Abnormal Automaticity in Canine Purkinje Fibers Focally Subjected to Low External Concentrations of Calcium

By Toyomi Sano and Tohru Sawanobori

ABSTRACT
The false tendon was isolated from the dog heart and mounted under a partition plate in the center of a chamber which was filled with Tyrode's solution. Two microelectrodes were positioned closely on either side of the plate and inserted into the false tendon. The partition did not alter repolarization of the action potential in normal Tyrode's solution. When EDTA was added to one side of the preparation, a low-plateau potential appeared after repolarization on the other side, which contained normal Tyrode's solution. This change occurred concurrently with a prolongation of the action potential in the calcium-free Tyrode's solution. The low-plateau potential was a reflection of the electrotonic potential from the nearby cells whose action potentials were prolonged. Repetitive firing of the action potential was frequently observed when abrupt differences in repolarization were recorded at close interelectrode distances. These results suggest that differences in repolarization of closely apposed cells can initiate arrhythmias.

KEY WORDS
coupled extrasystoles
low-plateau potential
arrhythmia due to repolarization difference
focal reexcitation
repetitive firing

Differences in the time course of repolarization between adjacent cardiac cells have been regarded as one of the important mechanisms of cardiac arrhythmias (1-3). Hoffman (4) hypothesized that the apposition of fully repolarized and not yet repolarized fibers could result in a flow of current capable of initiating excitation. However, it is not clear from earlier studies to what extent the observed arrhythmias were the result of electrotonic interaction rather than spontaneous, but independent, cellular activity. Recently Mendez et al. (5) have shown that the change in the duration of action potentials across the Purkinje-papillary muscle junction was not abrupt, but continuously graded. They pointed out that "during repolarization the current flowing between neighboring elements with intrinsically different repolarization times should minimize the disparity in action potential durations on the two sides of the junctional site." The present study was designed to examine whether marked differences in repolarization of closely apposed cells could initiate arrhythmias.

Methods
Mongrel dogs weighing 8–12 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). False tendons with the fewest branches were isolated from the right or left ventricle. They ranged from 1.0 to 1.3 cm in length and from 0.8 to 2.0 mm in diameter. Each preparation was positioned at the bottom of a Lucite chamber and placed centrally in a groove (2.5–3.0 mm in diameter) cut in paraffin. A celluloid partition plate (300μ thick) was coated with Vaseline and positioned over the tendon, thereby separating the chamber into two compartments. When junctional preparations were studied the partition was placed in the region between the terminal Purkinje fiber and the ventricular muscle. To test the completeness of chamber separation with the preparation in place, Evans blue dye was added to one of the compartments containing Tyrode's solution and the extent of dye exclusion was
monitored in the other compartment, which was also filled with Tyrode’s solution.

Usually one glass capillary microelectrode filled with 3M KCl was positioned on each side of the partition plate and inserted into the false tendon. The distance between these two microelectrodes was kept as small as possible, usually several hundred microns. On many occasions, a third microelectrode was inserted into the distal end of the preparation far from the plate. One of the recording microelectrodes close to the partition was occasionally used for passing current. Details of the microelectrode recording method have been reported elsewhere (6). Action potentials were recorded on both an oscilloscope and a direct-writing recorder. The false tendon was stimulated with bipolar Ag-AgCl electrodes by applying square waves of current, twice threshold intensity, 3–5 msec in duration, at a frequency of 1 cps.

The millimolar composition of Tyrode’s solution was: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.5, Na₂HPO₄ 4.6, Na₂HCO₃ 12.0, and glucose 5.5. Tyrode’s solution perfusing the muscle in the chamber was bubbled with 95% O₂-5% CO₂ and maintained at 33°C.

Results

In the absence of the partition plate two microelectrodes were inserted into the middle portion of the false tendon in normal Tyrode’s solution about 800 µ apart; the action potentials that were thus recorded repolarized at the same time (Fig. 1 [A + 1]). A hyperpolarizing current of 3 × 10⁻⁷ amp passed through one microelectrode was clearly recorded as an electrotonic potential by the other microelectrode (Fig. 1 [A + 2]). The action potential configuration did not change significantly when the Vaseline-covered partition plate was placed between the two microelectrodes (Fig. 1 [B + 1]). When 2–5 mM disodium ethylenediamine tetraacetate (EDTA) was added to one compartment of the muscle chamber, the action potential duration recorded in that compartment was prolonged and the action potential spike diminished in a few minutes (Fig. 2), as has been reported by others (7, 8). In the Purkinje fiber-papillary muscle preparation, EDTA was added to the compartment containing the Purkinje fiber. A similar phenomenon was observed in both cases illustrated in Figure 2. Note that the configuration of the action potential recorded from the papillary muscle in the compartment containing normal Tyrode’s solution (Fig. 2 [1B]) did not differ markedly from the control (Fig. 2 [1A]). However, a low-plateau potential appeared to continue after the repolarization and to end at the same time as the prolonged action potential recorded from the Purkinje fiber in the Ca-free solution (Fig. 2 [1B, 1C]). The time course of the repolarization phase could be clearly distinguished from that of the low-plateau potential (Fig. 2 [1, 2B]). Prolongation of the action potential recorded from the false tendon in the Ca-free solution was accompanied by repetitive firing of action potentials in both compartments (Fig. 2 [2B, 2C, 2D]). Since it was especially difficult to place the partition exactly between the terminal Purkinje fiber and the ventricular muscle, it was necessary to select the false tendon as the preparation of choice in the following experiments.

To question the existence and nature of a relationship between the prolonged action potential...
Action potential prolongation in Ca-free Tyrode's solution. 1: Terminal Purkinje fiber and ventricular muscle. One microelectrode was inserted on each side of the partition plate, which was placed between the terminal Purkinje fiber and the right anterior papillary muscle. The upper tracing is from the terminal Purkinje fiber and the lower tracing is from the ventricular muscle. A: Control—after insertion of the partition. B: About 2 minutes after 5 mM EDTA was added to the solution perfusing the Purkinje fiber. C: After 5 minutes of EDTA perfusion. The shape and duration of the action potentials from the ventricular muscle (lower tracings) do not differ markedly except for the appearance of the low-plateau potential in B and C. Note the calibration: the vertical bar represents 50 mv and the horizontal bars 200 msec.

2: False tendon. The results were obtained with a pen recorder. The upper tracing is from one compartment and the lower tracing is from the other compartment. A: Control—after insertion of the partition. B: About 20 seconds after 5 mM EDTA was added to the solution perfusing that portion of the false tendon in the compartment shown in the lower tracings. C: After 1 minute of EDTA perfusion. D: After 1.5 minutes of EDTA perfusion. The premature beat in B appears to occur earlier in the lower tracing than in the upper tracing, but when it is measured from the stimulus artifact, it occurs simultaneously in both tracings. Some Purkinje action potentials do not have a prominent plateau in normal Tyrode's solution, as is shown in 2A. The vertical bar represents 50 mv and the horizontal bar 2 seconds.

Potential and the low-plateau potential, it was necessary to record action potentials from a number of positions along the preparation. Since action potential configurations changed on a beat-to-beat basis after the addition of EDTA, potentials were compared from simultaneous measurements at three positions. It was noted that the magnitude of the low-plateau potential was larger when recordings were made close to the partition as compared with those obtained far from the plate (Fig. 3). Figure 3C is a semilogarithmic plot of the amplitudes at 300 msec and at 500 msec after the onset of the action potential against the distance between the microelectrode in Ca-free Tyrode's solution and one of the two microelectrodes in normal Tyrode's solution. The values obtained at three positions have been hand-fitted to approximate a straight line. If the low-plateau potentials decline with a space constant equal to that for similar
depolarizing pulses in normal Tyrode's solution, this would suggest an electrotonic etiology for the low-plateau potentials. To test this hypothesis, space constant measurements were made at rest in normal Tyrode's solution. In addition, special attention was focused on the repolarization phase by measuring changes in membrane resistance during stimulation in Ca-free Tyrode's solution.

Space constant (λ) measurements for the dog false tendon preparation were made in the resting state in normal Tyrode's solution. Rectangular hyperpolarizing current pulses, 60 msec in duration and $2.5 \times 10^{-7}$ amp in intensity, were applied through one microelectrode, and the electrotonic potential was measured at several points along a straight line by the other microelectrode. The values obtained were in the range 1.20 to 1.72 mm. This result is consistent with the value of 1.9 mm reported by Weidmann (9) for the sheep false tendon and that of 1.25 mm reported by Sakamoto and Goto (10) for the dog false tendon. A representative experiment is illustrated in Figure 4 (circles). The effect of the partition on the space constant measurements was also tested. When the center of the false tendon was pressed by the partition plate, no noticeable change in λ was observed (Fig. 4, crosses). To know whether electrotonic potentials recorded during the plateau phase were

![Diagram](https://example.com/diagram.png)

**FIGURE 4**

Electrotonic spread in the dog Purkinje fiber before (1 in the upper traces and circles in the lower graph) and after (2 in the upper traces and crosses in the lower graph) separation into two compartments. The $\lambda$ equals 1.58 mm before separation. After separation the electrotonic spread at 0.2 mm and 0.6 mm from the microelectrode passing the current could not be measured because of the presence of the partition plate. But, according to calculations from measurement at 0.8 mm and at more distant points, $\lambda$ is almost the same after separation. The top traces in 1 and 2 show the current pulse that was applied. The vertical bar represents 50 mv and the horizontal bar 60 msec.

**FIGURE 5**

Membrane resistance during the long-plateau phase and during the resting phase. Rectangular hyperpolarizing current pulses of $5 \times 10^{-7}$ amp and 50 msec in duration were passed at 10 cps through the second microelectrode. The result was recorded both by the oscillograph (1) and by the recorder (2). A: Control. B(1): About 30 seconds after EDTA was added to a final concentration of 5 mw. Beginning of prolongation of plateau recorded on an oscillograph. B(2): After 2 minutes. Further prolongation of the plateau shown on the recorder. Stimulus was given at the beginning and towards the end of the action potential, as shown by artifacts. The vertical bars represent 50 mv. The horizontal bar in 1 represents 100 msec and that in 2 represents 1 second.
comparable to those recorded at rest, the long plateau was produced by adding EDTA, and the membrane resistance of the Purkinje fiber was calculated from data obtained by passing hyperpolarizing current (3–5 × 10\(^{-7}\) amp) for 30–50 msec through the second microelectrode which was inserted several hundred microns from the first microelectrode (Fig. 5). In low calcium, the membrane resistance at any level of the repolarization phase was almost equal to that in the resting state. Although Weidmann (11) found a higher membrane resistance in the steep repolarization phase than in the resting phase, our results coincide with those of others (12, 13) who reported negligible differences between the membrane resistance during diastole and the prolonged plateau of the action potential induced by EDTA or low temperature.

The slope of the two straight lines shown in Figure 3C is about the same as the slope of the line shown in Figure 4. Specifically, the amplitude of the low plateau showed a diminution in magnitude with a space constant of 1.28 mm and 1.34 mm at 300 msec and 500 msec, respectively. The lower magnitude of the potential at position b in Figure 3C may be attributable to unknown geometrical changes caused by pressing the partition plate on the false tendon.

In many experiments repetitive firing occurred simultaneously with and on the prolonged plateaus. Analysis of the temporal sequence of depolarization in Figure 6 clearly shows that repetitive firing occurred in the muscle portion of the preparation in normal Tyrode’s solution. In the control, the onset of the action potential at position a was earlier than that at positions b and c (Fig. 6 [A2]); this finding is consistent with the fact that the stimulation was given in the compartment containing microelectrode a. After EDTA was added to the compartment containing microelectrode a, the plateau of action potential a (Fig. 6B) became prolonged as the low-plateau potential appeared. Subsequently, a rapid firing of small action potentials was seen superimposed on the high- (a) and low-plateau (b and c) potentials (Fig. 6 [C1]). Phase-4 depolarization was present on the high and low plateaus before each small action potential (Fig. 6 [C1, C2]). The onset of the superimposed small action potentials occurred earlier at positions b and c in normal Tyrode’s solution than it did at position a in Ca-free Tyrode’s solution (Fig. 6 [C3]).

Another type of repetitive firing probably was initiated by a different mechanism but also seemed to originate in the muscle portion in normal Tyrode’s solution. An example of this is shown in Figure 7. Many small localized or abortive action potentials were observed without phase-4 depolarizations. Small potential changes did not appear on the high plateau of the long action potential when abortive action potentials were superimposed on the short action potential (Fig. 7C, arrows 1 and 3). Only when rapidly depolarizing action potentials appeared after the short action potentials did the long action potential show a small electrotonic potential (Fig. 7C, arrow 2).
ABNORMAL AUTOMATICITY IN PURKINJE FIBERS

FIGURE 7
Occurrence of repetitive firing. Tracings a, b, and c were obtained by microelectrodes positioned as in Figure 3. A: Spontaneously beating control. B: About 1 minute after 5 mm EDTA was added to the compartment where microelectrode a was positioned. a: Initial change in Ca-free Tyrode’s solution. b and c: Initial change in normal Tyrode’s solution. C: About 2 minutes after EDTA. Note the extremely long action potential appearing spontaneously in a. For explanation of arrows 1, 2, and 3 see text. The vertical bars represent 50 mV. The horizontal bar for A and B represents 200 msec and that for C represents 400 msec.

Discussion
It has often been suggested that in cardiac fibers which fail to repolarize normally, closely coupled extrasystoles might be generated by a difference in potential between such fibers and adjacent cells which have repolarized (1-3). Differences in the time course of repolarization have been reported by a number of investigators (3, 14-17). Some were of the opinion that there was an abrupt change in the duration of action potentials of about 100 msec between the Purkinje fiber and the papillary muscle (14, 15). Others showed that the temporal dispersion of recovery of excitability in ventricular muscle was much exaggerated after the creation of myocardial ischemia as compared with the control state (17). But Mendez et al. (5) suggested that the difference in repolarization was observed because comparisons were made between distant cells. They reported that no major difference in the time course of repolarization should occur in neighboring cells under normal conditions.

Our findings show that significant differences in repolarization can occur at very close distances under abnormal conditions (focal low Ca²⁺), if the low plateau after the shortened action potential reflects the electrotonic decay of the plateau of the long action potential. It is conceivable that arrhythmias may be attributable to differences in the time course of repolarization, which support the development of focal reexcitation (repetitive firing) as well as reentry.

As for the origin of the observed experimental arrhythmias, two possibilities can be considered. (1) A potential difference exists between the prolonged action potential in Ca-free Tyrode’s solution and the short action potential in normal Tyrode’s solution. The cells of the short action potential repetitively fire and electrotonic potential changes appear on the high plateau of the long action potential. (2) Oscillatory potentials superimposed on the plateau of the long action potential induce potential changes on the low-plateau potential. On reaching threshold, the cells with short action potentials fire another action potential.

In some experiments, repetitive firing occurred in the muscle portion in normal Tyrode’s solution. In many others, however, the repetitive firing seemed to occur simultaneously in preparations bathed by normal Tyrode’s and Ca-free Tyrode’s solutions. Therefore, it was not excluded that repetitive firing can originate in the portion of muscle in the Ca-free Tyrode’s solution. It is of interest to note that Oikawa (18) showed abrupt differences in the time course of repolarization at the junction of normal and TEA-treated zones in the squid axon. He reported that repetitive impulse activity in the normal zone was preceded by subthreshold oscillatory potentials which were much larger in the TEA-treated zone than in the normal zone. Since our experimental conditions were different, it is difficult to compare and interpret these findings.

As for the possibility that long action potentials can induce repetitive firing in short action potentials because of differences in the time course of repolarization, two phenomena...
were observed which might indicate that different mechanisms are involved. In one, repetitive firing occurred with phase-4 depolarization (Fig. 6). At first, repolarization of the short action potential was followed by a low-plateau potential which in turn might induce repetitive firing in the manner described for the origin of oscillations in Purkinje fibers by Hauswirth et al. (19). They proposed that the oscillations were generated by time-dependent variations in an outward current component that was activated over the voltage range of -40 to -10 mV. Another mechanism might be considered based on the experiment shown in Figure 7. Weidmann (20) reported that calf Purkinje fibers failed to propagate in response to long cathodal stimuli until repolarization had restored the membrane potential to a value of -58 to -62 mV. Since the cells with short action potentials could reach this level sooner in repolarization, they may be triggered earlier and become the site of initial activation in our experimental preparations.

Acknowledgment

We wish to express our appreciation to Dr. Melvin Lieberman for his valuable advice and refinement on this manuscript.

References

Abnormal Automaticity in Canine Purkinje Fibers Focally Subjected to Low External Concentrations of Calcium

Toyomi Sano and Tohru Sawanobori

Circ Res. 1972;31:158-164
doi: 10.1161/01.RES.31.2.158

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1972 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/31/2/158