Depolarization and Action Potential Duration in Cardiac Purkinje Fibers

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ABSTRACT

The effect of depolarization on the duration of the action potential was studied in short cardiac Purkinje fibers. Membrane potential was altered by overdriving pacemaker fibers showing spontaneous diastolic depolarization (dog), by injecting constant-current pulses through an intracellular microelectrode (sheep), or by voltage clamping (dog). Test action potentials were elicited at the end of 50-300-msec depolarizations by extracellular stimuli in sheep fibers and by cathodal intracellular stimuli in dog fibers. In both species the duration of the test action potential was unchanged by subthreshold depolarization. In fibers showing spontaneous diastolic depolarization, action potential duration was not affected by the take-off potential within the range between —80 and —60 mv. In dog fibers depolarized from the threshold level (about —60 mv), action potential duration was shortened by 14-36%. After stronger depolarization (up to —10 mv), short action potentials could be elicited during the first 100-150 msec. This period coincided with the flow of net inward current. After longer suprathreshold clamps when the net membrane current reversed its polarity, the fibers were not excitable, and the clamp termination was followed by exponential decay of the membrane potentials. Our findings indicate that changes in take-off potential within the range between resting membrane potential and —60 mv do not alter action potential duration.

KEY WORDS voltage clamp slow inward current pacemaker potential potassium conductance take-off potential hyperpolarization sheep

Previous studies have not yet clarified the possible role of preceding depolarization in determining action potential duration. Hauswirth et al. (4) have reported that the premature action potentials evoked by suprathreshold stimuli before repolarization to —60 mv are no shorter than those evoked immediately after repolarization to —60 mv. Gettes et al. (5) have studied the effect of membrane potential at the onset of depolarization (take-off potential) on the duration of premature action potentials elicited during the terminal phase of preceding repolarization and have showed that action potential duration decreases progressively with increasing prematurity, i.e., with decreasing take-off potential. However, in these experiments the effect of close proximity on the duration of the premature action potential could not be distinguished from the effect of membrane potential at the onset of depolarization.

The purpose of the present study was to determine the effect of preceding depolarization on the duration of action potentials. Membrane potential changes were produced by polarizing constant-current pulses (current clamping) or by voltage clamping. In fibers showing spontaneous depolarization...
diastolic depolarization, membrane potential was changed by overdriving (6). In all experiments, short Purkinje fibers were used to minimize the possible effects of electrotonic interaction with neighboring fibers (7); moreover, it has been shown that cable complications can be eliminated by reducing the fiber length to less than 2 mm (8). The study was initiated by one of us (B.S.) in Bern, using sheep Purkinje fibers, and subsequently completed in Lexington. In this locality, sheep hearts were not available; therefore, we used dog Purkinje fibers.

**Methods**

Sheep hearts obtained at the slaughterhouse were carried to the laboratory in refrigerated (about 4°C) Tyrode's solution. Dog hearts were quickly excised from dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) and were immersed in cool oxygenated Tyrode's solution. The millimolar composition of Tyrode's solution was: NaCl 137, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, NaHCO₃ 11.9, NaH₂PO₄ 0.42, and glucose 5.0. Strands of Purkinje fibers were removed from both ventricles and were kept in Tyrode's solution at 35-37°C. A mixture of 95% O₂-5% CO₂ was bubbled through the solution.

Experiments were performed using short (1-2 mm) Purkinje fiber strands prepared by ligating the fiber with a fine silk thread near the cut end or by setting two ligatures in the center of the fiber (8). Some canine fibers were shortened by cutting both ends without ligation (8).

All current-clamp experiments were performed with sheep fibers, and all voltage-clamp experiments were performed using canine fibers. Membrane potentials were measured as potential differences between an intracellular and extracellular microelectrode. To pass current a second intracellular microelectrode was inserted at a distance of 0.1-0.5 mm from the first micelle (8). The resistance of the microelectrodes was 8-20 megohms. The recording microelectrodes were filled with 3x KCl, and the current-passing electrodes were filled with potassium citrate solution.

**CURRENT-CLAMP EXPERIMENTS IN SHEEP PURKINJE FIBERS**

The recording apparatus consisted of a push-pull cathode follower stage and a Tektronix 502 oscilloscope. The preparation was stimulated at a constant rate by a Grass S-4 stimulator through a pair of silver-silver chloride wire electrodes which were insulated except at their tips. These electrodes were placed in the bath on an edge of the preparation. Two other Grass S-4 stimulators were wired in series with channel 1 to stimulate through the same pair of extracellular electrodes and with channel 2 to deliver polarizing square current pulses. A 100-megohm resistor was put in series with the stimulator output and the current-feeding microelectrode. The delays between the first and the second extracellular stimulus and between the first stimulus and the polarizing current pulse could be varied independently of each other. All stimuli applied through the extracellular electrodes in the bath were monophasic pulses 3-5 msec in duration set at a voltage that was slightly higher than threshold.

**VOLTAGE-CLAMP EXPERIMENTS IN CANINE PURKINJE FIBERS**

Membrane voltage was controlled by the two-microelectrode technique described by Deck et al. (8). A feedback amplifier (Philbrick 1022) supplied the current necessary to keep the membrane potential at a chosen level (Fig. 1). Two two-channel Grass S88

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**FIGURE 1**

Block diagram of the experimental setup used in the voltage-clamp experiments. ISO = stimulus isolation unit. Other explanations are given in the text.

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stimulators were used (stimulators I-IV). Stimulator I was used to drive the preparation at a rate of 1 cycle/sec through a pair of extracellular platinum wire electrodes that were insulated except at their tips. The extracellular stimuli were 2–3 msec in duration, and the voltage was slightly higher than threshold. The level of membrane voltage was controlled by the command pulse from stimulator II. A battery-supplied bias voltage coupled with this stimulator could also be applied to change the holding potential when necessary. Switching of the clamp's circuit was controlled by a double-pole high-speed relay (time lag less than 0.2 msec) driven by a square pulse from stimulator III. The relay also controlled the premature stimuli delivered by stimulator IV through the intracellular current electrode. The cathodal pulses used to deliver the premature stimuli were 6–8 msec in duration, and they were set at a voltage two to three times higher than threshold. The interval between the end of the clamp and the onset of the premature cathodal stimulus was approximately 1 msec. No measurable change in the membrane potential occurred within this interval.

The measured membrane voltage was led to a Transidyne differential d-c preamplifier (MPA 6) and was displayed on one beam of the Tektronix 564B storage oscilloscope (CRO). The membrane current was recorded with an operational amplifier (Philbrick 1023) to maintain the membrane at virtual ground. This current was displayed on the second beam of the oscilloscope. The tracings were photographed with a Polaroid camera.

Experiments were performed only in fibers which had a resting membrane potential more negative than —65 mv and action potentials with a normal configuration, i.e., with a spike and a plateau. Introduction of large depolarizing clamps frequently decreased the resting membrane potential and changed the shape of the control action potentials. In such cases, the experiments were discontinued until the preparation recovered. If the resting membrane potential and the action potential configuration did not return to control, the preparations were discarded. The duration of an action potential was measured from the onset of depolarization to the point corresponding to 90% repolarization. A complete set of measurements was made in ten fibers in which both microelectrodes remained in place throughout the experiment and in which the resting membrane potential and the action potential configuration remained stable for more than 20 minutes despite repeated injections of the depolarizing currents. The Results include data from these fibers and from two other fibers in which the experiments yielded incomplete sets of clamps. Methods not common to all experiments will be described separately in the Results.

Results

CURRENT-CLAMP EXPERIMENTS

The effect of subthreshold depolarization was studied in 11 short sheep Purkinje fibers with resting membrane potentials ranging from —78 to —88 mv. Action potentials were elicited at the end of depolarizing current pulses 50–200 msec in duration. Such action potentials were not shorter than the action potentials elicited from the resting membrane potential even when the upstroke velocity was slow and the amplitudes of the initial spike and the highest plateau point were low (Figs. 2 and 3).

VOLTAGE-CLAMP EXPERIMENTS

Membrane Currents during Voltage Clamps.—A representative experiment in a short canine fiber is shown in Figure 4. The top traces show different clamp steps of 200-msec duration initiated from the resting membrane potential at the end of phase 3 of a basic action potential. At the moment of clamp termination, a cathodal stimulus was applied through the current electrode to produce a premature action potential. The bottom traces show the membrane currents during the clamps. During the hyperpolarizing clamp (A) the current was negative (inward), and during the subthreshold depolarizations (C and D) the current was positive (outward). On depolarization to threshold (E), a rapid inward current appeared. This current was initially negative, but it reversed polarity after 100–150 msec and rose slowly until the end of the
Tracings of four superimposed action potentials in a sheep Purkinje fiber stimulated at a rate 0.5/sec, before (1) and after 200 msec of increasingly stronger depolarization (2-4) by a constant-current pulse. After depolarization the amplitude of the action potentials decreased but the duration remained unchanged. Broken horizontal line at the top indicates 0 mv.

clamp. At the less negative clamp potential (F), the maximum initial negative current decreased, and the reversal of polarity occurred earlier. These tracings are similar to the currents described by Deck and Trautwein (9) in short Purkinje fibers of sheep. However, we did not record an initial transient outward current (see Fig. 1 in ref. 9) that has been attributed to chloride (10-12). We assumed that this current could be inactivated, because the full recovery of this current requires a rather long repriming time (0.7-1.0 second) (11).

Effect of Subthreshold Depolarization on Action Potential Duration.—The effect of subthreshold depolarization ranging in duration from 100 to 300 msec on the duration of the premature action potential was studied in 12 fibers. Typical results are shown in Figures 4C, 4D, 5B, and 5F. In Figure 4 the resting membrane potential was —68 mv, and the duration of the premature action potential was the same in fibers depolarized from —80 mv (hyperpolarization) (A), —66 mv (B), —62 mv (C), and —54 mv (D), although the upstroke velocity and the overshoot of the action potentials decreased progressively with increasing depolarization (13). The similar effect of subthreshold depolarization in a different fiber is shown in Figure 5A, B, E, and F. The same results were obtained in 8 of 12 fibers tested. In the remaining 4 fibers action potential duration was not changed by depolarization for 100 msec, but it was shortened by up to 20 msec when depolarizations lasted 150-300 msec.

To simulate premature action potentials that take off from a less negative membrane potential during phase 3 of the preceding action potential, depolarizing clamps were applied in two fibers before the full repolarization of the preceding action potential

FIGURE 3

FIGURE 4

Nonpremature (rate 1/sec) and premature action potentials (top traces) and currents (bottom traces) in a canine Purkinje fiber. In each section of the figure, the premature action potential was preceded by a voltage-clamp pulse 200 msec in duration. A: Hyperpolarization. B: Near the resting membrane potential. C and D: Subthreshold depolarizations. E and F: Supra-threshold depolarizations. See text for details.
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Nonpremature (rate 1/sec) and premature action potentials preceded by depolarizing clamps of increasing magnitude. Clamp duration was 100 msec in A-D and 200 msec in E-H, and a canine Purkinje fiber was used. Top traces show currents, and bottom traces show voltages. Note that the first measurable shortening occurred when depolarization exceeded the level of threshold potential (—60 mv) in C and G.

Effect of Suprathreshold Depolarization on Action Potential Duration.—Suprathreshold depolarization produced similar effects in all 12 fibers studied. Figure 5 shows a typical experiment with depolarizations of 100-msec (left) and 200-msec (right) duration. After the subthreshold depolarizations (B and F), action potential duration was the same as control. However, when the depolarization exceeded the level of approximately —60 mv (threshold potential for rapid sodium current in this particular fiber), marked shortening of the action potential occurred (C and G). The mean percent of shortening after depolarization to this level was 16.1% (range 14 to 17%) for the 100-msec clamp and 33.0% (range 30 to 36%) for the 200-msec clamp. With depolarizations to less negative potentials, the results were dependent on the clamp duration. Thus, after a 100-msec clamp (D) a short action potential was obtained, but after a 200-msec clamp to the same level of depolarization (H) the action potential was not elicited, and the membrane potential declined with a simple exponential decay. The relation between the take-off potential and the shortening of the action potential duration in the same experiment is plotted in Figure 7. This figure shows that subthreshold depolarization 100-200
Relation between the take-off potentials and the shortening of premature action potential duration (APD) obtained from the experiment in Figure 5. The shortening is expressed as the difference from control in msec (A and E in Fig. 5). Thus, a negative value on the ordinate indicates lengthening. The solid arrows point to the action potentials elicited at the smallest depolarization resulting in activation of inward current and correspond to action potentials depicted in Figure 5C and C. The open arrow points to the depolarization followed by exponential decay depicted in Figure 5H.

msec in duration does not shorten the action potential duration and that a 100-msec depolarization to a potential less negative than threshold (—60 mV) causes progressive shortening of the action potential duration. Figure 7 also shows that hyperpolarization by about 20 mV increases the action potential duration. This phenomenon was expected from the reported study in calf Purkinje fibers (1).

When the duration of depolarization was longer than 150 msec, it was usually difficult to find the critical membrane voltage for eliciting an action potential with a short plateau such as that shown in Figure 5C, because the transition from a nonshortened action potential (Fig. 5F) to an action potential spike followed by repolarization with an exponential decay (Fig. 5H) occurred abruptly after a minimal increase in depolarization. Such an abrupt change is shown in Figure 4D and E. In this preparation it was impossible to record an action potential with a short plateau.

**Spontaneous Membrane Potential Changes after Termination of a Suprathreshold Depolarizing Clamp.**—The experiment illustrated in Figure 5 suggests that the differences between the potential tracings after the termination of 100-msec clamps (D) and 200-msec clamps (H) could be due to the differences between the net currents flowing immediately before the termination of these two depolarizing clamps. Thus, in Figure 5D this net current is still negative, and in Figure 5H the net current is positive. These relations were further tested in five fibers in which depolarizing clamps ranging in duration from 20 to 200 msec were terminated without introducing a premature stimulus after the clamp. In Figure 8A-D, the clamp duration was increased stepwise from 30 msec to 150 msec. When the feedback circuit that controlled the voltage was suddenly opened during the flow of the net inward current (A–C), the membrane depolarized by about 55 mV (A), 35 mV (B), and 5 mV (C). Therefore, the magnitude of depolarization correlated with the strength of the net inward current flowing just before the end of the clamp. When the clamp was terminated after 150 msec (D), the net current was already positive; no depolarization occurred, but there was a simple

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**FIGURE 8**

Effect of termination of depolarizing clamp without premature stimulus in a canine Purkinje fiber. In each section of the figure, the first action potential is nonpremature (rate 1/sec). The clamp was initiated 350 msec after the onset of the nonpremature action potential and maintained for 20 (A), 50 (B), 100 (C), and 150 (D) msec. Note that even without stimulus, the action potential-like response occurs after terminations of the clamp in A, B, and C but not in D.
exponential potential decay similar to that shown in Figure 5H. Figure 8 also shows that the slope of the terminal repolarization phase was the same in A, B, and C but not in D. In all fibers depolarized to the approximate level of plateau (about $-30$ mv), the net current changed from inward to outward within 150–200 msec after depolarization, and stimuli applied during the flow of outward current resulted in a simple exponential decay of potential similar to that shown in Figure 8D.

**EFFECT OF MEMBRANE POTENTIAL CHANGE IN FIBERS SHOWING SLOW DIASTOLIC DEPOLARIZATION**

The effects of changes in membrane potential at the time of depolarization were studied in three short fibers showing spontaneous pacemaker activity. Figure 9A shows an action potential in one such fiber in which the spontaneous rate was 0.83/sec and the take-off potential was approximately $-55$ mv. To suppress the slow diastolic depolarization, the fiber was overdriven at a rate of 4/sec for several minutes. Thereafter, the stimulation rate was changed to equal the previous spontaneous rate (0.83/sec). Figure 9B shows the action potential recorded when the take-off potential was approximately $-80$ mv. In Figure 9C, the diastolic depolarization had become more prominent, and the action potential was recorded when the take-off potential was $-65$ mv. The superposition of these three tracings (D) revealed that action potentials elicited from three different levels of membrane potential were of the same duration. Similar results were obtained in the other two fibers at several constant rates ranging from 1.8–0.8/sec.

**Discussion**

Our current-clamp and voltage-clamp experiments showed that, following depolarization less than 150–200 msec in duration, the duration of action potentials was not affected by changes in the level of depolarization within the range from resting membrane potential to approximately $-60$ mv. We also showed that the action potential was not altered by changes in the take-off potential caused by spontaneous diastolic depolarization lasting up to about 1 second. Moreover, changes in upstroke
velocity and overshoot produced by depolarization had no effect on action potential duration.

Several conclusions can be drawn from these results. Our study does not support the hypothesis that depolarization itself is responsible for the shortening of action potential duration produced by increased extracellular potassium concentration (14). It does corroborate Weidmann's assumption (15) that changes in sodium conductance (referred to as rapid inward sodium current) provide no explanation for the potential course during repolarization. Our study also suggests that action potential duration is not affected by the voltage-dependent changes in the slow outward potassium current that is activated within the range of the pacemaker potential (3).

This conclusion is valid only for depolarizations of relatively short duration. However, it is possible that the slow outward current may contribute to action potential shortening after longer depolarizations. In 4 of 12 dog fibers, we observed shortening of the action potential duration by up to 20 msec after subthreshold depolarizations 150-300 msec in duration. This effect is compatible with the influence of an increase in the slow outward current due to delayed rectification (16).

To simulate premature action potentials occurring during phase 3 of the preceding repolarization, we changed the membrane potential during the terminal phases of spontaneous repolarization. In these experiments, depolarization did not change the duration of the action potentials, suggesting that the shortening of early premature action potentials arising in incompletely repolarized Purkinje fibers (3) is caused by proximity to the preceding repolarization (4, 5) rather than by decreased take-off potential (Fig. 6).

Our study showed that after depolarizing clamps, i.e., during the flow of net inward current, an action potential could be elicited from membrane potentials less negative than −60 mV (Fig. 5C, D, G). It seems improbable that an action potential elicited from low membrane potential is due to the rapid inward sodium current. Reuter (12) has reported that when the feedback circuit that controls voltage is suddenly opened during the flow of inward current in sodium-free solution, the membrane of Purkinje fibers depolarizes by about 20 mV. In our experiments in Tyrode's solution, opening the clamp circuit resulted in spontaneous depolarization only when the net current before termination of the clamp was inward. The magnitude of this spontaneous depolarization decreased with decreasing net inward current at the time of termination of the clamp. We assume that the short action potential elicited from a take-off potential less negative than −50 mV is initiated by a slow inward current, possibly the calcium current. In canine Purkinje fibers, Imanishi (17) has observed spontaneous repetitive discharge during depolarization to a potential less negative than −55 mV. This spontaneous discharge “depended markedly on extracellular Ca concentration” (17).

We showed that all action potentials elicited from a potential less negative than the threshold were of short duration. The shortening increased with increasing duration of depolarization. It is reasonable to attribute this shortening to one of the time-dependent or voltage-dependent outward currents activated within this range of membrane potentials (18, 19). Spontaneous depolarization from a potential less negative than the threshold and the short action potentials elicited by these depolarizations have been observed only in in vitro experiments. It remains to be determined whether this phenomenon occurs in the depolarized cardiac tissues in the living animal and whether it participates in cardiac arrhythmias.

In a recent review, Johnson and Lieberman (20) have pointed out that the voltage-clamp technique using a point source of current does not ensure a uniform potential distribution even in short Purkinje fibers. We cannot rule out the influence of possible nonuniform potential distribution on the duration of action potentials elicited at the end of suprathreshold depolarizing clamps. However, the following considerations suggest that our results cannot be attributed to a possible inadequate control of the membrane potential: (1) the current traces were smooth and conformed to the expected polarity and time course, (2) the phase 3 repolarization slope was normal and did not change following depolarizations of varying magnitude, and (3) the termination of clamp during the flow of net inward current produced in all experiments depolarization and shortening of plateau. If we assume that a noncontrolled action potential was present in some portion of this fiber, we can attribute to such an action potential either a depolarizing or a repolarizing effect but not both. In all experiments, the termination of clamp during the flow of a net outward current produced a repolarization course which decayed exponentially. Such a course would not be expected to result from the influence of a noncontrolled action potential in some portion of the fiber.
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