A STUDY OF THE MEMBRANE CONSTANTS IN THE DOG MYOCARDIUM

Yasuji SAKAMOTO AND Masayoshi GOTO

Department of Physiology, School of Medicine,
Kyushu University, Fukuoka, Japan

INTRODUCTION

WEIDMANN\(^{19}\) used cable analysis to obtain the membrane constants of the false tendon of the kid heart. Attempts to apply the cable theory have also been made on the terminal Purkinje fiber of the dog heart and an equivalent linear cable consisting of several component fibers attached side by side was analyzed by MATSUDA\(^{9}\). Experimental verification of the cable theory on the regular cardiac muscle, however, seems to be extremely difficult because of the complicated syncycium-like structure of the myocardial fibers. WOODBURY et al.\(^{21}\) proposed a planar model for the rat atrium, and TANAKA et al.\(^{15}\), a lattice model for the mouse ventricle. Both groups showed theoretically and experimentally that the extremely rapid spatial decline of a tonically applied potential can be expressed by a Bessel function. Thus, it seems strange that conduction of excitation in the ordinary cardiac muscle is known to occur with a high safety factor which is almost comparable to that of nerve and skeletal muscle fibers\(^{13,17,20}\). These facts strongly suggest that a group of myocardial fibers works as a functional bundle as just pointed out in the mammalian smooth muscle\(^{16}\).

KAMIYAMA and MATSUDA\(^{7}\), to circumvent these complexities in fiber structure, utilized a partition chamber method for polarizing the ventricular muscle fibers and showed that in these circumstances aggregates of muscle fibers could be regarded to behave as a one-dimensional cable. We used this partition chamber method to study the membrane constants and safety factors of propagation in various portions of the dog heart.

METHODS

Puppies were sacrificed under pentobarbital anesthesia, and three kinds of preparations were made: (i) the pectinate muscle bundle of the right atrium, (ii) the false tendon and (iii) the papillary muscle of the right ventricle. The pectinate muscle
MEMBRANE CONSTANTS OF DOG MYOCARDIUM

bundle and false tendon were about 5-10 mm in length, and the papillary muscle was isolated with its radix at the free wall of the ventricle. The thickness of these preparations was less than 1 mm.

Each preparation was mounted in a Lucite chamber and kept in Tyrode's solution aerated with 95% O₂ and 5% CO₂. Regular electrical stimulation at a rate of one per second was applied through small bipolar Ag-AgCl electrodes placed on one end of the preparation. Two microelectrodes were inserted in two myocardial cells 3 to 5 mm apart longitudinally and used to measure conduction speed and the rate of rise of the action potential. Microelectrode resistances ranged from 10 to 20 megohms.

The partition chamber method of Kamiyama et al. was used to measure the time and space constants of the myocardium (Fig. 1). The chamber made of Lucite, was divided into two sections (A and B) by three close fitting, slotted plates (P). The cut end of the specimen was put through the slots in the two outside partitions and the middle slotted partition put in from above. Both chambers were filled with Tyrode's solution but only the one (B) which contained the larger part of the specimen was aerated.

Polarizing current was applied between the two chambers through spiral Ag-AgCl electrodes put in each chamber. The close fitting partitions by vaseline minimized leakage around the sample. The electrotonic potential thus produced was recorded with a pair of microelectrodes (M), one inside and the other immediately outside of the muscle fiber. The polarizing current was monitored, simultaneously with the electrotonic potential, on a dual-beam cathode ray oscillograph and the features were photographed.

---

**Fig. 1.** Schematic diagram of the experimental arrangement. A and B: Separated chambers. P: Partition plates. M: Microelectrodes. Further explanations are in the text.
RESULTS

1. The surface membrane capacities

The membrane capacity of different portions of the myocardium was calculated from the propagation velocity and the time course of the foot of a propagated action potential\(^1,14,18\).

Fig. 2 shows the time course of the propagated action potentials in three different portions of the puppy heart together with semilogarithmic plots of the transmembrane voltage change with time during the foot. The foot rises exponentially and this suggests that these muscle fibers have cable-like properties. The time constant of the foot \((T_a)\) was 0.68 ± 0.17 msec and 0.88 ± 0.06 msec for pectinate fiber and papillary muscle respectively. In contrast, that of the false tendon was only 0.11 ± 0.02 msec.

![Graph showing conduction velocity and foot time course](image)

**Fig. 2.** Measurement of conduction velocity and time course of the foot of action potential in three types of fibers. Transmembrane action potentials were recorded with a pair of microelectrodes kept in two myocardial cells a few mm apart in longitudinal direction. Semilogarithmic plots of action potential foot are shown in the upper part. A: pectinate muscle of the atrium. P: papillary muscle. F: false tendon of the ventricle. Mean values of conduction velocity \((v)\) and time constant of spike foot \((T_a)\) are listed below the corresponding records of action potentials.
As expected from these data, the conduction velocity \( v \) was largest in the false tendon, \( 2.01 \pm 0.42 \); next largest in the papillary muscle, \( 0.78 \pm 0.13 \), and smallest in the atrium, \( 0.40 \pm 0.15 \) m/sec.

From the cable theory, the capacitance of the membrane \( (C_m) \) being filled by the foot of the action potential can be calculated from the following equation, which is similar to the formula used by Van Der Kloot\(^{18}\) and Fozzard\(^{3}\) but does not need the assumption such as \( v^2 \tau_m^2 > \lambda^2 \).

\[
C_m = \frac{a \cdot \tau_m}{2R_i \cdot T_a \cdot v} \left( 1 + \frac{\tau_m}{T_a} \right)
\]  

(1)

where \( a \) is the fiber radius (cm); \( \tau_m \) is the time constant of the membrane (sec); \( T_a \) is that of the foot of action potential (sec); \( R_i \) is the specific internal resistance (\( \Omega \) cm); and \( v \) is the conduction velocity (m/sec). The values of \( T_a \) and \( v \) were already measured on the different portions of the myocardium, and those of \( \tau_m \) of the muscle bundles were also determined as described later. Thus, the membrane capacity can be obtained from the equation.

Taking the value, \( a = 6 \mu, 15 \) or \( 60 \, \mu \), and \( 8 \, \mu \) for the atrium, false tendon and papillary muscle fibers respectively\(^{5,9,10}\), and \( R_i = 100 \, \Omega \) cm for all preparations assuming that the value is the same order as that of Purkinje fiber \( 105 \, \Omega \) cm\(^{19}\), the capacity being filled by the foot of action potential was calculated to be \( 2.61, 1.68 \) (6.75\(*\)) and \( 0.60 \mu F/cm^2 \) for the respective cardiac muscle fibers. The assumption of the constant value of \( R_i \), however, will raise a problem as will be discussed later.

2. The time and space constants

The time and space constants of the different heart muscle fibers were determined using the method of Kamiyama and Matsuda\(^7\). Figs. 3 and 4 show some of the results. Full penetration of the cell with microelectrode was ascertained not only by the size and steadiness of the resting potential but also by the appearance of full-sized action potential due to rectangular cathodal polarization (Fig. 3). The first step was to apply equal, weak cathodal and anodal polarizing currents. A symmetrical electrotonic potential was taken to indicate the absence of appreciable initial artifact. Next, a series of electrotonic potentials produced by anodal polarizing current of different intensities were recorded. These measurements were repeated at various distances from the partition (Fig. 4).

The amplitude of the electrotonic potential was largest near the partition and declined approximately exponentially with distance (Fig. 5, left). The space constants, \( \lambda \), thus determined were \( 1.24 \pm 0.09, 1.25 \pm 0.13 \) and \( 1.18 \pm 0.10 \) mm for the atrium, the false tendon and the papillary muscle respectively. It should be noted that these values are almost the same despite the great differences in fiber diameter or architectonics.

* A value of a "fused" bundle of the false tendon. See discussion.
Fig. 3. Transmembrane potential changes due to massive polarization. Effects of anodal and cathodal polarization on three types of fibers are shown. A: pectinate muscle of the atrium. B: false tendon. C: papillary muscle of the ventricle. Note differences in rate of rise, form and duration of the action potentials.

Fig. 4. Electrotonic potentials recorded with intracellular electrode at various distances from the partition (number by each curve). The uppermost curve is the polarizing current. A: pectinate muscle. B: false tendon. C: papillary muscle.

The half time of electrotonic potential rise increased linearly with the distance from the partition (Fig. 5, right). The slope of linear relationship is markedly less in the papillary muscle than those of the pectinate muscle and false tendon, the latter two being almost equal. Hodgkin and Rushton found an empirical trend that the slope of this curve approximated $2\lambda/\tau_m$, $\tau_m$ being the membrane time constant. The time constants obtained based on
3. Relative value of the safety factor of conduction as the muscle bundle

A few attempts were made to determine the safety factor of conduction in cardiac muscle fibers, however, most of these were performed under special conditions\textsuperscript{5, 17). The safety factor in the normal myocardium of the dog was attempted to elucidate, here, by utilizing the local circuit theory on the cable-like fibers. Blair\textsuperscript{1) and Offner et al.\textsuperscript{11}) derived an equation for the “safety factor” $S$,

$$S = \frac{v \tau_m}{\lambda}$$

(2)

where $v$ is conduction velocity, $\tau_m$ time constant of the membrane and $\lambda$ length constant. Using the values of $v$, $\tau_m$ and $\lambda$ for puppy cardiac muscle, the safety factors are 4.7, 24.1 and 2.1 for the pectinate muscle, false tendon and papillary muscle respectively.

This theory, however, is based on the assumption of a sharp boundary between resting and active regions, and according to Offner et al.\textsuperscript{11}), the value of $\lambda$ and $\tau_m$ of the active region should be used. Considering these limitations and propagation in a large volume, Katz\textsuperscript{8}) transformed above equation into,

$$S' = v C_m \sqrt{\frac{2 R_m}{R_m'}} / \sqrt{a}$$

(3)

which again can be expressed as,
where $R_m$ and $R_m'$ are the membrane resistance during rest and spike potential. It was attempted to determine the ratio of $R_m'/R_m$ as shown in FIG. 6. The mean value of five preparations appeared to be 87% of the resting membrane resistance in the pectinate muscle of the atrium and 86% in the papillary muscle, while the values was less than 10% in case of the false tendon. Taking these values, more reliable safety factors in the respective tissues were estimated. They are listed in TABLE 1.

FIG. 6. The membrane resistance at the crest of action potential. A: Measurement on the false tendon. A) Control record, B) application of anodal pulse, and C) superimposed tracing of a) and b). B: Papillary muscle. C: Pectinate muscle. In case of B and C, repetitive pulse were applied. The membrane resistance at the peak of action potential ($R_m'$) was determined to be 13% less than in the resting state ($R_m$) in the pectinate muscle, and 14% less in the papillary muscle. In the false tendon $R_m'$ was less than 10% of $R_m$. 

$$S' = \sqrt{\tau_m/\lambda R_m'/R_m}$$
Table 1 summarizes the value of various membrane constants obtained in these experiments on the puppy heart. The results depend apparently on the radius of the fiber (a). Though the radii of the single cells are measurable with a microscope, the functional radius during excitation may vary according to the complicated three dimensional structure of the muscle fibers. However, the partition chamber method makes the polarizing current flow uniformly through the fibers, presumably owing to mutual cancelling in effects of frequent branching and re-uniting of the fibers. Thus, in the work muscle of the atrium and ventricle, the real single cell radius can be used. However, in the false tendon individual cell contacts occur not only at end-to-end junctions but also at side-to-side ones. In this case the radius of the bundle rather than that of single cell should probably be used as the functional radius. The results in parenthesis of the table show the values of false tendon in which radius of the ‘fused’ bundle was used for comparison. The conduction velocities (v) observed coincide well with those determined.

\[ \text{Table 1. Membrane constants and safety factors of excitation conduction in three type of fibers.} \]

<table>
<thead>
<tr>
<th></th>
<th>Atrium</th>
<th>False tendon</th>
<th>Papillary muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>a* (assum.)</td>
<td>(\mu)</td>
<td>6#</td>
<td>15 (60)</td>
</tr>
<tr>
<td>v* (obs.)</td>
<td>m/sec</td>
<td>0.40±0.15</td>
<td>2.01±0.42</td>
</tr>
<tr>
<td>Ta* (obs.)</td>
<td>msec</td>
<td>0.68±0.17</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>R_i (assum.)</td>
<td>(\Omega \text{ cm})</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C_m (cal.)</td>
<td>(\mu F/cm^2)</td>
<td>2.61</td>
<td>1.68 (6.75)</td>
</tr>
<tr>
<td>C_m (assum.)</td>
<td>(\mu F/cm^2)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>R_i (cal.)</td>
<td>(\Omega \text{ cm})</td>
<td>261</td>
<td>168</td>
</tr>
<tr>
<td>(\tau_m)</td>
<td>msec</td>
<td>14.6±0.21</td>
<td>15.0±0.19</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>mm</td>
<td>1.24±0.09</td>
<td>1.25±0.13</td>
</tr>
<tr>
<td>(cal.)</td>
<td>mm</td>
<td>1.26</td>
<td>2.58</td>
</tr>
<tr>
<td>R_m (cal.)</td>
<td>(\Omega \text{ cm^2})</td>
<td>5593</td>
<td>8927 (2220)</td>
</tr>
<tr>
<td>S* (cal.)</td>
<td>Eq. II</td>
<td>4.7</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>Eq. IV</td>
<td>4.4</td>
<td>7.6</td>
</tr>
</tbody>
</table>

* a: fiber radius, v: conduction velocity, Ta: time constant of the foot of action potential, S: safety factor of conduction.

\# M ori\(^{10}\)
\$ M atsuda\(^{9}\)
by earlier workers (see 5,9); our value on the pectinate muscle of the atrium is rather small. The time constant ($T_a$) of the action potential foot of the false tendon is almost the same as that of the kid Purkinje fiber obtained by FOZZARD3. Accordingly, when the internal resistance ($R_i$) was assumed to be 100 $\Omega$ cm, the calculated value of $C_m$, 1.68 $\mu$F/cm$^2$, was not very different from his value (2.4 $\mu$F/cm$^2$). In the pectinate muscle the value of $C_m$ calculated with the same assumption (2.61 $\mu$F/cm$^2$) also appeared roughly comparable with those of the false tendon. In the papillary muscle, however, the value obtained (0.6 $\mu$F/cm$^2$) was extremely small, mainly because of the largeness of the time constant of the foot and the considerable rapidity of the conduction velocity. The causes of these characteristics are not apparent.

Anyhow, it must be noted that all these values of $C_m$ are conspicuously smaller than those measured by double penetration method3,19). As fully discussed by FOZZARD3 the difference between the two kinds of measurement suggests an existence of two components in the membrane capacitance, one parallel with the membrane resistance and the other in series with another resistance.

An alternative explanation, however, is that the internal resistance ($R_i$) itself is different depending on the portions of the myocardium. Marked differences in intracellular structures are known between the different portions, and the membrane capacitances we calculated as well as the surface membranes observed with electron microscope are similar to those of the skeletal muscles. Thus, if we can assume a constant $C_m$ of 1 $\mu$F/cm$^2$ for the different tissues, $R_i$ is calculated to be 261, 168 and 60 $\Omega$ cm for the pectinate muscle, false tendon and papillary muscle respectively. Actually the value of 261 $\Omega$ cm coincides well with that of the skeletal muscle8), and 168 $\Omega$ cm, with that of the cardiac Purkinje fibers2). In the papillary muscle, however, the internal resistance of 60 $\Omega$ cm is too small and almost comparable to that of the Tyrode's solution. This will hardly be the case.

Values of the membrane time constant ($\tau_m$) determined from the linear relation between the half-rise time and spread distance of electrotonic potential as observed in the partition chamber method agree well with those measured by the double penetration method on the kid Purkinje fibers3,19). With the partition chamber method, KAMIYAMA and MATSUDA7) first obtained a value of 2 msec on the dog ventricle. We have found values 14.6, 15.0 and 3.2 msec for the pectinate muscle, false tendon and papillary muscle respectively. Utilizing the double penetration method, on the other hand, MATSUDA9) reported time constants of 12.9, 3.7 and 1.5 msec for the dog false tendon, terminal Purkinje and ventricular muscle fibers respectively and we obtained values 10 and 3 msec for the dog and cat false tendon and the papillary muscle (unpublished observations). The agreement of the values obtained by two different methods is good, and these facts strongly suggest that the
separation chamber method increases effective $R_m$ and decreases effective $C_m$ due to elimination of the transverse current and hence produces almost the same $\tau_m$ to the values determined by the double penetration method.

Using the partition chamber method, the membrane space constant ($\lambda$) was also determined by Kamiyama and Matsuda\textsuperscript{7} on the dog ventricle and a value 1.35 mm was reported. We have found almost the same value (1.25\textasciitilde1.18 mm) in different portions of the heart examined. The values also coincide well with the calculated ones from $\lambda=v\sqrt{T_a/T_m}$, which was derived from the equation (1). On the other hand, greater differences with position were found with the double penetration method. Wiebmann\textsuperscript{19} reported 1900 $\mu$ for the kids false tendon and Matsuda\textsuperscript{9} 840 $\mu$ for the dog terminal Purkinje, while 130 $\mu$ was found in the rat atrium\textsuperscript{17}, and 70 $\mu$ and 500 $\mu$ in the mouse ventricle\textsuperscript{19}. The value of $\lambda$ obtained with the partition chamber method agree well with those obtained with the double penetration in specialized conduction system fibers, but partition chamber values are strikingly larger in the work muscle. Smallness of $\lambda$ determined with double penetration might be explained by the three dimensional spread of intracellularly applied polarizing current along frequent branching of the fibers as pointed out and modeled theoretically by Woodbury & Crill\textsuperscript{21} and Tanaka & Sasaki\textsuperscript{15}. However, since propagation of impulse in situ or in a large volume of tissue will occur massively in a functional unit rather than in an individual cell unit, the value of $\lambda$ determined with mass polarization method will have a significance in normal myocardial function.

The membrane resistance ($R_m$) can be estimated in two different ways, one from the equation of time constant ($R_m=\tau_m/C_m$) and the other from that of space constant ($R_m=2R_i\lambda^2/a$). Except the case of false tendon, agreement of two values obtained from these equations was good either when a constant $R_i$ of 100 $\Omega$ cm and variable values of calculated $C_m$ were taken or when a constant $C_m$ of 1 $\mu F/cm^2$ and calculated values of $R_i$ were adopted. With the latter assumption, however, unusually high values of $R_m$ and its large dispersions, depending on the tissues, (14600\textasciitilde2088 $\Omega$ cm\textsuperscript{2}) were produced. Moreover, since the equation of space constant contains two terms of estimations ($R_i$ and $a$), only the values which were calculated from the time constant equation and under the assumption of a constant $R_m$ of 100 $\Omega$ cm are shown in the table. Anyhow, it must be noted that in any of the assumptions and in any tissues the membrane resistance appeared larger than 2000 $\Omega$ cm\textsuperscript{2} and the values in the pectinate and papillary muscles are almost comparable to those of the skeletal muscle\textsuperscript{8}. In the case of the false tendon a markedly large $R_m$ of 8927 $\Omega$ cm\textsuperscript{2} was calculated simply because of the smallness of $C_m$ in the single fiber. However, when ‘fused’ fibers as a bundle were assumed, the value becomes 2220 $\Omega$ cm\textsuperscript{2} which coincides well with those obtained by the double penetration method\textsuperscript{5,19}. Thus, the differences in
value of $R_m$ between the mass and intracellular polarization methods as well as the agreement of $r_m$ and $\lambda$ between them strongly suggest that the false tendon fibers are functionally 'fused'.

SUMMARY

Using the partition chamber method together with microelectrodes, membrane constants of the dog heart were determined in the pectinate muscle of the right atrium (A), false tendon (F) and papillary muscle of the right ventricle (V). The membrane capacity calculated from the time course of propagating action potential foot and propagation velocity was 2.6, 1.7 (6.8) and 0.6 $\mu$F/cm$^2$ for A, F and V. The electrotonic potential and its spatial decline observed with the partition chamber conformed with the cable law. The space constant was 1.24 mm for A, 1.25 mm for F and 1.18 mm for V. The time constants were 14.6, 15.0 and 3.2 msec. The membrane resistance calculated from these values appeared about 5600, 8900 (2200) and 5300 $\Omega$ cm$^2$ for A, F and V respectively. The safety factor for excitation conduction was estimated to be 4.4, 7.6 and 2.0. Differences and agreements in value between the membrane constants determined by mass polarization and by intracellular polarization were discussed.

We wish to express our deep gratitude to Professor J.W. Woodbury for his criticism and correction of this manuscript. This investigation was supported by a grant from the Ministry of Education (Japan).

REFERENCES


