Electrophysiological Study of Human Heart Muscle

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Attempts to record membrane potentials from the human heart exposed during surgery have been reported by Woodbury et al.1 and Bromberger-Barnea et al.2 In these studies, rather large variation in the amplitude and time course of the potentials was observed, perhaps related to the circumstances of working with flexibly mounted micro-electrodes on an exposed, contracting heart. The present experiments were undertaken on the strength of the evidence that records obtained from suitably prepared and maintained tissue preparations excised from other mammalian hearts do not differ from records made in situ.3,4 Membrane potential changes of small preparations of human ventricular and atrial tissue, excised during open-heart surgery, were recorded in vitro, and the effects of physical and chemical influences on the resting and action potentials of single myocardial fibers were studied to test whether the basic concepts of the mechanisms underlying the action potential, automaticity and transmitter effects, derived from investigation of other cardiac tissue, are applicable to the human heart.

Methods

Small pieces of pectinate muscle from the right atrium, or trabeculae carneae from the right ventricle, were excised from the hearts of patients undergoing corrective open-heart surgery. Atrial specimens were obtained from five patients, aged 2, 10, 28, 32, and 53 years. The patients aged 28 and 53 had aortic incompetence and aortic stenosis, respectively; the others had atrial septal defects. Ventricular tissue was obtained from two patients, aged 4 and 6 years, who had ventricular septal defects. Immediately after excision, the tissues were immersed in cool Tyrode's solution (temperature about 30 C.) and carried from the operating room to the laboratory. Small muscle strips were then prepared by dissection and fixed in a tissue chamber through which Tyrode's solution flowed at a rate of 4 ml./min.; this was accomplished within 30 minutes after the tissue was excised from the heart. During the subsequent half-hour, the tissue was allowed to recover while stimulated electrically at a rate of 20/min. In the further course of the experiments, those preparations which did not beat spontaneously were driven electrically with a Grass stimulator at rates of 30 and 60/min., for ventricle and atrium, respectively.

The Tyrode's solution had the following composition (mM): Na, 153; K, 2.7; Ca, 1.8; Mg, 1.05; Cl, 145; HCO₃, 13.5; H₂PO₄, 2.4; and glucose, 5.5. The temperature of the bathing fluid was 36 to 38 C. The solution was equilibrated with a gas mixture of 95 per cent O₂ and 5 per cent CO₂, providing a pH between 7.2 and 7.4. A sodium-deficient solution, containing only 16 mM NaCl, was prepared by substituting an equivalent amount of choline chloride for the excluded NaCl. The acetylcholine chloride and epinephrine hydrochloride solutions used in these experiments were delivered into the tissue bath with a syringe and allowed to flow along the preparation in the stream of Tyrode's solution, having contact with the preparation for only a few seconds. The concentrations of acetylcholine and epinephrine, so diluted, were about 10⁻⁶ and 10⁻⁶ Gm./ml., respectively.

Membrane potentials were measured with glass micropipettes filled with 3M KC1, having tip diameters smaller than 0.5 μ, and resistances between 8 and 20 megohms. The recording system consisted of a cathode follower with negative capacitance (time constant 10 to 50 μsec.) and a Tektronix twin-beam type 502 oscilloscope.

Results

VENTRICULAR TISSUE

Resting and Action Potentials

An action potential recorded from a ventricular muscle fiber can be seen in figure 1. The rapid upstroke is followed by a plateau at a positive potential leading into repolarization to the resting potential. From 24 successful impalements at multiple sites in two preparations, resting potentials ranged be-
between -71 and -95 mv. The mean ventricular resting potential was -87 mv. (S.D. ± 5, S.E. ± 1). The action potentials varied between 92 and 125 mv., with a mean of 115 mv. (S.D. ± 9, S.E. ± 2).

**Upstroke and Conduction Velocity**

The sigmoid upstroke of the action potential (fig. 2) rose from 5 to 95 per cent of its amplitude in less than 1 msec. When the fiber membrane was gradually depolarized by increasing the extracellular potassium concentration, the upstroke velocity was successively reduced (cf. Weidmann). Conduction velocity was determined five times in one preparation. Two electrodes were impaled in a fiber within a longitudinal distance varying between 0.15 and 2.5 mm. The mean conduction velocity, determined from the latency between the known distances (fig. 2), was 1.3 M./sec., with calculated values ranging between 1 and 1.6 M./sec. The variation was not related to the distance between the recording electrodes. No action potentials with prominent spikes, typical of the faster conducting Purkinje system, could be detected in the areas where the conduction velocity was determined (cf. Draper and Mya-Tu). This relatively high conduction velocity, compared to that measured in the dog ventricle, may be due, in part, to hypertrophy of the fibers in the human preparations obtained from hearts having ventricular septal defects. Histological definition of the fiber diameter was not done, but ventricular hypertrophy was apparent when the hearts were exposed at surgery.

**Effect of Reduced Extracellular Sodium**

In the light of the sodium theory, it was of interest to test the effect of reduced extracellular sodium concentration on the overshoot and on the rate of rise of the action potential. No attempt was made to study this quantitatively, but figure 3(A) shows the effect of low external sodium in a qualitative manner (cf. Draper and Weidmann, Brady and Woodbury). After the control recording, the normal Tyrode's solution was replaced by one containing only 16 mM sodium. The overshoot and duration of the action potential were reduced successively and the upstroke velocity of the action potential was diminished (fig. 3B). The preparation became inexcitable after 15 minutes. The smallest action potential in figure 3(A) is one of the last responses to a strong stimulus and is preceded by a large stimulus artifact. As in other mammalian cardiac tissue, the effect of sodium depletion was quickly reversed when
(A) Superimposed tracings of three action potentials recorded with an intracellular electrode from a ventricular muscle fiber. The largest action potential was recorded when Tyrode's solution bathed the preparation. The two smaller action potentials were recorded 7 and 10 minutes after the Tyrode's solution (153 mM sodium) was replaced by one containing only 16 mM sodium. The stimulus intensity had to be greatly increased to reach threshold in low sodium (note the large stimulus artifact). (B) Tracings of upstrokes showing the effect of reduction in extracellular sodium concentration. The left upstroke was recorded in Tyrode's solution. The upstroke on the right shows a reduced rise velocity and was recorded in the low sodium (16 mM) bathing solution.

Tyrode's solution was readmitted to the bathing chamber.

Effects of Rate and Anoxia on the Plateau

Figure 4 depicts the shortening of the duration of the action potential when the stimulus rate was increased. The longest action potential was recorded at a rate of 24/min., after which the rate of stimulation was increased in five steps and the corresponding action potentials superimposed on film. Figure 5 shows the effect of anoxia on the action potential. When the Tyrode's solution was equilibrated with nitrogen, loss of plateau and shortening of the action potential duration occurred within 15 minutes. The shortest action potential, after 20 minutes nitrogen-anoxia, rises from a slightly lower potential level, causing a small reduction in overshoot.

Atrial Tissue

In contrast to ventricular muscle, it was more difficult to obtain recovery of excised atrial tissue. Two preparations showed oscillatory potential changes around low potential levels of -30 to -40 mv. Another preparation also exhibited spontaneous activity, and potential cycles with prominent diastolic depolarizations were recorded at all sites of electrode impalement (fig. 6). The maximum diastolic potentials in this preparation varied between -55 and -60 mv. These three preparations were excited from multiple foci and showed asynchronous small movements, sometimes at a fast rate, characteristic of fibrillation. Such preparations were excitable only early in diastole, when the membrane potential was relatively high (-50 to -55 mv.). Responses early in diastole had a larger amplitude and were often conducted through the entire muscle preparation, causing synchronized activity for one cycle. Responses to electrical stimuli later in diastole were of smaller amplitude and invaded only part of the preparation.

Since oscillatory potential cycles of low amplitude and low membrane potentials are frequently observed in deteriorated or drug-treated preparations of other mammalian hearts, it is unlikely that they represent the normal physiological state.

Resting and Action Potentials

Only two preparations had reasonably high resting and action potentials and constant diastolic membrane potentials. These were obtained from patients, aged 6 and 10 years,

Superimposed action potentials recorded during a single impalement of a ventricular fiber, showing the reduction in action potential duration as the stimulus rate was increased in the following steps: 24, 48, 60, 96, 120, and 192/min. Little change was recorded when the stimulus rate was increased in the step from 24 to 48/min. An action potential was recorded after adaptation to each change in driving rate.
with atrial septal defects. The resting potentials of 17 successful impalements at multiple sites varied between -60 and -80 mV, and the mean was -70 mV (S.D. ± 6, S.E. ± 1). From 19 successful impalements, the action potentials ranged between 64 and 90 mV, and the mean was 75 mV (S.D. ± 8, S.E. ± 2). Figure 7(A) shows an action potential of contour similar to that of atrial fibers of other mammals, having no plateau and repolarization following right after the upstroke. Figure 7(B) depicts an action potential with prominent spike and plateau at a negative membrane potential. This latter type of action potential was found commonly, and had a longer duration, although the resting and action potentials differed little from the recorded potential cycle of more normal contour.

Effect of Acetylcholine

Figure 8 shows the effect of acetylcholine on an electrically driven preparation. The resting potential increased to a level of greater negativity, the amplitude of the spike increased, and the action potential duration shortened.

Effect of Acetylcholine and Epinephrine on the Ectopic Pacemaker

On two occasions the preparations began to beat spontaneously in the bath, and were excited from a single pacemaker which could be located by multiple impalements at different sites in the preparation. At the locus of the pacemaker, typical pacemaker potentials were recorded, as shown in figures 9 and 10.
When acetylcholine was applied (arrow in fig. 9A), the ectopic pacemaker was strongly inhibited. The arrival of the drug at the site of electrode impalement caused a suppression of the pacemaker potential, the membrane potential increased and the preparation was arrested for about 40 seconds. After this, the effect of acetylcholine gradually disappeared, and slow depolarization occurred (fig. 9, B and C). The rate subsequently increased to that of the control (fig. 9D).

When the rate of the ectopic pacemaker slowed in the course of the experiment, the effect of epinephrine on spontaneity was tested (fig. 10). This also caused an initial suppression of the pacemaker, followed by arrest at the level of the antecedent maximum diastolic membrane potential (fig. 10, A and B). The inhibitory period lasted about 30 seconds; then the pacemaker resumed beating with increasing rate. The first three action potentials when the beat resumed are seen in figure 10(C) and show a successive increase in the amplitude of the spike. The pacemaker subsequently reached a high rate, which was maintained for several minutes. Figure 10(D) shows the excitatory period of epinephrine, during which the maximum diastolic potential was higher and the overshoot consequently greater than before epinephrine. An initial inhibitory effect was observed with each application of epinephrine in both of the preparations in which an ectopic pacemaker developed.

Discussion

The mean resting and action potentials of human ventricular fibers in vitro are higher than those recorded from the human heart in situ. It should be noted, however, that the largest potentials recorded in situ are close to those recorded in the present study. This suggests that the condition of the tissue in either case does not differ appreciably, insofar as the membrane properties are concerned. The shape of the action potential recorded in vitro, with long plateau, is corroborative evidence of the good condition of the preparation.

As with other mammalian cardiac fibers, the upstroke velocity of the action potential depends on the external sodium concentration, and excitability is lost when the sodium driving force is critically reduced.1, 10

The changes in action potential effected by alterations in beat-frequency or by anoxia are like those described for other mammalian cardiac fibers11-13 and reveal the sensitivity of the plateau duration to these factors.

The conduction velocity in human ventricle was found to be around 30 per cent higher than that determined by Hoffman in dog.7 The limited number of measurements must be considered in any attempt to reconcile this disparity. However, if the difference be real, it may be related to muscular hypertrophy, since the conduction velocity is proportional to the square root of the fiber radius (cf. Katz14).

In contrast to ventricular muscle strips, it was more difficult to obtain recovery and survival of excised atrial tissue. While an explanation for this difference in viability is beyond the scope of the present report, several considerations seem pertinent. The two muscle specimens obtained from the hearts...
of young patients (aged 6 to 10 years) gave satisfactory results, in contrast to the other three preparations excised from the hearts of adults having long-standing heart disease, two of whom were receiving digitalis for cardiac failure. The fibers of these latter preparations were difficult to impale and contained abundant connective tissue. Of special interest is the related observation that the fibers of atrial tissue excised from dog show a tendency to depolarize when they are not stimulated in vitro, an effect which is clearly reversible if the preparation is stimulated while it is still excitable.\(^{15}\)

The human atrial resting and action potentials recorded in this study are lower than those found in dog\(^{16}\) and rabbit.\(^{17}\) While action potentials with plateaus may be observed in dog atria, the plateaus characterizing the second type of action potential in the present study seem unusually prominent, and suggest a basic disturbance in the mechanism underlying repolarization. It is probable that the membrane conductance for potassium was reduced in these atrial fibers, especially in view of the striking hyperpolarization caused by acetylcholine. It is possible that these fibers lost potassium, whereby the driving force for repolarization was reduced. An alternate mechanism responsible for the prolonged plateau would involve the tendency to spontaneous rhythmicity.\(^{19}\)

The ectopic pacemaker activity in the atrial preparations was strongly inhibited by acetylcholine. The mechanism of inhibition, by virtue of increased potassium conductance of the membrane, is undoubtedly like that in other mammalian hearts.\(^{15}\)

The initial, transient hyperpolarization and inhibition produced by epinephrine was surprising. It is of related interest that hyperpolarization can also be seen in the arrested dog atrium in the presence of epinephrine,\(^{19}\) an effect uninfluenced by atropine. The inhibited sino-atrial node of dog or rabbit, epinephrine never causes inhibition, but shows only an excitatory effect.

**Summary**

Membrane potential changes in human ventricular and atrial muscle, excised from patients undergoing open-heart surgery, were recorded by micro-electrodes in vitro.

Mean ventricular resting and action potentials were \(-87\) mv. and \(115\) mv., respectively. The mean atrial resting potential was \(-70\) mv., mean action potential \(75\) mv. Two forms of atrial action potential were found, one having conventional contour, the other with prominent spike and plateau. A disturbance in repolarization is believed to underlie the latter type of atrial potential cycle.

The relation between the upstroke velocity of the action potential and the extracellular sodium concentration and membrane potential was shown to be similar to that in other mammalian cardiac tissue. The mean conduction velocity determined in ventricular fibers (1.3 M./sec.) was somewhat greater than that of the dog, and the possible relationship to hypertrophy of the cardiac fibers in the preparations studied is described.

The effect of increased rate and anoxia in reducing the action potential duration is like that found in the hearts of other mammals.

The conductance type of inhibition was produced by acetylcholine in spontaneously beating atrial tissue. The excitatory effect of epinephrine was preceded by a transitory inhibition.

The basic mechanisms underlying the action potential, automaticity and transmitter effects, derived from investigation of other mammalian cardiac tissue are applicable to the human heart.

**References**

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