Membrane Resting and Action Potentials of Single Cardiac Muscle Fibers

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An initial report is made on the electrocardiogram of a single heart muscle cell in vivo. The potential variations obtained by electrodes placed on opposite sides of the membrane of a heart muscle fibre are 50 to 100 times as large as those recorded by standard limb leads. The observations support the assumption that during activation the cell interior becomes positive with respect to its surrounding (depolarization, followed by polarization reversal). Induced alterations in shape and form of the action current of a single heart muscle fiber should provide further insight into the nature of the normal and abnormal electrocardiogram.

The development of the glass capillary microelectrode by Graham and Gerard and its subsequent improvement by Ling and by Hodgkin and Nastuk has made available a technic which permits the direct observation of the electrical events of single cells in multicellular tissues. It occurred to us that the recording of potential variations of individual heart muscle fibers would be of particular interest because information thus obtained would contribute to the understanding of basic problems of electrophysiology and the interpretation of the electrocardiogram. Using the following technic, we have recorded what we believe to be membrane resting and action potentials from single heart muscle fibers.

Leopard frogs were pithed and skinned; the heart was exposed, and filled and coated with melted 10 per cent Gelatin-Ringer’s solution at 26°C. The preparation was cooled to solidify the gelatin. This immobilized the heart so that only slight motion was visible under a magnification of 12X. Electrical and essentially isometric mechanical systole still occurred. After immobilization the entire preparation was covered with frog Ringer’s solution. The heart continued to beat for several hours so that no external stimulus was needed. Glass capillary microelectrodes made by the method of Graham and Gerard as refined by Ling and by Hodgkin and Nastuk were inserted into single ventricular muscle fibers by the use of a micromanipulator. The outer diameter of the electrode at the tip ranged from 0.5 to 2.0 micra.

The indifferent electrode was placed at convenience in the bathing medium. In later experiments, a 20 micra capillary electrode was placed on the surface within 0.5 mm. of the intracellular electrode. It served either as a return electrode or as an exploring contact for simultaneously recorded surface electrocardiograms. The electrodes were connected through calomel half-cells and a high input resistance DC amplifier to a millivoltmeter and a Cambridge film recording string galvanometer.

On the basis of experience gained in a series of preliminary experiments, the tip of the microelectrode was assumed to lie in the interior of a relatively undamaged fiber when the record obtained showed the following characteristics: (1) a series of monophasic action potentials showing (2) overshoot and (3) no appreciable change of shape or form during the series; (4) a resting potential of more than 50 millivolts; and (5) a disappearance of the monophasic action potential and the reappearance of the surface electrocardiogram on withdrawal of the electrode.

Resting potential. The average membrane resting potential in 175 measurements on 15 preparations taken at temperatures from 12 to 16°C, was 62.0 millivolts with a range of 50 to 90 millivolts. No corrections were made for junction potentials between the microelectrode and the cell interior. For an electrode filled with 3 molar potassium chloride solution, rough
calculations suggest that correction for this potential would increase the observed values by less than 3 millivolts.

**Action potential.** Typical membrane action potentials are illustrated in figure 1. It is apparent from the figures that the action potential exceeds the resting potential. This shows a reversal of the membrane polarity during activity. In 215 measurements of 15 preparations taken at 12 to 16 C. the action potential exceeded the resting potential by an average of 30.4 per cent. The mean value of 176 measurements of the action potential was 80.8 millivolts of the frog is as follows: During diastole a fairly constant voltage, the resting potential, exists between the inside and outside of the fiber with the inside negative to the outside. The onset of electrical systole is marked by a quick reversal of polarity of the membrane potential (depolarization and overshoot of the action potential). Following this reversal the membrane voltage, slowly at first and then more rapidly, returns to its resting value. In a small percentage of observations the membrane potential becomes greater than its resting value and then returns to normal (hyperpolarization).

**Fig. 1.**—Action potentials of single cardiac ventricular fibers. (M.P. Resting membrane potential, O.S. Overshoot, HP Hyperpolarization.) All measurements made from bottom of string shadow. Ordinates—millivolts; abscissae—time. Vertical lines 0.1 second apart.

A. Action potential of single ventricular fiber. Arrow indicates start of withdrawal of electrode from cell to show return of string to zero value and presence of small surface electrocardiogram. (QRS)

B. Action potential showing marked hyperpolarization during recovery.

C. Action potential of longer duration.

D. Action potentials of single fiber (below) and simultaneous surface electrocardiogram (above).

with a range of 65 to 115 millivolts. In 13 out of 276 records, hyperpolarization of the membrane following recovery occurred. The average value was 10 per cent, and the highest observed hyperpolarization was 12.7 millivolts.

The duration of the action potential varied from 0.4 to 1.0 second and increased approximately linearly with cycle length over this range of measurements.

**Discussion**

As may be seen from figure 1, the electrical sequence of events in a single ventricular fiber

In some experiments, surface electrocardiograms were recorded simultaneously with the internal events of single fibers and show the correspondence between the two. Figure 1, D shows that the QRS complex coincides with the sudden depolarization of the fiber and the T wave with the end of the repolarization process.

Because of the necessity for immobilization of the heart, no mechanograms have yet been obtained simultaneously with the electrical records of a single fiber. Visual observation, however, showed that contraction of the heart muscle occurred very shortly after the depolar-
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zation and that relaxation occurred shortly before or coincident with the end of repolarization.

The demonstration of overshoot of the action potential in heart muscle raises the question as to whether it may not be a general phenomenon in all excitable tissues. Curtis and Cole and Hodgkin and Huxley showed overshoot in the squid giant axon, and Hodgkin and Nastuk reported its occurrence in frog sartorius muscle. The latter authors give values for frog sartorius from which the overshoot can be calculated to be 32 per cent, a figure that compares well with our value of 30.4 per cent for frog heart.

Although the electrocardiogram is multiphasic when taken with indirect or semidirect leads or even on direct recording from the heart, the above results make it clear that the basic electrical event of the heart cell as seen by electrodes on opposite sides of the membrane of a single fiber is a simple monophasic wave. The rapid depolarization of the membrane coincides with the QRS complex as observed on a simultaneous surface record, and the end of repolarization coincides with the T wave (fig. 1, D). It may be speculated that the hyperpolarization is related to the U wave, but definitive evidence is lacking on this point.

The development of a satisfactory preparation in which single heart fiber potential can be recorded opens the way to investigations of the operation of the pacemaker and the effect of various physiologic and pharmacologic agents (such as electrolytes, epinephrine, acetylcholine and the cardiac glycosides) upon shape, size and duration of single fiber potentials. Work is being continued along these lines.

**Summary**

By the use of microelectrodes 0.5-2.0 micra in diameter, membrane resting and action potentials have been obtained from single fibers of the frog heart. Resting potentials averaged 62.0 millivolts and action potentials averaged 80.8 millivolts, giving an average overshoot of 30.4 per cent. The action potential was monophasic, with the beginning and end coincident with the QRS complex and T wave, respectively.

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