Refractory Period of the Dog’s Ventricular Myocardium Following Sudden Changes in Frequency

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ABSTRACT

In exposed hearts of anesthetized dogs with total A-V block, a sudden increase or decrease in heart rate changes the duration of refractoriness immediately. A steady state is established only after a few hundred beats. The time course of shortening of the refractory period caused by an increase in driving rate (“on” effect) has the following characteristics. The first beat shortens the refractory period, as determined from strength-interval curves, about 30% of the total shortening; the second beat shortens it 10% more. After the second beat, the rate of change in the duration of the refractory period suddenly becomes slower. Alternation of the refractory period begins with a slightly prolonged refractory period of the third beat and then diminishes rapidly in about the following 10 beats. These changes are in phase throughout the ventricular myocardium.

Following sudden transition to a slower rhythm, the time course of lengthening of the refractory period (“off” effect) is opposite to the “on” effect: the alternation that occurs is less marked. Changes in the duration of the functional refractory period near the stimulus site are parallel to those in the threshold curves.

These results demonstrate a long persistence of the effect on myocardial refractoriness of a previous cardiac frequency.

ADDITIONAL KEY WORDS cycle length premature beats cumulative effect functional refractory period A-V block

During the steady state of regularly driven hearts, the duration of the refractory period of cardiac tissue is inversely related to heart rate: a fast rate shortens, a slow rate lengthens this period (1, 2). The question whether the refractory period reaches a steady-state value immediately or gradually after a new frequency is initiated has not been studied extensively. Mendez et al. (3) measured the refractory period of dog hearts after one premature beat and compared the results with those obtained during regular driving of the heart with the same cycle length as the delay of the premature beat. Usually no differences were found, and they therefore concluded that the refractory period is determined by the length of the immediately preceding cycle. During stimulation of the ventricle there was evidence in some experiments for a cumulative effect of repetitive short cycles on the refractory period. Mendez et al. (3) assumed that in these experiments both myocardium and conduction system had been stimulated and that the conduction system, unlike atrial or ventricular myocardium, exhibits a cumulative effect of frequency. Carmeliet (4) measured the duration of the transmembrane action potential of frog ventricle at different frequen-
cies, and noted that after a change in heart rate, the duration of the action potential reached a stable value after 40 to 50 systoles. Since the duration of the refractory period is closely related to the duration of the transmembrane action potential, it seems plausible that the duration of the immediately preceding cycle is not the sole determinant of this variable.

In this present study we investigated the speed of adaptation of the refractory period to sudden change of the heart rate in canine hearts in situ. The results indicate that the refractory period changes quickly within the first two beats and then changes more slowly, reaching the steady-state value of the new frequency after a few hundred beats.

Methods

Mongrel dogs of both sexes, weighing 15 to 20 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Under artificial respiration a right-sided thoracotomy was performed. The pericardium was opened and a pericardial cradle constructed. Because of the need to explore longer cycle lengths than provided by the sinus rhythm, a total atrioventricular block was produced by electrocoagulation. For stimulation and recording, two or three multielectrodes were inserted into the left ventricular wall. They had a diameter of 0.9 mm and contained 10 terminals, each having a diameter of 0.1 mm. The interterminal distance was 2 mm (5).

Unipolar cathodal stimuli were delivered from the terminal closest to the epicardium, and bipolar complexes were recorded from the other terminals of the same intramural electrode and of those located in other parts of the heart. A specially designed stimulator, developed at the Laboratory of Medical Physics, enabled us to deliver various patterns of stimulation. All stimuli were rectangular pulses of 1 msec with a variable strength and separated from ground.

Unipolar and bipolar complexes were monitored and tape recorded on an adapted 14-channel DAS 100 Ampex physiological recorder, at a tape speed of 15 inches/sec and printed on line by a 16-channel Elema inkwriter. For the purpose of time measurements of the recorded complexes, the tape was played back at 1% inches/sec on the Elema writer, allowing a time resolution of 960 mm/sec at a frequency response of 8000 cps. Adequate control of heart and body temperature was provided by a d-c operated heating blanket wrapped around the animal and by covering of the thorax with cotton pads soaked in warm saline.

Results

Steady-state strength-interval curves were made at different driving frequencies to determine the range of refractory periods. The refractory period was considered to be in a steady state when the heart had been driven at a given frequency for at least 500 beats. Test stimuli were then given at selected intervals after every eighth beat, and the thresholds were determined. The duration of the "absolute" refractory period differed for the various frequencies; the shape of the resulting strength-interval curve for cathodal stimuli, however, was similar for every frequency tested. This also was found after 1, 2, 3, or a randomly chosen number of beats of the new rhythm. Therefore, the location of the curve in the cardiac cycle could be ascertained by determining one point only. For this purpose we chose a test stimulus strength 1.5 times diastolic threshold intensity.

In some experiments other stimulus strengths were used; the results are essentially the same. The earliest interval at which the test stimulus would evoke a propagated response will in this paper be referred to as the refractory period.

Figure 1 shows the data acquired in three dogs. At frequencies with a basic cycle length of 800 msec and greater the refractory period reached a plateau. At the fastest possible driving rate (basic cycle length 150 msec) the refractory period is about 100 msec shorter than at the slowest rate. At still faster rates ventricular fibrillation invariably occurs.

Shortening of the Refractory Period

A sudden increase in driving rate from 100/min (600-msec stimulus interval) to 200/min (300-msec stimulus interval) shortens the refractory period by approximately 50 msec. In preliminary experiments we observed that a maximal shortening does not occur in the first beats of the new rate, but develops gradually. To determine the time...
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Course of this shortening ("on" effect) the following procedure was applied. Immediately after changing the driving frequency from the slow to the fast rate, test pulses of 1.5 times diastolic threshold strength were given after every second driving stimulus. (Because of the properties of the stimulator, it was easier to give the extra stimulus after every second basic stimulus instead of after basic stimulus.) The intervals between driving and test stimuli were chosen to be shorter than the steady-state refractory period at the slow rate, but longer than the steady-state refractory period at the fast rate.

We counted the number of beats of the fast rate that were necessary to shorten the refractory period of the ventricle to such a degree that the test stimulus with this particular delay elicited a ventricular response. After allowing the heart to return to a steady state at the slow rate for at least 500 beats, this procedure was repeated for other test intervals. The shortest interval tested was equal to the steady-state refractory period at the fast rate. This entire procedure took about 1 hour to complete. In this way a curve was obtained that relates the progressive shortening of the refractory period after a sudden doubling of the driving frequency to the number of beats at the fast rate.

Figure 2 shows the results of experiments in three dogs in which the "on" effect for the frequency jump of 600 msec to 300 msec in basic cycle length was investigated. The differences between the two steady-state refractory periods were 48, 50, and 51 msec; the interval preceding the first successful test stimulus is expressed as a percentage of this difference. In the first 20 beats about 40 to 60% of the total shortening occurs; the final value is reached after 400 to 500 beats.

For several basic driving rates of 150, 100, 75, 60, and 50/min (equivalent to basic cycle lengths of 400, 600, 800, 1000, and 1200 msec) the "on" effect was investigated in three other dogs after doubling the driving frequency. Essentially the same curves were obtained (Fig. 3).

Influence of Subliminal Test Stimuli on the "On" effect.—To explore the possibility that the repeated application of subliminal test stimuli during the refractory period might influence the rate of change in refractory period, control experiments were undertaken in three dogs. After determining the "on" effect, by giving the test stimulus repeatedly after every second driving stim-
Figure 2

Top: Steady-state strength-interval curves for two driving rates (600-msec and 300-msec basic cycle length) in three dogs (A, B and C). Abscissa = interval following the basic driving stimulus. Ordinate = intensity of a test stimulus of 1 msec. In the area between the ascending parts of both curves, test stimuli of three intensities and with different delays, used for determining the progressive shortening of the refractory period following a sudden increase in driving rate from 600 to 300 msec basic cycle length ("on" effect), are indicated. Bottom: "On" effect: the number of beats of the faster rate after which the heart responds to a test pulse with chosen delay and strength. The delay of the test pulse is expressed as a percent of the difference between the steady-state refractory periods at the slow and fast rates. Note that 40% to 60% of total shortening of the refractory period is achieved after about 20 beats of the new rate, and that the steady-state value is reached after a few hundred beats.

Lengthening of the refractory period

A similar method was used to determine the "off" effect, i.e. the time course of the

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"On" effect for doubling the driving rate at various heart rates: the number of beats of the faster rate after which a test pulse of 1.5 times diastolic strength is followed by a propagated beat. The delay of the test pulse is expressed as percent of the difference between the steady-state refractory period at the slower rate and at the faster rate. 100% = steady-state refractory period at the slower rate (basic cycle length of 1200, 1000, 800, 600 or 400 msec). 0% = steady-state refractory period at the faster rate (basic cycle length of 600, 500, 400, 300 or 200 msec).

lengthening of the refractory period that occurs when the driving frequency is changed from a fast to a slow rate. Again, several test stimuli with a preceding interval longer than the steady-state refractory period at the fast rate were selected. In contrast to the method employed for studying the "on" effect, the test stimuli could not be applied repeatedly during the slow drive until the heart ceased to respond, because the occurrence of premature beats would counteract the effect of the slower driving rate on the refractory period. A more time-consuming procedure had to be followed, in which the heart, departing from a steady state (300-msec basic interval) was driven at a slower rate (600-msec basic interval) for a number of beats, after which the test stimulus was applied. If the heart still responded to the test stimulus, it was brought back to a steady state of the faster rate and then driven for a slightly larger number of beats at the slow rate before the same test stimulus was applied again. This sequence had to be repeated many times to find the number of beats at the slower rate, after which the refractory period would be lengthened to a point where the heart would not respond to the test pulse. Figure 5 shows the "on" and "off" curves for several frequency jumps (1200 → 600- and 1000 → 500-msec cycle...
“On” and “off” effects for two frequency ranges. Delay of test pulse expressed as percent of the difference between the steady-state refractory period at the slow rate (100%) and the fast (0%) rate is plotted against number of beats at the new (fast or slow) rate. Insert: Stimulation patterns used for determining “on” and “off” effects. For the “off” effect, the number of beats at the slower rate is indicated; then a test pulse is no longer followed by the heart. The curve indicates the progressive lengthening of the refractory period following a sudden change from a fast to a slow driving rate.

Immediate Changes in Refractory Period

The methods employed thus far do not give information about changes in refractory period brought about by the very first beats of the new frequency. Therefore another procedure was developed to measure accurately the refractory period after each of the first 10 (or more) beats of the new rate. To determine the refractory period after one beat of the new rhythm introduced during a steady-state frequency, a test pulse 1.5 times diastolic threshold strength was given after the premature beat. If the test pulse resulted in a ventricular response, the heart was brought back to the steady state and the procedure repeated. The interval between premature beat and test pulse was shortened each time by 1 msec, until the shortest interval was found at which the heart responded. In a similar procedure the refractory period after two short cycles was measured, by applying the test pulse after the second beat of the new rate. In this way the refractory period after any number of repetitive short cycles could be ascertained with an accuracy of 1 msec. The results obtained by this method were consistently reproducible. Each point of the curves shown in Figures 6 and 7 was determined twice during one series of measurements; in most cases identical values were found; no variation exceeded 2 msec.

Initial Part of “On” Effect.—Figure 6 shows the initial changes in refractory period in 3 dogs after doubling the driving rate, thus giving a basic interval of 300 instead of 600 msec. The refractory period is shortened considerably (about 27% of total shortening) by the first beat of the faster rate and up to 40 to 45% of total shortening by the second beat. After the first two beats the rate of change suddenly becomes slower and longer and shorter refractory periods alternate. Thus, the refractory period after the third beat is longer than after the second beat, the difference being sometimes as much as 10 msec.

When the initial part of the “on” effect was studied at several electrode terminals located in the ventricular myocardium, essentially the same curves were obtained, i.e. the changes in duration of the refractory period following sudden doubling of the heart rate were in phase throughout the ventricular musculature. Figure 7 shows the initial part of the “on” effect for several frequency jumps. Departing from a steady-state frequency of 600-msec basic interval, the driving rate was augmented to 400, 300, 250 and 230 msec. For each frequency jump essentially the same curve was obtained. It is evident that the faster the new rate, the greater the shortening by the first two beats and the more pronounced the oscillation between longer and shorter refractory periods after the second beat.

Initial Part of “Off” Effect.—To determine
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FIGURE 6
Immediate changes in refractory period upon sudden doubling of the heart rate in three dogs. Inset: stimulation pattern used for determining the refractory period after the first beat (top) and second beat (bottom) of the new rate. In each dog the "on" effect was determined twice, with a time interval of 2 to 4 hours; the same pattern was found at a slightly different length of refractory period. An alternation in refractory periods of subsequent beats occurs after the second beat, in varying degree in individual experiments.

FIGURE 7
"On" effect for the sudden transition to different, fast rates, departing from the same basal rate (600-msec cycle length). Symbols show cycle lengths: triangles = 400 msec; solid circles = 300 msec; open circles = 250 msec; crosses = 230 msec. The alternation increases as the rate becomes faster.
the lengthening in refractory period effected by one long cycle or any number of repetitive long cycles introduced during a steady-state fast rate, essentially the same method was employed. The beat with the long preceding cycle (or the last beat of a series of successive long intervals) was followed by a test pulse. In each instance the shortest interval preceding a successful test pulse was determined. As Figure 8 shows, the initial part of the "off" effect is not the exact mirror image of the "on" effect. The first long cycle has the largest effect (about 30% of total lengthening). After the first beat of the slow frequency the rate of change in refractory period is diminished, and each subsequent long cycle lengthens the refractory period about the same amount.

CHANGES IN FUNCTIONAL REFRACTORY PERIOD

The results described so far apply only to changes in the excitability as measured in threshold determinations; they do not contain any information about the recovery of conduction. The effect of frequency changes on the functional refractory period, i.e., the shortest interval between two propagated responses, was investigated. In these experiments the procedure was similar to that used to determine the "on" effect. In addition, bipolar complexes were recorded from terminals located on the intramural electrode 2, 4, 6, and 8 mm from the point of stimulation and from intramural electrodes located in other parts of the heart. Activation times were measured with an accuracy of about 1 msec.

Because of the technical impossibility of recording acceptable complexes from the electrode used for stimulation, the latency at the stimulus site itself could not be determined. Therefore the results do not indicate the changes in functional refractory period at the exact stimulus site. The shortest distance from the stimulus site at which it was possible to distinguish between intrinsic deflection and stimulus artifacts in bipolar records was 2 mm. This may introduce a slight error.

FIGURE 8
Immediate changes in refractory period when the driving rate is suddenly reduced from a cycle length of 300 msec to one of 600 msec in same dogs as in Figure 6. Inset: stimulation pattern.

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FIGURE 9

"On" effect for the functional refractory period as determined by the appearance of a propagated response 2 mm from the stimulation site. Solid circles = refractory period (shortest stimulus interval); open circles = functional refractory period (shortest interval between two propagated responses 2 mm from the stimulation site).

In records from the nearest terminals the bipolar complexes of the earliest responses display a slightly higher degree of notching than those occurring after longer intervals. The first clearly discernible fast deflection was taken as moment of activation. Bipolar complexes recorded at larger distances do not contain significant notching. The results demonstrate that the changes in the duration of the functional refractory period when the heart rate is suddenly accelerated, as observed in the close proximity of the stimulating electrode, are highly parallel to those of the threshold course of excitability. This is shown in Figure 9.

The response evoked by the test stimulus with the shortest possible preceding cycle length, arrives at the electrode terminal located 2 mm from the stimulus site, at most 5 msec later than the basic response, if test pulses with a strength of 1.5 times diastolic threshold were employed; for test pulses of 10 times diastolic strength this difference may amount to 10 msec. After the activation front has passed the electrode terminal which is located 2 mm from the stimulus site, propagation along the intramural electrode occurs at normal diastolic velocity. The lines, connecting the activation times of the terminals of the intramural electrode used for stimulation, run parallel to each other for every test stimulus and after any number of beats at a new, faster rate. At larger distances from the stimulation site, less uniform values were observed. These are subject to further investigation.

Discussion

An accurate quantitative description of the beat-to-beat variations in the duration of the refractory period following sudden alterations in heart rate has not yet been published. The present experiments show that a sudden increase or decrease in the driving
frequency of the heart immediately results in changes in the refractory period and that a new steady-state value of the duration of refractoriness is established after several hundreds of beats. This is in contrast to the results of Mendez et al. (3), who concluded that the duration of the refractory period was determined by the length of the immediately preceding cycle. In our opinion, the cumulative effect of repetitive short cycles on the refractory period cannot be explained by a cumulative effect on the conduction system, since in our studies driving and test stimuli were delivered in the outer layers of the ventricular muscle, where in the dog heart no specific conduction system is present (6).

Since a close correlation exists between the recovery of excitability and the time course of the transmembrane action potential (2), the results presented above are indirectly supported by observations of others. Carmeliet (4) noted that a stable value for the duration of the transmembrane action potential was established only after 40 to 50 systoles of a new rate. Brooks and associates (1) reported that when the driving rate is increased the action potential shortens slightly on each successive cycle and reaches a condition of equilibrium only after considerable lapse of time. Gibbs and Johnson (7) examined the changes in shape and area of the rabbit ventricular action potential brought about by changes of stimulus frequency and observed that the form of the action potential is in some way related to the history of the heart muscle. The determination of the changes of the refractory period in the transient period allows a high precision; differences of 1 msec are readily and reproducibly detected on a total duration of the refractory period between 100 and 200 msec, making for an accuracy of ½ to 1%. The records of transmembrane potentials by their nature preclude such precise measurements.

Hoffman and Suckling (8) stimulated a papillary muscle preparation after a period of quiescence and observed an alternation in the duration of subsequent action potentials; the second action potential was shorter than the first, the third slightly longer than the second, etc. Our observation of a more or less damped oscillation in the curve, depicting the duration of the refractory period in subsequent beats following the second beat of a suddenly initiated faster rate, indicates a similar alternation in the duration of the action potential. In the present state of knowledge no adequate explanation can be given for the mechanisms responsible for the time course of the adaptation of the refractory period to a new heart frequency. The fact that the refractory period, in the "off" effect of the first, and in the "on" effect of the first two, beats of a new rate is altered considerably, while the effect on subsequent beats is much smaller, suggests two different regulating processes, one acting on a short term, the other on the long term.

In the theoretical model of Noble (9), developed for Purkinje fibers, the K conductance (gK) rises during the action potential to a certain value at which repolarization is initiated. After the end of the action potential, gK decreases slowly. The first action potential of a new, faster rate will be shortened because gK is still high when this action potential starts, due to the short preceding diastole, and so less time is needed to reach the value required to initiate repolarization. Noble's computed action potentials after a sudden increase in frequency also show an alternation in the duration of successive action potentials: the second action potential is longer than the first and third action potentials. The diastole preceding the second action potential is longer than the diastole preceding the first action potential. Consequently, gK will be lower at the start of the second action potential, therefore it will take more time to reach the level at which repolarization starts, and the second action potential will be longer than the first one. This alternation goes on for an unspecified number of beats; the even action potentials are longer than the odd ones. It has to be noted, however, that this alternation is
not the same as the alternation we found in ventricular myocardium.

In our experiments, the refractory period of the second beat is still shorter than that of the first beat, and also shorter than that of the third beat; the odd action potentials are longer than the even ones. Evidently other factors besides the persistent increase of gK must be involved. One can postulate the presence of a process that also is determined by the length of the diastole, but acts with a delay of at least one beat, so that it affects the duration of the second consecutive action potential. Increase of the frequency then would result in the initiation of changes in both the “fast” factor (persistent increase of gK) and the “slow” factor; the net result for every beat would depend on the interaction of both factors. Slight variations in time constants of these factors may produce a large variability in the time course of the alternation. This would account for some deviations from the main pattern described above, that were occasionally observed in our experiments.

Carmeliet and Lacquet (10) suggested that the shorter duration of the action potential at higher rates was due to changes in ionic concentrations in the immediate neighborhood of the cell membrane. They calculated that each activation raises the extracellular K concentration by 0.6 mM and assumed that this high concentration is exponentially restored to the normal value. At higher rates, the K concentration outside the cell membrane would be higher than normal, and this would shorten the action potential. In fact, Carmeliet (11) demonstrated that a high extracellular K concentration increases gK. The considerable shortening of the refractory period by the first two beats of a faster frequency might therefore be explained by an accumulation of K outside the cell membrane, caused by an inability of the K-Na pump to restore the normal ionic concentrations during the suddenly shortened diastoles. It is also conceivable that the pumping mechanism is activated by the increased extracellular K concentration. This would tend to restore the ionic concentrations, and prevent a further accumulation of K. The effect of subsequent beats on the refractory period would therefore be diminished until eventually a new equilibrium is reached. Accumulation of Na inside the cell would have a similar effect. The long period needed to reach a new steady-state refractory period might represent the time required for the resetting of the Na-K pump and the restoration of the ionic gradients. Furthermore, during rapid driving more energy is used. This could initially result in a relative lack of substrate, slowly progressing as more “stores” are depleted. On the long run the metabolic processes may adapt themselves to an increased turnover rate.

One of the implications of the demonstrated “off” effect is that after abrupt termination of tachycardia, the heart can still respond to rapid frequencies, i.e., initiated by a fast-firing focus for a relatively long time, thus favoring the reestablishment of a tachycardia. The slight notching in early test responses recorded from the nearest terminals, indicates a certain degree of asynchronous recovery of adjacent groups of myocardial fibers close to the stimulus site. This is in agreement with the findings of Han and Moe (12).

It is important that no notchings are present in records from more distant terminals (4 to 8 mm); evidently at this distance synchronization of activation occurs, in other words the functional refractory period of the myocardium is in phase.

References

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