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Field-stimulation Can Elicit Membrane Action Potential in Mammalian Myocardium

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Abstract The feasibility that membrane action potential in mammalian myocardium can be produced by means of field-stimulation was investigated. Guinea-pig papillary muscle was driven by either point-or field-stimulation and transmembrane potentials were recorded simultaneously by two glass microelectrodes positioned at two different sites of the preparation. Both point-stimulation and weak field-stimulation produced a propagated response, while strong field-stimulation produced a membrane action potential. The present study demonstrated the feasibility of field-stimulation in an induction of membrane action potential in mammalian cardiac muscle.

Key Words: Action potential; myocardium, field-stimulation. Heart; myocardium, membrane action potential

Introduction

Both local anesthetics and class-1 type anti-arrhythmic drugs, including procainamide, quinidine, disopyramide and phenytoin, suppress the conductivity of the excitable membrane of nerve and cardiac tissue by virtue of selective blockade of the sodium channels. The suppression largely depends on both membrane potential and stimulation pattern[1]. Sodium current of the axonal membrane of a squid or a frog sciatic nerve has been qualitatively analyzed by means of a voltage clamp technique. However, analysis of cardiac tissues, except for frog atria, is not possible by the technique because of its difficulty in voltage control. Thus, studies of the actions of class-1 type drugs on sodium channels in the heart muscle have all been made by recording the maximum upstroke velocity ($\dot{V}_{max}$) as an index for the sodium current[2,3], even though some workers objected to the validity of $\dot{V}_{max}$ as a measure of sodium current[4].

When $\dot{V}_{max}$ is employed in the analysis of drug-sodium channel interaction, there arises another complicating problem, that is, whether the action potential elicited is a membrane action potential or a propagated action potential. The excitatory depolarization of the membrane proceeds simultaneously for the whole preparations in the former and only locally in the latter. The action potential thus elicited can and cannot propagate in the latter and in the former, respectively.

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The $\dot{V}_{max}$ in the propagated action potential is thereby affected more or less by the current loss in the longitudinal direction. This difficulty may be largely overcome by the introduction of sucrose gap technique[5]. However, this would result in a damage of the preparation by the sucrose solution, which impedes the recording of $\dot{V}_{max}$ during a long-lasting experiment. Ochi reported that with such an experiment, a stable action potential was obtained only within the first two hours (personal communication), which is not long enough for our study of the effects of antiarrhythmic agents. Membrane action potential is more suitable than propagated one for the precise analysis of drug-sodium channel interaction.

In the present study, the validity of field-stimulation for obtaining membrane action potential was tested as an alternative of sucrose gap technique.

Method

Details of the experimental animal and the procedures were described elsewhere[6]. Only the stimulation and recording techniques were slightly modified. Muscle fibers of guinea-pig right ventricles were horizontally mounted by stainless steel pins in an organ bath of 1 ml volume. Muscles longer than 5 mm were chosen in order to allow simultaneous insertion of two recording microelectrodes. A glass tube, containing Tyrode solution and placed at the septal wall-side of the mounted muscle preparation (Fig. 1), served as an electrode for point-stimulation. A pair of silver wires, 1 mm diameter and coated with AgCl, were placed on both sides of the muscle preparation, running 5 mm apart in parallel to each other (in Fig. 1). The wires were coated with varnish except for the side facing the preparation. They were used for field-stimulation. Intracellular potentials were recorded by two glass microelectrodes. One was placed about 1 mm apart from the point-stimulation electrode (Fig. 1). The other electrode was placed 3 to 5 mm distant from the former. The duration of square-wave pulse was 1 msec, unless otherwise stated. Recording was made at a fast sweep speed (2 or 5 msec/cm) in order to observe the change of membrane potential immediately after the stimulus artifact to the upstroke of cardiac action potential (Fig. 1 C). Action potential parameters involved in the present study are shown in Fig. 1 C.

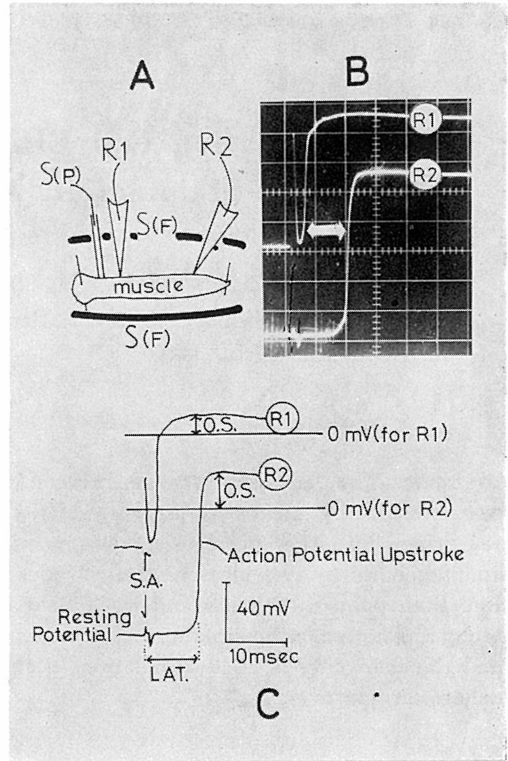

Fig. 1 Schematic presentation of stimulation and recording techniques (A) and original recording of the cardiac action potential (B) obtained from two different sites (only the upstrokes are shown). The action potential upstroke in B are retraced in C for the explanation of the technical terms. point-stimulation, S(P): field-stimulation, S(F); electrodes were maintained at proximal (R1) and distal (R2) recording sites in the septal sides of the muscle. S.A. and LAT in C denote stimulus artifact (S.A.) and latency (LAT). A time difference of about 7 msec between the upstrokes (white arrows) is seen. An electrotonic potential of the stimulus artifact at R1 was larger than that at R2 because R1 is closer to the stimulation electrode. The rate and duration of the stimulation: 1 Hz and 1 msec.

Results

Point-stimulation and propagated action potential

Intracellular potentials were recorded at two sites of the preparation (in Fig. 1A) which was electrically driven by point-stimulation S(P) in Fig. 1A. Only the initial parts of action potential, from stimulus artifact to action potential upstroke, were displayed on cathode ray oscilloscope and photographed. A typical case is presented in Fig. 1B. A time difference of about 7 msec between the upstrokes is seen. Such a time lag was almost independent of the intensity of stimulation, suggesting the propagation of impulse between the sites with a constant velocity.

Field-stimulation, intensity of the stimulation and membrane action potential

The upper panel (A) of Fig. 2 shows the propagated action potential elicited by the point-stimulation, the intensity of which was barely above the threshold. The upstroke of the action potential elicited by the field-stimulation and recorded at two different sites are shown in the middle panel (B). The intensity of the stimulation in B was also set barely above the threshold voltage. Contrary to the expectation of simultaneous firing at the two recording sites, there was still a time lag in the upstroke of the responses. When the intensity of the field-stimulation was set at twice the threshold voltage, firing occurred simultaneously at the two sites. These findings indicated that when the strength of a field-stimulation is higher than the threshold voltage but insufficient for all muscle cells to excite simultaneously, firing of a cell with the lowest threshold may occur to propagated to the surrounding cells, but that when the strength of the field-stimulation is sufficiently strong, membrane action potential may be yielded.

Intensity of stimulation, latency and graded response

Fig. 3 A shows gradual shortening of the latency-the distance between the stimulation shock and the upstroke of an action potential (Fig. 1C)-with increasing intensity of stimulation. Panel B in Fig. 3 shows a finding, obtained from a different preparation,

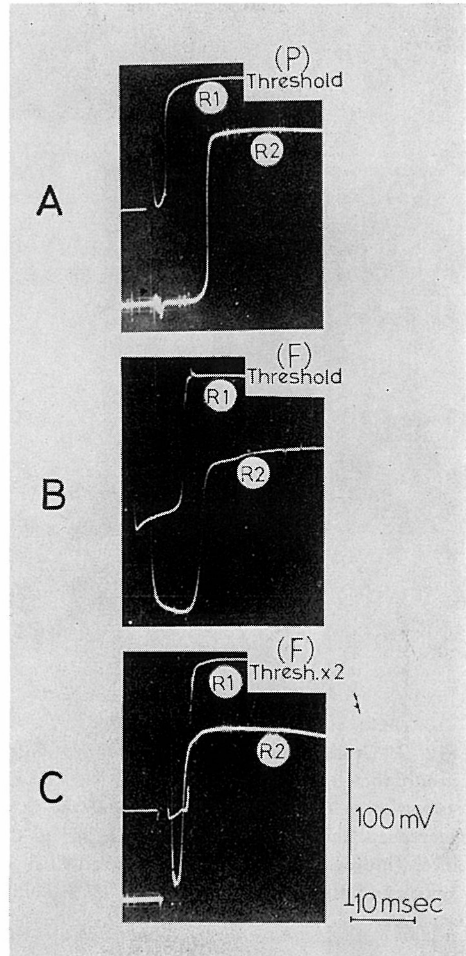

Fig. 2 Simultaneous recording of transmembrane action potentials at two different sites (R1 and R2) elicited by the point-stimulation at a voltage barely above the threshold (P threshold in A), field-stimulation at a voltage barely above the threshold (F threshold in B) and field-stimulation at a voltage twice the threshold (F thresh. ×2 in C). There is no delay in time between action potential upstrokes recording at R1 and R2 in the panel C.

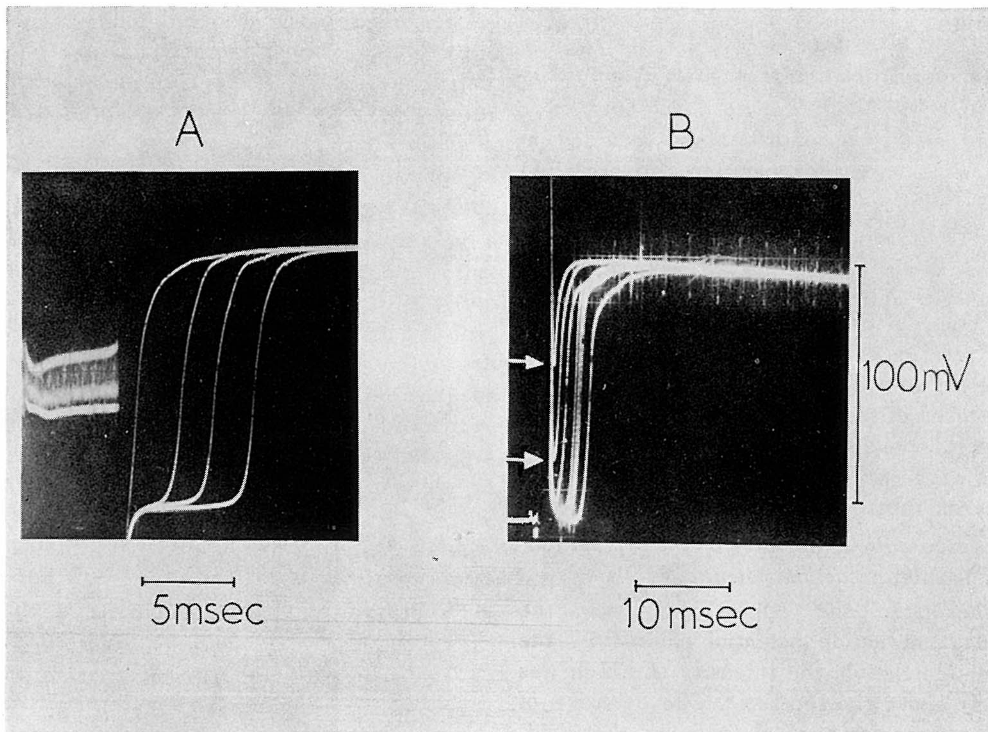

Fig. 3 Gradual shortening of latency (shift of the upstroke from right to left in the panel) in accordance with a progressive increase in intensity of stimulation (A). Shortening of latency and accerelation of the upstroke phase of the action potential (B). When the intensity of stimulus was increased, the membrane potential prior to the upstroke no longer returned to the resting potential level (indicated by arrows). Note gradual shortening in latency and gradual increase in height and velocity of the upstroke. Duration of stimuli was 5 msec in A and 1 msec in B.

similar to that in panel A. When relatively weak field-stimulation was applied, the membrane potential once returned to the resting potential level and the upstroke occurred from the level. However, when the intensity of stimulus was increased, the membrane potential prior to the upstroke no longer returned to the resting potential level. Under such a condition, both upstroke velocity and overshoot of the action potential were increasingly enhanced, whereas the latency was increasingly shortened. Such graded change in the action potential upstroke in response to the change of stimulation intensity is characteristic for the membrane action potential[7,8].

Discussion

In our present study, field-stimulation. when sufficiently strong, simultaneously fired the whole muscle preparation. The finding indicated the feasibility of field-stimulation in experimental induction of membrane action potential. Fig. 4 illustrates conventional sucrose gap. The central compartment in which sucrose solution flows is separated from the test compartment on one side and from the current injecting compartment on the other by rubber membranes. The preparation lies through a small hole in the rubber membrane. When the muscle in the test compartment is short as compared with the

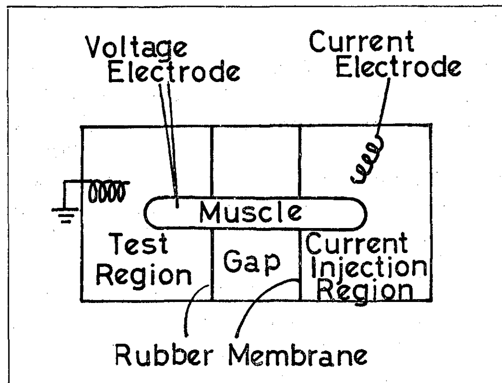

Fig. 4 Schematic illustration of a sucrose gap method. The central and side compartments are perfused with isotonic sucrose solution (electrically not conductive) and Ringer solution, respectively. The majority of the electric currents are forced to flow from the current injection pool through the myoplasm in the area of the test compartment due to the high extracellular resistance of sucrose solution[11].

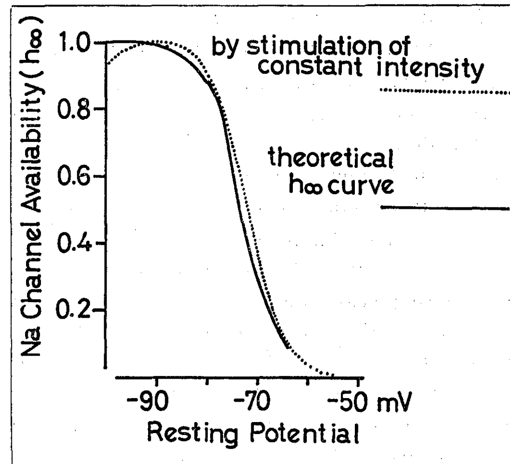

Fig. 5 Sodium inactivation curve (h ∞ curve) delineated on the basis of membrane potential–$\dot{V}_{max}$ relationship experimentally obtained (cited from ref. 9 with slight modification). When the stimulation of the constant intensity was applied, the latency was prolonged and $\dot{V}_{max}$ decreased at extremely hyperpolarized potentials (dotted line, at potentials negative to −90 mV). When the intensity of stimulation was altered to keep a constant latency, the $\dot{V}_{max}$–membrane potential relationship coincided with the theoretical h ∞ curve.

space constant** (see foot note), the potential gradient in the myoplasm of this compartment is small enough to reduce the current-flow in the myoplasm to zero. Because there is no current loss in the longitudinal direction during the action potential obtained by sucrose gap method, such will correspond exactly with the membrane action potential. The main advantages of the field-stimulation over the sucrose gap technique are the technical simplicity and the less damage to the preparation. Thus, stable data can be obtained throughout a long-lasting experiment. Its major disadvantage is that the amount of the electric current that flows through the preparation cannot be controlled. When the conventional point-stimulation method is used, there exists several factors which may affect action potential upstroke. For instance, the h ∞ curve (Fig. 5) obtained in the propagated action potentials, which is fired from an extremely negative membrane potential, was demonstrated to be lower than that predicted from the theoretical h ∞ curve, presumably due to an insufficient current supply[9]. In addition, since the conduction velocity in the nerve or heart is secondarily affected by primary changes of membrane properties (membrane capacity*, space constant** and membrane resistance),

*membrane capacity (Cm) : an excitable membrane behaves not only as resistor but also as capacitor. Cm can affect the action potential upstroke velocity ($\dot{V}_{max}$) as follows; $I_{ion} = Cm \times \dot{V}_{max}$, where I_{ion} denotes ionic current (almost excitatory Na current).

**space constant (λ): the distance with which the amplitude of electrotonic potential decreases to 1/e of the value at the stimulating site. $\lambda^2 = r_m/r_i$, where r_m and r_i denote the membrane resistance and internal resistance, respectively. Membrane potential can be adequately controlled by the external current-supply only within the space constant.

and since such changes, in turn, affect the action potential upstroke, we cannot attribute the change in $\dot{V}_{max}$ simply to that in sodium channel kinetics[10]. These defects on propagated action potentials are, to a large extent, overcome by the use of a membrane action potential. However, there remains an essential problem: that the value of $\dot{V}_{max}$ varies with an alteration of latency, associated with passive membrane properties, and that the length of latency can not be adjusted to a constant value before and after drug addition. The authors found that the latency was prolonged in the guinea-pig papillary muscles driven by field-stimulation when the class-1 type drugs or Ni^{2+} ion were added (unpublished observation). In particular, the length of latency in the premature action potentials are apt to prolong in comparison with that in the non-premature action potential. As stated above, the change in the latency thus produced may affect the $\dot{V}_{max}$ of the premature responses, therefore, a precise analysis of effects of class-1 drugs on $\dot{V}_{max}$ requires to maintain a constant length of latency throughout the whole time course of the experiment. In either way, this can not be done easily by the sucrose gap as well as by the field-stimulation. Nevertheless, our present study reveals that the field-stimulation may provide a methodical alternative to the sucrose gap method to obtain the membrane action potential.

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