Effect of Changes in Frequency of Stimulation Upon Rabbit Ventricular Action Potential

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It has been established that the duration of the action potential of cardiac muscle is a function of the frequency of stimulation. The changes in the shape and the area of the rabbit ventricular action potential upon alteration of the stimulus frequency have not, however, been examined. Such changes in action potential shape are of fundamental significance since, unlike changes in shape produced by drugs, temperature, and variation of extracellular ionic concentration, they take place without disrupting metabolic processes or diffusion gradients.

These initial experiments were designed to examine the magnitude and time course of changes in the shape of the rabbit action potential. They have shown that the ionic mechanism which produces the cardiac action potential is extremely flexible and can produce rapid changes in the time course of the individual ionic conductances.

Methods

Rabbits were killed by a blow on the neck. The heart was excised within 30 seconds and immediately transferred to a dish filled with aerated Krebs-Hensleit solution at 38°C; within a further 30 seconds the right ventricular wall was excised and quickly transferred to an organ bath maintained at 38°C ± 0.5°C. The organ bath was made of silver and was perfused with Krebs-Hensleit solution aerated with 95 per cent O₂ and 5 per cent CO₂.

The ventricular muscle was stimulated externally by two silver electrodes 2 mm. apart placed on one edge of the preparation. Membrane potentials were recorded by an intracellular microelectrode filled with 3 M KC1. The recording stage was the floating grid electrometer input stage described by Murray. Oscillographic traces of action potentials were photographed and appropriate measurements were made by projection of the records upon a calibration grid. Measurements of the area of action potentials were made with a planimeter and expressed in arbitrary units. A constant magnification of the oscillographic records and a constant planimeter setting were used throughout so that the arbitrary units of area are comparable. The duration of an action potential was obtained by measuring the interval between the upstroke of the action potential and the point at which repolarization was complete to within 5 mv. of the resting potential.

In certain circumstances, rabbit heart muscle does beat spontaneously and can average one such beat a second. Thus, in our experiments, care had to be taken at low frequencies of stimulation to see that the muscle was not beating spontaneously and thus producing erroneous results. It has been our experience that the preparation does tend to beat spontaneously when it has deteriorated, so that this phenomenon does occur toward the end of the day's experiment. It is also liable to be produced if the preparation is not excised quickly enough or is exposed to rapid changes in temperature.

We guarded against the previous possibilities and checked for the occurrence of such beats both optically through a Zeiss Opton stereomicroscope and by allowing any such beats to trigger the oscilloscope.

Results

Action Potential Duration and Action Potential Area as a Function of Stimulus Frequency

The ventricular muscle was stimulated at the following rates: 0.1, 0.2, 0.5, 1, 2, 4, 6, 8, and 10 sec⁻¹. After a change in the frequency of stimulation, action potentials reached a stable form within 60 seconds. In the 76 fibers in 16 experiments, the action potential area and duration were plotted against logarithm of frequency.

In all fibers both the area and the duration of the action potential were found to be maximal at a frequency ranging from 1 to 3 sec⁻¹. This can be seen in figures 1 and 2 in which, in five experiments, fibers have had both the duration and the area of their action potentials plotted against the stimulus fre-
frequency. At frequencies above and below this range the area decreased, and in the majority of fibers the duration also decreased. The decrease in duration at low frequencies was not as great as at high frequencies, and in a small proportion of fibers that had action potentials with a well defined plateau (as in fig. 3B) the duration remained at a maximum as the frequency was reduced.

It should be explained here that the action potentials with the prolonged plateaus were encountered in the endocardial network of pale tissue, the Purkinje network, and in the region of its termination in the myocardium. The extent of this network was never great and the width and number of the pale bands differed from preparation to preparation.

Action potential traces obtained from two typical fibers at differing frequencies of stimulation are shown in figure 3. In this figure, action potentials obtained at three different rates are superimposed to demonstrate that the changes in area of the action potential are the results of two different changes in shape. At high frequencies of stimulation (trace c), the decrease in area of the action potential resulted from an earlier "take off" of the final phase of repolarization while the plateau of the action potential remained at its normal level. At low rates of stimulation (trace b), the decrease in area was caused largely by a drop in the height of the plateau. In fibers which had a pronounced plateau (see fig. 3B), the drop in the height of the plateau at low frequencies was the only observable change. In other fibers, the drop in the height of the plateau was accompanied by an earlier "take off" of the final phase of repolarization and consequently a shortening of the action potential.

Sudden Changes in Stimulation Frequency

It was noticed in the preceding experiments that when the frequency of stimulation was changed suddenly, the action potential underwent quite striking changes in shape before stabilizing at the form characteristic of the fiber at the new frequency. These transient changes were demonstrated in the following experiments.

An extra stimulus was interposed between two driving stimuli. This procedure thus simulated a sudden increase in the frequency of stimulation, for the extra action potential so produced would be equivalent to the first action potential induced at a higher frequency. The interval between the extra stimulus and the preceding driving stimulus could then be expressed in terms of a new rate of stimulation.

The extra stimulus was introduced after every fourth driven action potential at frequencies below 1 sec.\(^{-1}\), and after every eighth...
VENTRICULAR ACTION POTENTIALS

Figure 3
Superimposition of action potential traces at three frequencies from two fibers (A and B). Fiber (A) shows a typical ventricular action potential; fiber (B) shows an action potential with a long plateau; trace (a): frequency 2 sec⁻¹; trace (b): frequency 0.1 sec⁻¹; trace (c): frequency 8 sec⁻¹. Note the depression of the plateau in trace (b) and the earlier take off of the final phase of repolarization in trace (c).

action potential at frequencies of 1 sec⁻¹ and above. This ensured that any changes produced by an extra stimulus had disappeared before the next extra stimulus was applied. The extra action potential and the preceding normal action potential were recorded at four driving frequencies (0.1, 0.5, 1, and 2 sec⁻¹) with varying intervals between the extra stimulus and the preceding driving stimulus. At all these driving frequencies, the extra action potential was greater in area than the normal, having a longer-lasting and more elevated plateau (see fig. 4). The extra action potential had its maximum area when the stimulus interval was at its shortest value. The area of the extra action potential diminished as the stimulus interval was increased. It was also found that the ratio of the area of the extra action potential to the area of the normal action potential diminished as the basic stimulus frequency was raised (see table 1).

In order to examine the exact sequence of changes in action potential shape at low driving rates upon alteration of the stimulus frequency, the tissue was stimulated at a slow rate (0.1 sec⁻¹) and one in every four of the driving stimuli was replaced by a burst of stimulation at a high frequency (5 sec⁻¹) lasting for two seconds. The changes in the form and area of the action potentials during and after the period of fast stimulation were recorded (fig. 5). The first action potential of the burst at 5 sec⁻¹ corresponded, of course, to a single extra action potential induced as in the previous experiment. The subsequent action potentials of the burst followed one of two courses. In the majority of fibers, the action potentials decreased in area after the first action potential, but in some fibers the first few action potentials had the same area and only the action potentials toward the end of the burst decreased in size. Thus, there was an initial rapid increase in area followed by a decrease which was slower in developing.

It was then decided to examine the effects of the high-frequency burst upon subsequent action potentials at the normal driving rate of 0.1 sec⁻¹. The first normal action potential at 0.1 sec⁻¹ recorded after the burst at 5 sec⁻¹ was no longer identical with the stable value previously recorded for this frequency. These action potentials (lower traces of figs. 5 and 6) had a much more depressed and elongated plateau. Each subsequent action potential at 0.1 sec⁻¹ showed a gradual return to the stable form (trace 1 in figs. 5 and 6).

As such a short period at a high stimulus
frequency could have such long-lasting effects, it was decided to see what effects were produced by a prolonged period of high-frequency stimulation. Therefore, the tissue was stimulated for one to two minutes at a frequency of 5 sec.\(^{-1}\) or higher. As soon as the stable action potential shape for this frequency was established, the frequency of stimulation was changed to 0.1 sec.\(^{-1}\) and the previous experiment, in which a short high-frequency burst was introduced between two of the driving stimuli, was repeated. It was found that the decrease in the areas of the action potentials that occurred at the end of the burst in the previous experiment was abolished or greatly reduced (fig. 6). The extra action potentials were now of identical shape to the stable form recorded at 5 sec.\(^{-1}\).

**Table 1**

<table>
<thead>
<tr>
<th>Basic stimulus frequency (sec.(^{-1}))</th>
<th>Ratio of area of extra action potential to area of normal action potential in the same fiber at various stimulus intervals</th>
</tr>
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<tbody>
<tr>
<td>0.1</td>
<td>2.42  2.40  2.25  2.20</td>
</tr>
<tr>
<td>1.0</td>
<td>1.70  1.66  1.49  1.39</td>
</tr>
<tr>
<td>2.0</td>
<td>1.34  1.26  1.24  1.18</td>
</tr>
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</table>

**Discussion**

One of the most striking features of these results is that in all the fibers which we have examined in the rabbit the action potential decreases in area at low frequencies of stimulation. This fact has not been reported before; indeed, no investigations have been made of the changes in area of the cardiac action potential upon alteration of the stimulus frequency. The majority of related reports are largely concerned with measurements of action-potential duration. The shortening of duration which develops at high rates of stimulation is well documented and our results are identical to those of other workers.\(^1-5\) At low rates of stimulation, the action-potential duration in other preparations remains at a maximum value.\(^3-5\) The three preparations used by these authors were cat papillary muscle, dog Purkinje fibers, and dog papillary muscle. All of these tissues, and especially the Purkinje fibers, have an action potential which has a long and elevated plateau. In action potentials of similar form (see fig. 3B), we observed the same absence of shortening at low frequencies. However, the majority of rabbit ventricular fibers have an action potential which has an ill defined plateau, and these were almost invariably shortened at low frequencies of stimulation.

Another interesting feature of our results is that the extra action potential induced between two normal driving stimuli is larger than the normal action potential. This procedure of inducing an extra action potential has been used by Hoffman and Suckling\(^6\) in investigations not directly concerned with changes in action potential shape. They did,
however, report that the extra action potential was slightly larger in area than the preceding normal action potential. It was not stated in their paper at what basic stimulus frequency these experiments were carried out. However, it is only at very low basic rates of stimulation that a very large difference between the area of the extra action potential and the area of the normal action potential is observable. In experiments with quiescent frog heart excited by two stimuli occurring at differing intervals, Carmeliet7 found that the action potential produced by the second stimulus was smaller in area than that produced by the first stimulus. He also found that the action potential produced by the second stimulus increased in both area and duration as the stimulus interval between the two action potentials was increased. This behavior is quite opposite to that found in rabbit ventricular muscle and can possibly be attributed to a species difference. This explanation does not seem unlikely, as is demonstrated by the results of Hoffman and Suckling8 and Trautwein and Witt.9 The former showed that reduction of the external calcium concentration in dog papillary muscle lengthens the action potential by prolonging the plateau and we have obtained similar (unpublished) results with rabbit ventricle. Trautwein and Witt have, however, shown that a decreased external calcium concentration shortens the plateaus of action potentials obtained from frog ventricle.

A peculiar feature of cardiac muscle is that the form of each action potential is in some way determined by the history of the muscle. In rabbit ventricle, the extra action potential always has an area greater than the normal action potential. However, the area of the extra action potential is dependent upon the basic driving frequency, the length of time at which the preparation has been beating at this frequency, and upon the interval at which the extra has been induced from the normal action potential.

In a similar manner, driving the tissue at a high frequency (5 sec.\(^{-1}\)) will cause the behavior of the muscle at low frequencies to be modified for some time afterward. Thus, if the muscle is now driven at a low frequency, the action potential produced will be smaller than usual, having a lowered plateau; also, the area of the action potentials will not follow its normal time course if the preparation is given a burst of high-frequency stimulation, a stable action potential area for this rate being produced immediately.

The experiments in which bursts of extra action potentials were induced between normal action potentials demonstrate another interesting fact, namely, that rapid reversible changes in action potential shape can be obtained at low frequencies, while at high fre-
quencies such changes are slow both in development and regression.

It is important to realize that any postulated ionic mechanism for the cardiac action potential must be capable of explaining these results. If the cardiac action potential is generated by changes in the relative conductance of the membrane to sodium and potassium ions and the movement of these ions down their electrochemical gradients, then the time course of these conductance changes must be capable of undergoing rapid alteration, and in some way they must be dependent upon the history of the preparation.

Summary

It has been demonstrated that in rabbit ventricular muscle, the area of the action potential is at a maximum at a stimulus frequency ranging from 1 to 3 sec. At frequencies on either side of this range the action potential decreases in area. The changes in action-potential shape at high rates of stimulation are caused by an earlier "take off" of the fast phase of repolarization, and at low rates of stimulation the changes in shape are predominantly caused by a drop in the height of the plateau. It has also been shown that the ratio of the area of the extra action potential to the area of the normal action potential depends upon the stimulus frequency and the interval between the normal and extra action potentials. Upon any change in the stimulus frequency, the area of the ventricular action potential overshoots its final value before stabilizing. The shape of the action potential is dependent upon the previous history of the muscle. By altering the frequency of stimulation, it has also been shown that the changes in action-potential shape at low frequencies of stimulation, in contrast to those at high frequencies, are very rapid and take place immediately when the basic stimulus frequency is altered.

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References

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