Note on the Time Course of Slow Inward Current of Sheep Heart Purkinje Fiber

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(Received February 16, 1972)

The application of voltage clamp to cardiac musculatures[1)2)3)] has enabled to elucidate the properties of membrane currents relating to the plateau phase of cardiac action potential. Of these currents, it has been reported that K^+ current and Ca^{++} current play important roles for maintaining the plateau, although Na^+ current has also a little effect on it. Noble et al.[4)] reconstructed a computed action potential of sheep heart Purkinje fiber based on the experimental data and pointed out that the role of K^+ should be emphasized to explain the plateau mechanism. Some other reports[5)6)7)] have described about the importance of Ca^{++}current in plateau. These authors seem to consider that the sodium current is not so much of importance for holding plateau level, especially in the late stage of plateau, because the current which may be carried by Na^+ disappears within 100 msec at longest. Giebisch & Weidmann[8)] have recently reported that a very long inward current can be recorded in the ventricular muscle of sheep heart and quite a few part of plateau potential may be maintained by Na^+ inward current. Vitek & Trautwein[9)] have also demonstrated that there is a different kind of sodium inward current whose duration is far longer than those shown by Noble et al.[4)], However the inward current reported by Vitek & Trautwein[9)] took a little different time course from that of Giebisch & Weidmann[8)] and decreases exponentially with the progress of time.

Results shown in Fig. 1 may suggest the reason why there are considerable differences in time course of Na^+ currents between these two reports[8)9)] recently published. Fig. 1 shows a series of voltage clamp carried out on a short strip of sheep Purkinje fiber which was crushed at interval of 2.2 mm. Two microelectrodes were inserted into the short fiber and membrane currents were measured. Fig. 1 shows the action potential elicited by the stimulation which was given through a pair of silver electrodes placed near the crushed part. The action potential in Fig. 1, 2 was elicited by intracellular current passing. Each action potentials show a considerably good shape as ordinarily observed in long preparations. Membrane currents recorded at various levels of membrane potential are shown from 3 to 11 in Fig. 1. Inward currents are progressively decreased as the membrane potentialis clamped at more depolarized level. Those inward currents diminish

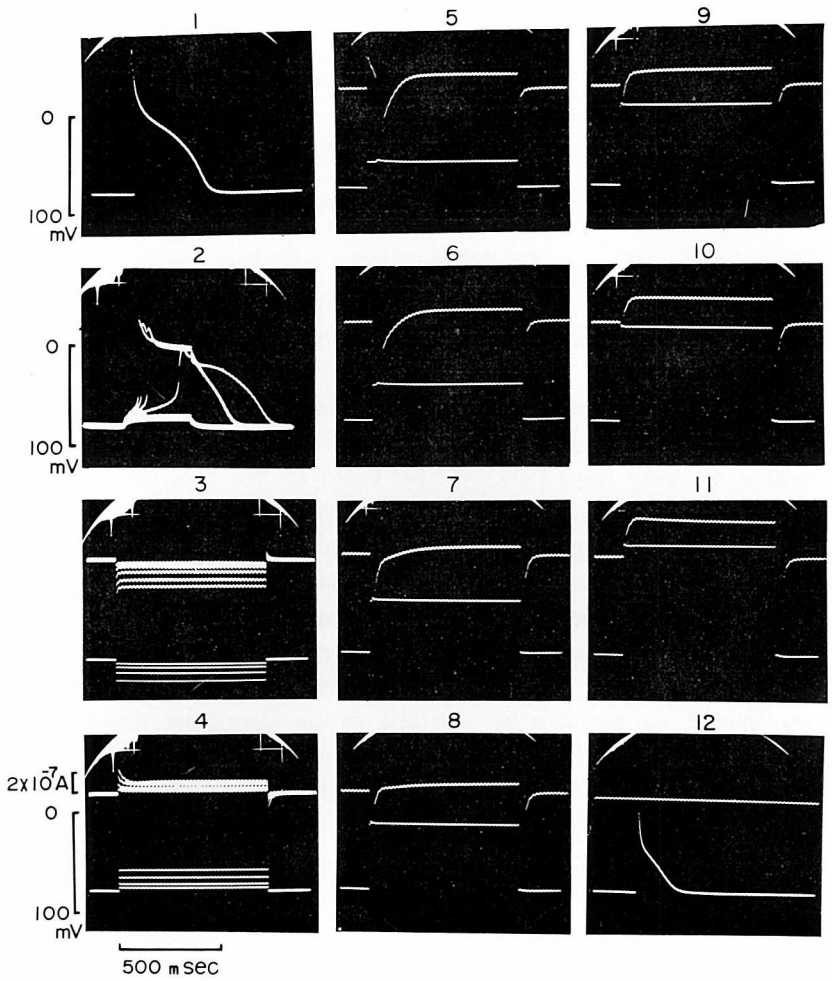

Fig. 1. Action potentials recorded from short Purkinje fiber and membrane currents at various clamped potential level.

1. action potential elicited by external stimulation.
2. action potentials elicited by intracellular current passing.
3. membrane current (upper trace) by hyperpolarizing clamp.
4—11. membrane currents when fiber is clamped at various depolarized level.
12. action potential elicited by external stimulation after voltage clamp experiment.

Note that the duration of action potential is greatly shortened after voltage clamp and note also that there can be seen a small notch on the potential recording when membrane is excited (5–9).

always smoothly from the peak to the end of clamp, but no distinct bend can be seen in any stages. Observations like this record have been often reported[2)3)5)6)] as representative time course of inward current in cardiac muscles. However, it is important to note that the configuration of action potential in Fig. 1, 12 differs remarkably from that in Fig. 1. 1 The action potential in Fig. 1. 12 was recorded immediately after the feedback circuit for voltage clamp was disconnected. It is very interesting that a distinct plateau phase can hardly be observed in this action potential and the duration of action potential are markedly shortened. The time courses of inward currents are all smooth and look simply exponential decay, it can not be thought that any sudden change happens to this preparation while the voltage clamp was being done. Hence this finding may lead a possible conclusion that the membrane property of this preparation was altered at the instance of the commencement of voltage clampand this alteration became gradually severer with the progress of time. Therefore it is also possibly assumed that the membrane currents in Fig. 1 are recorded from the preparation which merely show the so-called triangle action potential. Tracings shown in Fig. 2 were recorded from another short fiber and this experiment was done to examine the relationship between the length of action potential and that of inward current. Record shown in the left (from 1 to 4) are the action potentials elicited by the application of a short square pulse (100 msec in duration). It can be seen that the more intensity of current was given, the shorter duration of action potential was recorded. In both the middle and right, results of the voltage clamp which was modified for the purpose of this experiment were shown. First the membrane potential was held at the level of resting potential and the short square pulses were given to the fiber through the feedback system and the current passing electrode. It would be expected that the changes of membrane currents in the cardiac membrane shich is intracellularilly activated can be measured. It is clearly recognizable that the decay time of inward current is progressively shortened as the membrane potential is transiently held at depolarized level. When the membrane potential is held at a little depolarized level (about −50mV, 5 of Fig. 2) the inward current can be observed even in 400 msec after the onset of clamp and when held at about 0mV (10 in Fig. 2) the inward current disappears within 250 msec. This comparison may indicate that the sodium inward current can flow almost at the end of action potential and plays a considerably important part for maintaining the relatively late stage of plateau, as Weidmann[8)] has suggested and illustrated. This experiment may also suggests that the short inward currents previously reported may be obtained from whose membrane property was altered by the application for voltage clamp.

On the other hand, tracings of membrane current of some preperations of sheep heart Purkinje fiber show a slowly decreasing rate of inward current as issultrated in Fig. 3A. The time course of the slow inward current looks appearently like an

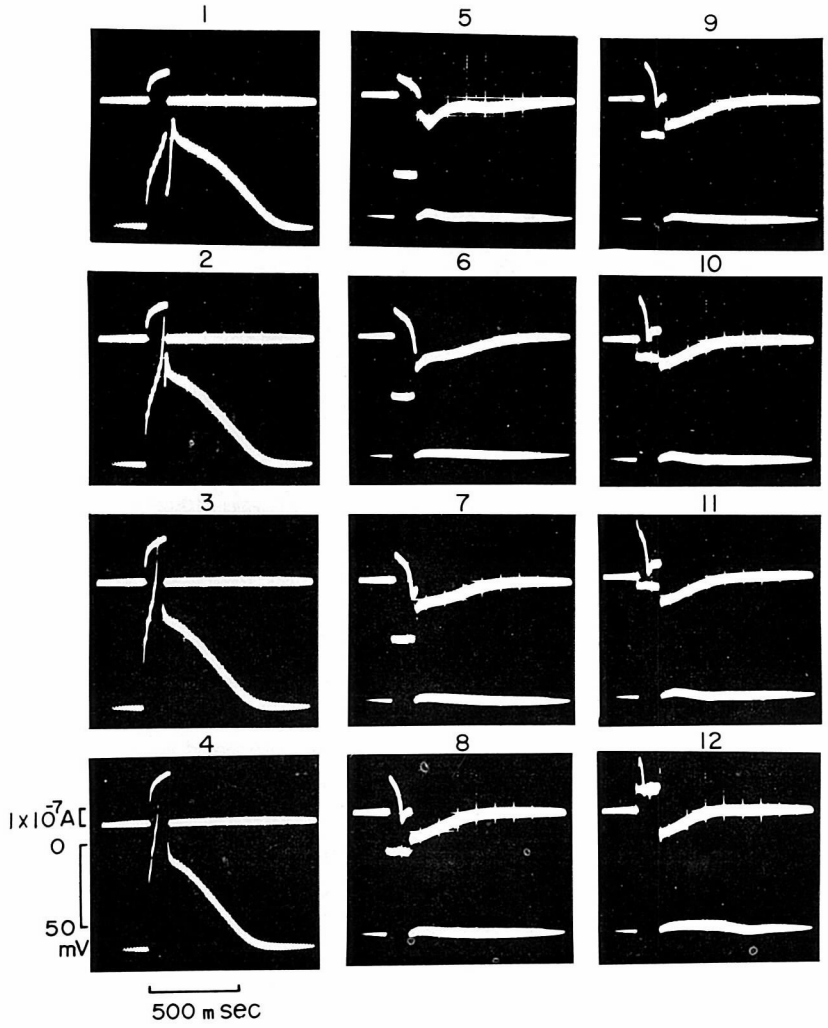

Fig. 2. Changes in configuration of action potential when the membrane is transiently (Ca, 100 msec) depolarized (1–4) and changes in time course of membrane current when the membrane is transiently (Ca, 100 msec) clamped (5–12). Note that the duration of action potential is progressively shortened as the membrane is gradually depolarized and the inward currents disappear earlier as the membrane is depolarized.
Current is shown in upper trace and potential is shown in lower trace.

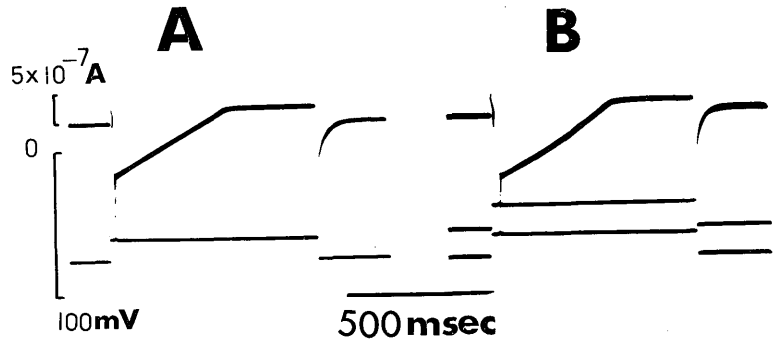

Fig. 3. A. The time course of membrane current which shows a relatively long inward component (upper trace). Configuration seems to be very similar to the inverted shape of cardiac action potential.

B. The membrane current (upper trace) and the clamped potentials rec orded from two electrodes which were very closely placed (middle and bottom). Note that there can be hardly recognized any difference between these two traces. Membrane potential was shifted from 74mV to 58 mV.

inverted shape of action potential and differs greatly from those of Fig. 1. However, good action potential could be recorded only from such a preparation whenever the feedback system was disconnected. It must be noted that the duration of inward current does not exced more than 200 msec and the slow change ends within about 300 msec by weekly depolarizing clamp when the action potential duration is more than 500 msec. These records are very similar to those depicted by Giebsch & Weidmann[8]. Weidmann[10] does not completely put his confidence on those records himself, although he still believes that there might be a relatively long inward current, because he thinks those records might be obtained from the preparations which were incompletely clamped. Then, in order to examine the completenes of voltage clamp, third electrode was inserted into the near part of second electrode. Membrane potentials were also recorded from the third electrode when the current for clamp was passed through feed back system. Each membrane potential recorded from both electrode (second and third) were hardly different in shape (Fig. 3B). This fact may indicate that the short fiber was clamped enough to elucidate the time course of membrane current.

One may argue that there might still be some current escape because the current tracing in Fig. 3A shows a small notch on the potential recording. However, the small notch can be also seen in the tracing recorded from the preperation showing a short current duration (Fig. 1). Therefore the existense of the small notch in potential recording does not seem to be directly related to whether the duration of membrane current is long or short and it can not always be concluded that the short membrane current can be obtained from the preparation which is

well clamped. Surveying the experimental data shown above, it is assumed that the altered properties of small fiber resulted in short membrane current and the long inward current should be recorded as long as the membrane properties is well maintained after pinching procedures.

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