Graded and Decremental Response in Heart Muscle Fibers

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ABSTRACT

KAO, C. Y. AND BRIAN F. HOFFMAN. Graded and decremental response in heart muscle fibers. Am. J. Physiol. 194(1): 187-196. 1958—Simultaneous records of the transmembrane potential from two points in a single cardiac fiber indicate that during the refractory period when repolarization is about half complete graded responses appear close to the site of stimulation. These responses to cathodal pulses vary in amplitude with stimulus intensity and may show spatial decrement even when an overshoot is present close to the stimulating electrode. The results obtained indicate that the transition from graded to all-or-none response does not depend solely on the restoration of membrane potential. In some experiments stimulation was accomplished by employing one lumen of a double microelectrode; in others single Purkinje fibers were stimulated by action potentials propagated from attached papillary muscle. Similar graded responses were elicited in both instances. The experiments employing propagating action potentials as stimuli suggest the possible occurrence of graded activity as a response mechanism in the intact heart.

Activity in excitable tissues is not always of an all-or-none character. In some instances graded responses, which vary in amplitude with the intensity of the stimulus, constitute the only type of activity occurring. In other tissues graded activity is an alternative which is normally superseded by the all-or-none response (1, 2). Examples of this are seen in the axons of several species in which graded activity appears when the all-or-none response is suppressed by refractoriness (3-5) or by microinjection of pharmacological agents (5). Under such conditions a response which reaches almost full spike amplitude but fails to propagate occurs close to the stimulating cathode. The purpose of this paper is to show that in mammalian heart muscle graded activity of this type can be demonstrated during the refractory period following a normal action potential.

The results to be described differ from those previously reported in one important manner. In axons and also in skeletal muscle (6) graded response can only be shown under certain experimental conditions. In preparations of heart muscle, on the other hand, advantage can be taken of the different durations of the action potentials in contiguous fibers of ventricular muscle and Purkinje system (7). Since the latter fibers have the longer action potential, propagated action potentials in ventricular muscle fibers can be timed so as to stimulate the Purkinje fibers at various intervals during the phase of repolarization. Under these conditions graded, local responses are elicited in the Purkinje fibers. It seems likely that a similar phenomenon may occur in the intact heart with appropriately timed ventricular extrasystoles.

MATERIAL AND METHODS

In the present experiments graded responses were elicited from ventricular muscle and Purkinje fibers during refractoriness following a previous response. To test whether responses were local and graded, simultaneous recordings from at least two loci were required. It was necessary for one to be as close as possible to the site of origin of the impulse and the other to be sufficiently distant to demonstrate

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the presence or absence of propagation (fig. 1).

Two methods of stimulation were employed. The more direct method was to use a double lumen microelectrode (8), one side of which was employed for stimulation and the other for recording (fig. 1B). These double lumen microelectrodes were made as follows: two hard glass tubes of approximately 6 mm o.d. and 0.5 mm wall thickness were softened in a gas flame, twisted once and fused. This fused region was further softened and pulled to an outside diameter of 1 mm. Microelectrodes were then made from these fused capillaries by hand pulling and were filled with 3 m KCl by boiling (6). Almost always the tips of these double microelectrodes were in immediate apposition and thus recordings were obtained from tissue at the immediate site of stimulation. The indifferent electrodes for stimulation and recording were in the external medium. An alternative method of producing graded responses in Purkinje fibers was by stimulating one end of a papillary muscle to which a bundle of Purkinje fibers was attached (fig. 1A). Action potentials of the papillary muscle lasted only 150 msec while those of the Purkinje fibers were approximately 250 msec in duration. This difference of about 100 msec allowed appropriately timed responses of the papillary muscle to stimulate the Purkinje fibers at various times during repolarization.

With both methods of stimulation a proximal recording electrode was placed at the site of initiation of the response (at the stimulating cathode or in the junctional tissue between papillary muscle and Purkinje fibers), and a distal recording electrode was placed several mm away from this site. Whenever possible, the distance intervening between the two recording electrodes was larger than the space constant of the fiber (2 mm for Purkinje fibers (10)), so that decrement of local responses could be clearly demonstrated.

The transmembrane recordings were led into a single gun switched beam oscilloscope through two independent cathode followers and direct-coupled amplifiers (11). Two sweep-synchronized square pulses, each independently variable in delay, amplitude, and duration, were used for surface or transmembrane stimulation. In studies employing the double lumen microelectrode, conditioning and testing pulses were usually delivered from the same electrode. On occasion a conditioning action potential was elicited from a pair of surface electrodes and the test shock alone was passed through the microelectrode. All records were calibrated by injecting known voltages between the preparation and the ground electrode. The base line, or line of zero potential difference, was checked immediately before and after each series of records by withdrawing and reinserting the microelectrode.

The modified Tyrode solution employed had the following composition, in millimoles per liter: NaCl, 137; KCl, 2.7; CaCl₂, 2.7; NaHCO₃, 12.5; NaHPO₄, 3.7; MgCl₂, 0.5; dextrose 5.5. In some experiments the concentration of Ca++ was decreased to two-thirds of the above to diminish mechanical activity of the papillary muscle (12). This procedure had some effect on the graded response. The Tyrode solution was aerated with a mixture of 95% O₂ and 5% CO₂ and together with the preparation was maintained at 37°C (10). Cardiac muscles of cats and dogs gave similar results but the records presented in this paper were all obtained from preparations of dog heart tissue.

**RESULTS**

The membrane potentials measured in these experiments were similar to those obtained previously (12, 13). Resting potentials averaged 90 mv while maximum height of the action potential was approximately 120 mv for papillary muscles and 130 mv for Purkinje fibers.
Stimulation by Propagated Action Potentials. Histologically, ordinary cardiac muscle fibers and Purkinje fibers are continuous, one merging into the other without morphological interruption (14). Furthermore, in spite of some differences in electrophysiological properties, excitation can cross the junctional region between the two types of fibers in either direction (15, 16). Figure 2 illustrates a case in which stimuli that were applied to the end of a papillary muscle (A), elicited first the characteristic muscle response (B) (top trace), and after an appropriate conduction delay, the response of the Purkinje fiber (bottom trace). The shorter action potential of the muscle fiber allowed it to be re-excited earlier than the Purkinje fiber (C), a fact which made possible eliciting responses during the repolarization phase of the Purkinje fibers (D). Contrasted with the conditioning responses, the test responses in the muscle that appeared before complete repolarization had taken place had several distinctive features. These consisted of a longer latency, slower rising phase, reduced peak amplitude (C) and shorter duration (C, D). These features are similar to those described by Weidmann (17) and have been explained by him on the basis of the recovery of the membrane potential and the availability of sodium carriers which the membrane potential controls.

Sometimes apparently paradoxical events occur when early re-excitation is produced. In figure 2F, when the testing response of the papillary muscle appeared shortly before complete repolarization of that muscle, the response of the Purkinje fiber appeared only after a long latency. Conversely, when the testing response fell later, at (E) or after (D) complete repolarization of the papillary muscle, the latency preceding the corresponding responses of the Purkinje fiber shortened. These latter responses of the Purkinje fiber (E, F) also had faster rising phases and higher amplitudes than the response in D, consistent with the demonstrated effect of membrane potential on inward sodium current (18). The cause of the change in latency is not apparent from these records. It might be presumed, however, that the delayed appearance of the propagated action potential in E and F was caused by a slowly rising local response in the junctional region (19) which does not appear in the records because of spatial decrement.

The existence of such local responses can be demonstrated when records of the transmembrane potential are obtained from a site close to the Purkinje fiber-papillary muscle junction. In the experiment shown in figure 3...
one electrode (top trace) was located as close as possible to the site of Purkinje fiber stimulation and the other (bottom trace) was placed 3 mm away in the same fiber. When stimulation by action potentials propagated from the papillary muscle occurred near the end of the phase of repolarization (C, D) the responses of the Purkinje fiber resembled those shown in figure 2. With progressively earlier re-excitation the response of the junctional region (top trace) rose more slowly, became smaller (E) and developed a distinct notch (F, G). At the distal recording site, the first part of these testing responses appeared as decremented step-like potentials preceding the propagated activities. The late, slowly rising but large response at this site appeared only after the peak of the local response and most likely represented excitation at some other, more excitable area. This, propagating in a retrograde direction, caused the second peak on the records obtained from the junctional area.

Graded, decremental responses to stimulation of the Purkinje fibers by means of propagated action potentials are shown more clearly in figure 4. In this case the bottom trace represents activity close to the Purkinje fiber-papillary muscle junction and the top trace shows records obtained at a distance of 2 mm from this area. When the action potential of the papillary muscle arrived at the junctional region progressively later during repolarization of the Purkinje fiber the response of the latter increased in amplitude (B–D). In each instance, however, the response of the junctional membrane was clearly decremental as indicated by the diminished amplitude at the more distal site (top trace). At times, without apparent alteration in the stimulus interval, propagated responses arose out of the local response (E, F). In some instances the local responses which failed to stimulate lowered the transmembrane potential to a level below the inflection point of the records showing a propagated response. Whether or not this indicates some fluctuation in excitability independent of the level of membrane potential is uncertain. In any event it is clear that, when stimulated at the appropriate time during repolarization by activity of adjacent tissues, the Purkinje fiber is capable of producing graded local responses.

Direct Stimulation. Ventricular muscle. The syncytial nature of the ventricular muscle precludes recording from the same fiber at two sufficiently separated sites. The two microelectrodes, therefore, were inserted into myocardial fibers in a narrow end portion of a papillary muscle in which all responsive fibers were excited by a stimulus several times stronger than threshold. Under these cir-
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Fig. 4. Graded responses in Purkinje fiber. Experimental arrangement similar to that in fig. 3, except that bottom trace is from the junctional region and top trace from 2 mm away. Time calibration 10 and 50 msec. Details in text.

In certain circumstances, graded responses in ventricular muscle were comparable with those in Purkinje fiber. Figure 5 illustrates two such cases in which graded responses were produced by varying the strength of stimuli applied at a fixed time during repolarization. In A–D, the proximal electrode (top trace) was located within 0.2 mm of the stimulating cathode, while the distal electrode (bottom trace) was 1.8 mm further away. In all cases the 20-msec. long test pulse, delivered shortly after half repolarization, was capable of producing a large response at the cathode, but only a decremental response at the distal locus. The proximal response was small when the test pulse was weak (B), and increased (C) reaching full amplitude (D) when the test stimulus was made stronger. Even when an overshoot was present in the proximal response (C, D), the distal locus produced only decremental activity. Thus, the test responses at the cathode varied from 85 mv (B) to 100 mv (D) but remained essentially local.

The decremental nature of the graded response was more obvious when the interelectrode distance was increased to 5.5 mm (fig. 5, E–H). The second stimulus was applied 20 msec. later than that in the previous case. When it was made stronger, the proximal response (top trace) underwent a gradual change from 54 mv (E) to 60 mv (F) and then to 77 mv (G and H). The corresponding responses at the distal site (bottom trace) changed more rapidly from 18 mv (E), to 85 mv (H). All the distal responses had a distinct local component which was most prominent in G. Without additional depolarization at the cathodal region such a response could abruptly turn into propagated activity (H). The transition from graded to propagated action potential was accompanied not by an increase of amplitude of the proximal response, but by an increase in its duration.

Single Purkinje fibers. Several disadvantages are inherent in the experimental procedure described above. In the case of the Purkinje fiber-papillary muscle preparation, exact localization of the microelectrode at the junction can be made only after probing the region several times. For this preparation as well as for papillary muscle additional difficulties may arise if the two recording electrodes are in different areas that have different rates of repolarization. While a suprathreshold surface stimulus capable of exciting all responsive fibers reduces some of the difficulties, more conclusive evidence can be obtained by using a single fiber. The syncytial nature of papillary muscle precludes its use in these experiments. Therefore, all experiments on single fibers were made on isolated bundles of Purkinje fibers from the left ventricular cavity. Single fibers in such bundles can often be traced for up to 1 cm. Into one such fiber were inserted both a double lumen microelectrode and another single microelectrode separated by several millimeters. One lumen of the double microelectrode served to carry stimulating current pulses, while the other one acted as the proximal recording electrode. Capacitative artefacts in the doubled pair did not seriously affect the records ob
Fig. 5. Graded responses in papillary muscle elicited by direct stimulation by surface electrodes. Details in text. A–D, interelectrode distance 1.8 mm. E–H, interelectrode distance 5.5 mm.

tained, because the pulses used were longer than 10 msec. Moreover, the presence of a delay between the end of the stimulus and the beginning of the graded response confirms the latter as resulting predominantly from tissue activity.

Effect of stimulus interval. When a test stimulus is introduced during the refractory period of whole hearts or bundles of cardiac muscle characteristic features occur in the response elicited (cf. 10, 13, 20). Figure 6 illustrates the changes occurring in a single fiber and recorded simultaneously at two loci. Figure 8A shows graphically the relation between response amplitude and stimulus interval. The top trace in figure 6 represents activity immediately at the cathode; the bottom trace, that 2 mm away. When the test shock was brought earlier, there was a gradual decline in the amplitude of the responses at the cathode and a more abrupt decline of that at the distal site. Detailed examination shows other interesting points: The earliest responses were clearly decremental as evidenced by the attenuated activity at the distal locus. The 5th 7th responses at the cathode were larger and of longer duration. At the distal site, these were much prolonged and notched, the second peak being equal in height to the first (5th response) or larger (6th and 7th responses). The first peak at the distal site corresponded to the peak of the response at the cathode and represented decremented activity. The second peak, however, must be attributed to local activity in a more excitable neighboring region. In the 8th 10th responses, the second peaks have increased further. With still later stimuli, all-or-none responses ensued (11th 13th) beginning closer to the distal site than to the stimulating electrode.

The influence of decremental responses upon the measurement of refractory period was recognized by Lewis and Drury (21). The significance of the effects of varying stimulus interval in this respect is discussed in conjunction with other experiments (7). Figure 8A shows the general relationship between response amplitude and stimulus interval. At the cathode a more gradual change of response height occurs depending on the time at which the test stimulus is introduced. At the distal site, the growth in amplitude is more variable; it may lag slightly, or it may be more rapid depending on the responsiveness of the impaled region. Invariably however, an abrupt change in amplitude occurs with the beginning of propagation.

Effect of stimulus intensity. In most of the preceding sections the existence of graded response in heart muscle has been shown by stimulating with a fixed strength in different states of repolarization. The rela-
A relationship between the strength of stimulus and response amplitude is shown in figure 7. The preparation and arrangement is the same as that shown in figure 6. A fixed interval, an inward current pulse passed through one side of the double microelectrode was progressively increased in amplitude. The pulse itself was registered as a distorted rectangular wave in a depolarizing direction. The capacitative surges on the make and break of the current were too fast to be photographed, but the latter went downwards and served as a clear demarkation from the tissue response.

Figure 7 shows that many steps of response amplitude can be produced by varying the stimulus strength. These responses may have a reversed membrane potential at the cathode but remained non-propagating as indicated by the decremented distal responses. In A the highest nonpropagating response at the cathode (top trace) was 60 mv (7th trace up) and in B it reached 62.5 mv (8th trace). The subsequent response in each case marked the beginning of propagation, but the transition was accompanied by an additional 3 mv and 1 mv of depolarization, respectively. In both cases propagation was initiated by activity of some area adjacent to the distal site as evidenced by the earlier appearance of the responses there. The relation between response amplitude and stimulus strength is shown in figure 7B in which stimulus intensity is measured in arbitrary units. At the cathode, large responses of different amplitudes can be produced by increasing the stimulus intensity without triggering propagation. The amplitude of the distal response again shows a sharp inflection upon the occurrence of propagation.

Effect of calcium ion concentration.

The results discussed so far were obtained from preparations immersed in a solution containing 1.8 mM Ca++. This amount was two-thirds that usually used for dog heart, and was employed to reduce mechanical activity (12). When a higher concentration of Ca++ (2.7 mM) was used, several differences were observed. Graded responses were less easily elicited in both Purkinje fiber (fig. 9A, B) and papillary muscle (C, D) because there tended to be a more abrupt change from small subthreshold responses to propagated ones. In the former tissue when stimulus interval was lengthened (A), relatively long-lasting re
Graded response is known to occur under certain conditions in excitable tissues which normally produce all-or-none activity (cf. 1, 2). In heart muscle, Lueken and Schütz (22) using monophasic recording from chilled frog hearts, have shown that it is present during refractoriness. They also showed the main features of such graded responses, that is, a decremental nature and the variation of amplitude with both stimulus interval and intensity. While decremental responses occur in heart muscle under a variety of conditions (23-25), the variation of the amplitude of these responses with the intensity of stimulus was studied only by Lueken and Schütz. Their preparation, however, contained many fibers; and although rejecting the possibility that their observation might be due to changes in the number of responding fibers they could not unequivocally establish the gradation in amplitude as occurring in single fibers. The results described in this paper confirm the observations of Lueken and Schütz, and clearly indicate that the phenomena are present in individual fibers. Thus, similar to results obtained in the electroplaques of electrophorus (cf. 2) and in the squid giant axon (5) transmembrane recordings in heart muscle show graded responses that vary in amplitude up to the full height of a normal action potential without becoming propagated.

The gradation of amplitude according to the degree of depolarization forms an apparent paradox to the ionic hypothesis concerning activity in the cardiac muscle fiber (10, 26). In contradistinction to the effect of increasing the membrane potential, progressive depolarizations may inactivate Na+ carriers and increase K+ outflow as in nerve (26). Both these factors oppose the realization of responses of increasing amplitudes as the stimulus increases. However, since the stimulating pulses were relatively short and since the time course of inactivation in heart muscle is not clearly known (10), proper evaluation of these factors is difficult. Within the general framework of the sodium hypothesis (26, 27), the mechanism of gradation of response has been proposed as resulting from a population phenomenon involving the sodium carriers (2). Individual carriers may have different thresholds, but once activated, respond in the all-or-none manner by transferring sodium ions from the outer surface of the excitable membrane to the inner surface without interruption. Since the spike, as recorded by a microelectrode, results from numerous such reactions in the active region, its form depends on the number of reactive carriers. Thus, for the all-or-none response, the critical depolarization is defined at a relatively constant level. Upon attainment of this membrane potential, because of a self-regenerating conductance change the sodium carriers effect a maximum amplitude and a rapid maximum rate of rise. However, if only a portion of all the carriers react to a certain depolarization, then the resulting response, like that appearing at low sodium concentrations (15, 27) would have a reduced amplitude and
Fig. 9. Effect of normal (2.7 mm) Ca++ ion concentration on graded response. A and B from Purkinje fiber. A, varying stimuli interval; B, varying stimulus strength. Inter electrode distance 2.5 mm. C and D from papillary muscles; C, varying stimuli interval. D, varying stimulus intensity. Inter electrode distance 5 mm.

slower upstroke (figs. 3, 6 and 7). Graded responses can then be viewed as the manifestation of the number of carriers activated by depolarizations of different magnitudes.

Nevertheless, a sequence of ionic conductance changes similar to that producing the upstroke of the action potential probably underlies the graded response since the latter has the general form of the all-or-none response and is also triggered by a decrease in membrane potential. The difference, however, is that the events do not necessarily displace the membrane potential to any fixed level (figs. 7 and 8.4). Assuming that the potassium outward current is relatively constant at any one moment after an action potential, the gradation in amplitude suggests that the inactivation and reactivation of sodium carriers may be more complex than has been postulated (26). Thus, not only may the thresholds of carriers differ but also the time-course of reactivation of individual carriers may show some variation. The observation that in a reduced Ca++ medium more steps of amplitude variation can be obtained suggests that the reactivation process is effected.

The change in the amount of Ca++ is not sufficient to produce any alteration in the conditioning response, i.e. in the resting sodium carriers that are being activated. However, carriers which are being reactivated may be more strongly influenced than those in the resting state. This agrees with the finding that Ca++ ions have a more profound effect on the Na+ carrying mechanism than on the resting potential (18). A change in the same direction occurs in frog skeletal muscle when the critical potential for firing becomes scattered in a medium containing little Ca++ (28). The observation that in a low Ca++ medium graded responses appear in larger areas is in agreement with a previous observation that cardiac muscles tend to produce multifocal pacemakers in this environment (12).

In the all-or-none response decrementless propagation is inseparably tied to the changes in membrane conductance by local circuit excitation. In the graded response when decrement occurs, the safety factor by definition is lowered. The threshold of the region in advance of the leading front of an impulse is, however, still low enough so that responses with overshoots may be produced there by local circuit current. But no propagation takes place unless the response has at least a minimum duration. This requirement is effected by contribution from adjacent areas (figs. 6, 7 and 9). Subthreshold responses were originally thought to be small depolarizations limited to a minute area (19, 29, 30). Since they also proceed membrane action potentials (31, 32) they are considered as essentially similar to propagated all-or-nothing responses (26). Whether graded responses are intermediary steps between subthreshold response and the action potential remains to be shown. The results in this paper suggest considering an area factor in initiating propagation (29). In support of this suggestion is the observation that cathodal graded responses may change only slightly in amplitude as the distal response changes from decremental propagated all-or-none activity.

REFERENCES
2. Grundfest, H. Prog. in Biophysics 7: 1, 1957.