Effects of Temperature on Membrane in Sheep Heart Pubkinje Fibers Currents

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ABSTRACT

Influences of temperature (32°C−38°C) on membrane currents of sheep heart purkinje fiber were examined by means of voltage clamp technique which was done by double microelectrode impalement into a short strip of the fiber. A slowly decreasing inward current following to an initial rapid inward current was observed in Na-Tyrode solution when the membrane potential was held between −20mV and −40mV. In Na-free solution (Choline-Tyrode), only outward current which gradually decays with the progress of time was observed in depolarizing clamp.

The time course of slow inward current is shortened in high temperature, but not much prolonged in low temperature. The time course of outward current is prolonged in low temperature but a little shortened in high temperature. The results suggest that the shortening of action potential duration in high temperature is mainly caused by the early disappearance of slow inward current carried by Na^+ and the prolongation is mainly caused by the slow decay of the outward current carried by K^+.

KEYWORDS voltage clamp slow inward current
voltage-current relationship plateau phase Ca^{++}

INTRODUCTION

The roles of delayed rectification of cardiac muscle[11)12)16)17)] calcium current, and slow inactivation of sodium have been discussed on mechanism fof maintaining a long-lasting plateau phase in action potential of the heart muscle. Noble and Tsien[11)12)] have pointed out tht the mechanism responsible for maintaining the plateau level can be explained through an analysis of slow outward and inward potassium currents. However, several recent investigations performed on the ventricular muscle of sheep heart[9)10)13)17)] have shown that a slow inward current lasts relatively long and the slow inward current is more greatly responsible for holding the potential level of plateau than the slow increase of outward current.

Trautwein of Vitek[20] have found a similar slow inward current in sheep heart purkinje fiber and have pointed out that the slow inward current is mainly carried by Na^+ and Ca^{++} does not much contribute to maintain the plateau potential in normal condition.

In the present paper, it will be confirmed that the above conclusions[20] are generally coincident with the experimental results which were independently obtained and it will be shown how these slow currents alter their time courses under the influence of temperature.

MATERIALS AND METHODS

Sheep heart Purkinje fibers taken from the left ventricle were used in all the experiments. The sheep were killed in a slaughter house, and the heart was very quickly taken out and immersed in a thermos filled with warm and well-oxygenated Tyrode solution. Transportation of the heart to the laboratory usually took thirty minutes. The Tyrode solution composition was Na, 149 ; K, 4.0 ; Ca, 1.8 ; Mg, 1.05 ; Cl, 135 ; in mMol/1. The solution was buffered by a phosphate system (HCO_3, 13.5 ; H_2PO_4, 2.4 in mMol/1). The NaCl in the Tyrode solution was replaced by re-crystalized choline chloride in equimolar weight, and 5×10^{-7} g/1 atropine was added to this Na-free Tyrode solution. The pH of the Na-free solution was adjusted by a TRIS-malonated buffer system. The Purkinje fibers were pinned on a rubber plate in a small plastic chamber and the test solutions described above were passed through them. The test solutions were permeated with a mixture of 95 % oxygen and 5 % carbon dioxide. Initially, strips of fibers were prepared by three methods ; pinching, ligation and severance. These three methods were in accordance with the methods described by previous workers[5)6)].

Fiber which could be well isolated electrically could show a good contour of action potentials was selected. After some experimentation it was found that crushing was the most suitable for our purposes.

For samples prepared by crushing, long strips of fibers (5mm–7mm) were crushed with small forceps at intervals of 0.5 mm to 2.0 mm. The temperature of the bathing fluid was carefully monitored by a thermister thermometer standardized by a mercury thermometer. A circuit diagram of the feedback system for the voltage clamp is shown in Fig. 1. This circuiting diagram is essentially the same as those of previous workers[5)6)] and PHILBRICK P65A and USA-4JX were used for feedback amplifiers. The recording electrodes were filled with 3Mol/1 KCl and their resistances are between 8 and 15 megohm ; the current-passing electrodes were filled with 2Mol/1 potassium citrate. The resistance of the second electrode was about the same as that of the recording electrode.

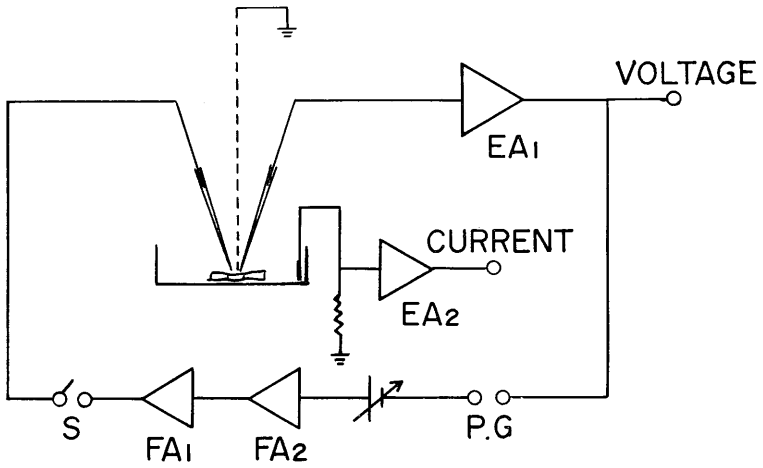

Fig. 1. Block diagram of circuiting for voltage clamp experiment. EA_1 ; electrometer amplifier for measuring voltage, EA_2 ; electrometer amplifier for measuring current.
FA_1 ; feed back amplifier (PHILBRICK-NEXAS P65A)
FA_2 ; feed back amplifier (PHILBRICK-NEXAS USA-4JX)
P. G. ; pulse generator (TEKTRONIX TYPE 162 and TYPE 161)

After careful examination we found that the short fiber made by pinching could not conduct action potential to the outside of the pinched section and stimulation from one was always ineffective in any type of action potential in the pinched part. Action potentials from such preparations can be elicited only when a field stimulating electrode is placed on the pinched part or current is passed through the second electrode.

RESULTS

Figure 2 shows changes in the action potential configuration as a result of the changing environmental temperature. These action potentials were recorded from one preparation already electrically isolated by pinching. It is clearly demonstrated that lower temperature results in longer action potentials and higher temperature results in shorter ones. Because of a relatively high concentration of K^+ in the Tyrode solution (4mM/1), spontaneous excitation did not occur when the temperature was lower than 39°C, but action potentials were obtained by the field stimulation as noted in the section describing method. When the temperature was higher than 39°C, spontaneous firing could be observed in some preparations. Even when the action potential could be elicited only by field stimulation, changes in the slope of the slow depolarization phase resulting from a change of temper-

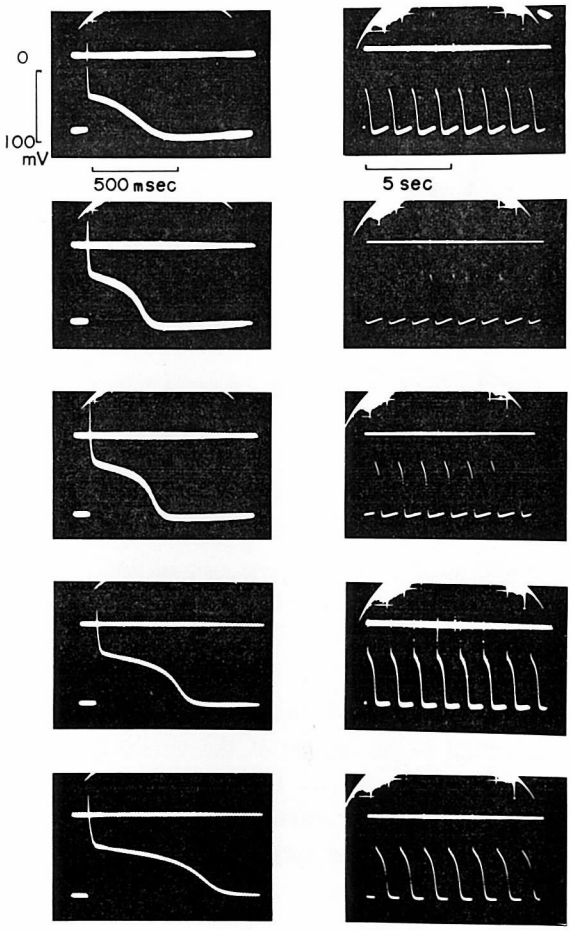

Fig. 2. Changes of action potential configuration at various temperatures. Note that the slope of diastolic depolarization is steepened in high temperature and action potential duration is very much prolonged in low temperature. Vertical mark shows 0–100mV and horizontal marks show 500 msec in left and 5sec in right respectivel.

ature were observed. As many authors have pointed out: the higher the temperature, the steeper the slope[3)4)9)18)19)20]. In a Na-free Tyrode solution, the membrane resting potential tended to decrease in magnitude as a result of temperature either higher or lower than 36°C.

In Fig. 3, the current change at various levels of clamped potential in NaCl-Tyrode solution at 36°C is traced as a control, using the depolarizing clamp, the outward current progressively increased. But when the potential was clamped at certain level (threshold) abrupt inward current flow could be observed. The inward current quickly decreased and began slow decay. It must be noted that

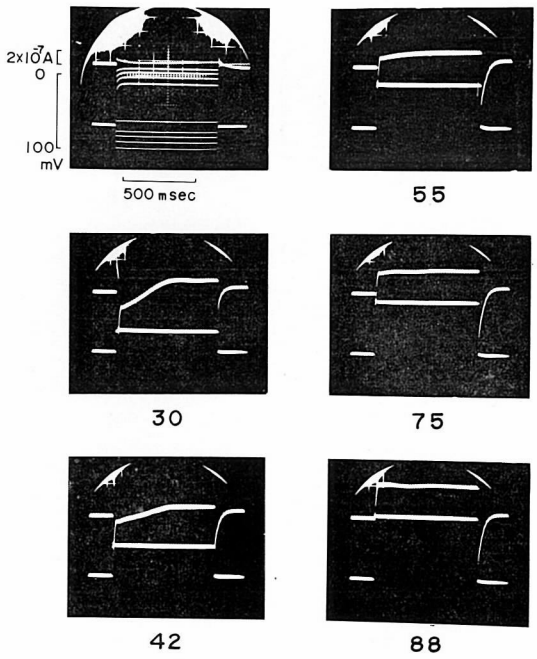

Fig. 3. Currents at various levels of clamped potential in Na-Tyrode of 36°C. Upper traces show current tracing and lower traces show holding potential. Downward is inward and upward is outward in current tracings. Note that the rapid inward current disappears in very early stage and this current begins to diminish relatively slowly with a distinct bend. Inserted marks show 0–100mV, 1×10^{-7} A in vertical and 500 msec in horizontal respectively. Calibrations are the same in Fig. 4 and Fig. 5. Each numbers show the holding potenial (the membrane potential is originally held at 70mV)

the abrupt inward current does not move smoothly on the follwing decay but that a distinct break can be recognized between the stages. This distinct break reveals that the slowly changing inward current may be carried by a system different from that does the initial abrupt one. When the clamp was applied at more depolarized level, the decay time of the slow inward current generally increased, but the intensity of the current gradually decreased. Consequently, the polarity of the current was finally reversed, and no slow inward current could be observed when the membrane potential was held at further depolarized level. In a hyperpolarizing clamp, the time course of the current is similar to that of a given voltage but a small notch of inward current in the very early stage just after onset of clamp.

Results obtained in a Na-Tyrode at 38°C using the same procedures are shown in Fig. 4. The time courses of the current are generally similar to those at 36°C. However, it should be noted that the decay time of the inward current

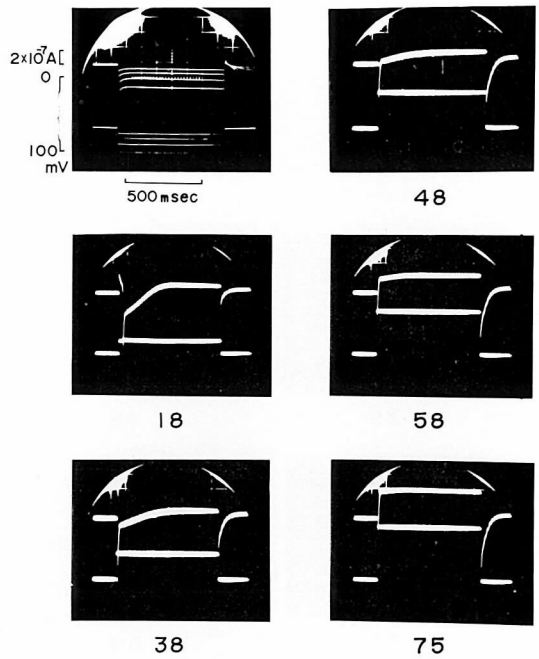

Fig. 4. Currents at various levels of clamped potential in Na-Tyrode of 38°C. Slow decay following to the rapid inward current diminishes a little faster than in 36°C. Each numbers show the holding potential (the membrane potential is originally held at 68 mV)

is a little shorter at 38°C, than at 36°C, although maximal value of the inward current during clamp at a given voltage does not differ from that of the control, except in the very early stage (within 50 msec).

Current changes in low temperatures (34°C and 32°C) are also shown in Fig. 5. The slow inward current does not decay in the relatively early stage of depolarizing clamp, but it could be found in a later stage than in the control. This tendency is more clearly found at 32°C than at 34°C. It is interesting that the slow inward currents by depolarizing clamp always disappear just before the time that the action potential is completely repolarized. In other words, although the disappearance of the slow inward current is delayed in low temperature, the prolongation of the action potential is much more evident than the delay of the decay time in current recording. This tendency is progressively clearer as the temperature is lowered. In hyperpolarizing clamp, it could be observed that inward currents increased in high temperatures and decreased in low temperatures. In order to investigate the relationship between current change and temperature change in more detail, the graphs in Fig. 6 and Fig. 7 were prepared. As shown in these graphs, although the intensity of inward current is greatest at 38°C and smallest

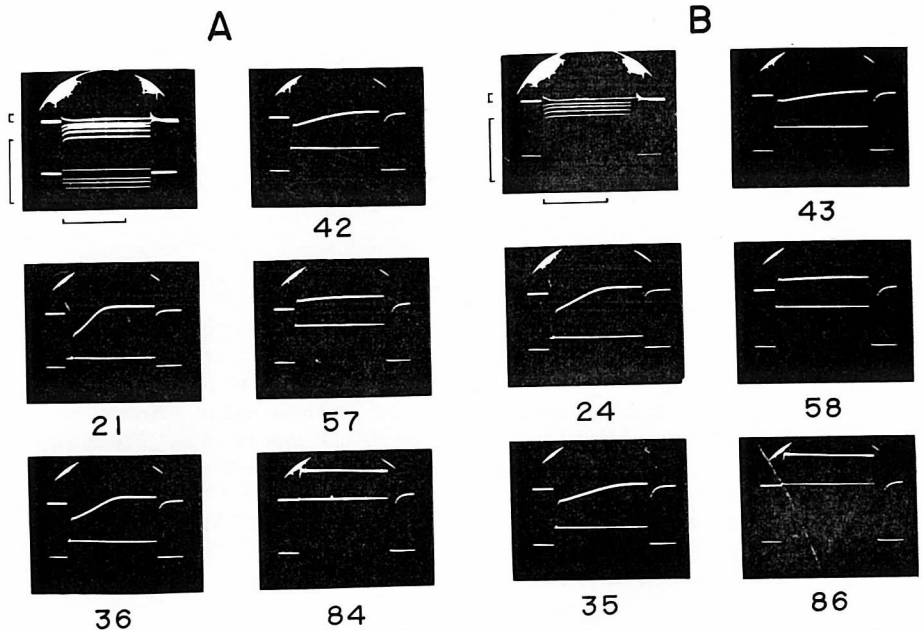

Fig. 5. Currents at various levels of membrane potential in Na-Tyrode solution of 34°C (A) and 32°C (B) are shown. Time course of slow inward currents does not seem to be greatly affected by temperature change. However, slight prolongation of decay time are observed when the membrane potential was held at about between -20mV and -30mV.
Each numbers show the holding potential (the membrane potential is originally held at 77mV in A and 76mV in B respectively)

at 32°C in 100 msec, there are not much differences between them. However, the decrease of inward current is progressively delayed as the temperature is lowered. In fact, the inward current can hardly be observed in 200 msec at 38°C, but it is still recognizable even in 200 msec at 32°C. These comparisons clearly show that the rate of decay is slowed in low temperatures ; this may indicate how the plateau phase of action potential is prolonged by low temperature and is shortened in high temperature.

Voltage clamp experiments in Na-free Tyrode solution were also carried out at 32°C, 36°C and 38°C, respectively, using the same procedures mentioned above (Fig. 8A). Current tracing in all cases showed no inward current in depolarizing clamp at any temperature range. Outward currents appearing in the onset of depolarizing clamp usually showed a gradual decay. In some ranges of clamped voltage, most frequently between -20 and -40 mV, a peculiar course of the current was observed in some preparations. As shown in Fig. 9A, outward current appearing at early stage decreased first and then showed gradual increase until the end of clamp. A similar time course of current tracing could be found even in the Na-Tyrode solution. Rougier et al.[16)17)] have reported similar observations

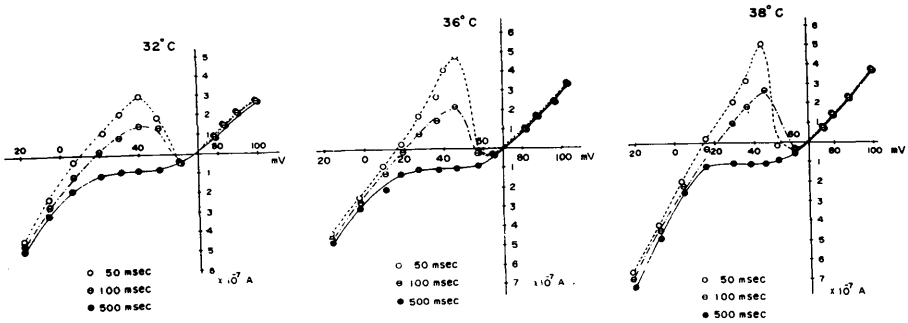

Fig. 6. Left; Voltage current relationship in NaCl-Tyrode of 32°C. It can be observed that the inward current is decreased in either 50 msec and or 100 msec.
Middle; Voltage-current relationship in NaCl-Tyrode of 36°C. The inward current is clearly found in 100 msec. Note that the outward current in 500msec is decreased at the level between –20mV and –40mV.
Right; Voltage current relationship in NaCl-Tyrode of 38°C. The inward currents in 100 msec is a little increased compared with 36°C, but they are almost the same in 50msec.

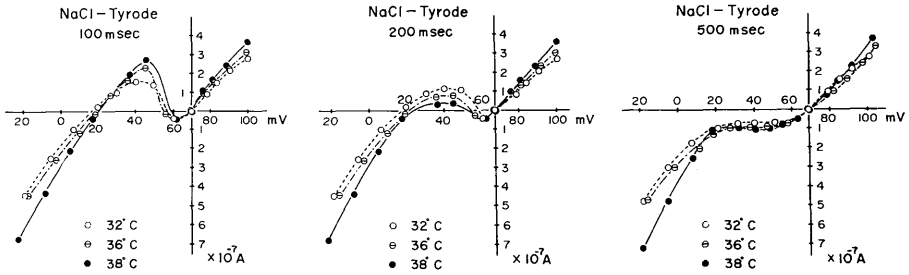

Fig. 7. Comparison of voltage current relationships in 100msec (left), 200msec (middle) and 500msec (right) after onset of clamp in Na-Tyrode solution of 32°C, 36°C and 38°C. At 38°C, largest inward current can be observed in 100 msec in depolarizing clamp. Note that inward currents in 200msec can be hardly found at 38°C, although they are clearly recognizable in either 36°C or 32°C. In 500msec, the largest outward currents at 38°C and the smallest outward currents at 32°C are observed in depolarizing clamp.

and have suggested that this slowly increasing current may be caused by delayed rectification property of cardiac muscles of the frog. However, in this experiment, the slowly increasing current can be seen only by adding a small amount of calcium (more than three times of the normal concentration, 5.4mMol/1) to the ordinary Tyrode solutions (Fig. 9 A). It must be noted that a more excessive amount of calcium (four times of the normal concentration, 7.2mMol/1) produced a slow inward current flow in a relatively early stage of clamp and the gradual increase of outward current become clearer (Fig. 9B). Thus we consider that the

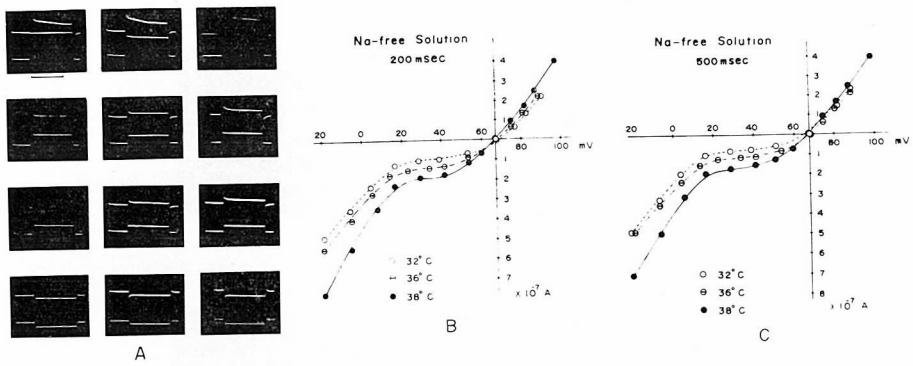

Fig. 8. A; Current ıracing s of voltage clamp performed in NaCl-free solution of 32°C (right), 36°C (middle) and 38°C (left). Marks show 2×10^{-7} A, 1–100mV and 500msec. The outward currents decay gradually just after onset of depolarizing clamp at any temperature ranges.

B and C; Voltage current relationships in 200msec (B) and 500msec (C) in Na-free Tyrode solution are shown. The largest outward currents are observed at 38°C in either 200msec and 500msec in depolarizing clamp. Note that the outward currents tend to be somewhat decreased between –20mV and –40mV, especially in 38°C. Any inward current could not be observed.

deflection of the inward current at 50–100 msec and following increase of the outward current may mostly result from calcium ions. This assumption seems to be supported by the fact that the addition of calcium makes the hump on plateau phase[9].

The outward currents by depolarizing clamp in Na-free Tyrode solution increased in high temperatures and decreaseed in low temperatures, although no remarkable difference in the decay time from initial peak between high and low temperature was found. Fig. 8B and C show a summary of the results obtained in a Na-free solution at 32°, 36° and 38°C. The outward currents in depolarizing clamp are clearly increased at 38°C and decreased at 32°C, either in 200 msec or in 500 msec. When the hyperpolarizing clamp was done, such remarkable influences by temperature change were not found in Na-free Tyrode solution. But a small increase of outward current at high temperature and a small decrease at low temperature were found. It could be observd that the outward current decreases at the potential levels between −20mV and −40mV in Na-free Tyrode solution. However the decrease of current is very small compared with the change of inward current obtainid in Na-free solution and no reversal of the direction of current could be observed.

DISCUSSION

Results obtained above suggest that the plateau in action potential of Purkinje

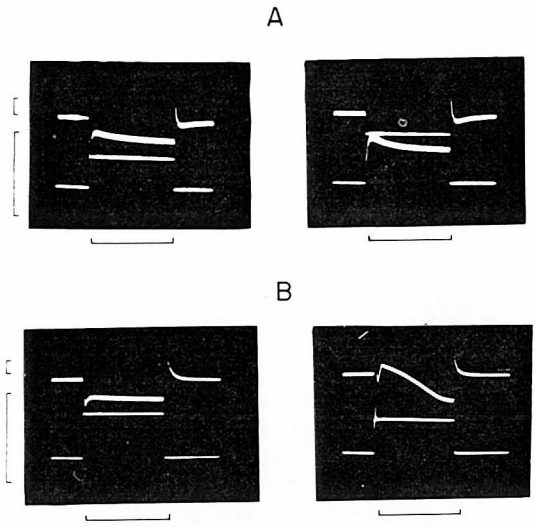

Fig. 9. A ; Current tracings by the voltage clamp performed in Na-free Tyrode containing excessive amount of Ca^{++}. Currents are recorded in reversed directions. upward ; negative, downward ; positive. The slowly increasing current in the later stage of clamp is found. Left ; current is recorded in normal Na-free Tyrode solution containing 3.6 mM/1 of Ca^{++} (twice the normal amount). Right ; Current is recorded in Na-free Tyrode solution containing 5.4 mM/1 of Ca^{++} (three times of the normal amount). The slowly increasing rate of outward current is enhanced by the excessive amount of Ca^{++}. Vertical short mark shows 1×10^{-7} A, vertical long mark shows 0–100mV and horizontal mark shows 500msec. (Calibration are the same in Fig. 9 B)

B ; Another example of current tracings by the voltage clamp in Na-free Tyrode solution. Currents are recorded in reversed directions, upward ; negative, downward ; positive. Left ; Current recorded in normal Na-free Tyrode solution containing 3.6 mM/1 of Ca^{++} (twice the normal amount). The outwartd current is slowly increased in the later stage. Right ; This slowly increasing current is clearly enhanced by 7.2mMol/1 of Ca^{++} (four times of the normal amount). Note that inward current can be observed in 50msec.

fiber is mainly held by the slow decrease of inward current rather than the slow increase of outward current and this slow inward current is mainly carried by Na^{+}, as Vitek & Trautwein have pointed out. A distinct break between the initial rapid current and the slow current may reveal that this slow inward current may be carried through a different channel from that responsible for the initial rapid one as some authors have suggested[7)11)21)]. Although the contribution of Ca^{++} to the plateau potential could not quantitaively measured, it can not be considered that the currents carried by Ca^{++} plays an important part to maintain the plateau in normal condition (Fig. 9). It should be considered that Ca^{++} affects the membrane properties and give some effects on the conductances for other ions,

for example, Na^+ and K^+.

The fact that the slow inward currents become short in high temperature (38°C) suggests that the shortening of action potential in high temperature is mainly brought about by the early disappearance of the slow inward currents.

Since a minute analysis of tail of the slow inward current could not be done, reasonable conclusion on the currents relating to the final stage of action potential can not be provided. However, it may be assumed that the slow decrease of outward current is more greatly responsible for the final stage than the slow inward current, because the duration of action potential is marked prolonged in low temperature (more than 750 msec at 32°C, Fig. 2), although the slow inward current diminishes in relatively early stage (within 300 msec at 32°C). Nevertheless the complex situations on the mechanism relating to the final stage of action potential remain unexplained, because this mechanism should be discussed in the connection with the combined effects of anomalous rectification and Na^+-inactivations as Giebrisch & Weidmann[8] have suggested.

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