

Membrane Currents in Mammalian Ventricular Heart Muscle Fibers Using a Voltage-Clamp Technique

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ABSTRACT Bundles of sheep ventricular fibers were voltage-clamped utilizing a modified sucrose gap technique and intracellular voltage control. An action potential was fired off in the usual way, and the clamp circuit was switched on at preselected times during activity. Clamping the membrane back to its resting potential during the early part of an action potential resulted in a surge of inward current. The initial amplitude of this current surge decreased as the clamp was switched on progressively later during the action potential. Inward current *decreasing* as a function of time was also recorded if the membrane potential was clamped beyond the presumed K equilibrium potential (to -130 mv). Clamping the membrane to the inside positive range ($+40$ mv to $+60$ mv) at different times of an action potential resulted in a step of outward current which was not time-dependent. The results suggest that normal repolarization of sheep ventricle depends on a time-dependent decrease of inward current (Na, Ca) rather than on a time-dependent increase of outward current (K).

INTRODUCTION

The action potential of mammalian heart ventricle consists of a rapid upstroke lasting about 1 msec, followed by a prolonged phase of repolarization lasting several 100 msec. The slowness of the change of membrane potential during repolarization suggests that there is almost no net ionic current through the membrane, and that outward current at any instant is slightly larger than inward current. On the concept that ionic currents are a function of membrane potential and *time* (Hodgkin and Huxley, 1952) the question arises whether a time-dependent increase of outward current (e.g. carried by K ions) or a time-dependent decrease of inward current (e.g. carried by Na or Ca ions) or possibly both mechanisms are responsible for the process of repolarization.

There is good evidence to show that in ungulate Purkinje fibers a time-dependent increase of outward current (K) is mainly responsible for repolarization (for references, see Noble and Tsien, 1969); and there is accumulating evidence indicating that in ventricular fibers repolarization is mainly brought about by a time-dependent decrease of inward current (for references, see Mascher and Peper, 1969; Beeler and Reuter, 1970 *b*). The present report is an extension of a preliminary communication (Giebisch and Weidmann, 1967). Since there is excellent agreement among three independent groups the present paper will be limited to a description of experimental procedures that are given no or little consideration by the other groups.

METHODS

Bundles of fibers from the right ventricle of sheep or calf hearts (0.4-0.6 mm diameter, 4-7 mm long) were mounted in a three-compartment chamber (Fig. 1). Thin rubber

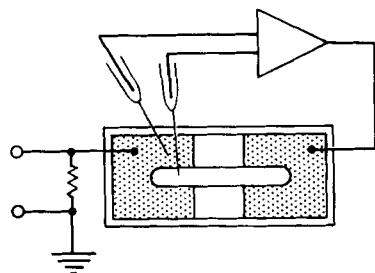


FIGURE 1. Block diagram showing myocardial preparation and voltage-clamp circuit.

membranes with punched holes (diameter about 0.1 mm smaller than diameter of preparation) separated the three compartments. A length of about 1 mm of the bundle extended into the test compartment. The middle compartment, 2 mm wide, was perfused by sucrose solution. The other two compartments were perfused by Tyrode solution containing 5.4 mM potassium. All experiments were carried out at 37°C.

Membrane potential was measured differentially between two Ling-Gerard electrodes, one of them intracellular, the other extracellular. Current was made to flow through the two Tyrode-perfused compartments; its strength was measured as a voltage drop across a series resistor. The feedback amplifier (USA 4, Philbrick/Nexus Research, Dedham, Mass.) received its command voltages (*a*) from the cathode follower output of one of the built-in amplifiers of a Tektronix 502 oscilloscope, (*b*) from a Grass square pulse generator, and (*c*) from a DC source. The USA 4 amplifier could be inserted into the feedback loop by relay-operated contacts, the relay being driven by another Grass stimulator. A third Grass stimulator provided driving stimuli (1 per sec) which were applied through an isolation unit and two 1 M Ω resistors. The circuit will not be described in greater detail since faster voltage-clamping of ventricular bundles is now possible by arrangements such as those described by Mascher and Peper (1969) and Beeler and Reuter (1970 *a*).

INADEQUACIES OF THE METHOD

Voltage clamping gives the clearest results if the fiber membranes can be kept at a uniform potential difference, and if the total current measured truly represents the current through the membranes under investigation. For several reasons these conditions were not ideally fulfilled.

(a) *Nonuniformity of Voltage Distribution* It is relatively easy to impose a preselected membrane potential to the site of the bundle where the transmembrane potential is controlled. The deviation of the internal potential in other parts of the bundle will depend on the internal longitudinal resistance, the strength, and the direction of membrane current (see Adrian, Chandle, and Hodgkin, 1970). In one experiment records of intracellular potential were taken simultaneously by two microelectrodes, one of them close to the diaphragm (0.3 mm) and the other near the end of the bundle in the test compartment (1 mm from diaphragm). Either one of these electrodes could be used as part of the voltage control system. Theoretical predictions were confirmed in all essential respects. Thus, the internal voltage near the end of the bundle was more negative than at 0.3 mm under all conditions of outward membrane current, e.g. during steady-state depolarization. In contrast, during the flow of inward current the internal voltage at 1 mm was more positive than at 0.3 mm. Deviations were small in the case of steady-state outward current (a few %), but appreciable during inward transients (see also Beeler and Reuter, 1970 *a*). For instance, at the onset of a clamp bringing the potential from its resting level to -20 mv (voltage control at 0.3 mm), the noncontrolled end of the bundle initially reached $+10$ mv. Upon clamping the membrane back to its resting potential (-80 mv) at the end of a pulse of 300 msec there was again an inward current transient, and the noncontrolled end initially reached an internal potential of only -60 mv.

(b) *Undesired Current Components* The preparations stayed in a better condition if KCl and CaCl_2 were added to the sucrose solution. While impulse conduction between the Tyrode compartments was blocked by the 2 mm sucrose gap, the ions present in the sucrose must have carried leak current during voltage clamping. Moreover, since the length constant (λ) in sucrose solution cannot be assumed to be negligibly small, some transmembrane current from fibers in the gap must have been added to transmembrane current entering or leaving the fibers in the test compartment. In general this component as well as the leak current would increase the measured current above the amount that crossed the fiber membranes in the test compartment.

(c) *Response Time* The clamp system was not sufficiently fast to hold a preselected membrane voltage under conditions of peak inward current, not even at the site of the control electrode. This problem is given an extensive treatment by Beeler and Reuter (1970 *a*).

For all the reasons listed under sections (a)–(c) no attempt will be made to relate peak current quantitatively to membrane potential, nor will any emphasis be placed on the time course of the transients. However, the conclusions regarding the *direction* of the major current components are thought to be valid in spite of all the limitations of the present method.

RESULTS

The general plan of the present approach was to interfere with a free running action potential at different times after its onset, forcing the membrane potential to a predetermined value, and measuring total membrane current. By looking at the relative amplitudes and the phasic changes of membrane current at selected voltage levels such as the presumed sodium equilibrium potential (E_{Na}) or the presumed potassium equilibrium potential (E_K) it should become possible to decide which of the current components increase or decrease.

In the experiment traced by Fig. 2 the membrane voltage was forced back to its resting level (-82 mv) at progressively later stages of the plateau. A

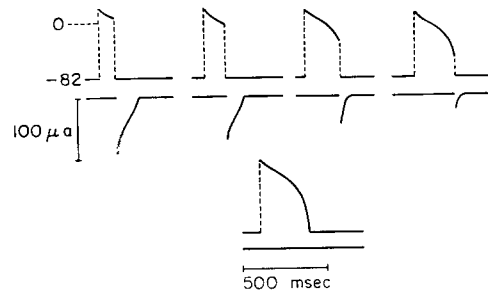


FIGURE 2. Tracings of membrane currents during voltage clamps to the level of the resting potential, applied at different times during the action potential. Note declining amplitude of inward transients as repolarization is initiated progressively later during the action potential.

large surge of inward current was observed when the potential was forced back to -82 mv early during the plateau; the amplitude of "inward tails" decreased when the clamp was switched on progressively later during the action potential. This result is readily explained by assuming a relatively large conductance of the membrane to an ionic species which has its equilibrium potential "above" the instantaneous voltage level (at inside more positive values). Repolarization by the "clamp" would greatly increase the driving force; the amplitudes of the tails would then be a relative measure of conductance (to Na and Ca) at the time of switching the clamp on (see Hodgkin and Huxley, 1952 *a*).

The result of clamping to *different voltage levels* is seen in Fig. 3 (left and right). The time course of the current was practically flat when the clamp took the membrane potential to highly inside positive values, into the region of the presumed sodium equilibrium potential ($+47$ mv, see Page and Solomon, 1960) and the calcium equilibrium potential ($+60$ mv, see Beeler and Reuter, 1970 *a*). The inward surges reached a higher amplitude when the voltage was

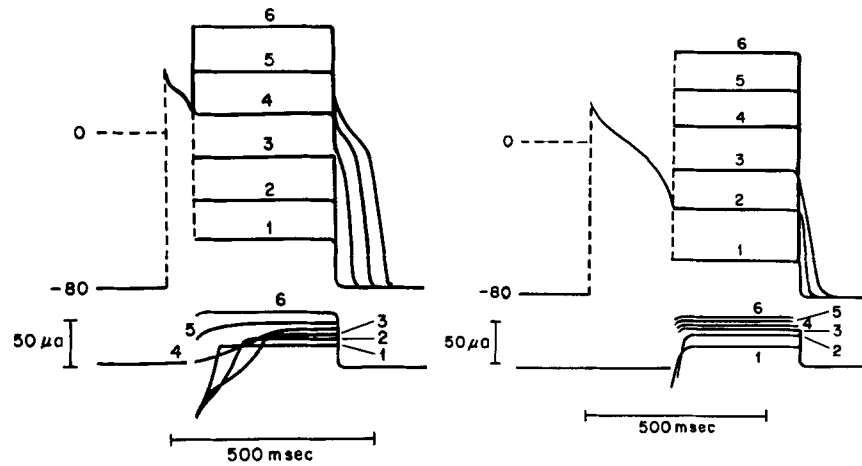


FIGURE 3. Membrane currents during clamps to different voltage levels. Left, clamp initiated 50 msec from onset of action potential; right, clamp initiated 225 msec from onset of action potential.

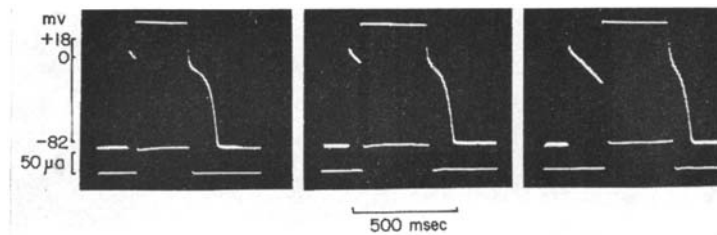


FIGURE 4. Membrane currents during voltage-clamp steps to the potential level of +40 mv applied 35, 75, and 175 msec from onset of action potential, respectively.

clamped to higher inside negative values; i.e., by a larger voltage step away from E_{Na} and E_{Ca} (Fig. 3, left). Again, as in Fig. 2, inward tails had smaller initial amplitudes when clamps were applied towards the end of the plateau. Inward tails still had the same direction (not illustrated) when the membrane potential was clamped from its plateau level to -130 mv which is considerably beyond the presumed K equilibrium potential, estimated at -92 mv by Page and Solomon (1960).

The result of clamping to the same strongly positive potential level at *different times* during the action potential is illustrated in Fig. 4. It is apparent that the magnitude of the flat outward current is independent of the duration of the preceding plateau phase of the action potential.

DISCUSSION

The conclusion that a decrease of inward current rather than an increase of outward current is mainly responsible for repolarization in mammalian ventricle rests on two kinds of observations: (a) When the membrane is clamped

to inside strongly positive potentials there is no clear indication of a slow increase of outward current as a function of time, as would be expected if the conductance of the membrane to an ion as, e.g. K with an equilibrium potential in the negative range slowly increased; (b) there is positive evidence to show that the conductance of the membrane to ions with an equilibrium potential in the positive voltage range decreases when natural repolarization proceeds (diminishing amplitudes of inward tails).

No attempt has been made to identify the carriers of charge for inward current. From experiments by Mascher and Paper (1969) and by Beeler and Reuter (1970 *b*) the conclusion seems justified that Ca ions rather than Na ions are involved during the main part of the action potential. A relatively small contribution to the total inward current by Na throughout the plateau seems not excluded since Délèze (1959) finds a shortening of the ventricular action potential in Na-poor (20% Na) solutions.

The deficiencies of the present method do not allow much significance to be attached to absolute amplitudes and to shapes of the recorded tails. Nevertheless, from the records traced in Fig. 2 it seems reasonable to suggest that inactivation of inward current becomes more rapid as the membrane repolarizes. This effect in combination with "anomalous rectification" would provide an explanation (see also Beeler and Reuter, 1970 *b*) for the typical shape of the cardiac action potential: a small rate of voltage change during the plateau, followed by a relatively rapid phase of repolarization.

The time course of the membrane potential after the "off" of a clamp such as is seen in traces 4, 5, and 6 of Fig. 3 (left) lends independent support to the idea that decrease of inward current depends on the level of membrane potential. Thus, by holding the potential at the plateau level (trace 4) or even by shifting it to more positive values during 300 msec (traces 5 and 6) the process of repolarization is markedly postponed.

Note Added in Proof Recent experiments by J. A. S. McGuigan (1970, *Experientia*, **26**: 682) in which ventricular bundles were voltage-clamped into strongly positive voltage ranges for several seconds indicate the presence of some delayed rectification which could play a role in the shortening of the action potential during rapid stimulation.

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