Distribution of Extracellular Potassium and Electrophysiologic Changes During Two-Stage Coronary Ligation in the Isolated, Perfused Canine Heart

Ruben Coronel, MD, Jan W.T. Fiolet, PhD, Francien J.G. Wilms-Schopman, Tobias Opthof, PhD, Alexander F.M. Schaapherder, MD, and Michiel J. Janse, MD

We studied the relation between \([K^+]_o\) and the electrophysiologic changes during a “Harris two-stage ligation,” which is an occlusion of a coronary artery, preceded by a 30-minute period of 50% reduction of flow through the artery. This two-stage ligation has been reported to be antiarrhythmic. Local direct current electrograms and \([K^+]_o\) signals from up to 48 intramural sites were simultaneously recorded in isolated, perfused dog hearts. A second period of one-stage ligation was compared with a consecutive two-stage ligation because reproducibility in \([K^+]_o\), and electrophysiologic changes are established only after the first period of ischemia. In control experiments, no difference was found between the second and third period of one-stage ligation in the electrophysiologic changes and in increases in \([K^+]_o\). After complete occlusion during two-stage ligation, activation block in the ischemic tissue occurred about 6 minutes earlier than during one-stage ligation, but the average potassium concentration at which block occurred was identical. This \([K^+]_o\), during total ischemia was achieved earlier during two-stage ligation than during one-stage ligation. No indication was found for a large decrease of intracellular potassium content during the period of low flow perfusion. Early activation block may explain the previously reported reduced incidence of ventricular fibrillation during two-stage ligation. (Circulation 1989;80:165–177)

In 1950, Harris described a method to study late cardiac arrhythmias after coronary artery ligation in the in situ dog heart. 1 Specifically, in this experimental model, the first phase of life-threatening arrhythmias after coronary occlusion was absent, thus reducing early mortality. The reduction of primary ventricular fibrillation was brought about by a 30-minute period of low flow ischemia preceding total ischemia of the myocardium supplied by the left anterior descending artery. This protocol is known as the “Harris two-stage ligation” and has been used by many investigators. 2–4

Kabell et al 5 measured regional myocardial blood flow during one-stage ligation of the coronary artery in one group of animals and during the second stage of a two-stage ligation in another. Although regional myocardial blood flow during stenosis was approximately 50% of normal, they did not detect differences in regional blood flow between the two groups of animals. Therefore, the antiarrhythmic effect of a two-stage ligation is not the result of an increased collateral blood flow during total ischemia.

In this study, we investigated the relations between \([K^+]_o\) and the electrophysiologic changes during a Harris two-stage ligation. The experiments were performed on isolated, perfused canine hearts and involved multiple potassium-sensitive electrodes.

We conclude that 1) during total regional ischemia preceded by low flow ischemia, the ischemic zone reaches the stage of activation block about 6 minutes earlier than during one-stage occlusion, and 2) the early activation block during two-stage ligation with respect to sudden ligation is the result of an increased \([K^+]_o\) at the beginning of the complete occlusion.

Methods

Thirteen mongrel dogs weighing 20–33 kg were anesthetized with pentobarbital (15 mg/kg i.v.)
heart was exposed through a midsternal thoracotomy. One liter of a modified Tyrode's solution and 5,000 IU heparin were infused through the femoral vein simultaneously. A 1:1 blood-Tyrode mixture (about 2 l) was collected from the anterior caval vein and used as a perfusion solution later. The heart was dissected free after ventricular fibrillation was induced by application of a direct current and was immersed in a 4°C Tyrode's solution as quickly as possible. The aorta was cannulated, and the heart was mounted on a Langendorff perfusion setup. The ventricles were vented. Subsequently, the heart was defibrillated by a DC countershock. The left anterior descending artery (LAD) was cannulated close to the bifurcation, and the cannula was connected to the perfusion apparatus. If this proved impossible because of the multiple branches emerging from the LAD close to the bifurcation, the circumflex artery (Cx) was cannulated instead (three hearts). Usually, the [K+] of the perfusate decreased by 1–2 mM after mounting the heart to the perfusion setup. This was counteracted by administration of potassium chloride to the perfusion solution.

The perfusion setup consisted essentially of two separate recirculatory systems that could be directed to the heart by a three-way stopcock placed above the aortic cannula. One of the recirculatory systems contained perfusate with a normal [K+]. The other contained perfusate with an elevated [K+] that was used to test the potassium-sensitive electrodes in situ and to defibrillate the heart if necessary. This method of defibrillation was used instead of a DC countershock to prevent damage to the potassium-sensitive electrodes and the amplifiers. Flow through the coronary cannula could be regulated by an occluder. Total coronary flow and flow through the cannulated coronary artery were measured by electromagnetic flow probes. The pH of the perfusate was regulated between 7.35 and 7.45. Temperature of the heart was 37°C. During total regional ischemia, the temperature of the ischemic zone decreased by 2°C at most.

We inserted 10–48 potassium-sensitive electrodes in the midmyocardium, 5 mm below the epicardial surface. Fabrication of the electrodes has been described before. Essentially, the electrodes consist of a potassium-sensitive terminal of the valinomycin type and a reference terminal. The potassium-sensitive electrodes were calibrated in vitro in two isotonic potassium chloride test solutions of 1 and 10 mM at room temperature.

Signals from the electrode pairs were differentially DC amplified, and the signal from the reference electrode was DC amplified against a common reference electrode attached to the root of the aorta. Thus, a potassium signal and a local DC electrogram could be recorded. Signals were passed to an A to D converter, were sampled every 4 msec, and were continually written into a circular buffer. The content of the buffer containing all data collected during the last 3 seconds could be stored on a disk at any moment during the experiment.

In four hearts in which the LAD was perfused at a low flow, the great cardiac vein was separately cannulated close to the bifurcation of the left coronary artery. During a period of 1 minute, blood from the vein was collected for determination of [K+], both during control perfusion and during flow reduction in the LAD. Simultaneously, arterial blood samples were drawn. Because blood sampled from this vein is not necessarily derived from the myocardium perfused by the LAD only and because [K+] in the great cardiac vein will be diluted by blood from the normal area, this method can only roughly approximate the amount of potassium lost by the ischemic myocardium.

The hearts were stimulated from the right ventricular outflow tract with rectangular current pulses of 2-msec duration at a stimulation strength of twice threshold and a basic cycle length of 350 msec. During the recordings, the basic cycle length was changed to 450 msec for a few seconds to more reliably identify the TQ segment. In three experiments, the site of stimulation was changed from the right ventricular outflow tract to a position on the normally perfused myocardium of the left ventricle during ischemia. For this purpose, the heart was instrumented with two pairs of stimulation electrodes. Changing the site of stimulation was instantaneous. For the analysis of the relation between monophasic electrograms and [K+]o (Figure 5), the site of stimulation was always on the right ventricular outflow tract.

**Experimental Protocol**

The coronary artery was first occluded for 6 minutes; no data were collected during this period because the first occlusion differs from all the subsequent periods of ischemia (see "Discussion"). This first ischemic period was used to identify the visual border. Then, the electrodes were inserted into the myocardium, and the heart was allowed to recover for at least 1 hour.

**Test perfusion.** A 4–5 minute period of perfusion with a high [K+] solution was performed in all hearts.

**One-stage occlusion.** After 20 minutes of perfusion with normal perfusion fluid, the coronary artery was occluded a second time for 8 minutes (one-stage occlusion) in all 13 hearts.

**Two-stage occlusion.** After the one-stage occlusion and a 15–20 minute period of recovery, the heart was made partially ischemic by adjusting the occluder on the coronary cannula until a 50% flow reduction in the cannula was measured (low flow ischemia or "stenosis") in seven hearts. This condition was maintained for 30 minutes. Immediately after this period of low flow ischemia, flow through the coronary artery was completely stopped for 8 minutes (two-stage occlusion).
Control experiments. After the one-stage occlusion and 40 minutes of reperfusion, a successive 8-minute period of one-stage occlusion was performed in six hearts.

All monitored variables returned to control value during the period of the occlusion. If ventricular fibrillation occurred before the end of the intervention, the occlusion was discontinued, and the heart was defibrillated by high [K+] perfusion.

Inclusion Criteria

Data from the potassium-sensitive electrodes were accepted for analysis only: 1) if the electrograms during control perfusion did not show more than 2 mV ST elevation, 2) if the calibration before the experiment showed a 55–61 mV per decade change of [K+] at 37° C, 3) if the response to a high [K+] perfusion was as large as calculated from the in vitro calibration, 4) if baseline drift was less than 5 mV/hr, and 5) if the reference electrode showed a stable recording.

For reference electrodes (recording local DC electrograms) only, criteria 1, 4, and 5 were used. Blood samples were drawn whenever required, and [K+] was determined during experiments with a commercially available K electrode (Philips, The Netherlands). Measurements of the latter were confirmed later by flame photometry. Potassium curves were corrected for baseline drift by fitting a straight line between points with an identical [K+].

The number of electrodes inserted in 13 hearts was 321, of which 213 (66%) were accepted for analysis of potassium curves at the start of the protocol. Of these electrodes, 158 were located in the ischemic zones, 59 in the six control experiments, and 99 in the two-stage experiments. Similarly, 248 reference electrodes, in the ischemic zones, were accepted, of which 97 were in the control experiments and 151 in the other experiments.

Definitions

The border is the line separating the ischemic zone from the normal zone (the tissue in which [K+] does not change during an occlusion). The central ischemic zone is arbitrarily defined as ischemic tissue at a 1-cm distance from the border, and the “border” zone is the tissue between the normal zone and the central ischemic zone.

A monophasic or “block” electrogram is an electrogram 1) that is entirely positive with respect to the TQ segment, 2) in which an initial, sharp negative deflection is absent, and 3) in which the repolarization wave cannot be distinguished from the depolarization wave.

Statistical Analysis

Statistical significance was tested with a t test and 0.05 was adopted as the level of significance when appropriate 95% confidence intervals were compared. Data are mean±SD unless stated otherwise.

Results

Subsequent Occlusions

When hearts are subjected to repeated short periods of ischemia, separated by a long recovery period, the time course and the magnitude of the electrophysiologic changes are identical from the second occlusion onward. K+ reproducibility is established after one or two periods of ischemia.

We performed six experiments to test reproducibility of the increase of K+ and of the electrophysiologic changes during subsequent periods of ischemia in our experimental setup. The time course of change of [K+] during the second and third one-stage occlusions is shown in Figure 1. Note that the increase in [K+] is similar during the two subsequent episodes of ischemia. In the central zone, a larger change of [K+] during the third than during the second period of one-stage occlusion can be observed after 8 minutes of ischemia. This difference is, however, small. Also, the sequence of activation (not shown) and the moment of occurrence of monophasic block electrograms (see Figure 5) were identical during subsequent periods of total ischemia separated by 40 minutes of reperfusion from the second occlusion onward. In two
control experiments, ventricular fibrillation occurred during the second period of ischemia, in one during the third period. The occurrence of other arrhythmias was not markedly different in the two subsequent periods of ischemia. This justifies the comparison of subsequent occlusions and allows every heart to serve as its own control from the second period of ischemia onward.

Low Flow Regional Ischemia

Figure 2 shows an example of the time course of change of \([K^+]_o\) during one-stage (left panel) and two-stage occlusion preceded by "stenosis" (right panel). One of the electrodes is in the central zone, the other is in the border zone. During low flow regional ischemia, the border zone electrode records a slow increase of \([K^+]_o\) until a maximum is reached after 15 minutes followed by a decline. The electrode in the central zone, however, records a steady increase of \([K^+]_o\) until a maximum is reached at the end of the period of low flow regional ischemia. Two-stage occlusion after low flow ischemia causes a rapid rise of \([K^+]_o\) in the central zone. Of the 99 potassium-sensitive electrodes in the ischemic area that were accepted for analysis (see "Inclusion Criteria"), 47 recorded an early maximum during the period of low flow regional ischemia. Fifteen of the 28 (54%) electrodes in the border zone recorded an early maximum of \([K^+]_o\) during low flow ischemia. Of the 71 electrodes in the central zone, 32 (45%) recorded an early maximum. Therefore, the characteristics of the rise in \([K^+]_o\) during low flow ischemia are independent of the location of the electrodes with respect to the border.

A map of distribution of the change of \([K^+]_o\), after 8 minutes of one-stage occlusion (left panel) and 30 minutes of low flow regional ischemia (right panel) is shown in Figure 3. The right panel shows a maximum of \([K^+]_o\), that is relatively low compared with the maximum \([K^+]_o\) in Panel A. Note that in both situations a lateral zone of intermediate values of \([K^+]_o\) is present. In each period of low flow regional ischemia, inhomogeneity of \([K^+]_o\) was estimated by calculating the coefficient of variation, that is, the standard deviation divided by the mean of the measurements of \([K^+]_o\), within the ischemic zone after 8 minutes of ischemia. Figure 3 shows the comparison of these values with those obtained during test perfusion with high \([K^+]\) perfusate and one-stage occlusion. During ischemia, values are presented only for electrodes in the central zone. In these five experiments (in two others, ventricular fibrillation started during one-stage occlusion), the...
TABLE 1. Variability of Change of [K+]o

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test perfusion</th>
<th>Central zone during regional ischemia</th>
<th>One-stage (8 min)</th>
<th>Stenosis (8 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ[K+]</td>
<td>CV</td>
<td>(n)</td>
<td>Δ[K+]</td>
</tr>
<tr>
<td>1</td>
<td>9.9</td>
<td>0.40</td>
<td>(19)</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
<td>0.20</td>
<td>(26)</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
<td>0.18</td>
<td>(15)</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>0.15</td>
<td>(28)</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>0.16</td>
<td>(12)</td>
<td>3.7</td>
</tr>
<tr>
<td>Mean CV</td>
<td>0.22</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>SD</td>
<td>0.10</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; n, number of electrodes involved.
Note that within the central zone a larger variability is observed during stenosis.

coefficient of variation is significantly larger during low flow ischemia than during no flow ischemia. During low flow ischemia, the coefficient of variation in the central zone was not statistically different from the coefficient of variation in the border zone, contrary to the situation in no flow regional ischemia. This indicates that during low flow ischemia the inhomogeneity in [K+]o in the central ischemic area is about as large as in the border zone.

Activation maps obtained in one particular experiment are shown in Figure 4. The upper panel (a) shows the activation sequence during control perfusion. The lower panel (a) shows the activation map after 30 minutes of stenosis. During low flow ischemia, the activation sequence of the ischemic area showed surprisingly little change despite the increase in [K+]o. Thus, only 19 of the 151 (13%) reference electrodes in the ischemic area recorded monophasic block electrograms after 30 minutes of low flow ischemia. In none of the nine hearts did ventricular tachycardia or ventricular fibrillation occur during low flow regional ischemia.

**Effects of Stenosis on Subsequent Occlusion**

**Activation times.** The upper panels of Figure 4 show the sequence of activation after 0, 4, and 8 minutes of one-stage occlusion. After 4 minutes of one-stage occlusion, none of the electrodes showed late activation or block. Although 4 minutes later, the number of electrodes recording block electrograms was increased, a large part of the ischemic area was still activated. By contrast, the lower.
panels show the sequence of activation after 0, 4, and 8 minutes of two-stage occlusion. Although the activation front travels relatively unimpaired through the ischemic region at 30 minutes of stenosis (i.e., the onset of two-stage occlusion), monophasic block electrograms were recorded from a large part of the ischemic area 4 minutes later. This area of block was increased in size after 8 minutes of two-stage occlusion.

Figure 5 shows the accumulated data from the seven two-stage experiments and six control experiments. The percentage of the electrodes in the ischemic region that recorded monophasic block electrograms is shown every 2 minutes of the second period of total ischemia (Panel A) and the third period of total ischemia (Panels B and C). Panel B presents the data from the seven two-stage experiments (with preceding stenosis), and Panel C shows the data of the control experiments (without preceding stenosis). A more pronounced increase of monophasic potentials is obvious during two-stage occlusion. Note that the time course of increase of the amount of block electrograms is identical during the third occlusion in the control experiments (right panel) and the accumulated one-stage occlusions (left panel). The direction from which the activation front approached the ischemic area was the same in each single experiment (see Figure 4) when stimulation was performed from the same site. Note also that the number of electrodes decreases in time because of the occurrence of ventricular fibrillation in four hearts. Two hearts were in the two-stage group during the one-stage occlusion. No ventricular fibrillation occurred during the occlusions after low flow ischemia. The other two hearts were in the control group. In one of these two hearts, ventricular fibrillation occurred in the third one-stage occlusion (right panel). Electrodes recording a monophasic electrogram in one-stage occlusion always recorded a monophasic electrogram in two-stage occlusion.

**Extracellular potassium.** Figure 6 shows potassium distribution maps corresponding to the activation maps of Figure 3. The maximum concentrations reached during two-stage occlusion were higher, and inhomogeneity of [K+]o during two-stage occlusion was not less than that during one-stage occlusion. The largest changes of [K+]o can be seen in the central zone.

Figure 7 shows the average time course of change of [K+]o, recorded by all potassium-sensitive electrodes in the ischemic zone during one- and two-stage occlusion. This figure shows that [K+]o was always elevated after 30 minutes of stenosis and
The early development of a large unexcited area during total regional ischemia after preconditioning with low flow regional ischemia may result from 1) an elevated \( [K^+]_o \), at the end of the period of preconditioning. The time needed to reach the concentration at which cells become inexcitable is thus reduced, and 2) depolarization during the period of low flow regional ischemia caused by a net loss of intracellular potassium. A small increase in \( [K^+]_o \) during the consecutive total regional ischemia would then bring the tissue to inexcitability.

The first possibility implies that the average \( [K^+]_o \) at which block electrograms occur is the same during one- and two-stage occlusions. The second alternative implies that block electrograms occur at a lower \( [K^+]_o \), during two-stage than during one-stage occlusion and that the net loss of potassium from the ischemic area during coronary stenosis is large enough to induce an initial depolarization of the cell membrane.

**Potassium Accumulation and Monophasic Electrograms**

Figure 9 shows the relation between the occurrence of monophasic electrograms and \( [K^+]_o \) in the second and third period of total ischemia. It shows the fraction of the electrodes that recorded block electrograms during one- or two-stage occlusion in classes of 1 mmol/l \( [K^+]_o \). We assume that when an electrode records a block electrogram at a certain potassium concentration it will also do so at a higher concentration. Figure 9 shows that the relation between \( [K^+]_o \), and block is unchanged in two-stage compared with one-stage occlusion. Specifically, there is not a leftward shift of the curve after preconditioning with stenosis. Therefore, a decrease of intracellular potassium during low flow ischemia probably is not a major cause for earlier development of monophasic block electrograms. At a \( [K^+]_o \) of 9-10 mmol/l, 50% of the electrodes recorded monophasic electrograms. Figure 7, therefore, indicates that a larger fraction of the electrodes recorded a monophasic electrogram during two-stage occlusion. Only 23 electrodes recorded a monophasic electrogram after both the one- and the two-stage occlusions. Individual absolute differences between the \( [K^+]_o \) at which a monophasic electrogram occurred after a one- and a two-stage occlusion were less than 1 mmol/l in nine electrodes and 1-2 mmol/l in eight electrodes. The average concentrations for these 23 electrodes were not different.

In a previous communication, we reported that monophasic electrograms are associated with a wide range of values of \( [K^+]_o \). This was also true in the present experiments (Figure 9). We suggested that the sequence of activation can influence the occurrence of block electrograms. This factor could offer an additional explanation for the relatively low \( [K^+]_o \) at which monophasic electrograms can be measured. In three experiments, we changed the site of stimulation from the right ventricular outflow

![Diagram](http://circ.ahajournals.org/)
tract to a position on the normally perfused portion of the left ventricle. Figure 10 shows electrograms recorded a few seconds after each other at 8 minutes of ischemia in one of those experiments. These electrograms were recorded at a relatively low $[K^+]_o$. Figure 10 shows that indeed local activation could be recorded that depends on the sequence of activation. In all three experiments, similar changes of the pattern of the electrograms were recorded by electrodes positioned at the margin of the area generating monophasic electrograms. The dependence of the electrograms on the sequence of activation was maximal at the end of the period of two-stage occlusion (after preconditioning) when the blocking area was largest. In these three experiments, 37 electrodes recorded monophasic electrograms when the heart was stimulated from the right ventricular outflow tract after 8 minutes of total ischemia after stenosis. Of all electrograms, 14 changed from a monophasic to a nonmonophasic complex or vice versa when stimulation was performed from the left ventricle. Thus, when the activation sequence changes, tissue that first generated monophasic electrograms can generate a sharp negative deflection, though of low amplitude. This illustrates that ischemic tissue that is excitable might not be activated when the activation front has to traverse severely depressed tissue first.

**Potassium Loss During Low Flow Ischemia**

If the decrease of intracellular potassium is the cause for earlier development of inexcitability, the following calculation can be made. To bring a cell to inexcitability, a depolarization by about 30 mV is required. For a depolarization of this magnitude, the intracellular potassium content has to decrease by approximately 66%. Based on estimations of wet weight of the ischemic region, wet weight to dry weight ratio, intracellular potassium content, and coronary flow during stenosis, we found that a continuous arteriovenous difference of about 6 mmol/l is required in our experimental conditions. This does not take into account the extracellular accumulation of potassium during stenosis or possible changes of

**FIGURE 7. Plots of average time course of increase of $[K^+]_o$ during one-stage occlusion (solid lines) and during two-stage occlusion (dotted lines) recorded by electrodes in the central zone (Panel A) and the border zone (Panel B). Bars indicate one-sided 95% confidence intervals. Numbers of electrodes are indicated next to the bars. At C, the mean $[K^+]$ at the onset of the interventions is indicated. Note similar rate of rise of $[K^+]_o$ and a large $[K^+]_o$ at the end of the period of low flow ischemia. Also note the absence of changes in the border zone electrodes.**
Coronel et al  \([K^+]_o\) and Electrophysiologic Changes  173

![Graph: Change of TQ-potential vs. duration of ischemia (min)](image)

**Figure 8.** Plots of average time course of change of TQ potentials during one-stage occlusion (solid lines) and two-stage occlusion (dotted lines) in the central zone (Panel A) and the border zone (Panel B). Bars indicate 95% confidence intervals. The more positive the value the larger the amount of TQ depression.

The intracellular volume. We have measured arterio-venous differences in \([K^+]\). Mean arteriovenous difference during control perfusion was 0.0±0.1 mmol/l. After 20 minutes of stenosis, when peak arteriovenous differences usually were measured, the mean difference was 0.2±0.15 mmol/l. Although arteriovenous differences during stenosis were always larger than during control perfusion, the difference was not significant when tested with a paired t test.

**Discussion**

In summary, the results show that 1) low flow regional ischemia is neither a homogeneous condition (Figure 3) nor a steady-state condition (Figure 2); 2) total ischemia after low flow ischemia (two-stage occlusion) is characterized by an early development of a large area of conduction block (Figures 4 and 5); 3) extracellular potassium concentration during total regional ischemia after low flow ischemia (two-stage occlusion) is larger, especially in the central zone and is equally inhomogeneous (Figures 6 and 7) with respect to total ischemia without preconditioning (one-stage occlusion); 4) the relation between \([K^+]_o\) and monophasic block electrograms is unchanged in a two-stage occlusion when compared with a one-stage occlusion (Figure 9); 5) the wide range of values of \([K^+]_o\) related to the occurrence of monophasic block electrograms (Figure 9) can be partially explained by the sequence of activation (Figure 10); and 6) the loss of intracellular potassium during low flow ischemia (50% flow reduction) is not large enough to depolarize the cell membrane and does not explain the early activation block.

The most important finding of this study is that during two-stage occlusion a large part of the ischemic zone becomes unresponsive at a much earlier time than after abrupt one-stage occlusion. A major factor contributing to the early development of a large area of block is an increase of \([K^+]_o\) in the ischemic zone during the period of low flow ischemia. Because the rate of rise of \([K^+]_o\) during one-stage occlusion is identical to that during two-stage occlusion, the \([K^+]_o\) at which conduction block occurs is reached much earlier in a two-stage occlusion.

**Methods**

The antiarrhythmic effect of a two-stage ligation has been amply documented before; in the original study, however, the comparison between a one- and a two-stage occlusion was made in a group of only four one-stage experiments (in which three
dogs died of ventricular fibrillation) and 60 two-stage experiments (one dog died). Kabell et al repeated the experiments in an accurately controlled manner and observed a significant difference in mortality due to early ventricular fibrillation between the two groups of animals.

The object of this study is to describe the relation between \([K^+]\) and the electrophysiologic changes during two-stage ligation and not to directly link early activation block to the antiarrhythmic effect of a two-stage ligation. Showing that a causal relation existed between our results and the reported antiarrhythmic effect of a two-stage ligation would have required the analysis of only first periods of ischemia, the use of large numbers of animals, and the simultaneous determination of regional myocardial blood flow, local activation times, and \([K^+]\). In our approach, each heart serves as its own control, thus simplifying the interpretation of the data. The limited number of experimental animals and the protocol used do not allow a statistical evaluation of incidence of ventricular fibrillation in animals subjected to one-stage and two-stage ligation.

The incidence of ventricular fibrillation in our preparation was four of 13 (31%) in the first 8 minutes of one-stage ischemia. Had the periods of ischemia been longer, a larger incidence would have been reported because of the Ib phase of arrhythmias. A similar incidence of ventricular fibrillation was reported by others. Yoshida and Downey showed that the overall arrhythmia score is identical after a first and a subsequent occlusion.

The overall occurrence of ventricular fibrillation was reproducible in the first four periods of ischemia in a study by Cardinal et al. In a single experiment, the occurrence of ventricular fibrillation in a first period of ischemia is not a good predictor for ventricular fibrillation after a subsequent occlusion. However, when experiments are pooled, there is a good reproducibility of the occurrence of ventricular fibrillation during repetitive occlusions.

The design of the experimental protocol in this study is based on the assumption that consecutive periods of reversible ischemia are electrophysiologically identical. Cardinal et al have measured electrophysiologic variables in subsequent 15-minute occlusions separated by 15 minutes of reperfusion in the isolated, perfused pig heart. They concluded that from the second period of regional ischemia onward consecutive occlusions show the same electrophysiologic changes, such as changes of the ST and the TQ segment, activation times, and the occurrence of arrhythmias. Fleet et al showed the same results for the change of extracellular potassium activity in the in situ working pig heart. In their experiments, however, reproducibility of potassium accumulation during repetitive short periods of ischemia was often only established after the third period of ischemia. For this reason, we performed six experiments studying reproducibility of \(K^+\) in our experimental conditions. In these hearts, the second and the third period of ischemia closely resembled each other, and therefore, the data recorded during the second period of ischemia were used as control values for those recorded in the third period of ischemia. The change of local pH decreases in consecutive periods of ischemia. We measured a larger area of activation block during...
the occlusion after low flow ischemia. Acidosis induces an additional depolarization.\textsuperscript{15,16} A decreased degree of acidosis therefore cannot explain the effect of a preconditioning period of low flow ischemia. Moreover, it can be expected that during low flow ischemia glycogen stores are depleted and that during a subsequent period of total ischemia the change of pH is much less.

**Low Flow Ischemia**

Watanabe et al\textsuperscript{17} have established that the increase of \([K^+]_0\) is one of the first signs of ischemia when blood flow is progressively reduced in the in situ pig heart. Our results show that 30 minutes of 50\% flow reduction do not have serious electrophysiologic effects; however, after 30 minutes of low flow ischemia, the \([K^+]_0\) in the central zone is raised to a level normally reached after 6 minutes of total ischemia (Figure 7). This is in line with the findings of Saito et al\textsuperscript{18} who described that conduction velocity only decreases at a \([K^+]_0\) in excess of 8 mM. The percentage of electrodes (13\%) recording block electrograms in the ischemic area at that particular potassium concentration is about the same as the percentage reached after 6 minutes of total regional ischemia (Figure 5). This may indicate that the relation between block electrograms and extracellular potassium is similar in low flow ischemia and total ischemia. Yet, low flow ischemia never precipitated ventricular arrhythmias. This may be explained by the patchy nature of low flow ischemia causing a discontinuous distribution of areas of block electrograms.

The inhomogeneous nature of regional low flow ischemia is illustrated by the distribution of extracellular potassium (Figure 3, and Table 1), which suggests that within the ischemic zone anoxic tissue is in close proximity to oxygenated tissue. The anoxic tissue is not necessarily deprived of blood flow but may be perfused with blood already depleted of oxygen. Because anoxic perfusion leads to contracture\textsuperscript{19} within 10 minutes, tissue perfused in this manner will be rapidly deprived of flow, favoring blood flow to and washout from neighboring myocardium. Steenbergen et al\textsuperscript{20} described the inhomogeneous distribution of NAD\textsuperscript{+} fluorescence in hypoxic myocardium. Wiersinga\textsuperscript{21} devised a computer simulation model of the capillary bed of the myocardium and suggested that even during normal perfusion blood flow through parts of the capillary bed may be zero. Perfusing the heart with a reduced flow can only increase the areas of zero perfusion, leading to partially normally perfused and partially ischemic tissue.

During total regional ischemia, \([K^+]_0\) is determined by the amount of cellular potassium loss and the flux of potassium toward the normal tissue.\textsuperscript{6} In low flow ischemia, an additional factor responsible for washout is residual blood flow. Because the change of \([K^+]_0\) in the border zone during the total occlusion after low flow ischemia is small (Figure 7B) and in some cases absent (Figure 2), the change of \([K^+]_0\) during low flow ischemia in the border zone may be primarily determined by flux of \(K^+\) toward the normal zone, whereas it may be mainly determined by the residual blood flow in the central zone. The residual blood flow probably is the main cause for the large inhomogeneity in \([K^+]_0\) in the central zone during low flow ischemia.

Our results also show that low flow ischemia even at a relatively small reduction of flow is not a steady state with respect to accumulation of extracellular potassium. The rising phase of \([K^+]_0\) can be protracted even more than in total ischemia (Figure 2). Therefore, total ischemia cannot be simulated with anoxic perfusion or a low flow.

**Preconditioning With Low Flow Ischemia**

In the four hearts that fibrillated during the control occlusion, fibrillation started early during ischemia (Figure 5) and was associated with a relatively small amount of tissue generating block electrograms. The early development of a large area of block electrograms after preconditioning with low flow ischemia could explain the reported decrease of occurrence of fibrillation during a two-stage occlusion. Apart from an area of block around which the activation wave can circle, another requirement for reentrant arrhythmias to occur is slow conduction.\textsuperscript{22} A large area of block implies a functional reduction of excitable myocardial tissue and consequently a reduction of the area in which slow conduction can occur. The result is a functionally “punched out” part of the myocardium almost directly neighboring normal tissue. These factors will reduce the probability for a reentrant arrhythmia to occur. A similar mechanism was proposed for the antiarrhythmic property of lidocaine.\textsuperscript{7}

In our experiments, we found a more pronounced response to total ischemia after pretreatment. A preconditioning stenosis on the following total occlusion causes a faster development of ischemic changes, resulting in an early functional separation between normal and ischemic tissue. In contrast to the protocol used in our experiments, preconditioning with four 5-minute occlusions separated by 5 minutes of reperfusion, showed a protective effect (reduction of infarct size) on an ensuing 40-minute period of ischemia.\textsuperscript{23} This effect was attributed to preservation of high-energy phosphates and a reduction of lactate accumulation.\textsuperscript{24} It is unlikely that the same mechanism is operative in the experiments described above because we did not reperfuse the hearts between the period of low flow ischemia and the subsequent occlusion.

**Role of Extracellular Potassium**

Larger \([K^+]_0\) is measured during total occlusion after low flow ischemia than without low flow ischemia. Figure 7 shows that \([K^+]_0\) in the central zone after 8 minutes of occlusion subsequent to stenosis is larger than the expected value of the
plateau. In global ischemia, a second rise of [K+]o after the plateau phase is associated with the irreversible phase of ischemic injury and the disruption of the cellular membranes. The increase of [K+]o during occlusion after stenosis possibly is associated with this phase. Supporting this idea is the observation that in all the experiments the no reflow phenomenon occurred after release of the occlusion subsequent to stenosis. This may be interpreted as an earlier occurrence of contracture. Direct proof, however, for such a mechanism is lacking. Additional support for this mechanism is that the increase in [K+]o was not associated with an increase in the amount of TQ depression, possibly indicating cellular uncoupling. An alternative explanation for the loss of potassium during the occlusion after stenosis includes a large flux of anions, such as lactate over the cell membrane. The latter is unlikely, however, because glycogen stores can be expected to be depleted during the period of flow reduction.

The potassium equilibrium potential approximates the resting membrane potential, measured with intracellular microelectrodes, also during ischemia. A change of the resting membrane potential is closely associated with a decrease of excitability due to the decreased availability of the fast sodium channels. This predicts a relation between inexcitability and [K+]o. In this study, we have used the occurrence of monophasic electrograms as an indicator for the absence of a local regenerative response. The approach is validated by the observation that at a [K+]o larger than 12 mM (or a depolarization by about 30 mV) almost all electrodes recorded monophasic electrograms. The large variability of [K+]o at which monophasic electrograms were recorded may have several origins. Monophasic electrograms may be recorded from tissue that is excitable. Also, factors other than extracellular potassium may play a role. Those factors that have been implicated include acidosis, altered lipid metabolism, free radicals, and the accumulation of lactate, catecholamines, and cyclic AMP. Catecholamines may explain why conduction persists in areas with a high [K+]o. Electrotropic coupling to Purkinje fibers (more resistant to ischemic injury than working myocardium) also may cause persisting excitability in tissue with high [K+]o. Our results take into consideration the influence of the activation sequence. A low [K+]o can be associated with a monophasic electrogram (Figures 4, 6, and 9). A change of activation sequence reveals that the tissue underlying the electrode is in fact not inexcitable but merely not activated.

Acknowledgment

We gratefully acknowledge the expert biotechnical support from Ch. Belterman.

References

24. Murry CE, Reimer KA, Jennings RB: Preconditioning with ischemia delays ATP depletion and limits lactate accumulation in severely ischemic canine myocardium. *Circulation* 1987;76(suppl IV):IV-228

**KEY WORDS** • inhomogeneity • activation • electrograms • ischemia • potassium
Distribution of extracellular potassium and electrophysiologic changes during two-stage coronary ligation in the isolated, perfused canine heart.
R Coronel, J W Fiolet, J G Wilms-Schopman, T Opthof, A F Schaapherder and M J Janse

_Circulation_. 1989;80:165-177
doi: 10.1161/01.CIR.80.1.165

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/80/1/165

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/