Membrane Characteristics of the Canine Papillary Muscle Fiber

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ABSTRACT Passive and active responses to intracellular and extracellular stimulation were studied in the canine papillary muscle. The electrotonic potential produced by extracellular polarization with the partition chamber method fitted the time course and the spatial decay expected from the cable theory (the time constant, 3.3 msec; the space constant, 1.2 mm). Contrariwise, spatial decay of the electrotonic potentials produced by intracellular polarization was very short and did not fit the decay curve expected for a simple cable, although only a small difference of time course in the electrotonic potentials produced by intracellular and extracellular polarizations was observed. A similar time course might result from the fact that when current flow results from intracellular polarization, the input resistance is less dependent on the membrane resistance. The foot of the propagated action potential rose exponentially with a time constant of 1.1 msec and a conduction velocity of 0.68 m/sec. The membrane capacity was calculated from the time constant of the foot potential and the conduction velocity to be 0.76 $\mu F/cm^2$. The responses of the papillary muscle membrane to intracellular stimulation differed from those to extracellular stimulation applied with the partition method in the following ways: higher threshold potential, shorter latency for the active response, linearity of the current-voltage relationship, and no reduction in the membrane resistance at the crest of the action potential during current flow.

INTRODUCTION

In the mammalian cardiac ventricle two kinds of muscle fibers exist: the Purkinje fiber and the ventricular fiber proper. The morphological differences between these fibers have been well-studied by many investigators. Weidmann (1952) and other investigators (Noble, 1962; Deck and Trautwein, 1964; Fozzard, 1966) have systematically studied the electrophysiological properties of the Purkinje fiber. On the other hand, there are few reports on the membrane characteristics of the ventricular fiber (Johnson and Tille, 1961; Tille, 1966), probably because its complicated architecture with extensive branching and anastomosing hinder the recording of reasonable electrotonic potentials. In an extensively branching tissue, the spatial decay obtained with an intracel-

lular microelectrode is sharper than that obtained with a large external electrode (Woodbury and Crill, 1961; Tille, 1966; Tanaka and Sasaki, 1966; Kamiyama and Matsuda, 1966). In canine papillary muscle, however, the same time course of the electrotonic potential was shown by Kamiyama and Matsuda (1966) with extra- and intracellular stimulation. In the smooth muscle of the guinea pig taenia coli, the spatial decay and the time course of the electrotonic potential obtained by intracellular stimulation are sharper than those obtained by external stimulation, as shown by Tomita (1967).

The present experiments were carried out to investigate the membrane properties of canine papillary muscle during the resting and active states. Until now there have been few reports about the propagated spike elicited by

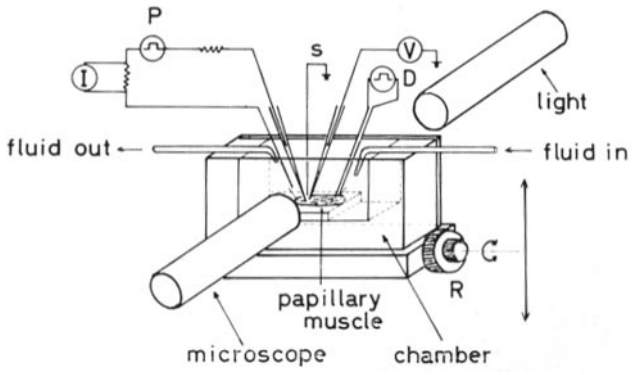

FIGURE 1. Recording and stimulating arrangements for the intracellular polarization method. Distances between the tips of the current and recording electrodes were adjusted to a given value by stereomicroscopy before penetration. The insertions of these two electrodes were made simultaneously by moving the stage of the preparation up and down under the microscope. *I*, current monitor; *P*, polarizing stimulator; *S*, shield; *V*, voltage monitor; *R*, manual rotor to move the chamber stage. *D*, extracellular stimulating electrodes.

intracellular stimulation in papillary muscle and the responses of the membrane have not yet been systematically investigated by intra- and extracellular stimulation methods.

METHODS

The isolated canine papillary muscle bundle, which has a 1.5–2.0 mm diameter, was used in these experiments. About a 5 mm length of the papillary muscle was placed longitudinally in the two compartments of the chamber so that most of the preparation was in the recording chamber. The two compartments were separated by a thin Lucite plate (about 0.1–0.3 mm in thickness) coated with vaseline. The extracellular polarizing current was applied across the two compartments through Ag-AgCl electrodes placed in both compartments (see Kamiyama and Matsuda, 1966). Both chambers were independently perfused with Tyrode solution oxygenated at 37°C. The

electrotonic potentials induced by the application of weak current were recorded along the tissue at various distances from the partition. In such an arrangement, the inputs from the two similar microelectrodes, one inside and the other immediately outside the surface membrane, were fed into a differential preamplifier to minimize the artifact induced by polarizing current. By adjusting the position of the extracellular electrode, the artifact could effectively be cancelled out, and then the microelectrode was inserted into the cell. For intracellular polarization two microelectrodes were used, one for passing current through the membrane and the other for recording. The two microelectrodes were inserted into the cell in the arrangement of equipment

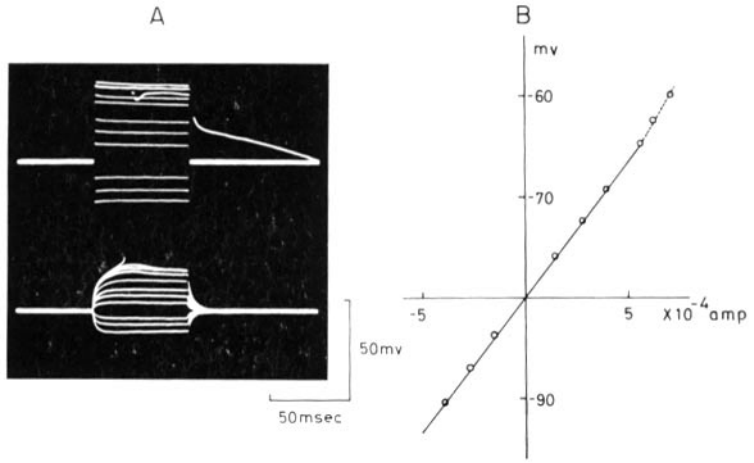

FIGURE 2 A. Electrotonic potentials produced by weak extracellular cathodal and anodal polarizing currents of different intensities. Upper traces show the current intensities and zero potential level. Bottom traces show a resting potential and a series of electrotonic potentials. The strongest depolarization elicited an action potential. 2 B. Current-voltage relationship obtained from the records (A). The relationship appeared linear over a limited range. Abscissa, current intensity shown by an arbitrary unit. Ordinate, membrane potential.

illustrated in Fig. 1; i.e., the distance between the tips of the current electrode and recording electrode was measured with the aid of a micrometer in one ocular of a stereomicroscope. The accuracy of this measurement was estimated to be $\pm 5\ \mu$. The resistances of the microelectrodes used were between 10 and 20 MΩ.

The control solution used for this tissue was Tyrode solution of the following composition (mM): Na, 152.0; K, 2.68; Ca, 1.8; Mg, 0.49; Cl, 144.06; HCO_3, 11.9; H_2PO_4, 3.3; with the pH adjusted to 7.2–7.3.

RESULTS

Electronic Potentials

The electrotonic potential in response to extracellular polarization was recorded intracellularly at five different distances (ranging from 0.1 to 1 mm)

from the partition toward the tendon of the papillary muscle. Only those data were used which were obtained when the full magnitude of the resting potential, as well as the spike, was generated by cathodal polarization. We also made sure that a weak cathodal or an anodal polarizing current evoked a symmetrical electrotonic potential without producing any initial artifact (Fig. 2). After these procedures, a series of electrotonic potentials produced by anodal polarizing current (hyperpolarizing current) of different intensities was recorded. In order to observe the relation between the amplitude of the electrotonic potentials, the hyperpolarizing current and the distance from the partition (Fig. 3), the spatial decay of the electrotonic potential (by a given current) was plotted on a logarithmic scale against the distance. As shown in Fig. 4, the experimental values could be fitted by a straight line, indicating

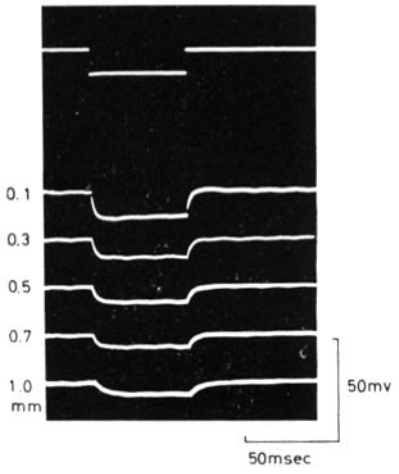

FIGURE 3. Decay of anodal electrotonic potentials along the fiber from the partition. The distance from the partition is shown to the left of each curve. The uppermost trace shows the intensity of anodal current applied.

that the amplitude of the electrotonic potential was reduced exponentially along the tissue. Consequently, the average value of the space constant (λ) was estimated to be 1.23 mm (SD = $\pm$0.11, n = 18). These results confirm those of Kamiyama and Matsuda (1966) in the canine papillary muscle. The above relationships between the amplitude of the electrotonic potential and the distance from the stimulating partition electrode were similar to those obtained by the partition method in canine atrial muscle and Purkinje fiber (unpublished observations).

The Time Course of the Electrotonic Potential

If the papillary muscle fiber has cable properties, the membrane time constant can be calculated by using the slope of $2\lambda/\tau_m$ (Katz, 1948). A nearly straight line with a slope of $2\lambda/\tau_m$ would be expected when the time to reach half-maximum of the electrotonic potential is plotted against the distance.

The time constant (τ_m) could be obtained from the experimentally obtained space constant, λ. As shown in Fig. 4, in the canine papillary muscle, the relationship was also linear and the time constant obtained by this method was was 4.2 msec (SD = ± 0.7, $n = 22$). Since the tissue showed cable-like properties, the time constant could also be calculated from the theoretical time course using the following cable equation (Hodgkin and Rushton, 1946; Katz, 1948).

$$V(x, t) = \frac{1}{2} V_{\substack{x=0 \\ t=\infty}} \left\{ e^{-x/\lambda} \left[1 + \operatorname{erf}\left(\frac{x/\lambda}{2\sqrt{t/\tau_m}} - \sqrt{t/\tau} \right) \right] + e^{x/\lambda} \left[1 - \operatorname{erf}\left(\frac{x/\lambda}{2\sqrt{t/\tau_m}} - \sqrt{t/\tau} \right) \right] \right\} \quad (1)$$

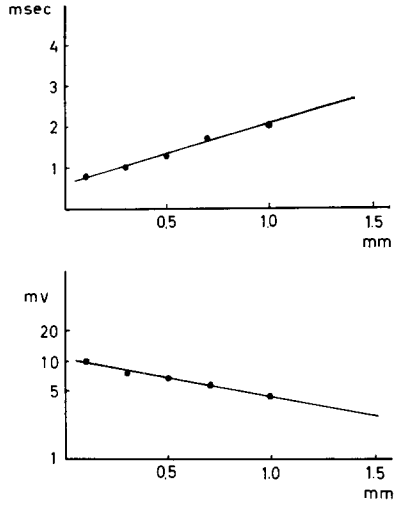

FIGURE 4. The upper graph shows an example of the linear relationship between the distance from the partition and the time to half-maximum of the electrotonic potential. The lower graph shows one example of the spatial decay of the electrotonic potential plotted on a logarithmic scale against the distance from the partition.

where $V(x, t)$ is the electrotonic potential which is a function of distance (x) from the partition and of time (t) after current is turned off; λ is the space constant; τ_m is the membrane time constant; erf is the error function; $V_{\substack{x=0 \\ t=\infty}}$, the potential at the steady state at the partition ($x = 0$). $V_{\substack{x=0 \\ t=\infty}}$ is obtained by extrapolating the relationships between the electrotonic potential and the distance from the partition to zero distance since when $t = \infty$, equation (1) becomes

$$V(x)_{t=\infty} = V(0)_{t=\infty} e^{-x/\lambda}. \quad (2)$$

The time course was usually examined at the make and the break of the hyperpolarizing currents. An appropriate τ_m was inserted into equation (1) and the best theoretical curve fitting the experimental results was found. After

finding the best fit of τ_m for one electrotonic potential at a given distance from the partition, the same τ_m was used to draw the theoretical curve for the potentials at different distances. The time course of the electrotonic potential should fit the theoretical curve obtained at any distance if the tissue shows cable-like properties. An example is shown in Fig. 5. The average value of the time constant obtained by this method was 2.5 msec (SD = ±0.8, n = 16).

Propagated Action Potential and Membrane Capacitance

According to Tasaki and Hagiwara (1957), if the impulse travels along a fiber with cable-like properties at a uniform velocity, a membrane capacitance can be calculated from the conduction velocity and the time constant of the foot

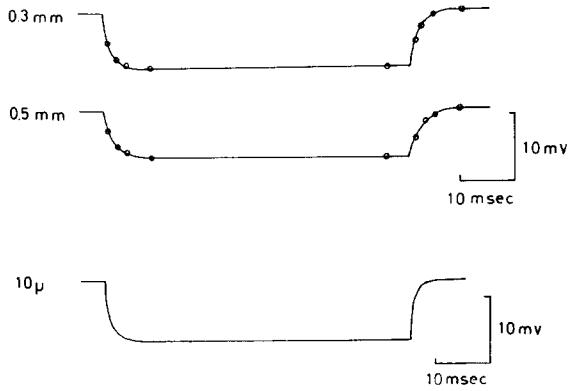

FIGURE 5. Upper two plots show the time courses of the electrotonic potential recorded at 0.3 mm and 0.5 mm from the partition, respectively. A current of the same intensity was applied to the tissue with the separation chamber method. Open circles show the theoretical values calculated from the cable equation inserting λ = 1.2 mm, and τ_m = 2.5 msec. Bottom graph shows the time course of the electrotonic potential due to intracellular polarization. The distance between the recording and current microelectrodes was 10 μ. Note a rapid development and decay of the electrotonic potential.

of the propagated action potential. The present study of the electrotonic potential of papillary muscle fiber suggests that it has cable-like properties; therefore it may be possible to apply the same equations which were used by Tasaki and Hagiwara (1957) to calculate the membrane capacitance. Fig. 6 shows the time course of the propagated spike in the canine papillary muscle and the semilogarithmic plot of the spike foot against time. The foot of the spike rose exponentially, which confirmed the previous observations that papillary muscle fiber shows cable-like properties. The time constant of the foot of the propagated action potential, T, was 1.13 msec (SD = ±0.2, n = 10) for the papillary muscle fiber of the ventricle.

In order to obtain the conduction velocity, two microelectrodes were inserted into two different cells (4–5 mm apart longitudinally). The value of the

conduction velocity was widely scattered when the two electrodes were separated less than 1–3 mm. As Abe and Tomita (1968) pointed out for the guinea pig taenia coli, the difference in latency in the two cells (i.e., conduction velocity) was not independent of the intensity of stimulus. We therefore measured conduction velocity by changing the stimulus intensity when the first recording electrode was close to the stimulating electrode; even if the distance between the first electrode and the second one was long, values of the conduction velocity were scattered. Therefore the first recording electrode was inserted into the cell at least 10 mm away from the stimulating bipolar electrodes and the second recording electrode was inserted 4–5 mm further away from the first one. The value obtained for the conduction velocity by that recording method was 0.68 m/sec (SD = ± 0.16, $n = 53$).

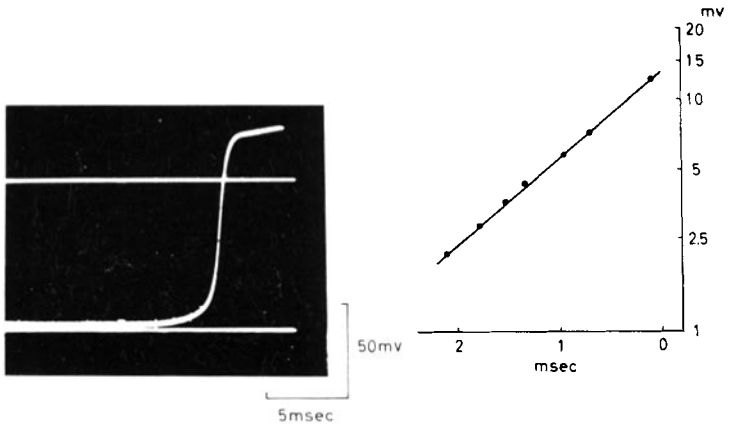

FIGURE 6. Left, the time course of the foot of the propagating spike. Right, semilogarithmic plots of the foot of the potential against time. The foot of the spike rises exponentially with a time constant of 1.1 msec.

The membrane capacity could be calculated from the following cable equation (see Tasaki and Hagiwara, 1957),

$$\frac{2C_m R_i}{a} = \frac{\tau_m}{T^2 U^2 \left(1 + \frac{\tau_m}{T}\right)} \qquad (3)$$

where U is conduction velocity, τ_m is the membrane time constant, C_m is the specific membrane capacitance, a is the fiber radius, and R_i is the specific internal resistance. Since the value of $\frac{\tau_m}{T}$ is greater than 1, the specific membrane capacitance, (C_m), is calculated by the following equation

$$C_m = \frac{a}{2R_i T U^2}. \qquad (4)$$

Taking the value $a = 8 \times 10^{-4}$ cm (Matsuda 1960; and Hoffman and Cranefield, 1960) for the papillary muscle and $R_i = 100\ \Omega$ cm for the preparation on the assumption that the value is of the same order as that of Purkinje fiber, 105 Ω cm (Weidmann, 1952); $U = 0.68$ m/sec (SD = ±0.16, $n = 53$); and $T = 1.13$ msec (SD = ±0.2, $n = 10$); the membrane capacitance of the papillary muscle fiber was calculated to be 0.76 μF/cm^2. This value was much smaller than that of 12 μF/cm^2 for the Purkinje fiber obtained by the square pulse method as well as by calculating from conduction velocity, the foot of the action potential and R_i.

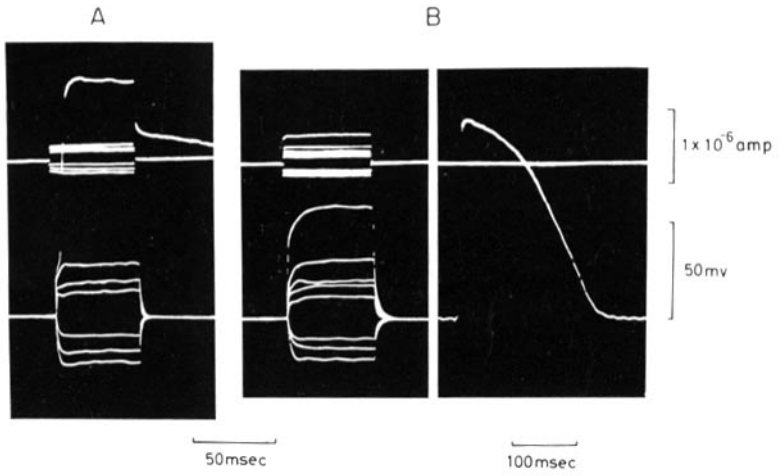

FIGURE 7 A. An action potential induced by intracellular stimulation about 10 μ from the recording electrode. 7 B. An example of failure to induce an active response by intracellular stimulation (left side). Extracellular stimulation, however, was able to elicit a propagated spike (right side). Note the sharp rise and fall of the electrotonic potential as well as the high threshold potential and short latency for the spike generation. Upper horizontal line shows zero potential level and the applied current intensities.

Response to Intracellular Stimulation

It was more difficult to induce the propagated spike in the papillary muscle fibers than it was in the Purkinje fiber by passing current through an intracellular microelectrode. The insertions of the current electrode and recording electrode were made in the vicinity of the tendon, because if the electrodes penetrated cells near the basal portion of the papillary muscle, the shape of the action potentials resembled those of the terminal Purkinje fiber rather than those of the papillary muscle (Matsuda, 1960).

Furthermore, when the microelectrodes were inserted into a superficial fiber of the subendocardium of the ventricular wall, an action potential of a shape intermediate between those of the Purkinje fiber and the ordinary

ventricular muscle fiber could be observed, as pointed out by Tille (1966). The induction of the propagated spike by intracellular stimulation of such fibers was relatively easy in comparison with papillary muscle fiber.

In some cells of the papillary muscle fiber (less than 10% of the whole experiments) a spike could be produced in an all-or-none manner as shown in Fig. 7 A. It is possible that the percentage of cells which produce an active response to intracellular stimulation would be increased by technical improvement, e.g. better electrodes or better insertion. The threshold potential for the spike was -30 to -48 mv and the threshold current was $1.2\text{-}2.1 \times 10^{-7}$ amp at resting potentials between -78 and -86 mv. This threshold current was smaller in value than that obtained by Johnson et al. (1958, 1961). Thus the threshold potential was greater than that obtained with extracellular stimulation (mean threshold potential, -60.3 mv, SD $= \pm 4.8$, $n = 32$).

Since the distance between the current electrode and the recording electrode was about 10 μ, the measured input resistance was regarded as the effective membrane resistance. This effective membrane resistance, calculated from the current-voltage relation, was 160-250 K Ω for weak currents, but the actual effective resistance was probably much greater.

In most cells (about 90%) no spike could be triggered by intracellular electrical stimulation even if the membrane was depolarized by more than 50 mv or the current intensity increased to more than 3.0×10^{-7} amp. However, in such a cell, a normally conducted spike was easily evoked by the application of external current as shown in Fig. 7 B, and the resting potential was the same as that for the cells in which the spike was elicited by intracellular depolarizing currents. There was no clear difference between the electrotonic potentials observed in the cells which produced, and those which did not produce an active response to intracellular stimulation. In all experiments, the second microelectrode was inserted within a 15 μ distance from the stimulating microelectrode because no spike and no electrotonic potential could be detected in any cell at a distance of more than 15 μ. This observation qualitatively suggested that the space constant of the papillary muscle fiber was very short, probably due to two- or three-dimensional spread of the current. When a weak current pulse was applied to the membrane through an intracellular microelectrode, an electrotonic potential was produced and the time constant to reach 63% ($1 - I/e$) of the steady level was 1.5 msec. The time course of the electrotonic potential evoked by the intracellular polarizing method was expressed neither by a simple exponential function nor by error function. On the other hand, when the time to reach 84% (erf 1) of the steady level was taken, it was about 3.0 msec. To investigate whether the electrotonic current spreads in a two-dimensional direction or a three-dimensional direction within the tissue, a lattice model was used for the case of the two-dimensional spread.

According to the lattice model, the relationships between the electrotonic potential (V) at the steady level and the distance (x) from the stimulating current electrode are expressed by the following equation under certain conditions (see Tanaka and Sasaki, 1966)

$$V(x)_{t=\infty} = A\left[(\log 2 - \gamma)\cdot I_o\left(\frac{x}{\lambda}\right) - \left\{I_o\left(\frac{x}{\lambda}\right)\cdot\log\left(\frac{x}{\lambda}\right) - \frac{\left(\frac{x}{\lambda}\right)^2}{4} \cdots\right\}\right] \quad (5)$$

where A is a constant, γ is Euler's constant with a value of 0.5772 . . . , and

$$I_o\left(\frac{x}{\lambda}\right) = 1 + \frac{\left(\frac{x}{\lambda}\right)^2}{2^2} + \frac{\left(\frac{x}{\lambda}\right)^4}{2^2\cdot 4^2} + \cdots \quad (6)$$

Therefore, when a current spreads in two dimensions, the spatial decay of the electrotonic potential produced by intracellular stimulation should be expressed by a Bessel function (Noble, 1962; Tanaka and Sasaki, 1966; Woodbury and Crill, 1961). As shown in Fig. 8, the measured amplitudes of the electrotonic potential plotted against the distances from the stimulating electrode were shifted completely from the theoretical curve drawn by the lattice model. The theoretical curve was plotted under the assumption of $\lambda = 100\ \mu$. When the current is applied by an intracellular microelectrode to papillary muscle, the electrotonic current might spread in three dimensions; therefore, the spatial decay of the electrotonic potential would be much sharper in this tissue than that in the lattice model in which current spreads in two dimensions as expected from the curve drawn in Fig. 8.

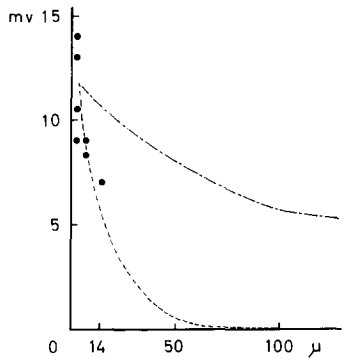

FIGURE 8. Spatial decay of the electrotonic potentials produced by intracellular polarization. Abscissa, interelectrode distance. Ordinate, electrotonic potential. Filled circles show experimental values. Dotted line shows the theoretical decay curve calculated as a Bessel function, assuming $\lambda = 100\ \mu$. Broken line shows the theoretical decay curve expected from the exponential decay with the same assumption.

Response to Extracellular Stimulation

When current was applied to the tissue through a large external electrode using the partition method, the passive and active responses of the muscle

membrane were quite different from those produced by intracellular polarization. The threshold potential for the spike observed at about 100 μ from the partition was -60 mv (SD $= \pm 4.8$, $n = 43$) which was similar to the threshold potential of Purkinje fiber (about -64 mv). When the record was taken at a longer distance from the partition, the apparent threshold potential was decreased due to decay of the electrotonic potential along the tissue. The latency for the spike generation at threshold potential was also prolonged. The space constant of the membrane measured by extracellular polarization was much larger than that measured by intracellular polarization, although the time constant of the electrotronic potential measured very close to the partition

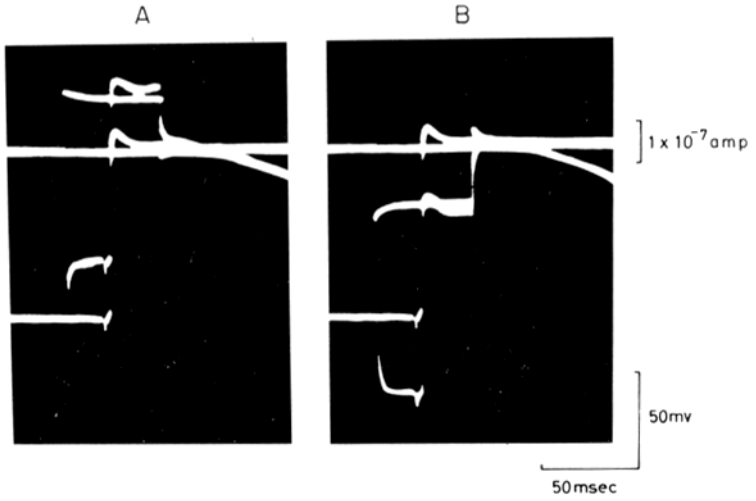

FIGURE 9. Effects on the spike amplitude of the membrane voltage displacement produced by intracellular polarization. The conditioning depolarizing (A) and hyperpolarizing (B) current pulses were applied about 25 msec before the onset of the action potential and maintained for 50 msec. The amplitude of the propagated spike was little influenced by the conditioning polarization or depolarization of the membrane.

showed nearly the same value by both methods. But the time constant measured by extracellular stimulation had a tendency to be longer than that obtained by intracellular stimulation. The possible explanation for this fact will be taken up in the Discussion.

Membrane Resistance Change at the Crest of the Action Potential

In order to investigate further the current spread in the syncytial structure, the membrane resistance change at the crest of the propagated action potential was estimated. Effects of the voltage displacement on the spike amplitude caused by the application of current through an intracellular microelectrode and by a large external electrode were measured. Conditioning

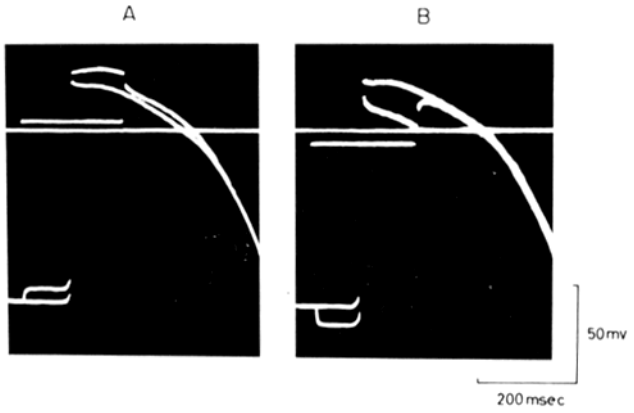

FIGURE 10. The effects on the spike amplitude of the membrane voltage displacement produced by extracellular polarization. The conditioning depolarizing (A) and hyperpolarizing (B) current pulses were applied about 100 msec before the onset of the action potential and maintained for 200 msec. The conditioning hyperpolarization of the membrane increased and the depolarization decreased the spike amplitude.

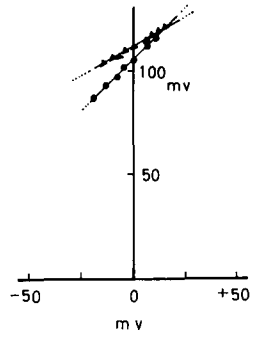

FIGURE 11. The relationships between the amplitude of the action potential and the voltage displacement of the membrane produced by intra- and extracellular polarizations. Abscissa, the voltage displacement produced by polarizing current pulses. Ordinate, the amplitude of the propagated spike during the conditioning current flow. The intracellular polarization produced a linear slope of 45° (closed circles) indicating no effect of the membrane displacement on the spike amplitude. Conversely in the case of the extracellular polarization (triangles) the slope became small indicating a causal relationship between the spike amplitude and the membrane potential level.

polarizing current pulses were applied for about 25 msec before the onset of the action potential for the intracellular polarization and 100 msec for the extracellular polarization. The conditioning currents were maintained for 50 msec (intracellular polarization) and 200 msec (extracellular polarization), respectively during the upstroke of every action potential. The records before and during current flow were superposed on the oscilloscope. Examples of

such records are shown in Figs. 9 and 10. Figs. 9 and 10 show the behavior of the active membrane observed during intracellular and extracellular polarization, respectively. The amplitude of the spike elicited by extracellularly applied current was not influenced by conditioning polarization of the membrane produced by intracellular polarization. Conditioning by extracellular polarization, however, enlarged the spike amplitude. These observations confirmed the results obtained by Cranefield and Hoffman (1958). This is seen more clearly when the relationships between the voltage displacement in the resting state and the amplitude of the action potential during the current flow are drawn. As shown in Fig. 11, with intracellular polarization, the relationship has a linear slope of 45° in the papillary muscle as observed by Johnson and Tille (1960, 1961) and Tille (1966); conversely with the extracellular polarization the slope is lower. The membrane resistance at the peak of the spike was reduced to about 25% of that in the resting stage. These results might indicate that in the interconnected syncytium, a decrease in membrane resistance at the crest was only caused by the application of current through a large external electrode. This reduction of the membrane resistance agreed well with that observed by Kamiyama and Matsuda (1966), and the interpretation of this effect has been discussed by them. But this reduction was not caused by the application of current through the microelectrode.

DISCUSSION

When current is applied to the preparation with the separation chamber method, polarization can be easily observed from every cell within a 2 mm distance from the partition, and the magnitude of the electrotonic potential decreases exponentially along the tissue with a space constant of about 1.2 mm. If all cells in the tissue lying in parallel at the same distance from the partition are polarized to the same extent, no current flows between the cells in the transverse direction but only in the longitudinal direction. The time course of the electrotonic potential at a given distance could be fitted fairly well to the theoretical curve obtained from the cable equation. Thus the results obtained by external polarization strongly indicate that the tissue has cable-like properties when current is applied with external electrodes.

On the other hand, when current is applied with an intracellular microelectrode, the spatial decay of the eelctrotonic potential is very sharp, and it becomes difficult to measure this potential at a distance of more than 20 μ. Current probably spreads in three dimensions through interconnections between cells.

In the preparation which has cable-like properties, the membrane area increases linearly with the distance from a given point. In an extensively branching preparation with three dimensional interconnections such as the cardiac syncytium, the membrane area increases more rapidly with the

distance. Therefore, the current density even very near the current-supplying microelectrode becomes very low. This may also be why, with intracellular polarization, the time course of the electrotonic potential is sharper than that of the electrotonic potential produced with large external electrodes. In the papillary muscle, however, not so large a difference in the time courses is observed with the two different methods of stimulation as was observed in smooth muscle (Kuriyama and Tomita, 1965; Tomita, 1966 *a*, *b*, 1967). There are several possible explanations of the difference between the papillary muscle of the dog and the smooth muscle of guinea pig taenia coli.

One possibility is that the shape of rectangular pulses may be distorted inside the tissue when currents flow at capacity in components of the tissue near the partition. This possibility can probably be discarded because the time course lf the electrotonic potential was not changed when the width of the partition was increased to more than 5 mm. Another possibility is that if the extracellular current is passing through an unknown resistance in series with the membrane resistance of the papillary muscle, it may affect the shape of the electrotonic potential. However, this series resistance may be negligibly small because a small artifact could be evoked by the various intensities of extracellular current as shown in Figs. 2 and 3.

Taking the value τ_m = 3.3 msec and C_m = 0.76 μF/cm^2, the specific membrane resistance (R_m) of the papillary muscle is calculated to be about 4000 Ω cm^2 and is one-tenth that of the smooth muscle of guinea pig taenia coli (Tomita, 1966 *b*; Abe and Tomita, 1968). In a leaky condenser model, the time course of the electrotonic potential is proportional to exp $(1/R_m)$, while in a core conductor model the time course of the electrotonic potential at the point of current injection is proportional to erf $(1/R_m)$. The observed results fitted those explained by the core conductor model. The slight dependency of the time course on the membrane resistance could also be explained by the core conductor model as being due to spatial decay of current density along the cable-like tissue. Furthermore, with a two- or three-dimensional model, the decay of current density would be expected to be much sharper than with a one-dimensional model (core conductor model) so that the time course would be much less dependent on the membrane resistance. For this reason, the time course of the electrotonic potential produced by the application of intracellular current would be nearly constant and independent of the specific membrane resistance (George, 1961; Berkinblit et al., 1965), while the membrane time constant obtained by an analysis of the electrotonic potential produced by the application of an external current is proportional to the membrane resistance. Therefore, in the papillary muscle, the time course of the electrotonic potential is the same when measured by the application of intracellular and external current because of a low membrane resistance.

Furthermore, recording of the electrotonic potential at some distance (about

10 μ) from the stimulating intracellular electrode may slow the time course due to the sharp spatial decay of the electrotonic potential. In the present experiment this could be another possible explanation for a small difference in the time courses with two different current applications.

When intracellular stimulation could not induce the propagated spike, the relations between current and voltage were nearly linear, while in the case in which intracellular stimulation could induce the spike, current-voltage relationships were nonlinear. The cable properties have the effect of reducing nonlinearities in the current-voltage relations (Burke and Ginsborg, 1956; Cole and Curtis, 1941). In the case of an extensively branching preparation the areas of membrane at a given distance from the stimulating electrode are larger than in a cable-like preparation and the region of membrane near the recording electrode would be shunted by that large area. Nonlinearity should therefore be reduced more.

When the applied currents are spatially nonuniform, in other words, when the membrane is not uniformly polarized, the geometry of the preparation greatly affects both excitation and propagation of the action potential, as discussed by Noble (1966). Thus the intracellular stimulation is not able to depolarize a large area of membrane, even if the recording electrode is placed in an area adjacent to the stimulating electrode. Furthermore, the inward ionic current generated by the membrane near the stimulating electrode will have to supply a local circuit current to discharge its own capacity. Even if the membrane near the recording electrode is depolarized beyond the threshold potential, it is not necessarily enough to produce the spike because more current might flow from the areas of membrane supplying outward current so that there is an excess of outward ionic current which recharges the membrane capacity. Therefore, to elicit the spike by intracellular polarization more inward current is required to overcome outward current flows in order to discharge the membrane capacity. This may be the reason for the difficulty in initiating a spike in papillary muscle and also the reason why the threshold potential level is much higher when intracellular stimulation is used than when external stimulation is utilized.

The average membrane capacitance is about 0.76 μF/cm^2, which is much smaller than those of the Purkinje fiber (Weidmann, 1955) and other excitable tissues.

The specific membrane resistance is calculated to be 3503 Ω cm^2 from cable theory (taking $\lambda = 1.2$ mm, fiber radius, $a = 8 \times 10^{-4}$ cm, and internal resistance, $R_i = 100\ \Omega$ cm). From this calculated specific membrane resistance and the time constant, the specific membrane capacity is calculated to be about 1 μF/cm^2. This value is nearly the same as the value calculated from the foot of the propagated action potential and conduction velocity. Recently the specific internal resistance, R_i, and specific membrane capacitance, C_m, were

calculated by Weidmann (1968) to be about 500 Ω cm and 0.81 $\mu F/cm^2$, respectively. If value R_i is taken to be 500 Ω cm, C_m will work out to be lower. Thus the accuracy of determination of the membrane capacity by the technique used in the present experiment is seriously limited by the difficulties of measuring the specific interval resistance, R_i, and the size and shape of the cross-section of the fiber. Therefore an impedance measurement with AC currents needs to be done in order to obtain the proper capacity of the membrane. In the future differences in membrane capacitance between sheep Purkinje fiber and canine papillary muscle should be clarified.

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