (non-ischemic) zone.

After electrode placement, systemic heparin (10,000-U bolus followed by 2,000 U/hour) was administered. A carotid artery-to-LAD shunt was created as previously described.\textsuperscript{16,17} Placement of the shunt took approximately 2–3 minutes, but the LAD flow was stopped for 5 minutes because successive occlusions produced similar metabolic and electrical changes.\textsuperscript{4} This carotid-coronary shunt was routed through a constant-flow roller pump, which permitted controlled reduction in flow from a control value of 1.2–1.5 mL per kg body weight/minute (30–50 mL/minute) to zero. Atrial pacing was used to maintain heart rate at 100 beats/min. Arterial blood pressure and the lead II electrocardiogram were recorded continuously during each experiment with a 12-channel Graphtec Linearcorder (Graphtec Corp., Yokohama, Kanagawa, Japan).

1.2. Ion-selective electrodes: Ion-selective plunge electrodes were fashioned and calibrated by methods described previously.\textsuperscript{18} Briefly, one end of a Teflon-coated silver wire (0.007-inch diameter) was chloridized by soaking it in sodium hypochlorite. The end was then covered with a cellulose acetate-titanium dioxide sponge. K$^+$-sensitive electrodes were made by covering the sponge with a polyvinyl-chloride-valinomycin membrane. Reference electrodes were fashioned similarly but lacked the ion-selective membrane. One K$^+$-sensitive electrode along with one reference electrode for recording [K$^+$]$e$ made up an electrode pair. Electrodes were calibrated before each experiment in standard solution (3 and 10 mmol/L KCl). Only electrodes that showed baseline stability to <1 mV/hour drift and 95–105% of the predicted Nernstian slope (56– to 62-mV shift per decade change in K$^+$ activity at room temperature) were used. After electrode insertion, in-vivo performance of the K$^+$ electrodes was tested by methods described previously.\textsuperscript{18} At the end of each experiment, the electrodes were removed from the heart and re-tested in vitro to confirm stable function throughout the experiment. The pair of electrodes was threaded into a 19-gauge hypodermic needle, which was used to insert them into the midmyocardium to a depth of 4–6 mm. The needle was then withdrawn, leaving the electrodes embedded in the myocardium. Up to 6 electrode pairs were used in each experiment.

1.3. Experimental protocol: The preparation was allowed to stabilize for at least 60 minutes after placement of the K$^+$-sensitive electrodes. Coronary blood flow through the roller pump then was reduced in a stepwise fashion at 5-minute intervals as follows: 50, 40, 30, 20, 15, 10, 5, 2.5, and 0 mL/minute, followed by return to control flow.

![Fig. 1 Diagram of the experimental preparation. LVP: left ventricular pressure.](image-url)
Fig. 2  $K^+$ electrode construction (left panel) and algorithm of simultaneous extracellular $K^+$ ($[K^+]_e$) and TQ potential (right panel). TQ-segment potentials were accomplished by using the $K^+$-reference as one pole of the unipolar, DC-coupled electrogram referenced to a common DC-electrode attached to the aortic root (right panel).

Calculation of potassium equilibrium potential ($E_K$)

$$E_K = 61.33 \text{ mV} \times \log \frac{[K^+]_e}{0.746}$$

$$100 - 0.33 \times \frac{[K^+]_e - [K^+]_o}{0.746}$$

$[K^+]_o = \text{extracellular } K^+ \text{ activity prior to occlusion}$

Fig. 3 Formula for calculation of potassium equilibrium potential (modified from Kleber AG. Circ Res 1983; 52: 442–450). 61.33 = Nernst slope for temperature of 37°; $[K^+]_e = \text{extracellular potassium concentration}$; 100 = assumed value of 100 mM for initial intracellular potassium activity; 0.33 = assumed extra- to intracellular volume ratio; 0.746 = activity coefficient that indicates the thermodynamically active fraction of the total ionic concentration ($[K^+]$) available to produce an electrochemical response.

1.4. Data collection and analysis: The amplified signals from all electrodes and the lead II electrocardiogram were digitized with an analog-to-digital converter (Phoenix Data Inc., Phoenix, AZ, USA) and simultaneously sampled (1,000 samples per second) every 15 seconds during myocardial ischemia with a MicroVAX II/GPX computer (Digital Equipment Corp., Maynard, MA, USA). Simultaneous acquisition of the change in TQ-segment potential (measure at the flat part of the PQ segment), i.e., change in TQ-segment potential from the control flow value ($\Delta TQ$), was accomplished by using the $K^+$-reference electrode as one pole for recording of a unipolar, DC-coupled electrogram referenced to a common DC-electrode attached to the aortic root (Fig. 2). The microvolt readings from the $K^+$-sensitive electrodes were first converted to $[K^+]$, and then to the change in potassium equilibrium potential, $\Delta E_K$ (Fig. 3). $\Delta E_K$ was plotted as a function of $\Delta TQ$ for each reduction in coronary flow.

1.5. Statistical analysis: Data are presented as mean ± SEM unless otherwise indicated. Differences in values were analyzed by Mann-Whitney U test or Fisher’s exact test, as appropriate, and correlation was tested by Spearman’s rank correlation coefficient. All statistical analyses were performed with StatView 5.0 software (SAS Institute, Cary, NC), and $P < 0.05$ was considered significant.

Results

$\Delta E_K$ plotted against $\Delta TQ$ for each reduction in coronary flow is shown in Fig. 4. The number in each coronary flow rate shows the total numbers of $K^+$-sensitive electrodes which met the calibration criteria. The reason for smaller numbers at 30 ml/min coronary flow was that in some pigs, $[K^+]$, did not change at
that flow rate, and for smaller numbers at 0 ml/min coronary flow was that in some pigs, ventricular tachyarrhythmias developed at 5.0 ml/min or 2.5 ml/min coronary flow, we quit to decrease the coronary flow further. The $\Delta E_k/\Delta TQ$ slope ($S$) became progressively steeper with the reductions in coronary flow up to 10 mL/minute, with corresponding improvement in the correlation coefficients ($R$ values). Thereafter, $S$ became less steep, and $R$ deteriorates. At 0 ml/min coronary flow, the two variables seem to have no significant correlation.

Fig. 4  Linear regression lines representing the $\Delta E_k/\Delta TQ$ relationship during graded coronary flow reduction. $S$ = slope; $R$ = correlation coefficient. Note that the $\Delta E_k/\Delta TQ$ slope becomes progressively steeper until coronary flow reduction to 10 mL/min with corresponding improvements in correlation coefficient. Thereafter, $S$ becomes less steep and $R$ deteriorates. At 0 ml/min coronary flow, the two variables seem to have no significant correlation.

Fig. 5  Individual data from $K^+$ electrode showing the time course of change for the two variables ($\Delta TQ$ and $\Delta E_k$). Note that three pattern of the $\Delta E_k/\Delta TQ$ data. Left panel shows the “TQ reversal” pattern, i.e., decrease in $\Delta TQ$ in spite of the increase in $\Delta E_k$, middle panel shows increase in $\Delta E_k/\Delta TQ$ slope pattern, i.e., the increase in $\Delta E_k/\Delta TQ$ slope as the increase in $\Delta E_k$, and right panel shows linear $\Delta E_k/\Delta TQ$ slope pattern.

<table>
<thead>
<tr>
<th>Flow Rate (ml/min)</th>
<th>$S$</th>
<th>$R$</th>
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<tbody>
<tr>
<td>30</td>
<td>0.338</td>
<td>0.725</td>
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<tr>
<td>20</td>
<td>1.636</td>
<td>0.819</td>
</tr>
<tr>
<td>15</td>
<td>2.641</td>
<td>0.891</td>
</tr>
<tr>
<td>10</td>
<td>3.235</td>
<td>0.791</td>
</tr>
<tr>
<td>5</td>
<td>1.641</td>
<td>0.494</td>
</tr>
<tr>
<td>2.5</td>
<td>1.488</td>
<td>0.482</td>
</tr>
<tr>
<td>0</td>
<td>0.321</td>
<td>0.115</td>
</tr>
</tbody>
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To better understand
the coronary flow rate-dependent changes in S and R, we examined the $\Delta TQ/\Delta E_k$ data for individual electrodes. Representative plots of data obtained from three centrally placed electrodes are shown in Fig. 5. The plot obtained from one electrode showed a linear relation up to a $\Delta E_k$ of 30 mV (right panel); the second showed a decrease in the $\Delta TQ/\Delta E_k$ line at $\Delta E_k > 20$ mV (middle panel); and the third showed a clear decrease in the $\Delta TQ$-segment potential at $> 30$ mV despite an increase in $\Delta E_k$ (left panel).

**Discussion**

In this study, we attempted to correlate the magnitude of changes in $E_k$ and $TQ$-segment potential in the midmyocardium at the center of the ischemic zone during graded reduction of LAD flow in the *in-vivo* pig heart. We postulated that the relation between changes in $E_k$ and $TQ$ potential at each stage of coronary flow reduction would be similar, but we found that the slope of the $\Delta E_k/\Delta TQ$ line changed as the reduction in flow progressed, and a plateau in $\Delta TQ$ potential was reached or a reversal in $TQ$ potential occurred despite an increase in $\Delta E_k$ in the context of high-grade coronary flow reduced-flow myocardial ischemia, and no-flow myocardial ischemia. Our results replicate those of a 1987 study indicating that $\Delta TQ$, regardless of the midmyocardial site, cannot be used as an index of local membrane depolarization or as an index of ischemic injury during acute no-flow myocardial ischemia.²⁰ There are several possible explanations for the large variability in the $\Delta E_k/\Delta TQ$ relationship during coronary flow reduction. First, factors other than $[K^+]_e$, such as extracellular pH, may alter resting membrane potential.²¹ Second, non-specific $K^-$-independent membrane currents may develop,²² and third, tissue anisotropy within the ischemic zone may alter injury currents in the center of the ischemic zone.²³ With respect to the latter possibility, $\Delta TQ$ is a result of differences between the transmembrane potentials of normal and ischemic cells during diastole.¹⁰ The actual magnitudes of extracellular potential changes are determined not only by the changes in resting membrane potential, but also by the changes in tissue resistances which influence the amount of the injury current. Extracellular and intracellular resistances are known to increase during *in vitro* and *in vivo* myocardial ischemia.²⁴ Intracellular resistances are known to be lower along the longitudinal fiber axis rather than transverse axis.²⁴ Furthermore, inhomogeneity of the $[K^+]_e$ rise in the midmyocardial area of the ischemic zone²⁰ and between the endocardial center and epicardial center of the ischemic zone may lead to flow of the injury current within the ischemic zone. Therefore, further quantification of the $\Delta E_k/\Delta TQ$ relationship during coronary flow reduction must include precise determination of the resistances, data on fiber orientation and of heterogeneities in the $[K^+]_e$, rise in the ischemic zone.

**Conclusions:** We found that local TQ-segment potentials cannot be used as indices of the severity of ischemic change in the setting of graded coronary flow reduction. Possible explanations for the dynamic character of the $[K^+]_e/TQ$ relationship include factors besides $[K^+]_e$ that might alter resting membrane potential, tissue anisotropy, and changes in extra- and intracellular resistance during each stage of myocardial ischemia.

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**Disclosure**

The authors have no conflicts of interest to declare.

**References**


