The Effect of Changes in Rate and Rhythm on Supernormal Excitability in the Isolated Purkinje System of the Dog

A Possible Role in Re-entrant Arrhythmias

By Joseph F. Spear, Ph.D., and E. Neil Moore, D.V.M., Ph.D.

SUMMARY
Microelectrode techniques were used to investigate the effects of rate and rhythm changes upon the period of supernormal excitability during repolarization in isolated canine right and left bundle branch and Purkinje fibers. The same microelectrode was used for both intracellular stimulation and recording. Excitability curves (strength-interval curves) were measured as the minimum depolarizing current required to re-excite the fiber during repolarization of a conducted action potential. During the supernormal period it required an average of 17.0 ± 4.6 SD % less than diastolic current to re-excite the fibers throughout the left and right bundle branch-Purkinje system. The supernormal period of excitability in the bundle branch-Purkinje system was voltage dependent, reaching minimum current requirements at 74.3 ± 5.8 SD mV. Excitability curves of conducted action potentials were determined at basic cycle lengths ranging from 1000 to 300 msec and following single and multiple premature beats. The decrease in action potential durations associated with shorter cycle lengths was not accompanied by a corresponding shortening of the supernormal period of excitability. Therefore, at shorter cycle lengths the supernormal period encompassed a greater proportion of the total action potential; in some cases as much as 50% of the total action potential duration exhibited a period of supernormal excitability. The supernormal period of excitability could be eliminated by perfusing the tissues in elevated potassium (5.0 and 7.5 mM) Tyrode’s solution. The possible implications for the generation of re-entrant arrhythmias are discussed.

VARIous LABORATORIES have investigated supernormal excitability in the heart using surface stimulation.1-4 In 1955 Weidmann5 first demonstrated a period of supernormal excitability in the isolated Purkinje fibers from sheep and calves using intracellular depolarizing current. The supernormal period of excitability occurred during late repolarization and was characterized by a decrease in the current required to re-excite the fiber. Weidmann’s experiments showed that the supernormal period was due to the fact that during the later phase of repolarization the threshold potential had recovered more completely than the membrane potential. The membrane potential therefore had to undergo a smaller degree of additional depolarization to reach threshold potential and this could be accomplished by a weaker depolarizing current. The purpose of the present study was to define the characteristics of the period of supernormal excitability in the canine Purkinje system during changes in action potential duration and configuration caused by changes in heart rate and rhythm.

Methods
Hearts were excised from anesthetized dogs (sodium pentobarbital 30 mg/kg i.v.) and right and left Purkinje fiber tissues were rapidly removed and placed in Tyrode’s solution. The composition of the Tyrode’s solution in millimoles per liter was: NaCl, 137.0; NaHCO3, 12.0; dextrose, 5.5;
KCl, 2.7; MgCl₂, 0.5; NaH₂PO₄, 0.9; CaCl₂, 1.6. The Tyrode's solution was equilibrated with 95% oxygen and 5% carbon dioxide and maintained at 37° centigrade. Transmembrane potentials were recorded using standard glass microelectrodes filled with 3 M KCl. The preparations were paced using silver bipolar surface electrodes. Our technique for stimulating and recording from a single microelectrode has been described previously. Briefly, it involves rapid relay circuits that electronically switch the microelectrode between record and stimulate modes. In the stimulate mode depolarizing current pulses 4 msec in duration were delivered through the microelectrode. The current intensity was measured as the voltage drop across a series 100 Kohm resistor. Following delivery of the current pulse the system was switched within 4 msec back to the record mode and the resulting changes in transmembrane potential recorded. The recordings were displayed on an oscilloscope and photographed on 35 mm film. The analog data were then projected on a film reader and the currents and potentials measured. The current values could be measured with an accuracy of 0.002 × 10⁻⁶ amps and the potential measurements with an accuracy of 0.5 mV.

Figure 1 is an example of the analog data. The upper three traces were obtained at one sweep speed of the oscilloscope (100 msec time dots top trace) while the simultaneously recorded lower two traces were obtained at a 100 times faster sweep speed (1 msec time dots lower trace). The transmembrane potential from a Purkinje fiber from the midportion of a false tendon is shown in the upper trace. At the arrow a threshold pulse of depolarizing current was delivered through the recording microelectrode.

![Figure 1](http://circ.ahajournals.org/)

Analog data demonstrating the technique of intracellular stimulation. Time marks indicate 100 msec intervals for the top three traces and 1 msec intervals for the lower two traces. The upper record is the transmembrane potential recorded from a fiber located in the midportion of a false tendon from the right ventricle. The first and third action potentials in this trace were evoked by stimulation through surface bipolar electrodes. Zero potential is indicated by the horizontal line near the peaks of the action potentials. The action potential indicated by the arrow was evoked by passing a 4 msec pulse of depolarizing current through the recording microelectrode. In the lower half of the figure the current record is displayed on an expanded time scale.

**Results**

**Characteristics of the Supernormal Period**

Table 1 summarizes the characteristics of the supernormal period of excitability measured in 24 Purkinje fibers in 12 separate preparations. The reduced current requirements for excitation always occurred during late repolarization (phase 3) before the membrane potential returned to its resting value. During constant pacing at a basic cycle length of 800 msec the Purkinje cells exhibited their minimum current requirements at an average potential of 74.3 ± 5.8 SD mV. At more depolarized states during repolarization (phase 3) the current requirements increased until the tissue became absolutely refractory. It required an average of 17.0 ± 4.6 SD % less current to excite the fibers during the minimum of the supernormal period than during diastole. The total duration of the period of supernormal excitability lasted 88.2 ± 23.6 SD msec.

**The Effect of Changes in Rate and Rhythm on the Supernormal Period**

The characteristics of the period of supernormal excitability were unaffected by changes in rate and rhythm. Figure 2 demonstrates the effect of increasing the rate of stimulation upon the period of supernormal excitability; all potentials were recorded in a single impalement in a single false tendon fiber. Above are shown superimposed tracings of records obtained during pacing at constant basic cycle lengths of 800 msec, 600 msec, and 400 msec. With increasing rates of drive the action potential durations decreased. At the lower left of the figure the excitability curves obtained during these three different rates of drive are shown. Notice that with increasing rates of drive the period of supernormal excitability was shifted earlier in time; however, there was little alteration in its configuration.
The effect of increasing the rate of drive on the period of supernormal excitability. At the upper left are shown superimposed tracings of action potentials obtained during pacing at basic cycle lengths of 800 msec, 600 msec, and 400 msec. The action potentials of shorter duration were recorded at the shorter basic cycle lengths. The excitability curves for each basic cycle length are shown in the lower left. At the lower right the threshold currents versus the membrane potential during repolarization (phase 3) are plotted.

Table 1

Electrophysiological Characteristics of the Supernormal Period

<table>
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<tr>
<th>Experiment #</th>
<th>Supernormal period potential (mV)</th>
<th>Supernormal period duration (msec)</th>
<th>Supernormal period % change from diastolic current</th>
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Mean and standard deviation: 74.3 ± 5.8

88.2 ± 23.6

17.0 ± 4.6

and duration. At the lower right of the figure the currents required to re-excite the impaled fiber are plotted versus their respective membrane voltages during repolarization. During increasing rates of drive the voltage dependence of the supernormal period of excitability was also unchanged.

Figure 3 is a similar experiment in which the excitability curves were measured in a single cell impalement during action potentials elicited at increasing degrees of prematurity. Above are shown superimposed tracings of the action potentials at increasingly premature cycle lengths. The longest duration action potential was obtained at a basic cycle length of 800 msec. The others were obtained during single premature beats evoked at cycle lengths of 700 msec, 600 msec, and 500 msec following periods of constant drive at the basic cycle length of 800 msec. With increasing degrees of prematurity the action potential durations were decreased. The associated excitability curves shown at the lower left demonstrate that while the period of supernormal excitability was moved earlier in time during the increasing degrees of prematurity, the configuration and duration of the supernormal period was relatively unchanged. The plot of the threshold current versus membrane voltage...
at the lower right demonstrates the unchanged voltage dependency of the supernormal period during increasing degrees of prematurity.

The effect of evoking several sequential premature beats upon the period of supernormal excitability was also studied. The results are shown in figure 4. Above are three superimposed action potentials. The longest duration action potential was at a basic cycle length of 800 msec. The next shorter action potential was due to a single premature beat elicited at a cycle length of 460 msec. The shortest action potential was obtained during a second premature beat following the first at a cycle length of 251 msec. Notice in the excitability curves at the lower left that although the supernormal periods were moved earlier in time that their duration and configuration still remained relatively unaffected. The voltage dependence of the supernormal period was also unchanged during the successive premature beats.

The fact that in a given cell the duration and configuration of the period of supernormal excitability is dependent upon membrane voltage during the later stage of repolarization and is independent of the total duration of the action potential implies that with cycle length dependent reductions in the action potential duration the supernormal period will comprise a greater percentage of the total duration of the action potential. This fact is demonstrated in figure 5. As the total refractory period was shortened in association with the successive premature beats, the supernormal period comprised a greater proportion of the total refractory period. At the shortest action potential duration the supernormal period comprised about half of the total action potential duration.

The pooled data from 39 determinations in 22 different cells are presented in B of figure 5. The total refractory periods were varied by changing the basic cycle length, the degree of prematurity, and by successive premature beats. While the behavior of the individual cell is obscured in this plot, the general trend for all of the cells was that at the shorter total refractory periods the supernormal period comprised a greater proportion of the total refractory period.

Moderate depolarization by increased external potassium concentration eliminated the period of supernormal excitability. This effect is demonstrated in figure 6. In normal potassium (2.7 mM) there was a prominent period of supernormal excitability. In the presence of 5.0 mM potassium the cell was depolarized from a resting potential of 65.5 mV to 83.5 mV. The diastolic current requirements were reduced from 0.355 to 0.210 microamps in the high potassium solution and the period of supernormal excitability was eliminated. At the lower right the

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The relationship between the supernormal period and the total refractory period. In A, the superimposed action potentials of figure 4 are shown. The shaded areas delineate the period of supernormal excitability; the x's within the shaded areas indicate the points of minimum current requirements. The end of the supernormal period is coincident with the end of the total refractory period. In A, histograms of the ratios of the supernormal period to the total refractory period (SNP/TRP) are plotted versus the total refractory period corresponding to each of the action potentials shown above. In B the pooled data from 39 determinations in 22 different cells are shown. As in A the data are plotted as the ratio of the supernormal period to the total refractory period versus the total refractory period.

**Discussion**

Weidmann et al. originally showed that the supernormal period in Purkinje fibers as measured by intracellular stimulation was due to the threshold potentials recovering more quickly than the membrane potential during repolarization. The present experiments

were a depolarization of 24.5 mV (93.0 mV versus 68.5 mV) and the diastolic threshold current requirement was increased from 0.275 to 0.350 micromamps. However, the supernormal period of excitability was also eliminated and the voltage dependence of the threshold current changed.

The effect of 5.0 mM extracellular potassium on the period of supernormal excitability. In the upper left of the figure are superimposed action potentials obtained in Tyrode's solution with a control potassium concentration of 2.7 mM contrasted with the action potential recorded in an elevated potassium concentration of 5.0 mM. Below the action potentials at the lower left are the corresponding excitability curves determined in normal and elevated potassium. At the lower right the minimum currents required to excite the impaled fiber are plotted versus their respective membrane voltages during repolarization (phase 3).

**Figure 7**

The effect of 7.5 mM extracellular potassium on the period of supernormal excitability. The data of this figure are arranged as in figure 6. The control potassium concentration was 2.7 mM and the elevated potassium concentration was 7.5 mM.
SUPERNORMAL EXCITABILITY

demonstrate that the supernormal period of excitability occurs at a specific membrane potential range within the bundle branch-Purkinje fiber system. In a given cell impalement, the voltage dependency of the supernormal period of excitability was unchanged by changes in rate and rhythm, even though these greatly modified the duration and configuration of the action potential (figs. 2, 3, and 4). This invariability of the supernormal period with large changes in the action potential duration and configuration is related to the fact that the action potential duration was modified primarily by a decrease in the duration of the plateau phase with little change in the time course of the phase of rapid repolarization. Since the supernormal period is voltage dependent and associated with the late part of the phase of rapid repolarization, its time course therefore is also unchanged.

Figure 5 demonstrates that because of this voltage dependency, as the total duration of the action potential decreased, the period of supernormal excitability became a greater proportion of the total duration of the action potential. While the direct role of supernormal excitability in the genesis of arrhythmias has yet to be established, the above finding does carry certain implications for a possible role in re-entrant arrhythmias. In most Purkinje fiber ventricular muscle preparations, re-entrant type beats often follow stimulated premature action potentials and these premature action potentials can be of quite short duration. In addition, the work of Mendez et al. and Sasyuniuk and Mendez showed that proximal to a site of conduction block in Purkinje fibers, the action potential duration is greatly abbreviated. Rapid recovery of the tissue at the site of block allows the possibility of early re-excitation of this tissue and therefore provides a local re-entrant pathway. Our experiments point out that the duration of the period of supernormal excitability is not proportionately abbreviated for short duration action potentials. Therefore, in these cases not only does the tissue recover rapidly, but it recovers with a proportionally long period of supernormal excitability. The increased period of supernormal excitability would facilitate reactivation of the tissue in situations where the re-entrant beat had a low safety factor for conduction (i.e., a slow rate of depolarization and low amplitude), and so may play a role in facilitating re-entry.

Our experiments demonstrate that the period of supernormal excitability can be eliminated by elevated extracellular potassium. The data of figures 6 and 7 indicate that excitability throughout the recovery of the action potential is modified under these conditions. The effects of varying the external potassium concentration on diastolic excitability of cardiac muscle have been described by other laboratories. In the high potassium solution, the voltage dependency of the threshold current is changed and not just moved along the control threshold current-membrane potential curve. It is therefore possible to modify supernormal excitability by external interventions. In Weidmann's experiments elevating the external calcium concentration did not eliminate supernormal excitability. The actions of antiarrhythmic drugs on the supernormal period of excitability as determined by intracellular stimulation is unknown but may provide an additional clue to their mode of action. This is especially true for those drugs effective against re-entrant type arrhythmias.

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References

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