On The Mechanism of Spontaneous Impulse Generation in the Pacemaker of the Heart

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ABSTRACT Rhythmic activity in Purkinje fibers of sheep and in fibers of the rabbit sinus can be produced or enhanced when a constant depolarizing current is applied. When extracellular calcium is reduced successively, the required current strength is less, and eventually spontaneous beating occurs. These effects are believed due to an increase in steady-state sodium conductance. A significant hyperpolarization occurs in fibers of the rabbit sinus bathed in a sodium-free medium, suggesting an appreciable sodium conductance of the "resting" membrane. During diastole, there occurs a voltage-dependent and, to a smaller extent, time-dependent reduction in potassium conductance, and a pacemaker potential occurs as a result of a large resting sodium conductance. It is postulated that the mechanism underlying the spontaneous heart beat is a high resting sodium current in pacemaker tissue which acts as the generator of the heart beat when, after a regenerative repolarization, the decrease in potassium conductance during diastole reestablishes the condition of threshold.

Recent studies suggest that the spontaneous heart beat depends on specific generator properties of cardiac pacemaker tissue. The membrane potential changes in pacemaker fibers are characteristic of a tissue exhibiting spontaneous excitation. At the pacemaker, the membrane potential in diastole is not constant, but shows a spontaneous, slow depolarization (pacemaker potential) to the threshold of the propagated impulse. Dudel and Trautwein (1958) have proposed that the pacemaker potential is the resultant of the interaction of two factors: (*a*) a significantly high membrane conductance for sodium, which is considered a specific property of pacemaker fibers responsible for the generation of the spontaneous heart beat; *b*) changes in potassium conductance during repolarization and diastole leading to slow diastolic depolarization, bringing the potential to the firing level. FitzHugh (1960) and Noble (1960) have computed solutions for the Hodgkin-Huxley nerve equa-

tions (Hodgkin and Huxley, 1952), with appropriate modification of the parameters of sodium and potassium conductance, finding long action potentials like those recorded from the heart. When the constants determining m (the variable describing the activation of sodium carriers) are changed further (Noble, 1960), the computed solutions show prominent pacemaker potentials.

Evidence suggesting that repetitive impulse generation is linked to an increase in sodium conductance derives from observations on other excitable tissues. In nerve (Hodgkin, 1948) and skeletal muscle fibers (Benoit *et al.*, 1955), repetitive discharge can be produced by a constant current stimulus. Altered sodium conductance may also be implicated in the production of repetitive activity observed in the squid axon (Huxley, 1959) and striated muscle (Bülbring, 1955) when the extracellular calcium concentration is reduced.

The present investigation sought further evidence relating the sodium conductance of the membrane to the generation of the spontaneous heart beat. Pacemaker activity in the heart was produced or enhanced by conditions which increased sodium conductance. Changes in membrane resistance during diastole were studied in order to elucidate the mechanism underlying the pacemaker potential.

METHODS

Bundles of Purkinje fibers and, in some cases, papillary muscle were excised from the ventricles of young sheep. The area of the right atrium including the sinus node was obtained from rabbit hearts. All preparations were mounted in a small plastic chamber through which Tyrode solution flowed at a rate of 4 ml per minute. The temperature of the bathing fluid was 37–38°C. The Tyrode solution had the following composition (mM): Na 153, K 2.7, Ca 1.8, Mg 1.05, Cl 145, HCO_3 13.5, H_2PO_4 2.4, glucose 5.5. The solution was saturated with a gas mixture of 95 per cent O_2 and 5 per cent CO_2, providing a pH between 7.2 and 7.4.

Sodium-deficient solutions were prepared by substituting equimolar concentrations of choline chloride for part or all of the sodium, and contained atropine in a concentration of 10^{-6} gm per ml. As a rule, the calcium concentrations in calcium-deficient Tyrode solution, representing reduction by 25, 50, 75, and 90 per cent were (mM): 1.35, 0.9, 0.45, and 0.18. The changes effected by calcium- or sodium-depleted bathing solutions were usually complete in 10 minutes, and were completely reversed when normal Tyrode solution was readmitted.

Most of the sheep Purkinje fibers did not beat spontaneously, despite membrane resting potentials around −90 mv. Such preparations were often driven during the course of an experiment by external electrodes from a Grass stimulator. The rabbit sinus preparations beat spontaneously.

The membrane potentials were measured by an intracellular glass microelectrode filled with 3 M KCl, and selected for a resistance of 10 to 20 megohms. A second

microelectrode, of similar resistance, was introduced into the same fiber to lead current through the membrane.

The recording system consisted of a cathode follower and a tektronix type 502 twin-beam oscilloscope.

A tektronix pulse generator (type 161) was used for currents up to 1 sec. duration; longer current pulses were provided by a battery and potentiometer device. The current was displayed and measured on the second beam of the oscilloscope.

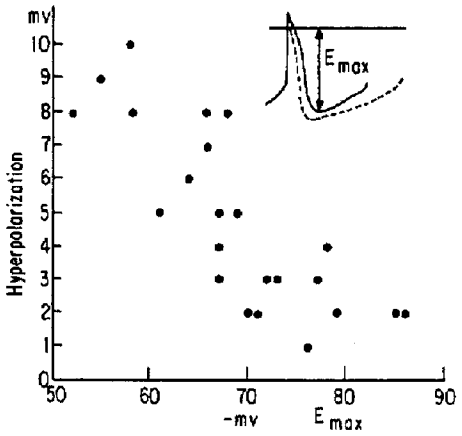

FIGURE 1. Hyperpolarization (increase in E_{max}) produced by reducing the sodium concentration in Tyrode solution from 153 to 16 mM, plotted as a function of the peak repolarization potential observed in the control solution. Spontaneously beating rabbit sinus. The solid curve in the inset is a potential cycle with control solution; dashed curve, one with low sodium. It can be seen that the magnitude of the hyperpolarization is generally greater the smaller the value of E_{max}.

RESULTS

Effect of Extracellular Sodium Depletion on the Maximum Repolarization Potential of Pacemaker Fibers

In spontaneously beating fibers from the sinoatrial node of the rabbit heart, the membrane potential at the end of repolarization (E_{max}) is about -65 mv. It was shown by Dudel and Trautwein (1958) that the potassium equilibrium potential (E_K) is about -80 mv, and the difference between E_K and E_{max} about 15 mv. When a fiber is arrested transiently by a small dose of acetylcholine or a slug of cool Tyrode solution, the resting potential (E) is a few millivolts more negative than the threshold potential, and $E - E_K$ is about 25 to 30 mv. These observations suggest that there may be a significant sodium conductance of the "resting" membrane which tends to keep the potential away from E_K. To test this, the effect of sodium depletion on the peak repolarization potential (E_{max}) was studied. When the Tyrode solution

bathing a beating rabbit sinus preparation was replaced by one containing only 16 mM sodium, the absolute value of E_{max} was observed to increase. It should be stated, parenthetically, that while changes in the membrane potential later in diastole were more pronounced, studies of the effect of external sodium depletion on this were vitiated by changes in threshold and frequent shifts of the pacemaker. Fig. 1 shows the hyperpolarization which occurred when the external sodium was reduced by 90 per cent, plotted as a function of the antecedent E_{max}. Hyperpolarization (increase in E_{max}) was an early effect, occurring within 2 minutes, and was followed by cessation of rhythmic activity.

In spontaneously beating Purkinje fibers of sheep, the maximum diastolic potential was only slightly increased (2 to 4 mv) when 90 per cent of the external sodium was replaced by choline chloride. When spontaneous activity ceased, the membrane potential was 5 to 10 mv above the previous firing level. Similar results have been reported by Draper and Weidmann (1951) in kid Purkinje fibers.

These results suggest that there is an appreciable resting or steady-state sodium current in pacemaker fibers of the heart.

Effect of Constant Current Stimulus on Pacemaker Tissue

Since it is known that membrane depolarization is accompanied by an increased sodium conductance, it was of interest to investigate the effect of a constant depolarizing current stimulus in generating repetitive activity in nonbeating Purkinje fibers of sheep hearts. In these experiments, threshold for a single discharge was determined for current pulses exceeding 100 msec. With shorter pulses, the responses were dependent upon the duration of the current pulse. With currents of successively greater suprathreshold intensity and several seconds' duration, three types of response were seen to follow the initial action potential (Fig. 2): (*a*) a series of damped oscillations which did not lead to propagated impulses; (*b*) incremental oscillations leading to successive, propagated responses; (*c*) repetitive discharge, which continued as long as the depolarizing current was maintained. As the current intensity was increased further, the rate of response increased. Curiously, a weak subthreshold current sometimes produced a series of damped oscillations similar to that seen when a suprathreshold current was applied (Fig. 2D).

Rather wide variability in the current intensity necessary to produce single or repetitive responses was found between different preparations, and between different impalements in the same preparation. In a series of 10 experiments, the current necessary to evoke a single action potential ranged between 0.3 and 2.3 microamps. The current needed to produce repetitive discharge ranged between 0.7 and 4.7 microamps, and was 1.7 to 5 times

that necessary for a single response. A number of quiescent preparations were found in which rhythmic beating could not be produced by a depolarizing current stimulus.

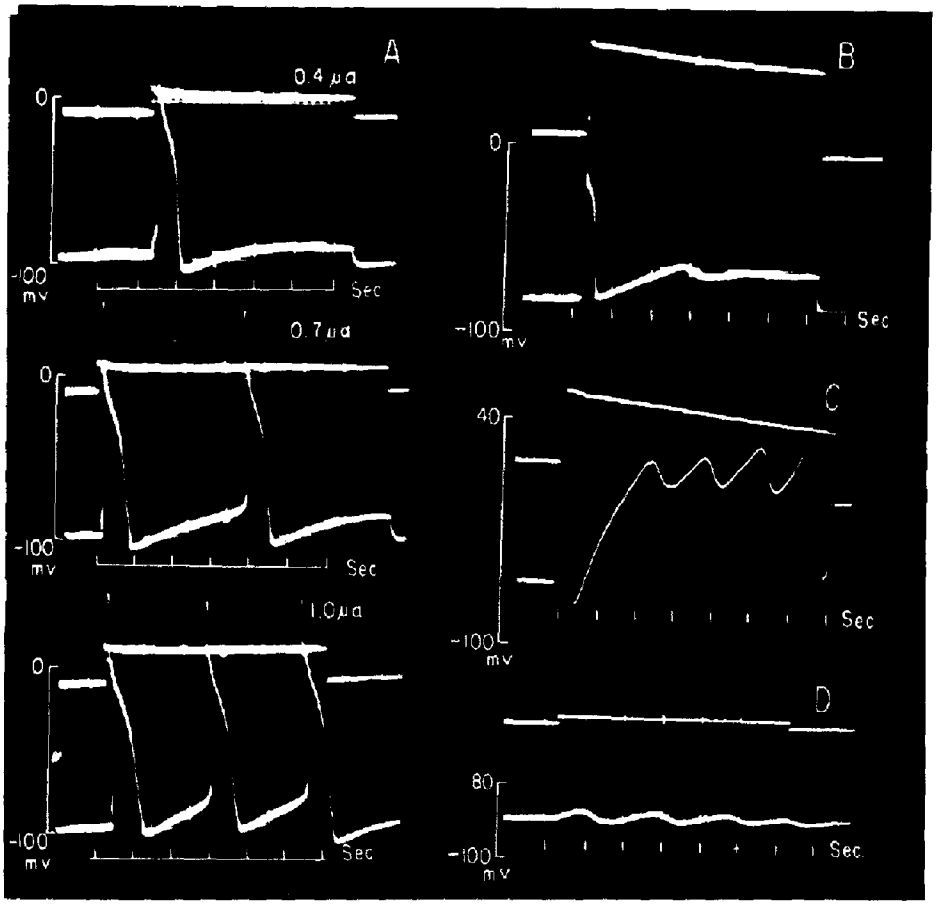

FIGURE 2. Non-spontaneously beating sheep Purkinje fiber. A, responses to constant depolarizing current stimuli of increasing intensity. Current strengths are given above the current recordings. Records are from the same electrode impalement. B, damped oscillations following an action potential during a depolarizing current stimulus. C, incremental oscillations from a different impalement produced by a depolarizing current stimulus of an intensity just below that which causes spontaneous firing; note the increased amplification. D, subthreshold oscillations during a weak depolarizing current stimulus.

In several experiments, the thresholds for single and repetitive discharges were determined at multiple sites in the same preparation, and the impalement maintained at the site of lowest threshold. It was observed that this became the locus of spontaneous activity (*i.e.* the pacemaker) when the

preparation was made to beat by the application of epinephrine or by depletion of calcium in the bathing solution (see below).

In spontaneously beating Purkinje or rabbit sinus preparations, a constant depolarizing current of a few seconds' duration enhanced the pacemaker activity (Fig. 3). The velocity of diastolic depolarization was faster, and the rate of discharge increased. The current intensities used were 1 to 3 times rheobasic

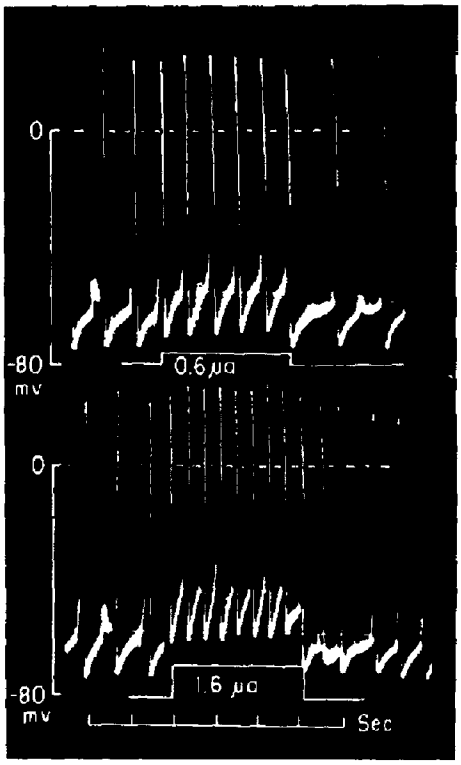

FIGURE 3. Spontaneously beating rabbit sinus. The effect of depolarizing current stimuli of two different intensities, producing an increased rate and velocity of diastolic depolarization. Note also the disappearance of the occasional diastolic oscillations during the current flow. The current recordings are redrawn below the records.

strength. When the stimulating current was not applied at the pacemaker, the depolarized area assumed pacemaker function. This shift of the locus of the pacemaker could be produced by currents of rheobasic strength or smaller. Successive increments in current intensity increased the rate further. Hyperpolarizing current decreased the slope of the diastolic depolarization or suppressed the autorhythmicity completely (Fig. 4). When this current was applied at the region of the pacemaker, the locus of the pacemaker shifted to another site.

One might assume that during the slow diastolic depolarization, especially

when the membrane potential is reduced by a constant current, inactivation of the sodium carrier system might be so large that the fiber would not respond regeneratively at the site of the current electrode. Although this situation did not obtain in the present experiments, the effect of inactivation was studied under these circumstances. The threshold for an action potential was determined with current pulses of 100 msec. at different levels of membrane depolarization. Fig. 5 shows clearly that inactivation occurs, in that the

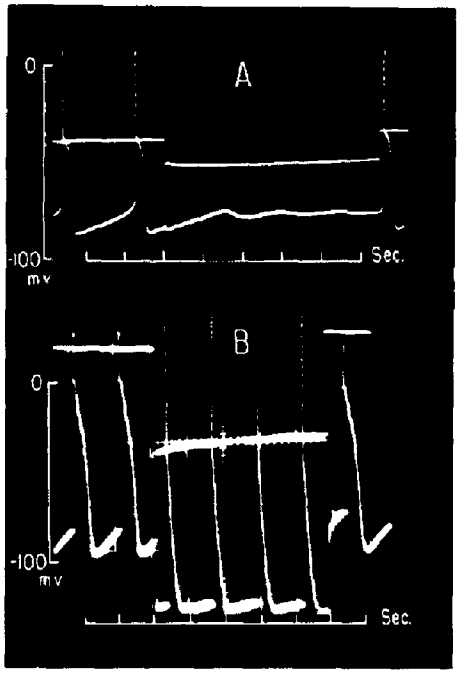

FIGURE 4. The effect of hyperpolarizing current on a spontaneously beating Purkinje fiber. A, locus of the pacemaker; B, non-pacemaker area. In A, the pacemaker activity is suppressed, and damped oscillations occur. In B, the pacemaker potential is flattened during the current flow, but the area is excited by conduction from an unaffected pacemaker several millimeters away.

threshold potential becomes progressively less negative as greater depolarizing currents are applied. It can be seen, however, that with successively greater depolarization, smaller additional depolarization is required to reach threshold, and when the constant depolarizing current is sufficiently great, "spontaneous" discharge occurs.

Effect of Reduction of Extracellular Calcium

The effect of external calcium depletion on lowering threshold and enhancing excitability in heart muscle has been described by Weidmann (1955 *a*). In

the squid axon, reduction of the external calcium results in a smaller depolarization being required to produce a given increase in sodium conductance (Frankenhaeuser and Hodgkin, 1957). Fig. 6 depicts graphically the decreasing current intensities necessary to produce a single response or repetitive discharge in Purkinje fibers when the calcium concentration of the bathing fluid was reduced successively.

No changes in membrane resistance were found by Weidmann (1955 *a*) when external calcium was reduced. From the present study, corroborative evidence that calcium depletion affects sodium and not potassium conductance is adduced from the determination of the current-voltage relationship which is unaltered by a calcium-deficient external medium (Fig. 8B).

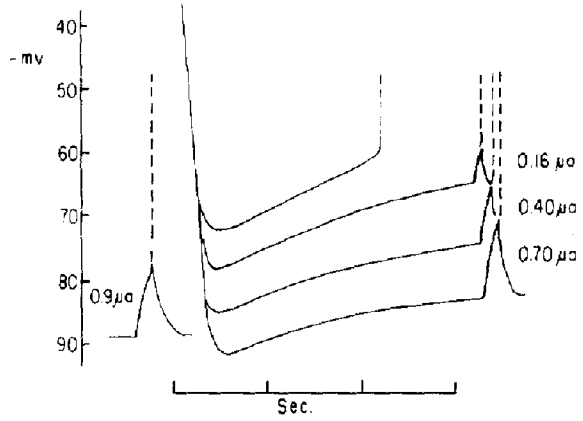

FIGURE 5. Threshold determinations at different levels of membrane depolarization in diastole. Quiescent sheep Purkinje fiber. Tracings of four diastoles are superimposed. Successively greater, constant depolarizing currents are applied. The greatest depolarization shown resulted in "spontaneous" firing. Current pulses of 100 msec. duration and given intensity were superimposed about 3 sec. after the end of repolarization to show the change in threshold with depolarization, compared with the threshold at the resting potential. The figures indicate the strengths of threshold pulses. See text for further explanation.

The number of experiments which could be completed was limited because of the frequent development of spontaneous beating when calcium was reduced to 25 per cent of normal. The experimental failures involved, particularly, those preparations in which high thresholds for impulse generation were found; in these fibers potential pacemaker areas existed which began to generate impulses.

When the threshold for repetitive impulse generation had been raised by external sodium depletion, an attempt was made to lower it by reducing external calcium. Repetitive activity could not be produced when the external sodium concentration was reduced to 50 per cent or less. Lesser sodium depletion raised the threshold which was inconsistently returned toward the

previous level by external calcium depletion. In general, preparations treated with both external sodium and calcium reduction deteriorated, and showed a tendency to arrest at the plateau when a depolarizing current stimulus was applied.

A few experiments were done on small trabeculae excised from the interior of the right ventricles of sheep. Such preparations never beat spontaneously. In contrast to Purkinje fibers, these myocardial fibers did not develop repetitive discharge when a constant depolarizing current was applied, even when the external calcium concentration was reduced to 25 per cent. In these

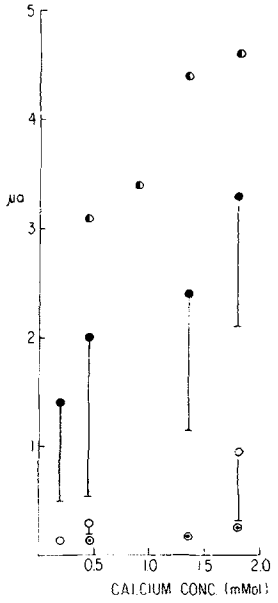

FIGURE 6. Threshold current intensities for repetitive discharge (ordinate) as a function of the extracellular calcium concentration (abscissa). The different circles identify separate experiments during which the same impalement was maintained. The intersections of the vertical with the horizontal lines indicate thresholds for single responses.

fibers, repolarization did not show an underswing, but ended at a membrane potential which remained constant as long as the depolarizing current was flowing (Fig. 7).

Membrane Resistance in Diastole

A high resting sodium conductance, acting as a constant stimulus, does not explain the rhythmic nature of the pacemaker. Dudel and Trautwein (1958) have shown that the potassium conductance decreases during diastole, and they believed this to be a function of time following repolarization. Weidmann (1955 *b*) and, more recently, Hutter and Noble (1960) have shown

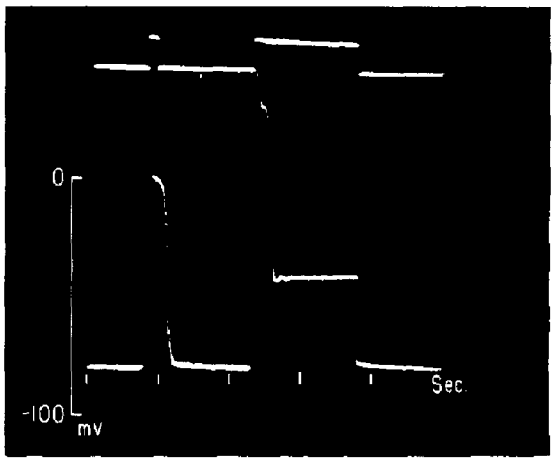

FIGURE 7. Effect of depolarizing current on non-beating sheep papillary muscle. A maintained, depolarizing current produces only a single action potential, and repolarization ends without an underswing at a depolarized level.

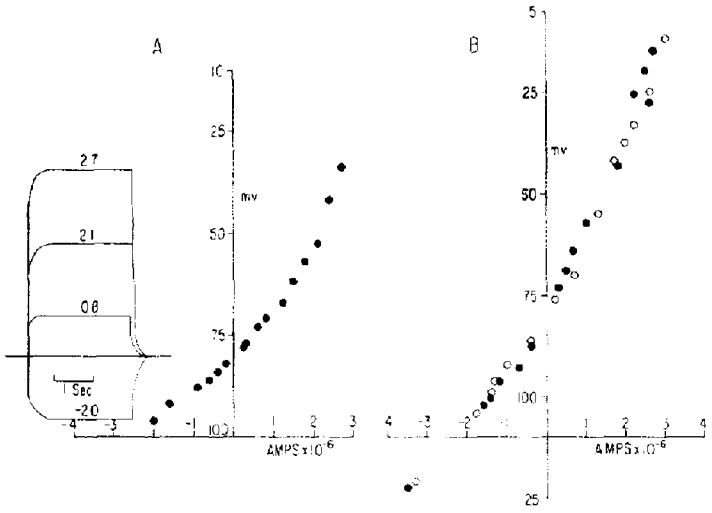

FIGURE 8. Current-voltage relationship in two Purkinje fibers bathed in sodium-free Tyrode solution. In A is included a tracing of electrotonic potentials for different indicated current intensities. Note that a depolarizing current produces a larger potential change than a hyperpolarizing current of nearly the same intensity. In B, the open circles represent the current-voltage relationship determined when the external calcium was reduced to 10 per cent. The filled circles show the current-voltage relationship from the same impalement with normal external calcium.

that membrane resistance is not constant, and increases appreciably when depolarization occurs. In the present study the experiments of Hutter and Noble were repeated to determine the alteration in potassium conductance

attending membrane depolarization, by defining the current-voltage relationship in Purkinje fibers bathed in sodium-free Tyrode solution. Current pulses of around 1 sec. duration were applied, and the resulting electrotonus measured.

Fig. 8A depicts the current-voltage relationship in a Purkinje fiber. It can be seen that the curve is alinear over the potential range measured, especially in the potential range of the pacemaker potential. Depolarizing currents produce larger potential changes than hyperpolarizing currents of the same

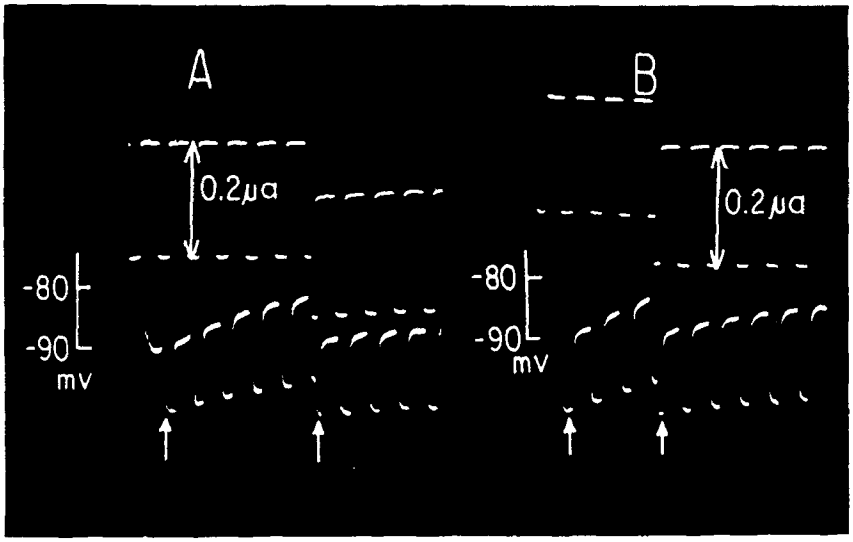

FIGURE 9. Anelectrotonic pulses in the course of diastolic depolarization. Sheep Purkinje fiber. Pulse duration 100 msec. In A, a steady depolarizing current is superimposed early in diastole. In B, a hyperpolarizing current is superimposed later in diastole. Note that the membrane resistance increases during the slow depolarization, but for the same membrane potential the electrotonus immediately after the end of repolarization is smaller than that seen later in diastole. Comparable electrotonic potentials are indicated by arrows. Further explanation in text.

intensity. The membrane resistance increases with depolarization. Since the contribution of chloride ions to the membrane conductance of cardiac muscle is small (Hutter and Noble, 1959), and the external fluid contained no sodium ions, it can be presumed that the changes in membrane resistance under these conditions reflect the potassium permeability of the membrane.

The current-voltage relationship was determined for papillary muscle and found to resemble that of Purkinje fibers, indicating that this alinearity is not a property peculiar to pacemaker tissue.

It was of interest to investigate whether a change in membrane resistance might occur in diastole as a function of time following repolarization, un-

related to the diastolic depolarization. For this purpose, the change in membrane resistance in diastole was estimated by leading rectangular current pulses into the fiber, and then superimposing a steady polarizing current so that resistances early and late in diastole could be compared for the same membrane potential. Fig. 9 shows a small, but significant, increase in electrotonus late in diastole, compared to the same membrane potential immediately after repolarization. In Fig. 9A the amplitudes of the two comparable electrotonic potentials, corrected for differences in the size of the current pulses when a polarizing current was applied, are 11.5 and 12.5 mv. Since the membrane resistance is proportional to the square of the electrotonic potential, the comparable values of 132 and 156 sq. mv indicate an increase in membrane resistance of almost 20 per cent. The corresponding values of Fig. 9B are 12.5 and 15 mv, or 156 and 225 sq. mv, indicating an increase in membrane resistance during diastole of about 45 per cent. In some of the experiments, smaller increases in resistance in the range of 10 to 20 per cent were noted. No attempt was made to determine the time course of the change in membrane resistance during diastole. These findings indicate that there is a time-dependent, as well as voltage-dependent, increase in membrane resistance during diastole.

DISCUSSION

In considering the sodium conductance alterations in pacemaker fibers, it is important to differentiate the "resting" membrane conductance from the "active" conductance which is a rapid function of membrane potential changes. No information is available regarding the role, if any, played by the carrier system in the resting conductance; nor is the magnitude of the resting conductance in cardiac pacemaker fibers known, but it seems reasonable to believe that it is appreciable in view of the diastolic instability of the membrane potential, where the sodium current must clearly exceed the potassium current.

In the present experiments, spontaneous rhythmicity was produced or enhanced when a constant cathodal current was led into the cell. The resulting depolarization shifted the level of resting or steady-state sodium permeability toward that at threshold, and thus increased sodium conductance.

The excitatory effect of external calcium depletion is presumed to affect steady-state sodium conductance in the same way as a depolarizing current. This lends credence to the implication that the resting sodium conductance is an important factor in the generation of spontaneous rhythmicity. Further corroboration is given by the observations that the maximum repolarization potential was increased transiently, and the pacemaker potential suppressed, when the sodium driving force was reduced by external sodium depletion.

The *sine qua non* of spontaneous, rhythmic excitation is the pacemaker potential. Following repolarization, the system becomes unstable, and slow depolarization leads to threshold. The ionic current relationships characterizing the pacemaker potential may be represented by the expression

$$I_{Na} > I_K; I_K = (E - E_K)\, gK$$

where I_{Na} and I_K represent sodium and potassium currents, E_K and E are the potassium equilibrium potential and membrane potential, respectively, and gK is the potassium conductance. The opposing currents would approach equality as the difference $E - E_K$ becomes larger, if it were not for a decrease in gK and an increase in gNa (and hence in I_{Na}) accompanying depolarization.

There is a voltage-dependent and, to a smaller extent, time-dependent decrease in gK during diastole. The increase in membrane resistance on depolarization can be considered to indicate a decrease in gK, as shown by the studies of Dudel and Trautwein (1958). Clearly, a regenerative repolarization which drives the membrane potential toward the potassium equilibrium potential is a precondition for the pacemaker potential. The observation of a reduced membrane resistance immediately after repolarization, when compared to the resistance for the same potential later in diastole, reflects the increased potassium permeability during repolarization. The time course of this increase seems to be so long that it exceeds repolarization, and the conductance change is detectable at the maximum diastolic potential. The increase in membrane resistance which follows may then serve to initiate the diastolic depolarization, which in turn causes a further increase in membrane resistance.

Opposing impulse generation is the inactivation of the sodium-carrier system which accompanies depolarization. However, a depolarizing current of sufficient intensity overcomes the effect of inactivation.

The current-voltage relationship of non-spontaneously beating papillary muscle was found to be similar to that of pacemaker tissues, showing a decrease in conductance with depolarization. These fibers did not, however, show repetitive discharge when stimulated by a constant cathodal current or when external calcium was reduced. When a depolarizing current was applied, repolarization ended without an underswing when inward and outward currents reached an instantaneous equilibrium. Thus, the full repolarization, related to an increase in gK, which is the precondition for diastolic depolarization in pacemaker fibers, does not obtain in myocardial fibers.

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