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**Alterations in Canine Myocardial Excitability during Ischemia**

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**SUMMARY** Changes in the ventricular diastolic excitability threshold following occlusion of the left anterior descending coronary artery (LAD) were studied in open-chest anesthetized dogs by using a new automatic threshold-following pacemaker (ATFP). The ATFP measures the diastolic excitability threshold by successively decreasing the duration of regularly occurring pacing stimuli until the ventricle fails to respond. Under control conditions, the threshold stimulus duration was 60 ± 4 (mean ± SEM) μsec. In the first 1-3 minutes following occlusion of the LAD, the diastolic excitability threshold in the ischemic zone (IZ) decreased to 51 ± 5 μsec and then rapidly increased to 600 μsec at 5 minutes. The initial decrease in excitability threshold at IZ could be abolished by elevating the serum K+ concentration prior to the LAD occlusion. These changes in excitability threshold at IZ could be prevented by infusing nonoxygenated solutions into the LAD at a site distal to the occlusion. As the excitability threshold increased in IZ during ischemia, the earliest time at which IZ could be reactivated by a stimulus with a voltage equal to twice the preligation diastolic voltage threshold was increased. In nine of 16 dogs, after 5 minutes of LAD ligation, the IZ to normal zone (NZ) activation time (when stimulating at NZ) exceeded the NZ to IZ activation time (when stimulating at NZ) by an average of 9 msec. We also found that in four dogs the NZ to IZ activation time exceeded the IZ to NZ activation time by an average of 10 msec. We conclude from these findings that a gradient of increasing excitability threshold exists as one moves from normally perfused toward more ischemic tissue, passing through a heterogeneous border zone that manifests some areas which have a decreased excitability threshold and other areas which have an increased excitability threshold, and that these changes in excitability importantly influence the determination of refractory period durations and conduction times.

**SEVERAL** investigators have examined changes in refractory period duration and conduction that accompany myocardial ischemia, but relatively few studies have dealt with alterations in myocardial excitability. Since changes in this parameter progress very rapidly after acute coronary occlusion, frequent and accurate measurements

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of the excitability threshold are mandatory to determine the time course immediately following myocardial ischemia. The development of a new pacemaker (Medtronic, Inc.), which automatically and continuously determines diastolic threshold, greatly facilitates such measurements and, in this study, permitted a new threshold measurement approximately every 4.5 seconds. The purpose of this study was to (1) measure temporal changes in excitability during myocardial ischemia at various epicardial sites and to investigate the role played by potassium in producing these changes, and (2) evaluate the effect these changes in excitability have on the determination of the refractory period duration and conduction time.

Methods

Experiments were performed on mongrel dogs of either sex weighing 15–30 kg, anesthetized with sodium secobarbital (30 mg/kg, iv, repeated as necessary to maintain anesthesia). Following intubation and ventilation with a Harvard respirator at a rate and tidal volume predicted from a nomogram, a left thoracotomy was performed at the level of the 5th intercostal space and the heart was suspended in a pericardial sling. A heating blanket was used to maintain normothermia. Ringer’s lactate (200 ml/hour) was infused into an external jugular vein to compensate for fluid losses incurred by the dog during the experiment. The sinus node was crushed to achieve a slower spontaneous heart rate. The left anterior descending coronary artery (LAD) was dissected free 2–3 cm distal to its origin and an umbilical tape was passed underneath. The blood pressure was monitored with a catheter inserted into the aortic wall. Three milliliters of blood or of lactated Ringer's solution with a varying concentration of KCl were injected performed by inserting a PE 10 cannula through the arterial wall. Three milliliters of blood or of lactated Ringer's solution with a varying concentration of KCl were injected with a hand-driven syringe over a period of 2 minutes. In seven dogs, we infused warmed, nonoxygenated saline or

tape recorder (Hewlett-Packard) for later replay and analysis.

Measurements of excitability and refractoriness were made during unipolar cathodal stimulation of the ventricular epicardium using one of the plaque electrode terminals. The anode was a metal disk (2-cm diameter) electrode located in the subcutaneous tissue over the chest wall. Ventricular refractoriness was determined by terminating a train of 10 regularly occurring stimuli (S1), delivered at a basic cycle length (BCL) of 450 msec, with a premature stimulus (S2), at progressively shorter S1-S2 intervals. Both S1 and S2 were delivered through the same electrode terminal to minimize changes attributable to conduction delays. With the same plaque electrode, the ventricular response was recorded from two terminals which were located not more than 1–2 mm from the stimulating electrode terminal. The longest S1-S2 interval at which S2 failed to produce a ventricular response was used to measure the duration of the refractory period at that site. Stimuli were square wave pulses of 2-msec duration, obtained from a pulse generator through an isolation transformer (Digipulser model 830, Isopulser model 850, W-P Instruments). Threshold and suprathreshold intensities were used (see Results). Strength-interval curves were measured from the Iz in three dogs before and 30 minutes after occlusion of the LAD.

Diastolic excitability threshold was determined by two methods. In one method, the threshold voltage required to depolarize the ventricle at a BCL of 450 msec was determined using square wave pulses of 2-msec duration. In the other method, a new pacemaker (automatic threshold-following pacemaker, ATFP 1476H, Medtronic) was used. This pacemaker automatically determines diastolic excitability threshold by altering stimulus duration; voltage and cycle length remain constant at preselected levels (2 V and 450 msec, respectively, for this study). The operating principle of this pacemaker is as follows: After each suprathreshold stimulus, the ATFP decreases the duration of each successive output pulse by 2 μsec until a stimulus fails to depolarize the ventricles. The output pulse immediately following this single subthreshold stimulus then increases duration by 20 μsec and, after regaining capture, the output pulse duration again decreases by 2 μsec in each succeeding stimulus. The ATFP detects ventricular depolarization (or its absence) by monitoring cardiac activity via a separate sensing myocardial electrode. The duration of the last stimulus that produced a response immediately preceding the subthreshold stimulus is displayed in digital form and taken as the diastolic excitability threshold, expressed in terms of stimulus duration. Measurement of the diastolic excitability threshold in this fashion is accurate to ±1.5 μsec. Use of this pacemaker permits a precise measurement of diastolic excitability threshold automatically every 10 beats or approximately every 4 1/2 seconds at a pacing cycle length of 450 msec.

In five dogs, nonocclusive cannulation of the LAD was performed by inserting a PE 10 cannula through the arterial wall. Three milliliters of blood or of lactated Ringer’s solution with a varying concentration of KCl were injected with a hand-driven syringe over a period of 2 minutes. In seven dogs, we infused warmed, nonoxygenated saline or
Tyrode's solution into the LAD during occlusion via a cannula inserted distal to the occlusive clamp. Rates of 0.4-4.0 ml/min were used. Following completion of the experiment, methylene blue was infused into the cannula to ascertain that the site in IZ at which we measured the excitability threshold was adequately perfused. Three dogs received an intravenous infusion of KCl (25 μEq/kg per min), and serum potassium concentration was measured from the arterial blood samples.

**Results**

**EXCITABILITY**

The diastolic excitability threshold, expressed in terms of the threshold stimulus duration, ranged between 40 and 75 μsec during control conditions [mean 60 ± 4 (SEM) μsec; 75 determinations in 27 dogs]. No statistically significant difference in diastolic excitability threshold was found between the anterior surface of the right and left ventricles. Following occlusion of the LAD, the diastolic excitability threshold in IZ varied in a consistent and reproducible fashion. During the first 1-3 minutes of LAD occlusion, the threshold stimulus duration in IZ decreased to 51 ± 5 μsec and then rapidly increased up to 600 μsec at 5 minutes. An example can be seen in Figure 1, which was obtained from one dog during four different episodes of LAD occlusion. Qualitatively similar changes occurred in BZ₁, but with a slower time course; changes in BZ₂, recorded from the same plaque electrode as BZ₁ but at a distance of 3 mm, showed only the initial decrease in threshold stimulus duration. NZ exhibited no change in threshold stimulus duration (not shown). All of these changes in threshold stimulus duration rapidly reversed within 1-2 minutes after reperfusion of the LAD. Of note was the absence of an undershoot in threshold stimulus duration.

This pattern of temporal and spatial distribution of myocardial excitability thresholds following occlusion of the LAD was observed in a total of seven dogs and was consistently reproducible in each dog. In an additional three dogs, changes in diastolic excitability threshold determined at the endocardial surface of IZ were compared with those changes determined at the epicardial surface of IZ during LAD occlusion. Excitability threshold varied similarly at both sites.

A similar spatial distribution in epicardial excitability threshold following occlusion of the LAD was found in six dogs after 20-30 minutes of LAD occlusion, at a time when the infarct had reached a more steady state (Fig. 2). The change in threshold stimulus duration measured at each terminal was averaged for each zone and paired analysis was performed on the mean values obtained. Threshold stimulus duration increased significantly in IZ and remained essentially unchanged in NZ. BZ had some areas in which threshold stimulus duration increased and some areas in which the threshold stimulus duration decreased. Those areas in BZ which required a longer stimulus duration were averaged separately from those areas which required a shorter stimulus duration. In these six dogs, changes in excitability as measured by the minimum voltage required to produce ventricular depolarization, using a stimulus of fixed duration (2 msec), were directionally similar to changes in threshold stimulus duration (Fig. 2).

If the initial decrease in excitability threshold during ischemia (Fig. 1) is due to a mild increase in extracellular potassium concentration as potassium is released from ischemic cells,12,15-18 this decrease in excitability threshold might be reduced or even converted to a rise if, prior to the ischemic event, we reduced the excitability threshold by increasing the external potassium concentration. Figure 3 illustrates the results obtained in one dog. The changes
in threshold stimulus duration observed during the control ligation of the LAD for 5 minutes are shown in the left panel. Prior to a second period of LAD occlusion (middle panel) KCl (25 µEq/kg per min) was infused intravenously for 12 minutes. This amount of KCl increased the serum potassium concentration from 3.4 to 5.1 mEq/liter and decreased the threshold stimulus duration by 20 µsec, an amount comparable to that initially produced by the first LAD occlusion. During the second LAD occlusion, the threshold stimulus duration declined by a very small amount and then the threshold stimulus duration progressively rose. The large initial decrease in excitability threshold which occurred following the first occlusion was prevented. After the threshold stimulus duration and the serum potassium concentration had returned to normal, a third LAD occlusion (right panel) produced a curve similar to that found in the left panel. Observations similar to those displayed in Figure 3 were demonstrated in three dogs.

In five dogs we attempted to duplicate the alterations in excitability brought about by ischemia by infusing various concentrations of potassium into the nonoccluded LAD. Blood (3 mEq of K+/liter) and Ringer’s lactate (4 mEq of K+/liter) alone failed to alter myocardial excitability. However, potassium concentrations of 8 and 12 mEq/liter decreased the threshold stimulus duration; more concentrated K+ solutions increased the threshold stimulus duration in a dose-dependent manner, resulting in an excitability threshold curve similar to that found in IZ (Fig. 1).

In an attempt to prevent the increase in excitability threshold during LAD occlusion, warmed nonoxygenated solutions of saline or Tyrode’s solution were infused into the LAD at a site distal to the occlusion. Response patterns were somewhat variable, probably depending on streaming and the rate of fluid infusion. However, in four dogs we decreased the ischemia-induced rise in excitability threshold at IZ when we perfused distal to the occlusion at a rate of 4 ml/min. As seen in Figure 4, during LAD occlusion without distal perfusion the excitability threshold rose as expected. When the LAD was perfused distal to the occlusion with nonoxygenated saline the rise in excitability threshold was markedly reduced. Failure to prevent the increase in excitability threshold during ischemia in three dogs occurred when flow rates less than 2 ml/min were used.

To investigate the influence of heart rate on changes in excitability following LAD occlusion, three dogs were subjected to ventricular pacing at a BCL of 600 msec and a BCL of 450 msec (Fig. 5). The time course for changes in excitability threshold was much more rapid and the magnitude of the increase in excitability threshold much greater after a 5-minute occlusion at a BCL of 450 msec than at a BCL of 600 msec. At BZ, both the initial decrease and the subsequent increase in threshold stimulus duration were observed at a BCL of 450 msec, whereas, at a BCL of 600 msec, only the initial decrease was observed (not shown).

**REFRACTORINESS**

Figure 6 illustrates the results obtained in one dog during two successive periods of LAD occlusion to measure changes in threshold stimulus duration (lower panel) and refractoriness (upper panel). The stimulus employed
during the refractory period determination had a voltage equal to twice the diastolic threshold (2 msec in duration) established before occluding the LAD. It can be seen that both values shift in a parallel fashion during the initial occlusion. However, on reperfusion, alterations in refractory period duration and excitability threshold became dissociated as the refractory period fell to values less than control, while threshold stimulus duration returned to control values. These findings were observed consistently in five dogs.

Five dogs were studied 20-30 minutes after LAD ligation, at a time when the diastolic threshold was more stable. Stimuli of two strengths were compared: During one determination of refractoriness the stimulus employed had a voltage equal to twice the diastolic threshold voltage obtained prior to LAD occlusion, while during the second determination the stimulus employed had a voltage equal to twice the diastolic threshold obtained 20-30 minutes after LAD occlusion. Stimuli were 2 msec in duration in both cases. Totally different results for the duration of refractoriness at the same site were obtained (Fig. 7). Using a stimulus voltage equal to twice the preocclusion diastolic threshold, refractory period duration was found to increase in IZ and in BZ1, but to decrease in BZ2. Using a stimulus voltage equal to twice the postocclusion diastolic threshold, refractory period duration was found to decrease in IZ and both areas of BZ.
The relationship between the excitability threshold and refractoriness can be further described by the conventional strength-interval curve, obtained in three dogs at an IZ site, before and 20–30 minutes following occlusion of the LAD (Fig. 8). In two dogs myocardial ischemia shifted the curve upward and to the left (top panel), increasing threshold voltage from 1.6 to 2.9 V and shortening the refractory period obtained at a voltage twice the postligation threshold, from 208 to 176 msec. However, if the refractory period following ischemia was determined using a stimulus which had a voltage twice the preligation threshold, then the value for the refractory period in this example lengthened to 246 msec. In one dog with only a small area of ischemia (lower panel), the diastolic threshold voltage was reduced from 2.4 to 2.1 V, a change similar to that usually found in some BZ areas (Fig. 1). In this dog the strength-interval curve shifted to the left but not upward. Because there was only a small decrease in excitability threshold at IZ, the effective refractory period for this dog shortened from 222 msec to 202 msec, regardless of whether the stimulus employed had a voltage twice the preligation threshold or twice the postligation threshold. The difference between these two curves emphasizes the role played by changes in excitability threshold and the voltage of the test stimulus when determining the effective refractory period.

**CONDUCTION**

Although ischemia results in delayed activation in the ischemic myocardium, alterations in excitability together with possible unidirectional conduction delay or block out of, or into, the ischemic region may result in greater conduction times in one direction than in the other. If this is so, then the site of stimulation can become a critical factor. To test this hypothesis, the NZ to IZ time interval when stimulating at NZ was compared with the IZ to NZ time interval when stimulating at IZ. Recording electrodes were within 2 mm of the stimulating electrodes. Comparisons were made in 16 dogs before and after 5 minutes of LAD occlusion.

As expected, the NZ electrogram did not demonstrate any appreciable change during occlusion of the LAD, and the IZ electrogram became progressively reduced in amplitude, increased in duration, and delayed in onset. The average conduction delay in IZ activation, when stimulating from the NZ at a BCL of 450 msec and after 5 minutes of LAD ligation, was 19 ± 12 msec. The average conduction delay in NZ activation, when stimulating from the IZ, was 22 ± 18 msec. In nine dogs, after 5 minutes of LAD ligation, the IZ to NZ activation time (when stimulating at IZ) exceeded the NZ to IZ time interval (when stimulating at NZ) by an average of 9 msec (range, 3–20 msec). In four dogs the NZ to IZ activation time exceeded the IZ to NZ activation time by an average of 10 msec (range, 5–15 msec), whereas in three dogs the conduction delay was comparable in both directions. The observations remained constant regardless of the intensity of the stimulus used in IZ and demonstrate asymmetry in activation times depending on the site of stimulation. In four dogs we attempted to map grossly the pathway traveled with recording electrodes placed at IZ and NZ, and at sites 1/3 the distance between IZ and NZ. No marked deviation from the expected sequence of activation during ischemia were noted when stimulation at IZ was compared to stimulation at NZ. That is, if the sequence of activation during ischemia recorded in four electrograms while stimulating at NZ was A-B-C-D, then the sequence of activation while stimulating at IZ was D-C-B-A. However, it is to be...
emphasized that such mapping in the intact heart represents a very crude attempt to determine pathways traveled and only offers gross approximations. Clearly both factors, i.e., unidirectional conduction delay and block, as well as different pathways, could account for the directional differences in activation times, and it would not be surprising to find differences in the sequence of activation in some dogs.

Discussion

The results from this study are consistent with the concept that a gradient of excitability exists during ischemia, from areas with reduced to areas with elevated excitability thresholds, determined by the severity of the ischemic injury. Potassium released from the ischemic cells may cause or contribute to these changes in excitability. Raising concentrations of potassium have been well documented to cause an initial decrease, followed by an increase, in cardiac excitability threshold, similar to that which occurred in IZ and which was duplicated by infusing potassium at increasing concentrations directly into the LAD. When potassium was infused intravenously prior to LAD occlusion, serum potassium concentration rose and excitability threshold decreased. At this time LAD occlusion resulted in little or no decrease in excitability threshold because extracellular potassium levels, already elevated by intravenous infusion, rose further following ischemia and achieved concentrations which increased excitability threshold. Preventing the ischemia-induced increase in excitability threshold by "washing out" the ischemic zone with nonoxygenated solutions suggests that accumulation of metabolic by-products of ischemia accounts for the excitability changes, but naturally cannot point to potassium as the cause.

The level of diastolic excitability threshold importantly contributes to the determination of other electrophysiological parameters, such as conduction time and refractory period duration. The intensity of the stimulus used influences the earliest time at which the heart can be reactivated. Under the present experimental conditions a decreased refractoriness occurred at IZ using a stimulus strength equal to twice the preocclusion threshold, but an increased refractoriness occurred at NZ using a stimulus strength equal to twice the postocclusion threshold. Decreasing refractoriness at IZ and recording a response at NZ exceeded the stimulus response time achieved when stimulating IZ and recording a response at NZ which exceeded the stimulus response time achieved when stimulating IZ and recording an increase or decrease in excitability threshold to LAD occlusion resulted in little or no decrease in excitability threshold because extracellular potassium levels, already elevated by intravenous infusion, rose further following ischemia and achieved concentrations which increased excitability threshold. Preventing the ischemia-induced increase in excitability threshold by "washing out" the ischemic zone with nonoxygenated solutions suggests that accumulation of metabolic by-products of ischemia accounts for the excitability changes, but naturally cannot point to potassium as the cause.

It is of interest that on reperfusion of the LAD the refractory period duration shortens to a value less than that of the control value, while the excitability threshold does not dip below control levels as it did initially following occlusion. The reason for this discordance is not clear at the present time. It is possible that a slight dip in excitability threshold was present but was too transient to be measured with the present techniques.

The site of stimulation influenced the stimulus-response times recorded during ischemia. Because of the increase in excitability threshold at IZ, stimulation at that site resulted in decreased latency of response and conduction delay so that, in the majority of experiments, the stimulus response time achieved when stimulating IZ and recording at NZ exceeded the stimulus response time achieved when stimulating at NZ and recording at IZ. However, because in some experiments the reverse was true, increased latency cannot be the only factor. Since the pathways involved are unknown and may not be the same for propagation in both directions, one cannot prove the presence of unidirectional conduction delay or block in this experimental preparation. The recorded sequence of activation provides support that the possibility exists. We can conclude, however, that ischemia lengthens activation times in an unequal fashion influenced by the site of stimulation.

References


Effects of Extracellular Potassium on Ventricular Automaticity and Evidence for a Pacemaker Current in Mammalian Ventricular Myocardium

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SUMMARY

Automaticity was induced in isolated guinea pig and cat papillary muscles by application of depolarizing constant current pulses. Increasing extracellular potassium from 1 to 15 mM caused a shift of pacemaker-like activity to less negative diastolic potentials and a decrease in maximum phase 4 slope. Membrane resistance, estimated from the relation of applied current to maximum diastolic potential, decreased when extracellular potassium (K+) was increased. Voltage clamps of cat papillary muscle demonstrated that action potentials activate a time-dependent outward current which has a reversal potential of -79.1 mV (±0.99 SE, n = 20) at an extracellular potassium concentration of 5 mM. The reversal potential of this current varies with extracellular K+ with a slope of 50-60 mV per 10-fold concentration change. The current is activated by voltage clamps or action potential plateaus in the range of -30 to +30 mV. It has a time constant of deactivation which increases from approximately 100 to over 400 msec as clamp potential is increased from -90 to -60 mV. It is proposed that this current is equivalent to I\textsubscript{K1}, demonstrated in other cardiac tissues and is responsible, in combination with inward currents, for automaticity in ventricular fibers.

It is now clear that under certain conditions ventricular myocardial cells are capable of automatic repetitive depolarization, i.e., pacemaker-like activity. Such conditions include depolarization by direct application of current and by exposure to barium ion. However, little information is available concerning the underlying ionic mechanisms for automaticity in this tissue.

Studies in Purkinje fibers and in frog atrial trabeculae have shown that automaticity in these cell types may be ascribed to the decay of time-dependent outward currents in the presence of inward currents of sodium (Na\textsuperscript{+}), calcium (Ca\textsuperscript{2+}), or both. Prior studies with ventricular preparations have provided evidence for the participation of Ca\textsuperscript{2+} and Na\textsuperscript{+} as inward charge carriers during automatic depolarization.

Recently, Imanishi and Sura-wicz reported that depolarization-induced automaticity in guinea pig ventricle is suppressed by increases in extracellular potassium (K\textsuperscript{+}). This observation suggests that K\textsuperscript{+} permeability is an important factor in ventricular automaticity.

The present study was therefore carried out to document the effects of extracellular K\textsuperscript{+} on ventricular automaticity and to study time-dependent currents present during pacemaker depolarization (phase 4).

Methods

Experiments with guinea pig papillary muscles were carried out in a single sucrose gap chamber as described previously. For studies of cat ventricular muscle, right ventricular papillary muscles were removed from cats (2–5 kg) anesthetized with pentobarbital (30 mg/kg, ip). The muscles were mounted and superfused as described for guinea pig tissue.

The solutions used included normal Tyrode's solution...
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