Magnitude and Time Course of Extracellular Potassium Inhomogeneities During Acute Ischemia in Pigs

Effect of Verapamil

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Prior studies have demonstrated the presence of inhomogeneities in myocardial [K+]e after serial 10-minute occlusions of the left anterior descending coronary artery in the pig, even within restricted locations of an ischemic zone. These inhomogeneities are thought to underlie the electrophysiological abnormalities responsible for lethal ventricular arrhythmias through reentrant and nonreentrant pathways, but a clear association has not been demonstrated. As a prerequisite to establishing this association, these studies were performed to establish measurement standards for [K+]e inhomogeneity, to quantify the magnitude and time course of these inhomogeneities, to determine whether the inhomogeneities are greater in the ischemic border where lethal ventricular arrhythmias are known to originate, and to assess the effect of a known antifibrillatory drug on [K+]e inhomogeneities. [K+]e (expressed as the change in potassium equilibrium potential, dE[K] (mV)) was measured in 15 preparations using an average of 17 closely spaced, critically calibrated K+-sensitive electrodes having stable response characteristics. A series of four 10-minute occlusions each separated by a 50-minute reperfusion period were performed in each study. In half of the studies, intravenous verapamil (0.2 mg/kg bolus followed by 0.0065 mg/kg/hr) was administered before the fourth occlusion. In nine studies (five control and four verapamil), electrodes were placed in the marginal ischemic zone (from 2 mm outside to 5 mm inside the visible cyanotic border). In six other studies (three control and three verapamil), electrodes were placed in the central ischemic zone (10–20 mm within the ischemic region). We determined that the standard deviation is the best measure of inhomogeneity and that 12 equivalent measurement sites are required to estimate it with a satisfactory degree of statistical confidence. We found that after 10 minutes of ischemia, mean dE[K] was 1.6 times greater in the central than in the marginal ischemic zone, whereas mean standard deviation at the same time was 1.5 times greater in the marginal than in the central ischemic zone. Verapamil reduced mean dE[K] and mean standard deviation in both ischemic zones for most of the occlusion by delaying the rise in [K+]e, and the inhomogeneity of that rise by 3–5 minutes. Comparisons of mean dE[K] with mean standard deviation revealed a steep linear relation in the marginal zone and a curvilinear relation in the central zone where higher mean dE[K] values were not accompanied by higher values for mean standard deviation. Furthermore, we determined that these relations were not altered by verapamil. We conclude that an inhomogeneous substrate for [K+]e exists in the marginal ischemic zone during acute ischemia and that this substrate may be of sufficient magnitude to initiate and sustain lethal ventricular arrhythmias. We found no evidence for a preferential reduction in mean dE[K] or mean standard deviation by verapamil in either zone. Instead, we found that the protective effects of verapamil with respect to potassium arise from the delay in the time course of change for both mean dE[K] and mean standard deviation rather than a disproportionate reduction in either. 

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rrhythmias that occur during the early stages of acute myocardial ischemia have been attributed to both reentrant and nonreentrant mechanisms. Inhomogeneities in conduction and refactoriness contribute to the development of reentrant arrhythmias, whereas injury currents generated by inhomogeneous changes in the resting potentials of cells within the ischemic zone may contribute to the development of nonreentrant arrhythmias. One possible explanation for these electrical inhomogeneities is the inhomogeneous rise in [K\(^+\)], after the onset of coronary occlusion. Potassium inhomogeneities have been observed within (microinhomogeneities) and between (macrinhomogeneities) discrete regions of the ischemic myocardium. We have hypothesized that the ionic inhomogeneities within and between ischemic regions are of sufficient magnitude to cause the observed electrical inhomogeneities. The recent study by Coronel et al., in which the inhomogeneities were determined at a single point in time and expressed semiquantitatively, supports this hypothesis.

In this study, we have attempted to delineate rigorously the magnitude and time course of the inhomogeneities of the rise in [K\(^+\)], in the center and margin of the ischemic zone after acute ligations of the left anterior descending coronary artery (LAD) in the anesthetized open-chest pig. We used multiple K\(^+\)-sensitive electrodes placed closely together within the center or at the lateral margin of the ischemic zone to measure the changes in [K\(^+\)]. Specific electrode calibration and performance criteria, including preexperimental and postexperimental calibration standardization and in situ response characteristics were developed to guarantee the accuracy of the data. Comprehensive statistical methodologies were applied to identify the most appropriate measure of the inhomogeneities and to determine the number of electrodes necessary to measure it. We also examined the effect of verapamil on these inhomogeneities because this drug, when administered before coronary occlusion, prevents ventricular fibrillation during the occlusion.

In brief, we found that 1) 12–20 equivalent measurement sites within an ischemic region of the heart are necessary to optimally estimate potassium inhomogeneity, 2) the statistical measure of standard deviation produces the most reliable, unbiased estimate of this potassium variability, 3) potassium inhomogeneity is greater in the lateral margin than in the center of the ischemic region, and 4) verapamil, an antifibrillatory agent, significantly attenuates the magnitude and variability of potassium change in both ischemic regions without altering the relations between them.

**Methods**

**Electrode Fabrication and Calibration**

Miniature K\(^+\)-sensitive plunge-wire electrodes were fashioned by methods previously described. In brief, cut ends of Teflon-coated silver wires (0.007-in. diameter) were chloridized and covered with a cellulose acetate–titanium dioxide sponge mixture. The K\(^+\)-sensitive electrodes were made by covering the tip with a polyvinyl chloride–valinomycin membrane and by hydrating them in 3 mM KCl. The reference electrodes lacked the polyvinyl chloride–valinomycin membrane. All electrodes were “hooked” by forming a 45–90° angle between the electrode wire and the ion-selective tip. Two K\(^+\)-sensitive electrodes and a reference were loaded into each 20-gauge hypodermic needle that was subsequently used to insert the electrodes into the myocardium. Withdrawal of the needle after insertion left the electrodes embedded in the myocardial tissue. Dual loading of the K\(^+\)-sensitive electrode limited myocardial damage during the placement of the large numbers of electrodes used in these studies.

Critical electrode assessments are mandatory to ensure the elimination of inaccurate estimates of potassium inhomogeneity introduced by differences in electrode response characteristics from one placement site to another. Therefore, the electrodes were calibrated and evaluated both in vitro and in vivo. The in vitro calibration was performed before the insertion of the electrodes into the myocardium. The in vivo verifications of electrode performance and calibration were performed while the electrodes were in the heart muscle.

The in vitro calibration of the K\(^+\)-sensitive electrodes was done before each experiment by alternately placing the electrodes in solutions of 3 and 10 mM KCl. Electrodes were inserted into the heart muscle only when they demonstrated a stable baseline (drift, <1 mV/hr) and a Nernstian slope ±5% (56–62 mV/decade change in [K\(^+\)] at 24°C) in the preexperimental in vitro calibration.

The in vivo performance of each K\(^+\)-sensitive electrode was determined before each control and verapamil occlusion by the rapid intravenous 1.5-mI bolus injection of a solution containing 2 meq KCl/ml through a catheter positioned near the terminal portion of the inferior vena cava. The response of implanted electrodes typically fell into one of the three categories identified as types A, B, and C (Figure 1). As shown in the figure, electrode types A and B responded within 5–10 seconds to the bolus injection, recorded a 2.0–3.5 mM rise in [K\(^+\)], and had a washout time constant of 2–3 minutes. Type A differed from type B electrodes by having a slightly faster rise time and a transient peak overshoot 30–75 seconds after the bolus injection. However, within 60 seconds after the bolus administration, the type A and B electrodes displayed similar response characteristics. We evaluated the [K\(^+\)]\(_e\) data from several hearts having both type A and B electrodes to determine whether the type A electrodes gave consistently higher millivolt readings than did type B electrodes. We were unable to demonstrate any response bias introduced by electrode type. We also performed a separate series of three experiments in which potassium chloride was infused into the LAD...
A midsternal thoracotomy was performed, and the heart was suspended in a pericardial cradle. The LAD was isolated distal to the first diagonal branch, and a snare of polyethylene tubing was placed around it to allow total, reversible occlusion. Temperature was continuously monitored using a temperature probe (Yellow Springs Instrument Co., Yellow Springs, Ohio). Isothermia was maintained at 36–39°C using a thermal blanket (Gaymar T/Pump, Orchard Park, N.Y.), and the open chest was covered with a polyethylene sheet or watch glass to maintain epicardial temperature (36–39°C) and moisture. During an initial 30-second occlusion of the LAD, the visible cyanotic border was identified to facilitate the midmyocardial placement of as many as 56 ion-selective electrodes approximately 1–2 mm apart in the midmyocardium (i.e., more than 3 mm from both the subendocardium and subepicardium). Electronic amplification limitations dictated that these electrodes were either placed in the center of the ischemic zone, which was defined as the region 10–30 mm inside of the visible cyanotic border, or along the lateral margin, which was defined as the region from 5 mm inside to 2 mm outside the visible cyanotic border (Figure 2). Typically, center placements were in three to five rows of 8–14 electrodes covering an average area of 186.6±39.9 mm² and marginal placements were in one to three rows of 9–18 electrodes covering an average area of 47.4±18.0 mm². The arterial pressure, a lead II electrocardiogram, and 10 ion-selective electrode data channels were monitored continuously with a 12-channel graphtec linearorder (Western Graphtec, Irvine, Calif.).

After the electrodes were in place for 50 minutes, a series of four 10-minute LAD occlusions was performed. Each occlusion was followed by 50 minutes of reperfusion, and no anesthesia was administered less than 10 minutes before an occlusion. In eight experiments (three in which the electrodes were located in the center and five in which the electrodes were located in the margin), there was no intervention between the third and fourth occlusions. These occlusions are labeled C₁ and C₄. In seven experiments (three having only centrally placed electrodes and four having only marginally placed electrodes), verapamil was given as an intravenous infusion of 0.2 mg/kg during a 25-minute period after the third occlusion. A continuous intravenous infusion of 0.0065 mg/kg/hr was then maintained throughout the fourth occlusion. This is similar to the dose regimen of verapamil used in our prior experiments. These occlusions are labeled C₁V and V. Right atrial pacing was used as needed after the verapamil administration to match the spontaneous heart rate of the previous control occlusion. The number of experiments performed for each electrode location and experimental protocol are summarized at the bottom of Figure 2.

The amplified signals (gain, 50; band pass, 0–1 Hz) from all ion-selective electrodes were sampled every 15 seconds by a PDP-11/03 minicomputer (Digital
Equipment Corp., Marlboro, Mass.) during the ST segment of the cardiac cycle. Each value of \([K^+]_e\) was calculated from measured millivolt changes using the in vivo calibration curve of each electrode, the temperature, and the systemic \([K^+]\) determined from an arterial blood sample obtained immediately before the occlusion. Each value of \([K^+]_e\) was then converted to the equilibrium potential for potassium \((E_K)\) by the methods outlined by Kleber,\(^{14}\) which assume an intracellular potassium activity of 100 mM. We expressed our data as the changes in \(E_K\) rather than changes in \([K^+]_e\), because this linear transformation effectively normalizes the data against the starting levels and is correlated to the anticipated change in resting potential that results from the rise in \([K^+]_e\).

Electrode placements and estimates of the extent of myocardial ischemia were determined by injecting thioflavin S (1 g) intravenously into the heart with the LAD occluded for 30 seconds before death. Subsequent dissection permitted the determination of electrode positions within and in relation to the ischemic border. Electrodes that were within 3 mm of the endocardium or epicardium and those not satisfying the location restraints were eliminated from further study. On average, 17 electrodes in each experiment fulfilled the calibration and location requirements. In four randomly selected preparations from both protocols, we divided the heart ventricles into left or right and ischemic or nonischemic portions. We found no statistical differences between the two protocols for the degree or distribution of the ischemia produced by LAD ligation.

**Statistical Analysis**

We compared the results of the third and fourth sequential control occlusions by placement location for the control protocols \((C_3\) and \(C_4\) for central and marginal electrode locations) to demonstrate the reproducibility of the results in the fourth occlusion. We then compared the results of the preverapamil control occlusion \((C_{VW})\) to the subsequent occlusion performed after the administration of the drug \((V)\). To verify the equivalency of the third occlusion between those animals given drugs and those not treated, we compared the results of the \(C_{VW}\) occlusions to the \(C_3\) occlusions and the results of the \(C_4\) occlusions to the \(V\) occlusions in the two protocols by placement location. Statistical analysis was performed with \(t\) tests for paired data within a protocol \((C_3\) versus \(C_4\) or \(C_{VW}\) versus \(V)\) and two-way analysis of variance for unpaired data between protocols \((C_3\) versus \(C_{VW}\) or \(C_4\) versus \(V)\). A probability value less than 0.05 was considered statistically significant in all tests. Without exception, the statistical significance or insignificance of \(t\) tests on mean data at each minute within a protocol (see "Results") was confirmed by analysis of variance between protocols using the averaged data collected during the entire occlusion. The summary data shown (Figures 4, 5, and 7–10) were calculated as follows: the mean
change in $E_K$ and the corresponding standard deviation of that change was calculated for each preparation at each minute during the occlusions using only the electrodes that met our calibration and placement criteria. Coefficients of variation (i.e., the standard deviation divided by the mean change in $E_K$) were also calculated for each of these time periods. These means, standard deviations, and coefficients of variation were then averaged across animals by electrode location and experimental protocol to produce a mean of the mean change in $E_K$ ($\pm$SD), a mean standard deviation of the mean change in $E_K$ ($\pm$SD), and a mean coefficient of variation ($\pm$SD).

## Results

The values for serum [K+]*, systemic arterial pH, heart rate, and mean aortic pressure before each occlusion are shown in Table 1. There were no significant differences in these values before $C_3$, $C_4$, or $C_{SV}$. The only significant difference ($p < 0.05$) was a decrease in the mean arterial pressure after the administration of verapamil (i.e., before $V$).

Figure 3 demonstrates the results of representative experiments in the central and marginal ischemic zones in the control group. In the central ischemic zone, the change in $E_K$ recorded from the 16 acceptable electrodes 10 minutes after the start of the $C_3$ occlusion ranges from 25 to 35 mV. Some electrodes demonstrate the onset of a plateau in the change in $E_K$ as early as 5 minutes into the occlusion, whereas others show a continuous rate of rise that slows during the second half of the occlusion. These changes were reproduced in $C_4$. At the lateral margin of the ischemic zone, the maximum change in $E_K$ recorded from the 15 acceptable electrodes during $C_3$ ranges from 0 to 30 mV. Most of the electrodes demonstrate a plateau in the change in $E_K$ at 5–6 minutes after the start of the occlusion. These changes were also reproduced in $C_4$.

The mean changes in $E_K$ in the center and the margin of the ischemic zone during $C_3$ and $C_4$ are shown in Figure 4. As mentioned previously, the change in $E_K$ at each time interval was first averaged for each animal at 1-minute intervals, and the mean of the averages was then calculated to obtain the values shown. For the center of the ischemic zone, the maximal mean changes in $E_K$ are $31.5 \pm 1.5$ and $29.5 \pm 1.6$ mV for $C_3$ and $C_4$, respectively. The values do not differ significantly at any time during the occlusion. For the margin of the ischemic zone, the mean changes in $E_K$ during $C_3$ and $C_4$ also do not differ at any time. Maximum values for the mean changes in $E_K$ are $19.5 \pm 3.6$ and $19.1 \pm 4.3$ mV for $C_3$ and $C_4$, respectively.

The average standard deviation of the change in $E_K$ at each time interval during $C_3$ and $C_4$ is shown in Figure 5. In the center of the ischemic zone, the peak average standard deviations of the change in $E_K$ are $6.6 \pm 0.2$ and $5.9 \pm 0.5$ mV for $C_3$ and $C_4$, respectively. These peak values occur between 4 and 5 minutes after the onset of the occlusion. Thereafter, the average standard deviations decrease significantly to $4.8 \pm 0.7$ and $4.2 \pm 0.9$ mV for $C_3$ and $C_4$, respectively. There were no significant differences between the mean standard deviations of the change in $E_K$ at any time during $C_3$ and $C_4$. In the margin of the ischemic zone, the peak average standard deviations of the change in $E_K$ are $8.2 \pm 2.3$ and $7.9 \pm 3.0$ mV for $C_3$ and $C_4$, respectively. These peak values occur between 9 and 10 minutes after the start of the occlusion; however, unlike the standard deviations for the central zone, those for the marginal zone at 4 minutes into the occlusion are not different from these maximum values ($7.4 \pm 1.3$ and $7.3 \pm 1.7$ at 4 minutes in $C_3$ and $C_4$, respectively). There were no significant differences between the average standard deviations of the change in $E_K$ at the margin at any time during $C_3$ and $C_4$.

The results shown in Figures 3–5 confirm the reproducibility of the changes in $E_K$ and the standard deviation of the change in $E_K$ at the center and margin of the ischemic zone. Figures 6–8 show the data from the verapamil experiments that compare the preverapamil control ($C_{SV}$) and the verapamil (V) occlusions. Representative experiments for central and marginal zones are shown in Figure 6. In the center of the ischemic zone, the maximal changes in $E_K$ during $C_{SV}$ recorded by the 26 acceptable electrodes range from 20 to 35 mV. These are similar to

### Table 1: Hemodynamic Comparison of the Four Experimental Groups Before the Third and Fourth Serial Occlusions

<table>
<thead>
<tr>
<th>Protocol</th>
<th>[K+] Concentration</th>
<th>pH</th>
<th>HR (beats/min)</th>
<th>MP (mm Hg)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>$C_3$</td>
<td>$C_4$</td>
<td>$C_3$</td>
<td>$C_4$</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Center</td>
<td>4.4±0.7</td>
<td>4.6±0.8</td>
<td>7.39±0.04</td>
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<tr>
<td>Margin</td>
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<td>4.3±0.5</td>
<td>7.41±0.02</td>
<td>7.41±0.04</td>
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<tr>
<td></td>
<td>$C_{SV}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Center</td>
<td>4.0±0.4</td>
<td>4.2±0.5</td>
<td>7.40±0.04</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>Margin</td>
<td>3.8±0.5</td>
<td>4.1±0.4</td>
<td>7.42±0.03</td>
<td>7.41±0.01</td>
</tr>
</tbody>
</table>

* $p < 0.05$ vs. $C_{SV}$.
the changes recorded during C₃ of the control series. In this experiment, verapamil reduces the maximum changes in $E_K$ to 13–22 mV. The differences in the change in $E_K$ between the C₃V and V occlusions are significant from 2 to 10 minutes after the start of the occlusion. In the margin of the ischemic zone, the changes in $E_K$ recorded by the 12 acceptable electrodes during C₃V range from 2.5 to 29 mV. These values are also similar to those recorded during C₃. As in the center of the ischemic zone, verapamil considerably reduces the maximum and intermediate changes in $E_K$. The differences in the changes in $E_K$ for C₃V and V from 2 to 7 minutes after the start of occlusion in this experiment are significant.

**FIGURE 3.** Plots of typical potassium results from two preparations in the control protocol. Panel A: Preparation with electrode placements in the center of the ischemic zone during a third (C₃) and fourth (C₄) period of acute ischemia ($n=16$ electrodes). Panel B: Preparation with electrode placements in the margin of the ischemic zone ($n=15$ electrodes). Change in $E_K$ (mV), increase in the potassium equilibrium potential compared with initial, preocclusion values.

**FIGURE 4.** Bar graph of the change in the potassium equilibrium potential ($E_K$) for preparations in the center ($n=3$) and margin ($n=5$) of the ischemic zone during the third ($E_KC₃$) and fourth ($E_KC₄$) serial, 10-minute control occlusions of the left anterior descending coronary artery. Values are mean change in $E_K±SD$ for each minute of the occlusion.
The mean changes in $E_k$ before and after verapamil administration from all experiments are shown in Figure 7. In the center of the ischemic zone during $C_{3V}$, the mean changes in $E_k$ reach a maximum of $31.1\pm6.7$ mV, similar to that observed in $C_3$ ($31.5\pm1.5$ mV). After verapamil administration, the mean changes in $E_k$ increase more slowly and reach a maximum of $21.9\pm5.3$ mV. The differences between $C_{3V}$ and $V$ were significant from 1 to 7 minutes after the beginning of the occlusion. In the margin of the ischemic zone, the mean changes in $E_k$ during $C_{3V}$ reached a maximum of $15.9\pm1.0$ mV. This value...
is similar to that observed during C3 (19.5±3.6 mV). After verapamil administration, the mean changes in $E_K$ increase more slowly and reach a maximum of 14.6±2.9 mV. The differences between $C_{SV}$ and V are significant between 2 and 7 minutes after LAD ligation.

The average standard deviations of the change in $E_K$ at each time interval before and after verapamil administration are shown in Figure 8. In the center of the ischemic zone during $C_{SV}$, the mean standard deviations of the change in $E_K$ reach a maximum of 4.1±1.3 mV at 3 minutes after the beginning of the occlusion, are sustained until 6 minutes, and then decrease gradually to 3.7±0.8 mV at the end of the occlusion. This pattern is not substantially different than that recorded during C3. After verapamil administration, the maximum mean standard deviation of the change in $E_K$ is also 4.1±2.6 mV. However, it occurs 6 minutes after the start of the occlusion. Thereafter, mean standard deviations decrease to 3.2±0.9 mV by the end of the occlusion. Between 2 and 4 minutes after LAD ligation, two-way analysis of variance reveals that the standard deviations were significantly reduced by verapamil. In the margin of the ischemic zone during $C_{SV}$, the standard deviations were at their maximum 5 minutes after the start of occlusion (8.5±1.1 mV) and remained at this level throughout the remainder of the occlusion. This is also similar to the pattern of change that was recorded during C3. After verapamil administration, the maximum standard deviation was 7.4±0.9 mV. However, this maximum value was not observed until 9 minutes after the start of occlusion. The differences in the mean standard deviations for $C_{SV}$ and V were significant during the first 6 minutes of the occlusion.

We determined the coefficients of variation for $C_{SV}$ and V (i.e., the standard deviation divided by the mean) at each minute during the occlusion. These results are shown in Figure 9. In the absence and the presence of verapamil, the coefficients of variation were greatest in the first minute after occlusion and then decreased curvilinearly during the remainder of the occlusion. In the center of the ischemic zone, the coefficients of variation before verapamil administration average 0.72±0.15 at 1 minute after the beginning of the occlusion and decreased to 0.12±0.01 at 10 minutes. After the administration of verapamil, the coefficients of variation at 1 minute after the start of the occlusion averaged 2.3±0.35. This value is significantly greater than that during $C_{SV}$. The coefficients of variation then decreased to an average value of 0.15±0.03 at 10 minutes, a value not significantly different from $C_{SV}$. The significant increases in the coefficients of variation induced by verapamil in the center of the ischemic zone during the first minutes of the occlusion were not observed at the margin of the ischemic zone. In $C_{SV}$, the coefficients of variation in this region averaged 0.85±0.29 at 1 minute and 0.55±0.11 at 10 minutes after the start of the occlusion, whereas in the verapamil occlusion, the values averaged 0.86±0.17 and 0.51±0.13, respectively. The coefficients of variation during $C_{SV}$ and V at the margin were not significantly different at any time during the occlusion.

A different view of the relation between the mean change in $E_K$ and the mean standard deviation of the change in $E_K$ before and after treatment with verapamil in the two ischemic zones is provided in Figure 10, where these two parameters have been plotted against each other without respect to time. In the
center of the ischemic zone, initial increases in the mean change in $E_K$ are accompanied by similar increases in the mean standard deviations of the change in $E_K$. However, the standard deviations reach a maximum when the mean change in $E_K$ is about 15 mV. Therefore, the continued rise of the mean change in $E_K$ to 30 mV is not accompanied by additional increases in the mean standard deviation of the change in $E_K$. In the margin of the ischemic zone, a linear trend between these two parameters is clearly evident within the range of values observed. Verapamil has no effect on the relation between mean $E_K$ and mean standard deviation of the $E_K$ for either ischemic zone other than reducing the upper limit of the mean change in $E_K$ observed in the center and margin and slightly reducing the upper limit of standard deviations observed in the margin.

**Discussion**

In these studies, we attempted to quantify the magnitude and time course of the microinhomogeneities of the change in $E_K$ in the midmyocardium of the central and lateral margin of the ischemic zone during acute 10-minute occlusions of the LAD in the in situ pig heart. Also, we assessed the effects of verapamil on these parameters. We postulated that these inhomogeneities will provide insight into the cause of previously described electrical inhomogeneities and that these inhomogeneities may underlie the critical events that lead to ventricular fibrillation and sudden cardiac death.

**Existence of Microinhomogeneities**

The presence of differences in the rate and magnitude of the accumulation of $[K^+]_e$ within and between discrete regions of the ischemic zone was first suggested by Hill and Gettes. Their findings have been supported by several more recent studies. However, the magnitude and the time course of the microinhomogeneities existing within discrete regions of the ischemic zone have not been quantified. Our results indicate that the changes in $E_K$ at the same point in time after coronary occlusion range from 0 to 30 mV in the margin and from 20 to 35 mV in the center of the ischemic zone. Using standard deviation as a measure of the microinhomogeneity, we showed that the inhomogeneity is greater in the margin than in the center and that verapamil reduces the inhomogeneity of the $E_K$ change in both zones.

Our confidence in these results is strengthened by our application of strict electrode calibration, response, and location criteria, thereby eliminating the possibility that the inhomogeneities are due to differences in electrode response or inappropriate electrode placement. We did not accept electrode data that deviated by more than 5% from the Nernstian calibration response in vitro or by more than 15% from the Nernstian calibration response in vivo. We
used the in vivo calibration slope of each individual electrode to calculate the changing $E_K$ recorded from that electrode rather than assuming a typical calibration slope. We rejected data from electrodes that did not demonstrate a reproducible response to repeated in vivo boluses of potassium. Last, the postexperimental determination of the location of each electrode within the myocardium allowed us to exclude electrodes that were inappropriately placed. The application of these calibration, response, and placement criteria eliminated 50% of the total electrode data but substantially improved our confidence in the existence of the inhomogeneities.

**Measurement of Microinhomogeneity**

Several statistical parameters can be used to assess microinhomogeneities. These include the standard deviation, variance, standard error, and the coefficient of variation. We chose to use standard deviation because it has a time course that closely resembles the changes in $E_K$, uses the same units of measure, and provides a direct correlation to the changes in resting membrane potential. Our conclusions concerning the magnitude and time course of the inhomogeneities would have been somewhat different had we selected an alternative index, although the reproducibility of the results and the effect of verapamil would have remained essentially the same. The use of variance would have introduced a squared term, thus changing the units of measure to millivolts squared and “exploding” the data at higher $E_K$ values. The use of standard error would have attenuated the measurement through the division of the standard deviation by the square root of the number of electrodes used, thereby sensitizing the measure to the number of electrodes used in each study. We also calculated the coefficient of variation by dividing the standard deviation at each minute by the change in $E_K$ at that time. The effect of this manipulation is to express the variability as a fraction of the mean, thereby allowing comparisons between ischemic zones when neither the change in $E_K$ nor the standard deviation of the change have the same order of magnitude. This method produces a dimensionless value in which the absolute magnitude of the numerator and denominator are lost. Although the coefficient is of value in relating the mean and standard deviation near the end of the ischemic episode, we found the coefficient to be limited, if not misleading, during the initial phase of the occlusion. For example, the high value of the coefficient of variation recorded 1 minute after the start of the occlusion in the center and the margin of the ischemic zone during the control and verapamil occlusions is due to the relatively small change in $E_K$ rather than to a large change in standard deviation. Thus, this numerical expression of variability obscures the fact that both the change in $E_K$ and the standard deviation of the change are quite small initially and are likely to have little physiological significance. For these reasons, we do not consider the coefficient of variation to be a useful index of inhomogeneity in most circumstances. Rather, we believe that the standard deviation of the change in $E_K$ when viewed in light of the magnitude of the $E_K$ change, is the most useful and the most physiologically significant measure of the inhomogeneity in $[K^+]_o$ associated with ischemia.

The calculation of the minimum number of measurement sites required to accurately estimate inhomogeneity is not direct. The ratio of measured to true variance has a $\chi^2$ distribution as shown below:

$$
\chi^2_{0.05}(n-1) \leq \frac{s^2}{\sigma^2} \leq \chi^2_{0.95}(n-1)
$$

where $\chi^2$ is the upper and lower $\chi^2$ value for a specified probability interval (e.g., 90%, $\alpha=0.05$), $n$ is the number of measurement sites, $s$ is the measured standard deviation, and $\sigma$ is the true standard deviation. Through this probability interval for the $s^2/\sigma^2$ ratios, the $\chi^2$ tables can be used to calculate the expected percentage of overestimation or underestimation that $s^2$ represents relative to the true variability for selected values of $n$ and specified probability levels. We calculated these percentages for $n$ ranging from 5 to 36 at probability levels of 90% ($\alpha=0.05$) and 80% ($\alpha=0.10$). These percentages are summarized in Figure 11.

Examination of this figure reveals two details: 1) As the number of measurement sites used to calculate variability becomes larger, the range of overestimation and underestimation of true variability becomes narrower. 2) As the probability level allowed becomes smaller, fewer measurement sites are required to attain a given range of overestimation and underestimation of true variability. After an $n$ of 12, the improvement in the estimate of true variability at larger sample sizes begins to level off. For example, at $n$ equal to 12, estimates of true variability are about $\pm 25\%$ for the 0.80 probability level and $\pm 35\%$ for the 0.90 probability level. At $n$ equal to 20, these probability levels decrease to $\pm 20\%$ and $\pm 25\%$, respectively. In cases where the true variability is less (e.g., in the central ischemic region or after

**Figure 11.** Plot of the percentage of overestimates and underestimates of variance with increasing number of electrodes at probability levels of 80% ($\alpha=0.10$) and 90% ($\alpha=0.05$) for marginal electrode placements at 10 minutes after left anterior descending coronary artery occlusion.
the administration of verapamil), narrower ranges of underestimation and overestimation of variability will occur for each value of $n$ because of the reduced variability of the population for which the estimate is being calculated. In summary, our results suggest that 12 equivalent measurement sites are necessary to accurately estimate the $[K^+]$, microinhomogeneity. Although additional measurement sites will improve the accuracy of that estimate, practical limitations imposed by the experimental conditions may prevent the accumulation of these data.

We found that the changes in $E_K$ and in the standard deviation of the changes in $E_K$ were reproducible in the third and fourth control occlusions. This finding was expected on the basis of our earlier studies.\(^4\) However, fewer electrodes were used in those earlier studies, and the procedures used to calibrate the electrodes were less rigorous. For these reasons, we believed it important to confirm the reproducibility of the inhomogeneities before assessing the effects of verapamil. We confirmed the finding that verapamil lessened the change in $E_K$ during the occlusion. We also observed that verapamil reduced the inhomogeneity of the change in $E_K$ during the first 4 minutes of the occlusion in the center and during the first 6 minutes in the margin of the ischemic zone.

This work directly follows from our previous study,\(^7\) which was designed to relate the inhomogeneities in $[K^+]$ to electrophysiological variables. In that study, inhomogeneity during regional ischemia was determined at a single point in time measured 7–10 minutes after occlusion and was expressed as the coefficient of variation. In addition, 10-mV $E_K$ response bands were used to map the changes in $E_K$. In the present study, our intent was to quantify the magnitude of the microinhomogeneities and to determine the time course of their development. For these reasons, we did not partition the changes in $E_K$ into 10-mV bands, and we determined the inhomogeneities at each minute during the occlusion. In both studies, data were expressed as the change in $E_K$, and special care was applied in validating the performance of the electrodes, including an in situ calibration. We now conclude that standard deviation is a more appropriate and better physiological marker of the inhomogeneity than is the coefficient of variation, especially during the early portion of the occlusion, provided that an adequate number of electrodes are used in each region. Moreover, we conclude that the coefficient of variation masks a more subtle relation between the mean change in $E_K$ and the mean standard deviation of the change in $E_K$, thus indicating that the variability of changes in $E_K$ must be viewed in light of the magnitude of the change in $E_K$.

**Cause of the Inhomogeneities**

The mechanisms underlying the development of the microinhomogeneities, particularly those in the center of the ischemic zone, are not intuitively obvious. Several mechanisms can be postulated. These include the diffusion of potassium into regions having less potassium efflux, the presence of varying changes in $P_O_2$ and $P_CO_2$ within the sampled areas, the presence of varying proportions of ischemic and nonischemic or partially ischemic cells within the regions sampled by each electrode, and varying rates at which the myocardium reacts to the acute deprivation of nutrient blood flow.

In the study by Coronel et al,\(^7\) infusions of a high $[K^+]$ solution into the nonischemic vascular bed altered the characteristic changes in $E_K$ observed in the margin of the ischemic zone, converting the changes in $E_K$ to those more characteristic of and even greater than those observed in the center. These experiments provided evidence that the diffusion of the potassium from the margin of the ischemic zone to the normal myocardium accounted at least in part for the observed $E_K$ changes in the margin. The subendocardial border zone demonstrated by Wilensky et al\(^5\) is also partially explained by the diffusion of potassium into the ventricular chamber. Diffusion may also contribute to the microinhomogeneities at the margin. In the areas closest to the normal myocardium, the margin serves as a potassium source, and the change in $E_K$ is, therefore, lower than anticipated. In the area closest to the center, the border serves as a potassium sink; thus, the changes in $E_K$ are higher than anticipated.

The anatomic nature of the border may also contribute to the microinhomogeneities that we have observed. It is known that the margin is composed of interdigitating islands of normal and ischemic cells.\(^16,17\) The electrodes labeled as “margin” on the basis of their anatomic relation to the lateral cyanotic border may sample regions from both normal and ischemic cells. Those in or close to normal cells will record lower levels of $[K^+]$, than those in or closest to ischemic cells. This mechanism is obviously more applicable to the inhomogeneities observed in the margin than to those observed in the center and, in combination with the effects of diffusion, may help to explain the greater standard deviation of the changes in $E_K$ observed at the margin than in the center of the ischemic zone.

The efflux of potassium from ischemic cells was recently linked to the rise in $P_CO_2$ in the ischemic region.\(^18\) In areas closest to the lateral margin and to the ventricular chamber, carbon dioxide will diffuse away from the ischemic zone. This factor will lessen the rise in $[K^+]$, regardless of any effect of potassium diffusion. Again, this effect would be expected to contribute to the lower changes in $E_K$ at the lateral margin and in the subendocardial border region. In addition, the diffusion of oxygen into each of these regions would be expected to lessen the effects of ischemia and lead to a lesser rise in $P_CO_2$ and a lesser rise in $[K^+]$. Although these events may help to explain the differences in the changes in $E_K$ between the center and margin and may contribute to the microinhomogeneities present in the margin, they do not readily explain the microinhomogeneities in the
change in $E_K$ present within the midmyocardium of the center of the ischemic zone.

A possibility that cannot be excluded on the basis of our study is that the cells in the midmyocardium of the center of the ischemic zone respond to the abrupt cessation of coronary flow at slightly different rates, leading to slightly dissimilar rates of the free energy of ATP hydrolysis,\textsuperscript{19} the rate of development of intracellular acidosis,\textsuperscript{4} or the appearance of ionic or metabolic compartments.\textsuperscript{20} Because the rise in intramyocardial $[K^+]_e$ has been attributed to these mechanisms, minor differences in the time course of these events may explain the microinhomogeneities in the changes in $E_K$ that we have observed in the center of the ischemic zone and may contribute to the microinhomogeneities in the margin.

**Effect of Microinhomogeneities**

Our findings indicate that within the midmyocardium of the center of ischemic zone, the changes in $E_K$ after coronary occlusion may range from 20 to 35 mV. In the lateral margin, the changes may range from 0 to 30 mV. Kleber\textsuperscript{14} showed that during ischemia, the changes in $E_K$ predict the changes in resting membrane potential. If the resting potential of the myocardial cells is assumed to be $-85$ mV in the control preocclusion period, then resting potentials ranging from $-65$ to $-50$ mV will occur simultaneously in closely spaced cells within the center of the ischemic zone and from $-85$ to $-55$ mV in the cells within the lateral margin. These differences in resting potential will result in significant differences in the magnitude of the inward sodium current, the rate of rise of the action potential upstroke, the time constant of the recovery of excitability, and the action potential duration. In addition, the rates at which intracellular and extracellular resistances change may also vary.\textsuperscript{21} As a result, significant differences in conduction velocity and refractoriness within closely spaced regions will occur. These differences may contribute to the development of the intramural reentry circuits recently described by Pogwizd and Corr.\textsuperscript{22} If the changes in the subendocardial border described by Wilensky et al\textsuperscript{15} are similar to those that we and Coronel et al\textsuperscript{17} observed at the lateral margin, then macroreentry and microreentry circuits may be expected to occur in the lateral and subendocardial borders. The differences in $E_K$ (and resting potential) between closely spaced myocardial elements may also lead to local injury currents that either add to or detract from the more global injury currents generated across the various border regions.\textsuperscript{2,23} These local injury currents may contribute to the lack of correlation between the changes in $E_K$ and changes in the TQ potential of the local electrogram.\textsuperscript{6,7} They may also contribute to the development of spontaneous depolarization and triggered activity within the center of the ischemic zone, and thereby be one cause of the nonreentrant spontaneous beats and rhythm observed.\textsuperscript{22}

The magnitude of the microinhomogeneities as expressed by the standard deviations of the change in $E_K$ peaked within the first 5 minutes of the occlusion then decreased in the center or remained constant at the margin. This finding provides at least a temporal correlation between the development of inhomogeneities and the onset of ventricular arrhythmias that occur in the first 5 minutes of a coronary occlusion as determined by Kaplinsky et al.\textsuperscript{24} By so doing, it lends credence to the argument that the microinhomogeneities may be one cause of these arrhythmias.

**Effect of Verapamil**

In this study, as in our previous work,\textsuperscript{12} we found that verapamil slowed the rate of rise of $[K^+]_e$ after LAD occlusion in the center and the margin of the ischemic zone. Using an isolated papillary muscle preparation, Casco et al\textsuperscript{25} showed that verapamil also postpones cell-to-cell uncoupling, the secondary rise in $[K^+]_e$ and the onset of ischemic contracture while decreasing the rate of uncoupling and contracture. This study adds to these observations by showing that verapamil also slows the rate at which the inhomogeneities in the change in $E_K$ develop. Whether this represents an important mechanism underlying the known antibrillitarian effect of verapamil,\textsuperscript{8,9} cannot be determined from this study because calcium channel blockade exerts several additional effects that may be of equal or greater significance. These include the slowing of the rate of rise of $[K^+]_e$,\textsuperscript{12,26} a decrease in the fall in pH,\textsuperscript{12} suppression of action potentials dependent on the calcium inward current,\textsuperscript{27} the preservation of energy stores,\textsuperscript{20} and mitochondrial protection.\textsuperscript{29} Our observation that verapamil does not alter the relation between the mean change in $E_K$ and the mean standard deviation of the change in $E_K$ (Figure 10) strongly suggests that the antiarrhythmic effects attributed to verapamil are more likely due to the time shift of the change in $E_K$ and the standard deviation of the change in $E_K$ than to the actual reduction in the magnitude of either parameter. Indeed, previous work using this same animal model\textsuperscript{30} has shown that the plateau levels of $[K^+]_e$ during 60-minute occlusions is the same in both ischemic zones after treatment with verapamil compared with those of the control, although verapamil significantly delays the time at which the plateau occurs. Nevertheless, if the microinhomogeneities in $E_K$ do contribute to the early arrhythmias after coronary occlusion, then it is reasonable to speculate that the lessening of these inhomogeneities is an important antiarrhythmic effect.

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**References**


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