Refractoriness in Cardiac Muscle

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ABSTRACT

HOFFMAN, BRIAN F., C. Y. KAO AND E. E. SUCKLING. Refractoriness in cardiac muscle. Am. J. Physiol. 190(3): 473-482. 1957.—A preparation consisting of a papillary muscle and attached bundle of Purkinje fibers has been employed to study refractoriness of single cardiac fibers of the dog heart. Transmembrane stimulation of single fibers and records of the transmembrane potential have been used to compare the stimulating efficacy of applied current pulses and normally propagated action potentials. The absolute and effective refractory period and full recovery time of the single cardiac fiber are outlined in a similar manner by both applied cathodal stimuli and the propagated action potential. Two factors, a local response and a change in the local action potential, have been shown to contribute to the latency of response to stimuli applied during the relative refractory period. These studies have also demonstrated a considerable safety factor in propagation in cardiac muscle.

A number of techniques are currently employed to measure the recovery of excitability in cardiac muscle and several of them delineate quite different aspects of refractoriness. The minimum possible interval between two ventricular responses both of which were propagated from the atrium is employed to measure the functional refractory period of the atrio-ventricular conduction system (1). Similarly, the maximum frequency at which either the atria or ventricles will follow directly applied suprathreshold driving stimuli is often taken as an indication of the duration of refractoriness in either chamber (2). In experiments of this type any effective alteration of mean heart rate (sometimes associated with the testing procedure) will affect the results (3, 4). Furthermore, when driving stimuli are applied directly to a chamber the stimulus strength selected, usually some multiple of 'threshold,' determines whether the end-point represents the total or absolute refractory period (5). Even when test stimuli of known strength are applied infrequently at selected intervals after the R wave of a regularly driven heart the apparent duration of the absolute refractory period depends in part on the duration, polarity and maximum strength of the testing stimulus (6, 7).

The use of moderately long (up to 10 msec.) and strong (up to 30 ma) test pulses has been criticized on the basis that such stimuli are not within physiological limits (8). This implies that some arbitrary limits for duration and current strength should be selected in studies of refractoriness in cardiac muscle.

In light of these considerations it is of interest to compare the refractoriness of cardiac muscle outlined by applied current pulses to that defined by a propagated action potential of normal amplitude and duration. This comparison should indicate the validity of results obtained from use of electrical stim-
ulii. It should also demonstrate the responsiveness of heart muscle to naturally occurring stimuli. In this type of study several advantages are provided by the use of intracellular microelectrodes both for stimulation of single cardiac microfibers (9) and for recording the transmembrane potential of these single units (10, 11). By this technique the effects of asynchronous fiber activation are largely eliminated, changes can be observed directly at the site of stimulation and stimulus polarity is easily controlled. In addition, the recovery of excitability following activity can be related to repolarization of the membrane as shown in the transmembrane action potential. This relationship has been studied by Weidmann (12) who has shown that the membrane of a single Purkinje fiber from calf heart fails to give a propagated response to long cathodal stimuli until repolarization has restored the transmembrane potential to a value of $-58$ to $-62$ mv. The same stimuli do not elicit a response of normal rise velocity and amplitude until repolarization is almost completed. These results have been interpreted in light of the ionic hypothesis (13) and presumably demonstrate the relationship between transmembrane potential and sodium transport mechanism in cardiac muscle (13, 14).

In the work to be described the normally propagated action potentials of papillary muscle were employed to stimulate single Purkinje fibers at various times during their phase of repolarization. The excitability of a single Purkinje fiber determined in this manner was compared to that obtained by transmembrane stimulation of the same fiber with rectangular current pulses of known strength and polarity. The excitability of papillary muscle was studied by similar techniques. Transmembrane action potentials were recorded by means of intracellular glass capillary microelectrodes (15) both in the immediate vicinity of and at varying distances from the site of stimulation. Some observations were also made on the nature of conduction delays and latency of response during the refractory period.

**METHODS**

The tissue employed consisted of a papillary muscle and attached bundle of Purkinje fibers isolated from the right ventricle of dog hearts (16). The preparation was immersed in Tyrode solution of the following composition: NaCl—137 mM; CaCl$_2$—2.0 mM; KCl—2.7 mM; NaHCO$_3$—12.5 mM. Dextrose—5.5 mM; MgCl$_2$—0.5 mM. The solution was maintained at 38°C and aerated with a mixture of 95% O$_2$ and 5% CO$_2$. The volume of the muscle chamber was less than 5 ml and the flow rate was maintained at 80–100 drops/min.

The preparation was driven at a regular rate by means of threshold stimuli applied to the tendinous end of the papillary muscle through two pin electrodes. These electrodes were also employed to elicit extrasystoles in the papillary muscle. Transmembrane action potentials were recorded from both papillary muscle and Purkinje fibers by means of intracellular capillary microelectrodes filled with 3 M KCl (15, 17). The single-ended cathode followers employed had a rise time of approximately 100 μsec and a grid current of less than 10$^{-13}$ amp. Action potentials were displayed on a splitbeam, 12-inch, long-persistence oscilloscope. Voltage calibration was accomplished by injecting known voltages between the tissue bath and ground.

Single fibers were stimulated by passing current pulses of selected duration between an intracellular microelectrode and ground. In some experiments one barrel of a double-lumen microelectrode was employed for stimulation and the other for recording transmembrane potential at the stimulus site (18). In other instances two or more single lumen microelectrodes were located in a single fiber. One electrode was used for stimulation and the others for recording membrane potential. The stimulus current strength was recorded by amplifying the voltage drop across a 10KΩ resistor placed between the preparation and ground and displaying this deflection on one beam of the oscilloscope. The zero potential reference for all records of transmembrane potential was determined at frequent intervals by withdrawing the microelectrode from within the fiber and subsequently reinserting it in the same location.
Figure 1 is a diagrammatic representation of the arrangement employed for stimulation and recording. Additional description of methods is given in various sections of the results.

RESULTS

A. Stimulation by Means of Propagated Action Potentials. 1. Purkinje fibers. It has been demonstrated that the duration of the transmembrane action potential of a single papillary muscle fiber is considerably less than that of a Purkinje fiber from the same heart (16, 19). This difference in time required for repolarization enables one to employ the action potential of the papillary muscle to test the recovery of excitability in single Purkinje fibers by the method shown in figure 2. Conditioning action potentials in the papillary muscle (top trace, fig. 2), elicited by regular driving stimuli, are propagated from that tissue across a junctional area (20) to the Purkinje fibers. The commencement of the transmembrane action potential of a single papillary muscle fiber recorded from within 0.1 mm of the junctional area gives the instant at which the specialized conducting fibers are stimulated. Similarly the action potential of a single Purkinje fiber (bottom trace, fig. 2), recorded several millimeters distant from the junctional area, shows the occurrence of propagated activity in that tissue and absence of any appreciable conduction delay at the junction (fig. 2A).

When test stimuli are applied to the papillary muscle at selected intervals after the driving, or conditioning, stimuli the records obtained show that the resulting ‘test’ action potentials reach the junction between the two fiber types at varying times during repolarization of the Purkinje fibers. As seen in figure 2B when the test action potential reaches the junction early during repolarization of the Purkinje fibers, there is no propagated response in the latter. A test action potential arriving somewhat later during repolarization gives rise to a propagated response, but only after a considerable latent period (figure 2C and D).

Finally, when the test action potential reaches the Purkinje fiber at the end of its phase of repolarization, conduction occurs without appreciable delay (fig. 2E).

The results obtained from this type of experiment permit determination of two of the classical parameters of refractoriness in the Purkinje fibers. The first of these, the ‘full recovery time’ has been defined as the time at which the delay between the test and response becomes constant (21). The second, the end of the ‘effective refractory period’ is defined as the earliest moment during recovery when a response to the test is conducted throughout the muscle (22, 23) and corresponds to the end of the absolute refractory period of other investigators (5). From enlarged tracings of records similar to those seen in figure 2 it can be determined that the full recovery time of a single Purkinje fiber is coincident with the completion of repolarization or, in cases where the resting potential is greater than 90 mv, with attainment of this level of transmembrane potential. This result is expected from the earlier studies of Weidmann (12). Similarly, the effective refractory period ends when repolarization has restored the transmembrane potential to a value of $-55$ to $-65$ mv.

However, since in these experiments the transmembrane potential of the Purkinje fiber was recorded several millimeters from the actual junction with the papillary muscle, the absolute refractory period, as defined by Lewis and Drury (23), could not be ascertained. Also, the possibility exists that in the junctional region where stimulation of Purkinje
fiber by papillary muscle action potential occurs the time-course of repolarization in the former might differ from that shown in figure 2. To check on the absolute refractory period and to determine the transmembrane potential of the Purkinje fiber at the point of stimulation a somewhat different experiment was performed. In this case one microelectrode was inserted in a single papillary muscle as before but the other microelectrode was located in a Purkinje fiber in the immediate junctional region. Location of this region was based on recordings of graded activity in the Purkinje fiber and has been described elsewhere (20, and manuscript in preparation by Kao and Hoffman). In addition, a third microelectrode was located in the same Purkinje fiber 2 mm or more from the junction to determine the presence or absence of propagation of the junctional responses.

As shown in figure 3, records obtained by a microelectrode located in a junctional Purkinje fiber show considerable variation in the form and amplitude of responses to the test action potentials. In the experiment shown in this figure one series of records (A-D) pairs the action potentials of the papillary muscle (top trace) with those recorded from a junctional Purkinje fiber (bottom trace); another series (F-I) pairs records from the junctional Purkinje fiber (top trace) with those obtained from the same fiber at a distance of several millimeters from the junction (bottom trace). When the test action potential of the papillary muscle reached the junction towards the end of repolarization of the Purkinje fiber the latter responded with an action potential of good amplitude and rise velocity (fig. 3A). Stimulation earlier during repolarization elicited Purkinje fiber responses which showed a decreased amplitude and slower rise (fig. 3B). Both these responses, however, were propagated to the site of the distal electrode (fig. 3F and G). When the test action potential arrived slightly earlier during repolarization of the Purkinje fiber, records from the junctional area showed graded responses (fig. 3C and D) which appeared at the distal site as decremented electrotonic potentials (fig. 3H and I).

A similar series of experiments employing a different muscle is shown in figure 4. In this case the records from junctional Purkinje fiber (top trace) and papillary muscle (bottom trace) are shown in A–D; junctional Purkinje fiber (top trace) and distant Purkinje fiber (bottom trace) appear in E–G. The early junctional responses shown in B and C show clear decrement in F, while the response in D appears as a normally propagated action potential in G. Enlarged tracings of records obtained from seven experiments of this type were projected on calibration curves (similar to those shown in fig. 3E and J) and measurements made first, of the level of membrane potential at which the earliest propagated response appeared in the junctional Purkinje fiber and, second, of the level of membrane potential.
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in that shown in fig. 3 with the exception that records from papillary muscle are on bottom trace in A-D. Voltage calibration (steps of 10 mv), time calibration (10- and 50-msec. intervals) and zero reference levels shown in II.

potential at which a clearly regenerative response first appeared. From these measurements the effective refractory period was found to end when repolarization had restored the membrane potential to a value of -60 mv. and the absolute refractory period maintained until recovery had reached a value of -45 to -50 mv. These values are comparable to earlier results obtained by Weidmann (12).

2. Papillary muscle fibers. The refractoriness of single papillary muscle fibers was outlined in a similar manner but by using a different sequence of stimuli. Conditioning (fig. 5A) and testing stimuli (fig. 5B) were applied to the papillary muscle as before and the test stimulus was introduced progressively earlier until a propagated response to the test action potential of the papillary muscle failed to occur in the Purkinje fiber. This failure of excitation left the Purkinje fibers excitable to direct stimulation through an additional pair of electrodes (fig. 5C). In this way normal action potentials could be elicited in the Purkinje system which arrived at the junction with papillary muscle at selected times during the repolarization of the latter (fig. 5C and D). In these experiments the transmembrane action potentials of the Purkinje fiber were recorded within 0.2 mm of the junctional area in order to provide an accurate indication of the time at which the papillary muscle fibers were stimulated. Records from the papillary muscle were usually obtained from two locations, one near to and one far from the junction, to demonstrate the propagation of responses.

It was not possible to record clearly graded activity of the papillary muscle fibers in these particular experiments and thus the determination of the absolute refractory period was not possible. However, records similar to those in figure 5 show that it was possible for a Purkinje fiber action potential to elicit a propagated response in papillary muscle prior to completion of its repolarization and suggest that the effective refractory period of the papillary muscle ended when the transmembrane potential had attained a level of -62 to -67 mv. Similar to the results obtained for Purkinje fibers, the full recovery time of the papillary muscle coincided with the completion of repolarization.

B. Latency of Response. Experiments in which Purkinje fibers were stimulated by action potentials propagated from the papillary muscle afford some information concerning the nature of conduction delays in cardiac muscle. In figure 6A and B are records obtained from papillary muscle (bottom trace) and Purkinje fiber (top trace). As the test action potentials in the papillary muscle arrive at the junctional area progressively earlier during repolarization of the Purkinje system, the responses of the latter, recorded at a distance from the junction, are displaced earlier in time up to a certain point (fig. 6A). When the test action potentials are still more premature (fig. 6B), the response of the Purkinje fiber appears at a fixed time and the latency between test and response increases considerably.

Two components responsible for this delay become apparent when records are obtained from a single Purkinje fiber in the localized junctional area (fig. 6C). In this instance one electrode (bottom trace) is located quite near to the junction and the other (top trace) is somewhat farther removed as is indicated by the local response shown in figure 6D (20). With this electrode arrangement it can be seen that the action potential elicited from the
Fig. 5. Effective refractory period of papillary muscle. Records of transmembrane potential of a single Purkinje fiber (top trace) and a single papillary muscle fiber (bottom trace). In A and B the conditioning and testing stimuli, applied to the papillary muscle, elicit propagated responses in both tissues. In C the test action potential of the papillary muscle reaches the Purkinje fiber earlier during repolarization and fails to excite. Under this condition direct stimulation of the Purkinje fibers gives rise to action potentials which reach the junctional region too early (C) or just late enough during repolarization (D) to cause a propagated response in the papillary muscle. Time calibration in 10- and 50 msec. intervals in A. Upstrokes of action potentials indicated by dotted lines.

juncional area early during repolarization of the Purkinje fibers arises out of a local response (figure 6E). Moreover, this local response rises quite slowly to its maximal depolarization. Thus the time lapse from the beginning of the local response to the start of the upstroke of the action potential may amount to as much as 50 msec. or more. Secondly, the local action potential shows a slow rise velocity and decreased amplitude and is propagated at a reduced rate because of incomplete repolarization of adjacent membrane (fig. 6F). A similar local response and delayed origin of the action potential is also seen when cathodal and anodal current pulses are employed as stimuli.

1. Cathodal stimuli. The stimulating efficacy of the propagated action potential was compared in a roughly quantitative manner to that of applied pulses of cathodal current. One double-lumen electrode was inserted into a single Purkinje fiber in the junctional region. Rectangular current pulses of 10-20 msec. dura-

tion were passed through one lumen and the local transmembrane action potential was recorded through the other. An additional microelectrode was inserted into the same fiber at a distance from the site of stimulation to check on the occurrence of propagation. Stimulus strength was adjusted to a value approximately five times threshold and stimuli were applied progressively later and later during the phase of repolarization of the Purkinje fiber until a propagated action potential appeared at a distant recording electrode.

The results obtained with a cathodal stimulus (i.e. outward or depolarizing current) are shown in figure 7. When the stimulus fell early during the phase of repolarization graded local responses were recorded at the stimulus site (top trace) and also, with appropriate decrement, at the distant electrode (bottom trace). Stimulation later during repolarization elicited a slowly rising depolarization locally and, at the distant recording site, a more abrupt depolarization in the form of a premature action potential. Still later the local depolarization increased in amplitude and rise velocity and the response at the distant electrode appeared as a normal propagated action potential. These results are quite similar to those reported previously by Weidmann (12) and indicate that the effective refractory period was quite comparable to that delineated when the propagated action potential was employed as a stimulus. The level of membrane potential at which propagation uniformly occurred ranged from −58 to −65 mv.

2. Comparison of applied and intrinsic stimuli. In several experiments refractoriness both to propagated action potentials and to applied cathodal stimuli of 10-20 msec. duration was studied employing the same single fiber. An example of results obtained by this technique is seen in figure 8. In this case three microelectrodes were employed. One (top trace, fig. 8A–C) was inserted in the papillary muscle near to the junctional area and employed to record the transmembrane potentials of a single fiber of that tissue. The second electrode (bottom beam, fig. 8A–J) was inserted in a single Purkinje fiber in the immediate junctional region, as shown by the graded activity recorded in figure 8D and E. The
third microelectrode was located in the same Purkinje fiber several millimeters farther from the junction than the second and was employed, first, to check on propagation of responses to intrinsic stimuli arising in the papillary muscle and, secondly, to apply cathodal current pulses to the same fiber (fig. 8F–I). Under these latter conditions the relative strength of the stimuli was displayed on the top trace as an upward pulse (fig. 8F–I, see METHODS).

In the top row of figure 8 it can be seen that action potentials originating in the papillary muscle elicited a propagated Purkinje fiber response in B and C, but earlier during repolarization gave rise to graded, decremental activity (D, E). Somewhat suprathreshold cathodal stimuli applied at various times during repolarization elicited propagated responses in G and H, but failed to excite, in spite of a marked increase in strength, in J. The earliest propagated responses to both type of stimuli (C and H) obviously arise at a similar time and similar level of transmembrane potential. In three experiments of this type the membrane potentials at the end of the effective refractory period for intrinsic stimuli and applied cathodal pulses did not differ by more than 3–8 mv.

From this type of experiment it was possible to form some estimate of the extent to which the propagated action potential exceeds the threshold requirement for a fully repolarized fiber. This was done by comparing the cathodal stimulus current requirement at the end of the effective refractory period and after complete repolarization. In three experiments the earliest effective cathodal stimulus was 4.5–6.5 times the threshold current requirement; this suggests that the propagated action potential has a safety factor of at least a similar order of magnitude.

**DISCUSSION**

A. Terminology Describing Refractoriness.

These attempts to test the recovery of excitability of single cardiac fibers by means of propagated action potentials warrant some discussion of the applicability of various terms describing refractoriness to this particular study. In earlier reports from this laboratory (5) the recovery of excitability of the heart was described in terms of an absolute, relative, and total refractory period. The end of the ‘absolute’ refractory period was defined by the earliest propagated response to test stimuli applied during the recovery phase. ‘Absolute refractory period’ thus is analogous to ‘effective refractory period’ as employed in this paper. The latter term appears to be preferable because it differentiates between local response and propagated activity and in this sense is probably the most significant measure of refractoriness. The absolute refractory period is best defined in terms of the presence or absence of local, active membrane response in the immediate vicinity of the test electrode (22, 23) and can be studied by means of either surface or transmembrane recording techniques. This measurement is less precise than the effective refractory period because of the
difficulty encountered in differentiating active from passive changes in membrane potential when the total change is small. However, it would seem that the absolute refractory period can be outlined equally well by either cathodal stimuli or propagated action potentials.

The relative refractory period and total refractory period are defined by the stimulus strength required in relation to the threshold requirement of the fully recovered tissue. These periods thus have no direct counterpart in tests made with propagated action potentials of fixed amplitude and duration. However, it is clear from records of the transmembrane potential (see fig. 3, 4 and 8) that during both the supernormal period (12) and the relative refractory period the incomplete recovery of the fiber can be recognized from a decreased rising velocity and small amplitude of the potentials resulting from stimulation during this time. The total refractory period is usually said to end when the threshold attains the resting value. This measurement gives a somewhat erroneous impression of the recovery of excitability, since records of transmembrane action potential clearly show that the supernormal period, which follows the end of the total refractoriness, results from incomplete repolarization of the fiber membrane. Responses at this time show a lowered rate of depolarization and decreased overshoot (12). In terms of the transmembrane potential, for a given set of conditions the recovery of excitability is complete at a time when the resting potential has been fully restored and the response to a test has regained a normal amplitude and configuration. This point in recovery seems to be most closely related to the older concept of the full recovery time (21).

B. Relationship Between Recovery and Repolarization. In the present paper the recovery of excitability and the limits of the absolute and effective refractory periods and the full recovery time have been described in terms of the level of membrane potential attained at the particular instant under consideration. This treatment differs from the usual method which describes refractoriness in terms of the time between the onset of activity and attainment of a certain degree of recovery. It is perhaps preferable to employ both time and voltage parameters in descriptions of the recovery of excitability; however, the latter measurement presents several advantages. In the first place there appears to be a fairly consistent relationship between transmembrane potential and ability of the membrane to give an active response (13). This relationship has been determined for cardiac fibers (12) and clearly demonstrates the actions of numerous factors which influence the recovery of heart muscle, such as calcium, antifibrillatory drugs, and local anesthetics (25) even though in the concentrations employed these agents may give rise to little change in the duration of refractoriness. Furthermore, when the limits of the absolute and effective refractory period and full recovery time are given in terms of the level of transmembrane potential, direct comparisons between tissues with action potentials of different duration are greatly facilitated. Similarly, changes in the duration of refractoriness resulting from altered heart rate, temperature and other influences which act primarily on the duration of the plateau of the action potential are clearly seen to leave the relationship between membrane potential and response largely unaltered.

C. Comparison of Cathodal Stimuli to Propagated Action Potentials. The results obtained in this study indicate that rectangular pulses of cathodal current of reasonable duration (10-20 msec.) give an accurate portrayal of the limits of the absolute and effective refractory period and the full recovery time and cardiac muscle. These stimuli, therefore, are
FIG. 8. Stimulation of a single Purkinje fiber by propagated action potentials of papillary muscle (A–E) and by cathodal current pulses (F–J). Note similarity of effective refractory period for both types of stimuli (C, H). Time calibration in A in 10- and 50-msec. intervals. Voltage calibration in steps of 10 mv and zero reference level (heavy line) shown in J. See text for description.

not unphysiological (8). With respect to the maximum intensity of cathodal stimuli which should be employed in studies of the recovery of excitability, the relationship demonstrated for heart muscle between reactivation of sodium carrier and recovery of membrane potential (12), as well as the present studies, clearly indicates that excessive stimulus strength is important only in relation to the occurrence of stimulation at a distance from the electrode site.

Although no attempt has been made to determine the safety factor of the cardiac action potential, the present studies show that, as has been reported previously on a different basis (26, 27), the cardiac action potential has a considerable margin of safety. At junctional areas it is able to stimulate the repolarizing membrane prior to recovery of the ability to give an immediate propagated response. The limit, therefore, is imposed not by the stimulating efficacy of action potential but by the processes associated with the recovery of excitability.

In a consideration of the effective stimulus strength of the action potential two additional points should be considered. Records obtained from the actual area of stimulation often show that the propagated action potential elicits only a local response or local depolarization which in turn gives rise to an action potential only after an additional lapse of time. It is quite likely that the propagated all-or-none response recorded at a distance originates in some area slightly removed from the stimulus site (20, and manuscript in preparation by Kao and Hoffman) and that in this latter area recovery has proceeded beyond the level shown in the record obtained at the location of the stimulating electrode. A second factor to be considered is the occurrence of a phase of supernormality in the recovery of the Purkinje fiber (12). During repolarization, when the membrane potential is between the level of the threshold potential and that of the full resting potential, the stimulus requirement for cathodal current is considerably less than after the normal resting potential has been attained. If there is an appreciable difference in the action potential duration at the stimulus site and in the immediately adjacent membrane this period of supernormality may assume considerable importance.

D. Latency of Response. Some mention should perhaps be made of the studies of conduction delay encountered when premature action potentials were conducted from papillary muscle to Purkinje fibers. The role played by the local response in the production of this delay facilitates the explanation of long latencies often encountered when recording from intact heart. This matter is discussed elsewhere (20, 24, and manuscript in preparation by Kao and Hoffman) in considerable detail and it is tempting to speculate on the operation of a similar mechanism in the production of the normal, long delay encountered in atrioventricular conduction.
REFERENCES