EFFECT OF STROPHANTHIDIN ON INTRACELLULAR Na ION ACTIVITY AND TWITCH TENSION OF CONSTANTLY DRIVEN CANINE CARDIAC PURKINJE FIBERS

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ABSTRACT Intracellular Na ion activity (a^i_{Na}) and twitch tension (T) of constantly driven (1 Hz) canine cardiac Purkinje fibers were measured simultaneously and continuously with neutral carrier Na^+-selective microelectrodes and a force transducer. The a^i_{Na} of 8.9 ± 1.4 mM (mean ± SD, n = 52) was obtained in the driven fibers perfused with normal Tyrode solution. Temporary interruption of stimulation showed that a^i_{Na} of the driven fibers was ~1.5 mM greater than that of quiescent fibers. The constantly driven fibers were exposed to strophanthidin of 10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, and 10^{-6} M for 5 min. No detectable changes in a^i_{Na} and T were observed in the fibers exposed to 10^{-8} M strophanthidin, and the threshold concentration of the strophanthidin effect appeared to be ~5×10^{-8} M. With concentrations >5×10^{-8} M, strophanthidin produced dose-dependent increases in a^i_{Na} and T. An increase in a^i_{Na} always accompanied an increase in T and after strophanthidin exposure both a^i_{Na} and T recovered completely. During onset and recovery periods of the strophanthidin effect the time course of change in a^i_{Na} was similar to that of change in T. A plot of T vs. a^i_{Na} during the onset and recovery periods showed a linear relationship between T and a^i_{Na}. These results indicate strongly that the positive inotropic effect of strophanthidin is closely associated with the increase in a^i_{Na}. Raising $[K^+]_0$ from 5.4 to 10.8 mM produced decreases in a^i_{Na} and T, and restoration of $[K^+]_0$ resulted in recoveries of a^i_{Na} and T. During the changes of $[K^+]_0$ the time course of change in a^i_{Na} was similar to that of the change in T. A steady-state sarcoplasmic Ca ion activity (a^i_{Ca}) of 112 ± 31 nM (mean ± SD, n = 17) was obtained in the driven fibers with the use of neutral carrier Ca^{2+}-selective microelectrodes. Temporary interruption produced 10–30% decreases in a^i_{Ca}. No detectable changes in a^i_{Ca} were observed in the fibers exposed to strophanthidin of 10^{-7} M or less; 5×10^{-7} and 10^{-6} M strophanthidin produced 1.3–1.6 and 2–3-fold increases in a^i_{Ca}, respectively. This result is consistent with the hypothesis that an increase in a^i_{Na} produces an increase in a^i_{Ca}, which enhances Ca accumulation in the intracellular stores.

INTRODUCTION

The mechanism of inotropic action of cardiac steroids on heart muscle is still controversial, although it has been studied extensively and much information is available (for reviews, see Lee and Klaus, 1971; Schwartz et al., 1975; Akera and Brody, 1978; Noble, 1980). There are two main views regarding the mechanism of cardiac steroid action. One view is that an increase in contractile force (positive inotropic effect) is causally related to Na-K pump inhibition by cardiac steroids. This predicts a necessary correlation between the increase in intracellular Na ion activity and the increase in contractile force. The increase in intracellular Na ion activity reduces the transmembrane Na^+ activity gradient and thereby the driving force of Na^+ into the muscle cells. A reduction of the driving force produces a rise in intracellular calcium by retardation of Na-Ca exchange that leads to an increase in contractile force. The other view is that an increase in contractile force is not necessarily correlated with the Na-K pump inhibition. This view is supported by the experimental data showing a temporal dissociation between positive inotropy and Na-K pump inhibition and a more negative reversal potential for K^+ during exposure to ouabain (for a review, see Noble, 1980). In addition, it has been shown that cardiac steroids increase the slow inward calcium current (Weingart et al., 1978).

Lee et al. (1980*a*) have shown a close correlation between the increase in intracellular Na ion activity and the increase in twitch tension of sheep cardiac Purkinje fibers exposed to dihydro-ouabain. However, it appears that, in contrast with cardiac steroids such as ouabain and strophanthidin, dihydro-ouabain does not produce a stimulatory effect on the Na-K pump (Ghysel-Burton and Godfraind, 1977; Noble, 1980). It was reported that ouabain and ouabagenin at low doses induced a stimulatory effect on the Na-K pump, while dihydro-ouabain exerted only an inhibitory effect (Ghysel-Burton and Godfraind, 1977). Therefore, the present experiments were designed to study the effect of the cardiac steroid strophanthidin on intracellular Na ion activity and twitch tension of

canine cardiac Purkinje fibers. We also measured steady-state intracellular Ca ion activity of the fibers exposed to strophanthidin. Our improved techniques permit us to measure simultaneously and continuously intracellular ion activities and twitch tension of constantly driven fibers. The results indicate that the increase in intracellular Na ion activity is closely associated with the increase in twitch tension. This suggests that the level of intracellular Na ion activity is an important factor in determining contractile tension. To test such a role further, intracellular Na ion activity and twitch tension were measured during a reduction of intracellular Na activity. This reduction in intracellular Na ion activity was produced by inactivation of fast Na channels by raising the extracellular K ion concentration. The results also show close correlation between intracellular Na ion activity and twitch tension.

METHODS

Tissue Preparation and Solutions

Bundles of Purkinje fibers were dissected from the left ventricle of canine heart and transferred into a tissue bath containing oxygenated Tyrode solution. The dissected fiber bundles had diameters of 0.4–0.9 mm and lengths of 6–8 mm. A fiber bundle was mounted in a narrow channel of the muscle chamber shown in Fig. 1. One end of the fiber bundle was fixed by an insect pin on the Sylgard floor of the narrow channel. The stimulating electrodes were placed close to the insect pin. Another insect pin, L-shaped, was used to minimize movements of the fibers during contraction as shown in Fig. 1. This second pin was placed ~1.5 mm away from the stimulating electrodes. The other end of the bundle was connected to the tension transducer by a silver wire 50 μm in diameter. Oxygenated Tyrode solution was perfused through the narrow channel at a constant rate. The persusion rate was adjusted so that solution exchanges around the fiber bundle could be completed within 2–3 s. Concentrations (mM) in the Tyrode solution were: NaCl, 137; KCl, 5.4; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.45; $CaCl_2$, 1.8; dextrose, 11.1. Tyrode solution was saturated with 97% O_2 + 3% CO_2. The temperature of the perfused solution was kept at 36°C throughout the experiments; the pH of the solution was 7.3–7.4.

Stock solutions of 10^{-3} and 10^{-4} M strophanthidin (Sigma Chemical Co., St. Louis, MO.) were prepared. These stock solutions were diluted with oxygenated Tyrode solution for use in each experiment. Strophanthidin concentrations used in this study were 10^{-8}, 5×10^{-8}, 10^{-7}, 5×10^{-7}, and 10^{-6} M.

Tension Recording

The Purkinje fibers were stimulated with square pulses delivered to the electrodes from a stimulator (WPI model 301-T, W. P. Instruments, Inc., New Haven, CT) through a stimulus isolation unit. The stimulus voltage was 20–40% above threshold voltage. The fibers were driven continuously at the rate of 1 Hz throughout the experiments. Twitch tension of the fibers was measured with a force transducer (Cambridge Technology, Inc., Cambridge, MA, model 405). The maximum twitch tension was determined by stretching the fibers in steps. The fiber length was reduced until the twitch tension was ~80% of the maximum tension. As shown in Fig. 1, the output of the force transducer was connected to the channel 1 (*C* 1) of the chart recorder 2 (*R* 2) (Gould Inc., Cleveland, Ohio, model 280) and the oscilloscope (Tektronix, Inc., Beaverton, Oregon, model 5113; *OS* I of Fig. 1). Thus the twitch tension was recorded on the chart recorder and displayed on the oscilloscope together with the action potential. The force transducer was linear with a sensitivity of 60 mV/1 mg.

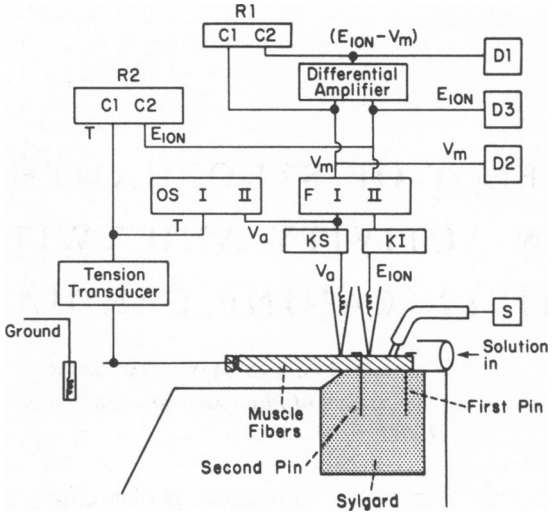

FIGURE 1 Schematic diagram of experimental system for the simultaneous measurements of intracellular ion activity, twitch tension, and action potential of cardiac Purkinje fibers. A part of the muscle chamber is shown. *S*, stimulator; KI, Keithley amplifier (model 604); KS, KS700 dual microprobe system (W.P. Instruments); *FI II*, low pass filters; OS *I II*, channels *I* and *II* of oscilloscope; *D1*, *D2*, *D3*, digital voltmeters; *C1*, *C2*, channels *1* and *2* of recorders *1* and *2*; V_a, conventional microelectrode potential; E_{ION}, ion-selective microelectrode potential; V_m, filtered potential of V_a.

Measurement of Intracellular Na and Ca Ion Activities

Intracellular Na ion activities were measured with neutral carrier Na^+-selective microelectrodes. The construction and calibration of the Na^+-selective microelectrodes have been described (Dagostino and Lee, 1982). The selectivity coefficient (k_{NaK}) of each microelectrode was determined using calibration solutions of 10 and 100 mM NaCl, and 150 mM KCl by a simple method described previously (Lee, 1981; Dagostino and Lee, 1982). It has been shown that k_{NaK} values do not depend significantly on the methods used and the Na^+ and K^+ activity levels in the physiological range. The k_{NaCa} was determined from the potential measured with 1.8 mM $CaCl_2$. Using the k_{NaCa} value determined with 1.8 mM $CaCl_2$, the potential of a Na^+-selective microelectrode in a solution containing 10^{-5} M Ca^{2+} was calculated by the Nicolsky equation. The calculated potential agreed with the microelectrode potential measured with the solution. This suggests that k_{NaCa} values do not depend significantly on Ca^{2+} activity levels. Therefore, the variation of k_{NaCa} with Ca^{2+} activity levels is not likely to affect significantly the measurement of intracellular Na^+ activity. The selectivity coefficients of the microelectrode used were constant throughout each experiments for 10–15 h. Intracellular Na ion activities (a^i_{Na}) of the fibers were calculated by the following equation (Nicolsky equation):

$$E^i_{Na} - V_m = E_0 + S \log [a^i_{Na} + k_{NaK}\, a^i_K + k_{NaCa}\, (a^i_{Ca})^{1/2}]. \quad (1)$$

E^i_{Na} represents the transmembrane potential measured with a Na^+-selective microelectrode with respect to the potential of a reference electrode in external bathing fluid. V_m is the membrane potential measured with a conventional microelectrode. E_0 is a constant potential of Na^+-selective microelectrode and electrochemical system used, and can be determined routinely (Lee, 1981). S is the slope of a Na^+-selective microelectrode that was determined empirically. S values were close to the theoretical values of 59 and 61 mV at temperatures of 25° and 36°C, respectively, within 1 mV deviation. k_{NaK} values were in the range of 0.01

to 0.02 (Dagostino and Lee, 1982). The range of k_{NaCa} values was 1.7–2.5. The intracellular K ion activity of the fibers a_K^i was measured using K^+-selective microelectrodes made with a K^+-selective liquid ion exchanger (W. P. Instruments, Inc., New Haven, CT). The a_K^i measured from five hearts was 120.7 ± 6.6 mM (SD, n = 12). In the present study the changes in a_K^i do not significantly affect the measurements of a_{Na}^i because of low k_{NaK} values. a_{Ca}^i is sarcoplasmic Ca^{2+} activity and was measured with Ca^{2+}-selective microelectrodes. In most experiments changes in a_{Ca}^i do not influence significantly the measurements of a_{Na}^i because of small changes in a_{Ca}^i. Significant changes in a_{Ca}^i were found only in fibers exposed to 10^{-6} M strophanthidin, in which case appropriate corrections were made.

After 5 min exposure to 10^{-6} strophanthidin, a_{Ca}^i increased on the average 2.4-fold (range 2–3, see Results). This increase was equivalent to a 0.3 mM change of a_{Na}^i calculated by $k_{NaCa}\,(a_{Ca}^i)^{1/2}$ in Eq. 1. The 0.3 mM (an interference) was used in corrections for maximum a_{Na}^i changes in the fibers exposed to 10^{-6}M strophanthidin. It was assumed that the change in a_{Na}^i was proportional to that in a_{Ca}^i. It should be pointed out that the interference (0.3 mM) was a relatively small fraction of the a_{Na}^i changes (2–3 mM) produced by 10^{-6} strophanthidin. In the experiments with 5×10^{-7} M strophanthidin, on the average 1.4-fold increase of a_{Ca}^i was equivalent to 0.1 mM (calculated by $k_{NaCa}\,[a_{Ca}^i]^{1/2}$) which might not significantly influence the a_{Na}^i changes (1–2 mM). Therefore, the interference by changes in a_{Ca}^i has not been corrected except for the a_{Na}^i changes produced by 10^{-6} M strophanthidin. For accurate measurements of a_{Na}^i, Na^+-selective microelectrodes and conventional microelectrodes must measure the same transmembrane potential when different cells are impaled by the two microelectrodes. This is difficult when the cells of a fiber bundle have different membrane potentials. However, cardiac muscle cells are connected electrically. Therefore, errors by variation of cell membrane potential in a fiber bundle may be not significant. Eq. 1 can be rewritten in the following form (Lee, 1981):

$$E_A + V_A - V_m = E_0 + S \log [a_{Na}^i + k_{NaK}\,a_K^i + k_{NaCa}\,(a_{Ca}^i)^{1/2}] \quad (2)$$

where E_A is the Na^+-selective microelectrode potential contributed by intracellular ion activity. V_A is the transmembrane potential measured with the Na^+-selective microelectrode. V_m is the transmembrane potential measured with a conventional microelectrode, and V_A must be equal to V_m so that the Na^+-selective microelectrodes measure intracellular Na ion activities accurately. The two membrane potentials may be different when impaling a cell with ion-selective microelectrode produces an electrical shunt. In this study no significant shunts were produced by ion-selective microelectrodes (see Discussion).

Sarcoplasmic Ca ion activities (a_{Ca}^i) were measured with neutral carrier Ca^{2+}-selective microelectrodes. Construction and some properties of the Ca^{2+}-selective microelectrodes have been described (Dagostino and Lee, 1982). The microelectrode potentials drift considerably for 1–2 h after construction. Ca^{2+}-selective microelectrodes were aged for 2–3 h before use so that the potential drifts could not produce a significant error in the measurements of a_{Ca}^i. Sarcoplasmic Ca ion activities were determined using calibration curves. A Ca^{2+}-selective microelectrode was calibrated with solutions of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M Ca^{2+}. These solutions contained 140 mM K^+, 10 mM Na^+, and 1 mM Mg^{2+}, pH 7.0.

The apparent stability constant of 2.51×10^6 M^{-1} for the Ca-EGTA complex was used to calculate free Ca^{2+} concentrations. The solutions containing 10^{-5}, 10^{-4}, and 10^{-3} M Ca^{2+} were made by dilutions of 10^{-1} M $CaCl_2$ solution (Dagostino and Lee, 1982). Ca^{2+}-selective microelectrode potentials were plotted against Ca^{2+} activities of the solutions calculated using an activity coefficient of 0.32 (Lee, 1981). The potential measured with Tyrode solution (0.58 mM Ca activity) was set arbitrarily as zero. E_{Ca} was measured by impaling a cell with a Ca^{2+}-selective microelectrode. Sarcoplasmic Ca^{2+} activities were determined from a plot of $E_{Ca} - V_m$. The criteria for accepting a measurement will be described later (see Discussion). Ca^{2+}-selective microelectrodes were usually calibrated at room temperature (22°C). In separate experiments, some microelectrodes were calibrated at 22°C and ~35°C using Ca^{2+} buffered solutions adjusted for these temperatures. The two temperatures produced a difference of ~3 mV (cf. Marban et al., 1980). This potential difference was corrected for the microelectrodes calibrated at room temperature. The 3-mV difference corresponded to 20–40 nM in a_{Ca}^i.

In this study, two identical low-pass filters (*F I* and *II* in Fig. 1; active filters, model APVLP -43-5, A.P. Circuit Corp., New York, NY) were used to remove voltage fluctuations caused by action potentials of constantly driven Purkinje fibers. The filters had a fixed frequency of 0.24 Hz. Without the filters, it is not possible to record a potential level corresponding to an intracellular ion activity of a constantly driven fiber that generates action potentials. This potential level is the voltage difference between the potential measured with an ion-selective microelectrode and that measured with a conventional microelectrode (Eq. 1). Both microelectrodes actually measure potential fluctuations of action potentials. The amplitudes of the potential fluctuations recorded by the microelectrodes depend on the resistance and capacitance of the microelectrodes (see Appendix). It should be pointed out that the input voltages of the potential fluctuations to both microelectrodes are the same. With the low-pass filters (see Fig. 1) the potential fluctuations were completely removed without a ripple. The filtered potential (V_m) of potential fluctuations measured with a conventional microelectrode should be the same as the filtered potential (V_A in Eq. 2) of potential fluctuations measured with an ion-selective microelectrode as shown by measuring changes in filtered potentials of a square wave signal that was applied to both conventional and Na^+-selective microelectrodes (see Appendix). Therefore the use of the filters does not distort the measurements of intracellular ion activities.

RESULTS

Intracellular Na Ion Activity of Driven Fibers

Cardiac muscle cells generate action potentials and develop twitch tensions (heart beat). During each action potential Na ions enter the cell through Na channels. In the present study, we measured simultaneously and continuously a_{Na}^i and twitch tension (T) of contantly driven canine Purkinje fibers at the rate of 1 Hz. Fig. 2 shows the measurements of a_{Na}^i, T, and transmembrane potential of such a fiber. The driven fiber had been equilibrated in Tyrode solution for ~1 h. A cell was then impaled with a Na^+-selective microelectrode as shown in Fig. 2*B*. The potential of the Na^+-selective microelectrode in Tyrode solution was set arbitrarily as zero in the potential recording. It should be pointed out that the Na^+-selective microelectrode potential in Tyrode solution was not used for determining a_{Na}^i. The impalement resulted in a large change (~120 mV) of the Na^+-selective microelectrode potential (E_{Na}). After the E_{Na} reached a stable level, a cell was impaled with a conventional microelectrode (Fig. 2*A*) and the measured transmembrane potential (V_m) was subtracted electronically from the E_{Na} (Fig. 2*B*). Both Na^+-selective and conventional microelectrodes actually measure potential fluctuations of action potentials because the fiber was driven at a constant rate. The potential levels of Fig. 2 *A* and *B* except the period labeled "filter off" were recorded after filtering both electrode signals with identi-

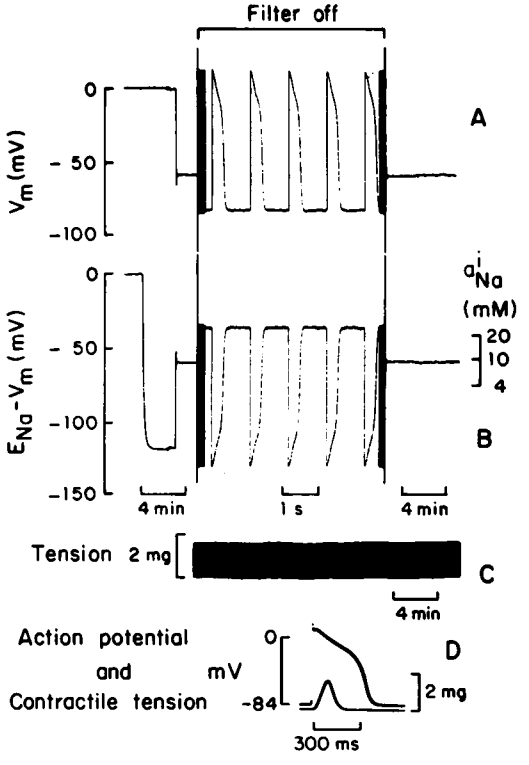

FIGURE 2 Simultaneous and continuous measurements of transmembrane potential (V_m), intracellular Na ion activity (a^i_{Na}), twitch tension (*Tension*), and action potential of canine cardiac Purkinje fibers driven at a constant rate (1 Hz). During the period the filter was off, speed of the chart recorder (*R 1* in Fig. 1) was increased. At the beginning and end of the time the filter was off, speed of the chart recorder was slow. The filter connected to the Na^+-selective microelectrode was not turned off.

cal low-pass filters (frequency of 0.24 Hz) as seen in Fig. 1. E_{Na} represents the potential by a^i_{Na} plus the filtered potential of action potential. V_m is the filtered potential of action potentials. The potential fluctuation can be seen when the filter connected to the conventional microelectrode is turned off. The potential fluctuations during the period the filter was off are not related to a^i_{Na}. When the filter was turned on again, the potential levels of Fig. 2 *A* and *B* were identical to those before it was turned off. The potential level after subtraction of V_m from E_{Na} represents a^i_{Na}. Using the fast speed of the chart recorder, no ripples were seen on the potential recordings during the period the filter was on. The a^i_{Na} values in Fig. 2 *B* were calculated by Eq. 1. Fig. 2 *C* shows twitch tensions measured continuously from the same fiber. At the same time, action potentials and twitch tension of the fiber were displayed on an oscilloscope as shown in Fig. 2 *D*. The action potentials displayed on the oscilloscope were not filtered as shown in the diagram of Fig. 1. In this way, the shape of action potentials and the twitch tension were accurately measured. The intracellular Na ion activity of the driven Purkinje fibers was found to be 8.9 ± 1.4 mM (mean ± SD, 52 measurements from 36 hearts).

Intracellular Na Ion Activity of Quiescent Fibers

Fibers beating continuously at a constant rate were used to measure a^i_{Na} and twitch tension in the present study. Under these conditions Na ions may enter the cells through the Na channels during each action potential (Vassalle, 1970). Therefore it is interesting to know whether the a^i_{Na} of beating fibers differs from that of quiescent fibers. Fig. 3 shows the measurement of a^i_{Na} when stimulation of a fiber was interrupted for ~4 min. The fiber was driven at the rate of 1 Hz for ~1 h before the interruption of stimulation. Fig. 3 *A* and *B* represent a Na^+-selective microelectrode potential (E_{Na}) and a conventional microelectrode potential (V_m), respectively. Fig. 3 *C* shows a^i_{Na}, i.e, the difference between E_{Na} and V_m. Twitch tensions of the fiber were measured simultaneously as shown in Fig. 3 *D*. When the stimulation was turned off, E_{Na} and V_m were hyperpolarized initially. The initial hyperpolarization of V_m is sharper than that of E_{Na} because the response time of a conventional microelectrode to a sudden change in membrane potential is faster than that of Na^+-selective microelectrodes. After the initial hyperpolarization, the membrane potential (V_m) reached a resting potential. This resting potential of the quiescent fibers was −77 mV. Vassalle (1970) has shown that after the cessation of overdrive the hyperpolarization subsided gradually. The change in V_m after the interruption of stimulation (Fig. 3) is consistent with the observation by Kline et al. (1980). They reported that, on cessation of stimulation following a period of overdrive, the membrane potential of canine cardiac Purkinje fibers is hyperpolarized. E_{Na} also decayed

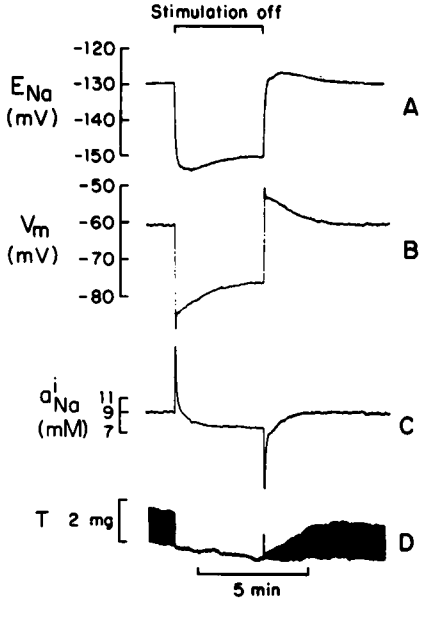

FIGURE 3 Measurement of intracellular Na ion activity (a^i_{Na}) during interruption of stimulation. Note that on the restimulation the twitch tension of the first beat is large.

to a stable potential level after an initial hyperpolarization (Fig. 3*A*). The difference between the stable potentials of E_{Na} before and after the stimulation was turned off (Fig. 3*A*) was 2–5 mV greater than that between the stable potentials of V_m (Fig. 3*B*). This indicates that the intracellular Na ion activity decreased during the interruption of stimulation. Otherwise, the difference between the stable potentials of E_{Na} might have been equal to that between the stable potentials of V_m. After the stimulation was turned on again, E_{Na} and V_m were depolarized initially and then returned to the levels before the interruption. On interruption of stimulation, the potential recording of Fig. 3 *C* representing a^i_{Na} showed an initial spike for a duration of ~10 s. This initial spike reflects the difference of the response time of Na^+-selective microelectrode from that of a conventional microelectrode (see Appendix). After the initial spike, a^i_{Na} decreased from 9.0 to 7.4 mM. The decreased a^i_{Na} levels were stable during the periods of no stimulation. When the stimulation was turned on again, an initial spike is seen in the recording of a^i_{Na}. After the initial spike, the decreased a^i_{Na} returned to the initial level. Seven measurements from three experiments resulted in a decrease of a^i_{Na} from 9.1 ± 1.2 mM to 7.6 ± 1.0 mM (mean ± SD). This result indicates that the a^i_{Na} in beating fibers at the rate of 1 Hz is greater than that in quiescent fibers. This is consistent with the observation by Cohen et al. (1982) and also indicates that the Na^+ equilibrium potential of quiescent fibers is more positive than that of beating fibers. The Na^+ equilibrium potential calculated using the a^i_{Na} value of 9.1 mM is +67 mV, while the Na^+ equilibrium potential calculated using the value of 7.6 mM is +72 mV. After the interruption of stimulation (Fig. 3), the decrease in a^i_{Na} appears to be approximately exponential, reflecting the rate of Na^+ extrusion by the Na-K pump. Similar observations were reported when the Na-K pump was reactivated after its inhibition (Deitmer and Ellis, 1978; Gadsby and Cranefield, 1979; Eisner et al. 1981*a*) or when intracellular Na concentration was increased by injection (Thomas, 1969).

Effect of Strophanthidin on Intracellular Na Ion Activity and Twitch Tension

Intracellular Na ion activity (a^i_{Na}) and twitch tention (T) were measured simultaneously and continuously in canine cardiac Purkinje fibers exposed to strophanthidin for 5 min. At the concentration of 10^{-8} M strophanthidin, 11 measurements from five preparations showed no detectable changes in either a^i_{Na} or T. Fig. 4 shows the changes in a^i_{Na} and T of the same fiber exposed to 5×10^{-8}, 10^{-7}, and 5×10^{-7} M strophanthidin. The threshold concentration for the effect of strophanthidin on a^i_{Na} and T appears to be around 5×10^{-8} M (Fig. 4 *A*). After 5 min exposure, both a^i_{Na} and T recovered to the initial levels. In the right panel of Fig. 4 *A*, the action potential and T before the drug application were superimposed with those at the peak of T

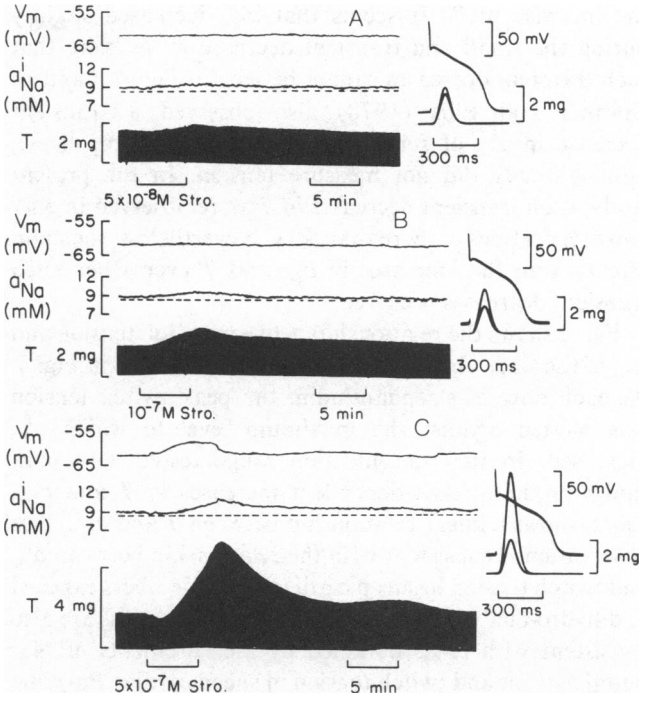

FIGURE 4 Effect of strophanthidin (*Stro.*) on intracellular Na ion activity (a^i_{Na}) and twitch tension (T) of canine cardiac Purkinje fibers. The fiber was exposed to strophanthidin of 5×10^{-8} M (*A*), 10^{-7} M (*B*), and 5×10^{-7} M (*C*). V_m represents filtered transmembrane potential of constantly driven fiber.

after the drug application. A small increase in T can be seen on the superimposed T recordings, while the shape of action potentials does not change. At the concentration of 5×10^{-8} M, four preparations out of seven showed small increases in both a^i_{Na} and T. Fig. 4 *B* shows increases in a^i_{Na} and T of the fibers exposed to 10^{-7} M strophanthidin. The increases in a^i_{Na} and T were greater than the increases in a^i_{Na} and T of the fiber exposed to 5×10^{-8} M strophanthidin. The superimposed action potential and T are shown in the right panel of Fig. 4 *B*. A distinct increase in T can be seen, while there is no apparent change in the shape of action potentials. At the concentration of 10^{-7} M strophanthidin, most preparations showed increases in a^i_{Na} and T. Fig. 4 *C* shows increases in a^i_{Na} and T of the fiber exposed to 5×10^{-7} M strophanthidin. The increases in a^i_{Na} and T were much greater than those in a^i_{Na} and T of the fiber exposed to 10^{-7} strophanthidin. The superimposed action potential and T are shown in the right panel of Fig. 4 *C*. The increases in a^i_{Na} and T of the fiber exposed to 10^{-6} M strophanthidin (not shown) were greater than those in a^i_{Na} and T of the fibers exposed to 5×10^{-7} M strophanthidin. In the range of strophanthidin concentrations tested, an increase in a^i_{Na} always accompanied an increase in T. During the onset and recovery of the drug effect, the time course of a^i_{Na} change was similar to that of T change as shown in Fig. 4.

In Fig. 4 *C* a transient decrease in T can be seen before

the increase in T. It seems that a^i_{Na} decreased slightly during the small and transient decrease in T. Note that such transient decreases cannot be seen in Fig. 4 *A* and *B*. Deitmer and Ellis (1978) also observed a transient decrease in a^i_{Na} of some Purkinje fibers of sheep heart, although they did not measure tension. In the present study, such transient decreases in T were observed in only two preparations of more than fifty. Nevertheless, the main effects were the increases in a^i_{Na} and T even when such transient decreases occurred.

Fig. 5 shows the relationship between twitch tension and a^i_{Na} of the fiber used in the experiment illustrated in Fig. 4. At each dose of strophanthidin, the peak twitch tension was plotted against the maximum level to which a^i_{Na} increased. In the concentration range tested, strophanthidin produced dose-dependent increases in T and a^i_{Na}. Fig. 5 shows a linear relationship between T and a^i_{Na}. This observation is consistent with the relationship between a^i_{Na} and twitch tension in sheep cardiac Purkinje fibers exposed to dihydro-ouabain (Lee et al., 1980*a*). The results are also consistent with those obtained by measurements of Na-pump current and twitch tension in sheep cardiac Purkinje fibers (Eisner and Lederer, 1980 *b*).

Correlation between a^i_{Na} and T during Onset and Recovery of Strophanthidin Effect

The results shown in Figs. 4 and 5 indicate that the increased levels of a^i_{Na} and T strongly correlate during the onset and recovery of the strophanthidin effect. To examine closely the relationship, T was plotted against a^i_{Na} during onset and recovery of the positive inotropic effect of strophanthidin. Fig. 6 illustrates one set of results obtained

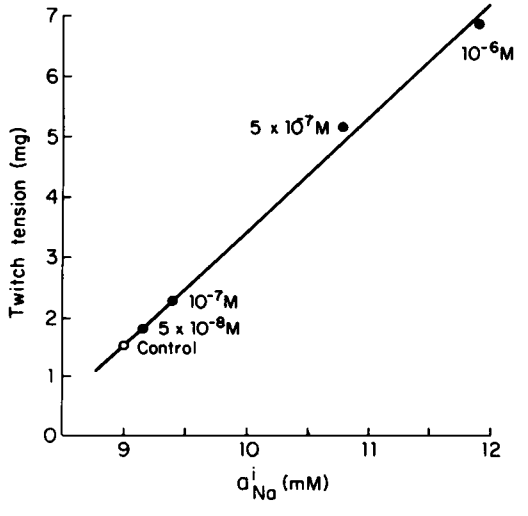

FIGURE 5 Relationship between the peak twitch tension and the maximum value of intracellular Na ion activity (a^i_{Na}) in the fiber exposed to strophanthidin of 5×10^{-8}, 10^{-7}, 5×10^{-7}, and 10^{-6} M. The open circle represents the twitch tension and a^i_{Na} before the exposure of the fiber to strophanthidin.

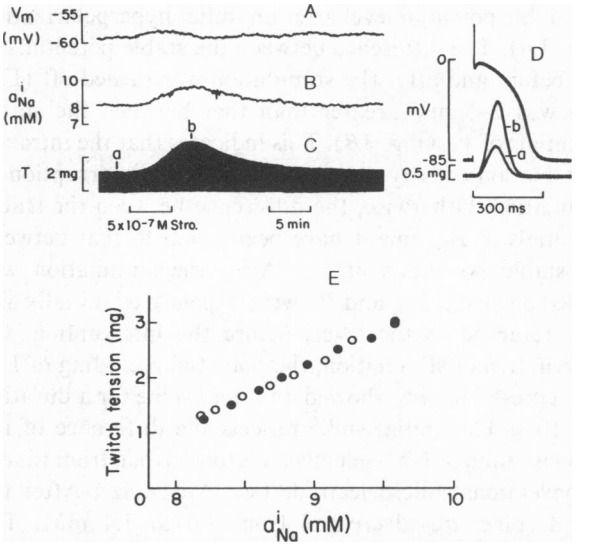

FIGURE 6 Changes in filtered transmembrane potential, V_m (*A*), intracellular Na ion activity, a^i_{Na} (*B*) and twitch tension, T (*C*) of constantly driven (1 Hz) fiber exposed to 5×10^{-7} M strophanthidin (*Stro.*) for 5 min. *D* shows superimposed action potentials and twitch tensions (*a* and *b*) at the times indicated by *a* and *b* in *C*. *E* shows relationship between twitch tension and a^i_{Na} during onset (●) and recovery (○) periods of strophanthidin effect.

from the fiber exposed to 5×10^{-7} M strophanthidin for 5 min. Fig. 6 *B* and *C* show a^i_{Na} and T, respectively, during onset and recovery of the strophanthidin effect. There was an increase in a^i_{Na} from 8.2 to 9.6 mM and a return to the original level after the exposure. The time course of a^i_{Na} change is similar to that of T during both onset and recovery periods. The shape of action potentials did not change at the time indicated by b (Fig. 6*D*), while T and a^i_{Na} increased distinctly. The relationship between T and a^i_{Na} is linear during both onset and recovery periods (Fig. 6*E*). Both T and a^i_{Na} recovered completely after the drug exposure. There was no apparent dissociation between a^i_{Na} and T during onset and recovery periods, and no apparent hysteresis. With the strophanthidin concentration of 5×10^{-7} M, five additional experiments (five tests) showed results similar to those of Fig. 6.

Fig. 7 shows a^i_{Na} and T of another Purkinje fiber exposed to 10^{-6} M strophanthidin for 5 min. During onset period of the drug effect, a^i_{Na} increased from 8.5 to 10.7 mM (Fig. 7 *B*) and T increased in a similar time course (Fig. 7 *C*). After the exposure of strophanthidin, a^i_{Na} and T recovered completely to their initial levels with a similar time course. In some experiments, with 10^{-6} M strophanthidin, the shape of action potentials at the time of maximum T and a^i_{Na} changed slightly as seen in Fig. 7 *D*; the plateau level was more negative, and the action potential duration shortened. At the same time diastolic potential was more positive . The relationships between T and a^i_{Na} during onset and recovery periods are linear and similar to that shown in Fig. 6 *E*. With the strophanthidin concentration of 10^{-6} M, three additional experiments (3 tests) showed results simi-

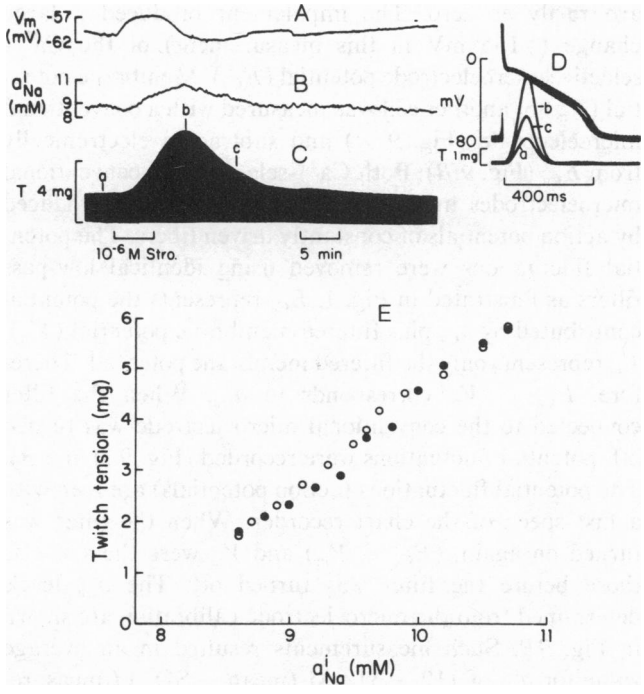

FIGURE 7 Changes in filtered transmembrane potential, V_m (*A*), intracellular Na ion activity, a^i_{Na} (*B*) and twitch tension, *T* (*C*), of constantly driven (1 Hz) fiber exposed to 10^{-6} M strophanthidin (*Stro.*) for 5 min, (*D*) superimposed action potentials and twitch tensions (*a*, *b*, and *c*) at the times indicated by *a*, *b*, and *c* in *C*. (*E*) relationship between twitch tension and a^i_{Na} during onset (●) and recovery (○) periods of strophanthidin effect.

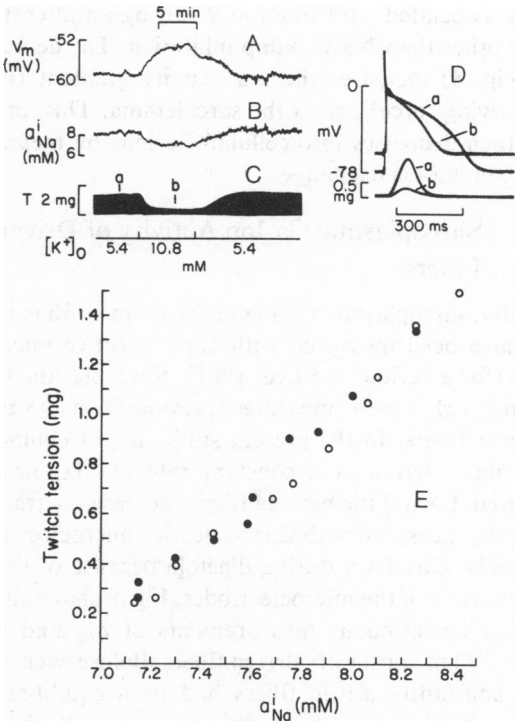

FIGURE 8 Effects of 10.8 mM $[K^+]_0$ on filtered transmembrane potential, V_m (*A*), intracellular Na ion activity, a^i_{Na} (*B*), and twitch tension, *T* (*C*), of constantly driven (1 Hz) fiber. *D* shows superimposed action potentials and twitch tensions (*a* and *b*) at the times indicated by *a* and *b* in *C*. *E* shows relationship between twitch tension and a^i_{Na} during increase (●) and restoration (○) of $[K^+]_0$.

lar to those of Fig. 7. The results (Figs. 6 and 7) indicate that the change in a^i_{Na} is closely associated with the change in *T* during both onset and recovery of the strophanthidin effect. This suggests that the increase in a^i_{Na} by Na-K pump inhibition is causally related to the positive inotropic effect of strophanthidin.

Effect of High External K^+ Concentration, $[K^+]_0$ on a^i_{Na} and *T*

The results have so far indicated that a^i_{Na} increases produced by strophanthidin play an important role in the enhancement of contractile force of canine Purkinje fibers. A question arises whether a^i_{Na} plays a similar role during a decrease in a^i_{Na}. The a^i_{Na} may be decreased by a reduction of Na^+ entry through inactivation of Na channels. The fast Na channels can be inactivated by depolarization of membrane potential. Fig. 8 shows simultaneous and continuous measurements of a^i_{Na} and *T* when $[K^+]_0$ was increased from 5.4 to 10.8 mM and restored. In Fig. 8 *A*, membrane potential (filtered action potential) became depolarized during the increase in $[K^+]_0$ and hyperpolarized during the restoration of $[K^+]_0$. The increase of $[K^+]_0$ produced a decrease of a^i_{Na} from 8.2 to 7.1 mM (Fig. 8 *B*) and a fall of *T* from 1.3 to 0.3 mg (Fig. 8 *C*). When $[K^+]_0$ was restored, the decreased a^i_{Na} and *T* recovered to the levels similar to the initial a^i_{Na} and *T*. In Fig. 8*D*, action potential and *T* at 5.4 mM $[K^+]_0$ (indicated by *a*) were superimposed with the action potential and *T* at 10.8 mM $[K^+]_0$ (indicated by *b*). At 10.8 mM $[K^+)_0$, diastolic potential (resting membrane potential) depolarized from −78 to −59 mV. At the high $[K^+]_0$, the action potential did not have an overshoot and the phase 0 depolarization was slow. This indicates that the fast Na channels are inactivated by the depolarization of diastolic potential. The high $[K^+]_0$ shortened the duration of action potential, but the initial phase of the plateau did not alter substantially. The decrease of a^i_{Na} at high $[K^+]_0$ is primarily due to a reduction of Na^+ entry caused by the inactivation of the Na channels, although Na-K pump stimulation by high $[K^+]_0$ and a decrease of passive Na^+ leak during diastole cannot be completely ruled out (see Discussion). At high $[K^+]_0$, the activity of the Na-K pump may be reduced because of low a^i_{Na}. Fig. 8 *E* shows a plot of T vs. a^i_{Na} during increase and restoration of $[K^+]_0$. The filled circles represent *T* and a^i_{Na} during increase of $[K^+]_0$. The open circles represent *T* and a^i_{Na} during restoration to the original $[K^+]_0$. The relationship between *T* and a^i_{Na} during increase and restoration of $[K^+]_0$ is approximately linear. Two experiments (six tests) showed results similar to those of Fig. 8. The results of Fig. 8 represent the relationship between a^i_{Na} and *T* in a negative inotropy (decrease in twitch tension) of cardiac Purkinje fiber. The results show that the changes in a^i_{Na} are

closely associated with those in T during an alteration in a^i_{Na} by other than Na-K pump inhibition. The decrease in a^i_{Na} (Fig. 8) increases the Na activity gradient (inward Na^+ driving force) across the sarcolemma. This, presumably, then decreases intracellular Ca ions by means of a change in Na-Ca exchange.

Sarcoplasmic Ca Ion Activity of Driven Fibers

Recently, sarcoplasmic Ca ion activities in cardiac muscle cells have been measured with Ca^{2+}-selective microelectrodes (for a review, see Lee, 1981). Sarcoplasmic Ca ion activities (a^i_{Ca}) were measured previously in electrically quiescent fibers. In the present study, a^i_{Ca} of canine Purkinje fibers driven at a constant rate (1 Hz) has been measured. Even if the beating fibers increase a^i_{Ca} transiently, the a^i_{Ca} measured with Ca^{2+}-selective microelectrodes is the steady-state level during diastole because of the slow response time of the microelectrodes. Fig. 9 shows simultaneous and continuous measurements of a^i_{Ca} and twitch tension (T) of constantly driven fibers. Before each experiment, constantly driven fibers had been equilibrated in Tyrode solution for about one hour. A cell then was impaled with a Ca^{2+}-selective microelectrode as shown in Fig. 9 *B*. The potential of Ca^{2+}-selective microelectrode in Tyrode solution (1.8 mM Ca^{2+} concentration) was set arbitrarily as zero. The impalement produced a large change (~165 mV in this measurement) of the Ca^{2+}-selective microelectrode potential (E_{Ca}). Membrane potential (V_m) of another cell was measured with a conventional microelectrode (Fig. 9 *A*) and subtracted electronically from E_{Ca} (Fig. 9 *B*). Both Ca^{2+}-selective and conventional microelectrodes measure potential fluctuations produced by action potentials of constantly driven fibers. The potential fluctuations were removed using identical low-pass filters as illustrated in Fig. 1. E_{Ca} represents the potential contributed by a^i_{Ca} plus filtered membrane potential (V_m). V_m represents only the filtered membrane potential. Therefore, $E_{Ca} - V_m$ corresponds to a^i_{Ca}. When the filter connected to the conventional microelectrode was turned off, potential fluctuations were recorded (Fig. 9 *A* and *B*). The potential fluctuations (action potentials) are seen with a fast speed of the chart recorder. When the filter was turned on again, ($E_{Ca} - V_m$) and V_m were the same as those before the filter was turned off. The a^i_{Ca} levels determined from the microelectrode calibration are shown in Fig. 9*B*. Such measurements resulted in an average value for a^i_{Ca} of 112 ± 31 nM (mean ± SD, 17 measurements from 7 preparations) when the apparent stability constant ($2.51 \times 10^6\ M^{-1}$) of Allen and Blinks (cf. Fabiato and Fabiato, 1978) for the Ca-EGTA complex was used. The a^i_{Ca} value of 112 nM gives the Ca equilibrium potential of about +114 mV across the cell membrane.

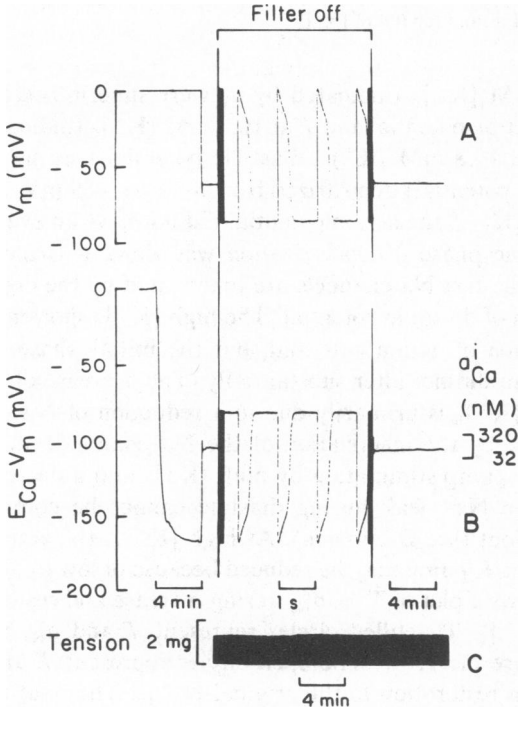

FIGURE 9 Simultaneous and continuous measurements of transmembrane potential (V_m), sarcoplasmic Ca ion activity (a^i_{Ca}), and twitch tension (*Tension*) of canine cardiac Purkinje fiber driven at a constant rate (1 Hz). During the period the filter was off, the speed of the chart recorder (*R 1* in Fig. 1) was increased. At the beginning and end of the time the filter was off, speed of the chart recorder was slow.

Sarcoplasmic Ca^{2+} activities were measured when stimulation of the fibers was interrupted as shown in Fig. 10. Upon the interruption, $E_{Ca} - V_m$, representing a^i_{Ca}, showed a transient spike of ~10 s due to the difference in the response times of the Ca^{2+}-selective microelectrode and the conventional microelectrode. After the spike, the potential declines and reaches a stable level. This potential level is ~3 mV more negative than before the interruption of stimulation. This indicates that a^i_{Ca} decreases from ~110 nM to 85 nM owing to the interruption. Three additional tests (two experiments) showed that a^i_{Ca} decreased by 10–30%. After the fiber was stimulated again, the decreased a^i_{Ca} returned to the initial level after a transient

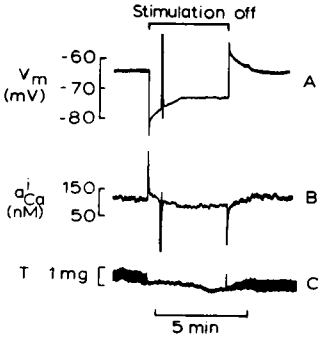

FIGURE 10 Measurement of sarcoplasmic Ca ion activyt (a^i_{Ca}) during interruption of stimulation. Note that on the restimulation the twitch tension of the first beat is large, and ~40 s after the interruption the conventional microelectrode came out of the cell temporarily.

spike as shown in Fig. 10*B*. The decrease in a^i_{Ca} is consistent with an operation of Na-Ca exchange because a^i_{Na} decreased after an interruption of stimulation (Fig. 3).

Effect of Strophanthidin on Steady-State Sarcoplasmic Ca Ion Activity

Strophanthidin produced dose-dependent increases of intracellular Na ion activity and twitch tension of canine Purkinje fibers (Fig. 4–7). This suggests that strophanthidin increases the intracellular Ca^{2+} level. Steady sarcoplasmic Ca^{2+} activities (a^i_{Ca}) were measured when the fibers were exposed to strophanthidin of 10^{-7}, 5×10^{-7}, and 10^{-6} M for 5 min. At the strophanthidin concentration of 10^{-7} M, no detectable changes in a^i_{Ca} were observed. At the concentrations of 10^{-6} and 5×10^{-7} M, however, a^i_{Ca} increased. With 10^{-6} M strophanthidin (Fig. 11), a^i_{Ca} increased ~2.2-fold at the peak, and after the exposure the a^i_{Ca} almost recovered. As a^i_{Ca} increased, twitch tension and diastolic tension increased. Four tests (two experiments) resulted in a 2–3-fold peak increase in a a^i_{Ca}. In some experiments, a small increase in diastolic tension was observed. At 5×10^{-7} M strophanthidin, five tests (two experiments) gave a 1.3–1.6-fold peak increase in a^i_{Ca}. In these experiments, no detectable increases in diastolic tension were observed. The results described above are consistent with the hypothesis that an increase in a^i_{Na} by inhibition of the Na-K pump raises sarcoplasmic Ca ion activity by means of Na-Ca exchange. An increase in sarcoplasmic Ca ion activity might then enhance Ca^{2+} accumulation in the sarcoplasmic reticulum.

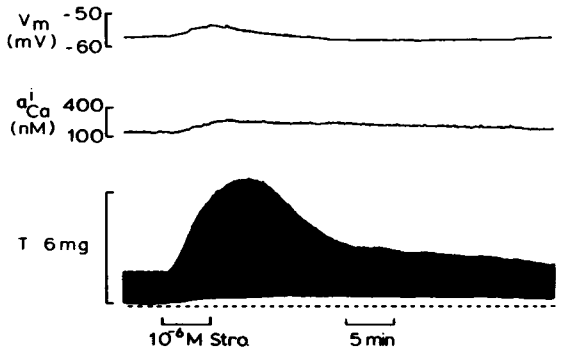

FIGURE 11 Effects of 10^{-6} M strophanthidin on filtered transmembrane potential (V_m), steady-state level of sarcoplasmic Ca ion activity (a^i_{Ca}), and twitch tension (T) of constantly driven (1 Hz) cardiac Purkinje fiber.

DISCUSSION

Intracellular Na Ion Activity of Constantly Driven Fibers

Simultaneous and continuous measurements of a^i_{Na} and twitch tension of constantly driven fibers have not been done in cardiac muscle, although a^i_{Na} has been measured in quiescent fibers (Lee and Fozzard, 1975; Ellis, 1977; Fozzard and Sheu, 1980; Sheu et al., 1980; Lee et al., 1980*a*; Glitsch and Pusch, 1980) and in voltage-clamped fibers depolarized by 0.5 s voltage pulses at a rate of 0.1 Hz (Eisner et al, 1981*a*). Cohen, Fozzard and Sheu (1982) have measured a^i_{Na} using $E^i_{Na} - V_m$ values during diastole of cardiac muscle fibers stimulated at 0.2 and 0.5 Hz. During normal heart function, cardiac muscle cells generate action potentials and beat at a regular interval so that measurements of a^i_{Na} of beating fibers is necessary for an understanding of electrophysiological and contractile properties of cardiac muscle.

Our study shows that a^i_{Na} in fibers driven at a rate of 1 Hz is 8.9 ± 1.4 mM (n = 52). Further we found that a^i_{Na} in driven fibers is, on average, 1.5 mM greater than that of quiescent fibers. This results has a few electrophysiological consequences. First, a significant amount of Na ions enters the cell during each action potential. Upon stimulation intracellular Na ions increase with time and then reach a steady-state level (Fig. 3). Second, the rate of Na-K pump in the driven fibers is higher than that in quiescent fibers. It has been shown that the Na-K pump rate in cardiac muscle cells depends on the level of intracellular Na^+ (Deitmer and Ellis, 1978; Gadsby and Cranefield, 1979; Eisner and Lederer, 1980*a*; Glitsch and Pusch, 1980; Eisner et al., 1981*a*). Upon cessation of stimulation membrane potential is transiently hyperpolarized and a^i_{Na} declines to a resting level (Fig. 3). This suggests that the rate of Na-K pump in the beating fibers remains enhanced. Third, Na equilibrium potential (V_{Na}) in beating fibers is different from that in quiescent fibers. In calculation of V_{Na} for the overshoot of action potentials, the a^i_{Na} value of driven fibers should be used. Our study shows that the V_{Na} of the driven fibers is +67 mV, while the V_{Na} of quiescent fibers is +72 mV. Although the V_{Na} of the driven fibers is lower than that of quiescent fibers, the V_{Na} of +67 mV is still more positive than the overshoot (+30 to +40 mV) of cardiac action potentials.

We have been concerned about a possible shunt produced by poor sealing around the ion-selective microelectrode tip during cell impalement. In Eq. 2, V_A (membrane potential measured with an ion-selective microelectrode) must be the same as V_m (membrane potential measured with a conventional microelectrode) so that a significant error could not be involved in measurements of intracellular ion activities. Two types of methods were used to test possible shunts by cell damage in impalements with Na^+- and Ca^{2+}-selective microelectrodes. One method is to change membrane potential by an interruption of stimulation of fibers as shown in Figs. 3 and 10. When membrane potential (V_m) is changed, the potential change (ΔE_{ion}) measured by an ion-selective microelectrode must be nearly identical to the potential change (ΔV_m) measured by a conventional microelectrode. This is reflected by no significant change in differential voltages, V_{diff} (representing intracellular ion activities) before and after the

change in membrane potential. The ΔE_{ion} was compared with the ΔV_m during the period of 10–15 s after V_m was suddenly changed by an interruption of stimulation. This is because the Na^+- and Ca^{2+}-selective microelectrodes used in this study have a response time of 10–15 s to a voltage change (see Appendix). As shown in Figs. 3 and 10, a sudden change in V_m produced an initial spike of the differential voltage (V_{diff}) with a duration of 10–15 s, and then a slow change in V_{diff}. The V_{diff} before a change in V_m was compared with the V_{diff} at the end of the initial spike after a change in V_m. If the V_{diff} before a change in V_m agreed with the V_{diff} at the end of the spike within 2 mV, the measurements were accepted.

In another method, membrane potentials were measured with the micropipettes (filled with 3 M KCl) similar to those used for making Na^+- and Ca^{2+}-selective microelectrodes. The micropipettes filled with 3 M KCl had resistance of 7–16 MΩ. Membrane potentials measured with these micropipettes were essentially the same as those measured with the conventional microelectrodes used in this study. This supports the conclusion that significant shunts were not produced when cells were impaled with the ion-selective microelectrodes.

Effect of Strophanthidin on Intracellular Na Ion Activity

There are two important questions regarding the controversial issue of cardiac steroid effect on heart muscle. The first question is whether cardiac steroids stimulate the Na-K pump. The second question is whether the time course of the change in a^i_{Na} differs from that of the change in twitch tension particularly during the recovery of the cardiac steroid effect. Deitmer and Ellis (1978) reported that with 1.5×10^{-8} M strophanthidin a^i_{Na} slowly decreased and was maintained at a lower level. In their study, sheep cardiac Purkinje fibers were exposed to strophanthidin for 15 min. In our study, no detectable changes in a^i_{Na} and twitch tension were observed in the fibers exposed to 10^{-8} M strophanthidin for 5 min.

The apparent discrepancy cannot be explained satisfactorily, although experimental conditions were different. The fibers used by Deitmer and Ellis (1978) were quiescent; the fibers used in our study were driven at a rate of 1 Hz. Deitmer and Ellis (1978) observed that with strophanthidin concentrations $\geq 1.5 \times 10^{-7}$ M a^i_{Na} increased after a small transient decrease. Recently Grupp et al. (1982) have reported that low concentrations of ouabain (10^{-10}, 10^{-9}, and 10^{-8} M) had no effect on the contractile force of papillary muscles of guinea pig, rabbit, and cat, and higher concentrations of ouabian produced an increase in contractile force without a transient decrease. In our study such transient decreases in a^i_{Na} and twitch tension were very rare. Cohen et al. 1976 studied effects of ouabain on the reversal potential of the pacemaker current i_{K_2} in sheep Purkinje fibers. They observed that low doses ($10^{-8} - 5 \times 10^{-7}$ M) of ouabain produce an increase in the reversal potential rather than a decrease. This indicates that ouabain at low doses stimulates the Na-K pump and increases K^+ gradient across the cell membrane. Ghysel-Burton and Godfraind (1977 and 1979) reported an increase in K content and a corresponding decrease in Na content of guinea-pig atria exposed to ouabain lower than 10^{-8} M.

There are reports that the time course of change in cellular ion concentration or content does not parallel the time course of change in twitch tension. Gadsby et al. (1971) investigated the relation between cellular potassium content and twitch tension of frog ventricle exposed to 5×10^{-7} M 3-acetyl strophanthidin. They found that the time courses of the fall in potassium content and of the rise in twitch tension were different. Okita (1977) described results supporting dissociation between digitalis inotrophy and Na^+, K^+-ATPase inhibition, particularly during the recovery period of the drug effect. Recently Barry et al. (1981) reported that after ouabain and dihydro-ouabain were washed out the positive inotropy of chick embryo ventricle recovered completely, but the bulk cellular Na concentration remained increased. This also indicates a dissociation between the Na-K pump inhibition and positive inotropy. In our experiments, intracellular Na^+ activity and twitch tension were measured simultaneously and continuously in the same fibers. No apparent dissociation between intracellular Na^+ activity and positive inotropy has been observed during onset and recovery periods of the strophanthidin effect (Figs. 4–7). The discrepancy between our results and the results of others cannot be explained readily. However, it should be pointed out that it is difficult to draw inferences about cellular ion transport processes from measurement of total cellular ion content or concentration, as these measurements may not reflect the relevant ion activity (Lev and Armstrong, 1975; Walker and Brown, 1977; Lee, 1981).

Recently Eisner et al. (1981*a*) studied the relationship between a^i_{Na} and twitch tension of sheep Purkinje fibers during Na-K pump inhibition and activation by changes in $[Rb^+]_0$. They reported that, in general, changes in a^i_{Na} produced by altering Na-K pump activity are associated with inotropic effects. When the relationship during Na-K pump inhibition were compared with that during Na-K pump reactivation, however, they found in many fibers a hysteresis in the relationship between a^i_{Na} and both twitch and tonic tension. We hardly ever observed a hysteresis. Although the reason for the different results is not clear, it is worth pointing out the different experimental conditions in the two studies. In the study by Eisner et al. (1981*a*), the Na-K pump was inhibited and reactivated by removal and re-adding of external Rb^+ in K^+-free solution, whereas in our study the Na-K pump was inhibited and reactivated by exposure to, and washing out of, strophanthidin, with a constant $[K^+]_0$ of 5.4 mM. The reactivation process in their study was much faster than that in our study, and the recessed-tip Na^+-selective glass microelectrodes used in

their study may have had a slower response time than the neutral carrier Na^+-selective microelectrodes ($t_{0.5}$ = 2–3 s, see Appendix) used here. Tonic tension changed in many experiments of their study and hysteresis was more pronounced in the change of tonic tension than in that of twitch tension. In our experiments, diastolic tension did not change in the fibers exposed to strophanthidin of 5×10^{-7} M or less.

Barry et al. (1981) have reported that the a^i_{Na} in a subsarcolemmal space may differ from that in the bulk sarcoplasm. Eisner et al. (1981*a*) suggested that such an inhomogeneous a^i_{Na} in sarcoplasm is unlikely because of the same kinetics of the electrogenic Na pump current and the a^i_{Na} in sarcoplasm. Our results are consistent with a homogeneous distribution of a^i_{Na} in sarcoplasm because the time course of the change in a^i_{Na} agrees well with that of the change in twitch tension.

Our study has shown a strong correlation between a^i_{Na} and twitch tension when a^i_{Na} was changed by altering of Na-K pump activity by exposure to and washing out of strophanthidin. This indicates that a given a^i_{Na} level is important for setting a given magnitude of twitch tension. Although a quantitative description of such a relationship may not be simple, the following qualitative interpretation may be possible. An increase in a^i_{Na} produces a rise in steady-state sarcoplasmic Ca^{2+} activity by retarding Ca^{2+} extrusion via Na-Ca exchange. It has been shown that the steady-state (basal) level of sarcoplasmic Ca^{2+} activity increased in fibers exposed to strophanthidin (Fig. 11). An increase in sarcoplasmic Ca^{2+} activity might enhance Ca loading in intracellular stores (sarcoplasmic reticulum). Thus the amount of Ca in intracellular stores would depend on a Ca^{2+} activity level in the sarcoplasm. The magnitude of twitch tension in mammalian cardiac cells is believed to depend on the amount of Ca in intracellular stores available for activation of contractile proteins by transient release during an action potential. The relationship between Ca^{2+} concentration and tonic tension of skinned cardiac muscle cells is linear except at the extremely low and high range of Ca^{2+} concentrations tested (Fabiato, 1981). Quantitative description of the linear relationship between a^i_{Na} and twitch tension may be more complicated. It may depend on the Na-Ca exchange process across sarcolemma and Ca accumulation in the intracellular stores and its release, which are relatively unknown.

Effect of High $[K^+]_0$ on a^i_{Na} and Twitch Tension

An approximately linear relationship between a^i_{Na} and twich tension was observed when a^i_{Na} was decreased and recovered by rise and restoration of $[K^+]_0$. Thus we have shown a strong correlation between a^i_{Na} and twitch tension when a normal a^i_{Na} was either increased or decreased by Na-K pump inhibition or by high $[K^+]_0$. The rise of $[K^+]_0$ from 5.4 to 10.8 mM may stimulate Na-K pump because Ellis (1977) observed decreases in a^i_{Na} by raising $[K^+]_0$ in the range of 4 to 25 mM. However, Gadsby (1980) has shown that Na-K pump in canine Purkinje fibers was half maximally activated at 1 mM $[K^+]_0$. This suggests that in canine Purkinje fibers a decrease in a^i_{Na} by a change in K stimulation of the Na-K pump may not be significant in the range of 5.4 to 10.8 mM $[K^+]_0$. As judged by the early phase of plateau of the action potetial recorded in 10.8 mM $[K^+]_0$ (Fig. 8*D*), slow Ca^{2+} current may not be altered during the exposure to raised $[K^+]_0$. This suggests that a decrease in intracellular Ca^{2+} (decrease in twitch tension) may not be due to a reduction of slow Ca^{2+} current. A change in the slow inward Ca^{2+} current may directly affect intracellular Ca^{2+} and thereby alter twitch tension. Under this condition, a correlation between a^i_{Na} and twitch tension may not be expected. Another factor influencing a^i_{Na} may be transmembrane potential. Eisner et al. (1981 *b*) have shown that depolarizing the membrane potential decreased a^i_{Na} and hyperpolarization increased it. At 10.8 mM $[K^+]_0$ (Fig. 8*D*), the diastolic potential depolarized 20 mV while the membrane during action potential (except the early phase) hyperpolarized. The overall potential change was about 6 mV depolarization as shown by the filtered V_m in Fig. 8*A*. It is not clear that such changes in transmembrane potential affect significantly a^i_{Na}. Such membrane potential changes may affect Na^+ and Ca^{2+} transport by the Na-Ca exchange if it is electrogenic (Sheu and Fozzard 1981). At present it is difficult to account for such effects.

As seen in Figs. 6*E*, 7*E*, and 8*E*, the slopes of T change for a^i_{Na} change varied from one fiber to another. In the T and a^i_{Na} ranges investigated in this study, the slopes (T change / 1 mM a^i_{Na} change) were between 1 and 2 mg mM^{-1}. This variation might be reflecting individual Purkinje fiber bundles used in each experiment. Although the T changes for a given dose of strophanthidin varied considerably from one fiber bundle to another, in the same fiber, T changes per a^i_{Na} changes obtained at different doses of strophanthidin were similar (Fig. 5). At present, it is difficult to know whether some other factor was contributing to such a variation.

Effect of Strophanthidin on Sarcoplasmic Ca^{2+} Activity

Steady-state level of sarcoplasmic Ca^{2+} activity (a^i_{Ca}) of constantly driven fibers was measured with Ca^{2+}-selective microelectrodes. This measurement may be subject to error because of cell injury by impalements and drift of the microelectrode potentials. However, the errors by such problems may not be significant because of the criteria used to accept the measurements. As discussed, the measurements were accepted if V_{diff} ($E_{Ca} - V_m$) agree within 2 mV before and after a change in V_m. The measurements were rejected if the microelectrode potentials differed by more than 5 mV in the calibration solutions of pCa 7 and 6

before and after an intracellular measurement. The a^i_{Ca} values obtained in this study are in the range similar to those reported previously. Marban et al. (1980) reported 260 nM Ca^{2+} concentration (ferret ventricular muscle cells), which corresponds to the a^i_{Ca} of 83 nM. Lee et al. (1980*b*) obtained 38 nM a^i_{Ca} (rabbit ventricular muscle cells), which corresponds to 87 nM when corrected for the stability constant value of $2.51 \times 10^{-6} M^{-1}$. Coray et al. (1980) reported sarcoplasmic Ca^{2+} concentrations of 170 and 360 nM, in sheep Purkinje fibers and ventricular cells, respectively. The a^i_{Ca} of sheep Purkinje fibers estimated by Dahl and Isenberg (1980) was 83 nM. Sheu and Fozzard (1981) measured the a^i_{Ca} of 99 nM in sheep ventricular muscle cells.

We have measured changes in a^i_{Ca} when stimulation of the fibers was interrupted. $E_{Ca} - V_m$ representing a^i_{Ca} was 1–3 mV negative during an interruption of stimulation (Fig. 10). This indicates that a^i_{Ca} decreased by 10–30%. This a^i_{Ca} decrease can be related to the fall in a^i_{Na} seen when stimulation of the fibers was interrupted (Fig. 3). A fall in a^i_{Na} increases the rate of Na-Ca exchange and so may cause a reduction in a^i_{Ca}. It cannot be excluded that the loss of the Ca^{2+} entry associated with action potentials may cause the observed fall in a^i_{Ca}. In some experiments, $E_{Ca} - V_m$ remained unchanged. It should be pointed out that the measurements of decrease in a^i_{Ca} are difficult. This is because the Ca^{2+}-selective microelectrode responses for changes in a^i_{Ca} in the low range are small and are subject to error, for example due to defective impalements. No detectable changes in a^i_{Ca} were observed in fibers exposed to strophanthidin concentrations of 10^{-7} M or less, although twitch tension increased slightly in 10^{-7} M strophanthidin. This may be due to small voltage signal of the microelectrode for small change of a^i_{Ca} in that range. The 5×10^{-7} and 10^{-6} M strophanthidin produced small increases in a^i_{Ca}. This is consistent with an operation of Na-Ca exchange across sarcolemma as discussed above. An increase in a^i_{Ca} may enhance Ca accumulation (loading) in the intracellular stores from which Ca is released during twitches.

APPENDIX

We have used two identical low-pass filters to remove action potentials of canine cardiac Purkinje fibers driven at a constant rate of 1 Hz (see Methods). Filtering the potential signals resulted in steady-state levels of E_{Na} (Na^+-selective microelectrode potential) and V_m (conventional microelectrode potential) without potential fluctuations, so that a steady-state level of $E_{Na} - V_m$ could be measured (see Fig. 2). A question arises of whether the filtering of the signals distorted the measurements of a^i_{Na}, particularly when the filtered potentials were changed by alterations of action potentials (changes in amplitude and duration of action potentials). This is because response times of ion-selective microelectrodes are much slower than those of conventional microelectrodes. One simple method to test the question is to apply a square wave to both ion-selective and conventional microelectrodes placed in the tissue bath and change the shape of the wave. Fig. 12 shows the result obtained from such a test. Na^+-selective microelectrodes tested were similar to those used in the measurements of a^i_{Na}. They had tip diameters <1 μm and resistances of

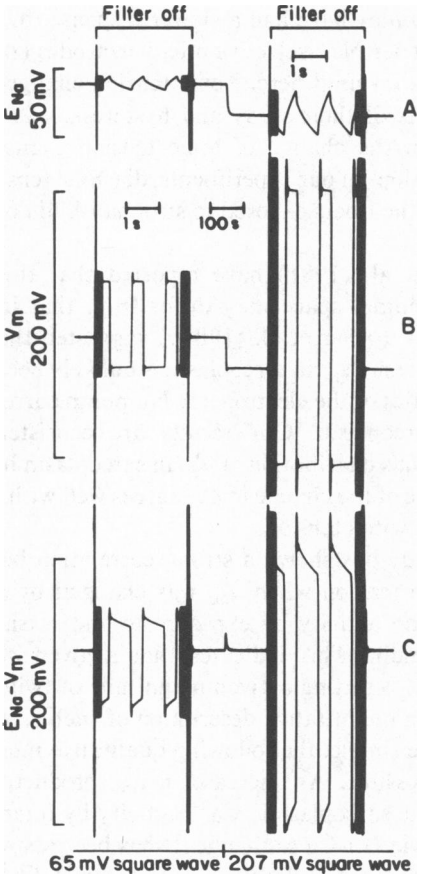

FIGURE 12 Responses of a Na^+-selective microelectrode (E_{Na}) and a conventional microelectrode (V_m) to a square wave signal in the tissue bath, and the differential voltage ($E_{Na} - V_m$). Note that the amplitude of the square wave was increased by more than three times at the time indicated below the *C* tracing. At the beginning and end of the time the filter was off, speed of the chart recorder was slow. Both filters connected to the Na^+-selective and conventional microelectrodes were turned off during the periods labeled filter off.

$\sim 5 \times 10^{10}$ Ω. A Na^+-selective microelectrode and a conventional microelectrode were placed in a tissue bath containing Tyrode solution. Then the square waves with 65 mV amplitude and 350 ms duration were applied to the microelectrodes at the rate of 1 Hz. This can be seen in the potential recording (V_m) of the conventional microelectrode (Fig. 12*B*) when the filters were turned off and the speed of the chart recorder was increased. However, the Na^+-selective microelectrode recorded voltage fluctuations of only 10 mV as seen in the potential recording (E_{Na}) of Fig. 12*A*. This is because the Na^+-selective microelectrode has a high resistance. Such small potential fluctuations of ion-selective microelectrodes were observed in constantly driven Purkinje fibers. The potential recording of Fig. 12*C* shows the difference ($E_{Na} - V_m$) of E_{Na} and V_m. After the filters were turned on again, the filtered potentials of E_{Na}, V_m, and $E_{Na} - V_m$ were the same as those before the period when the filters were off. During the period that the filters were on, the filtered potentials of E_{Na} and V_m were changed by an increase of the amplitude of the square waves at the time indicated below the potential recording *C*. The amplitude was increased from 65 to 207 mV as indicated below the potential recording *C*. This amplitude increase can be seen in the potential recording *B* when the filters were turned off again and the speed of the chart recorders was increased. On increasing the amplitude during the period the filters were on, the filtered potentials of E_{Na} and V_m changed by 24 mV as seen in the potential recordings *A* and *B*. On the other hand, the differential potential

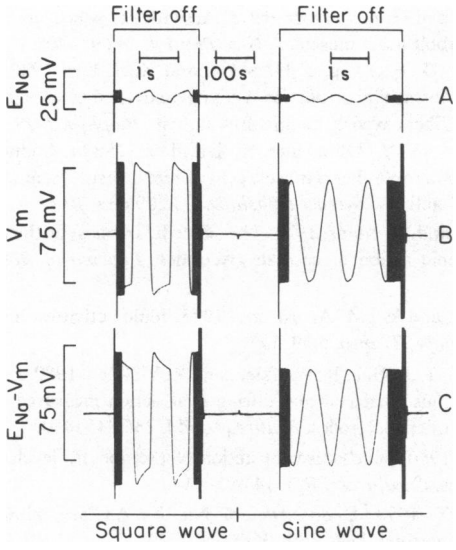

FIGURE 13 Responses of a Na^+-selective microelectrode (E_{Na}) and a conventional microelectrode (V_m) to a square and sine wave signal in the tissue bath, and their differential voltage ($E_{Na} - V_m$). Note that the square wave was changed to the sine wave at the time indicated below the *C* tracing.

($E_{Na} - V_m$) did not change except for a brief spike of an ~10 s duration as seen in the recording *C*. This spike is similar to the initial spike of Fig. 3*C* that was recorded on the interruption of stimulation of the Purkinje fiber. The brief spike is due to the difference between the response time of the Na^+-selective microelectrode and that of conventional microelectrode as indicated already. This is clearly shown by the potential recordings *A* and *B* of Fig. 12. When the amplitude of the square wave was suddenly increased, the V_m change (Fig. 12*B*) was faster that the E_{Na} change (Fig. 12*A*). The results (Fig. 12) have a few important consequences. First, no change in the differential potential except the brief spike indicates that the filtering of action potentials did not produce errors in the measurements of a^i_{Na} of constantly driven Purkinje fibers. Second, any steady-state changes in electrical signals such as action potentials did not distort the measurements of a^i_{Na} changes. Third, one can determine the electrical response times of ion-selective microelectrodes using the change in steady state potential of Fig. 12*A*. It has been described that slow response times of ion-selective microelectrodes may be due to electrical component (resistance and capacitance) rather than the chemical component (Lee, 1981). We have determined the electrical response times of neutral carrier Na^+-selective microelectrodes. The response time was estimated by 10 times magnification of the recorded potential (Fig. 12*A*) of Na^+-selective microelectrode. The applied potential changes were 15 to 25 mV. The 12 tests with five Na^+-selective microelectrodes gave 2.5 ± 0.2 s (mean ± SD) for 50% change of the total response and 8.0 ± 0.8 s (mean ± SD) for 90% change. This result indicates that ~10 s estimated for the spike duration is reasonable. Furthermore, such delays in electrical response times might not distort the measurements of a^i_{Na} changes in this study except for a 10 s period after the sudden interruption of stimulation. Upon interrupting stimulation, there was an initial spike of ~10 s duration (see Figs. 3 and 10). This 10 s delay might not dominate the slow changes in a^i_{Na} (2–3 min). In all other experiments, the changes in V_m were small (~0–6 mV) and slow (see Figs. 4–8, and 11). Therefore, the delays in electrical response times of ion-selective microelectrodes did not distort the measurements of a^i_{Na} changes.

The effect of small changes in V_m and E_{Na} on $E_{Na} - V_m$ was tested with a Na^+-selective microelectrode as shown in Fig. 13. In this test the square waves with ~80 mV amplitude and ~500 ms duration first were applied to the Na^+-selective and conventional microelectrodes at a rate of 1 Hz. This can be seen during the increase of speed of the chart recorder after the filters were turned off (Fig. 13*B*). The amplitude of the square wave recorded by the conventional microelectrode was ~80 mV, while the amplitude of the wave recorded by the Na^+-selective microelectrode was only ~7mV. During the period the filters were on, the square wave was changed to sine wave at the time indicated below the recording *C*. This change can be seen when the filters were turned off again and the speed of the chart recorder was increased. On changing the wave form, the changes in filtered V_m and E_{Na} were small (~1 mV). No change in $E_{Na} - V_m$ was observed, and there was no apparent delay in the $E_{Na} - V_m$ signal except a small and momentary spike. Four tests (two Na^+-selective microelectrodes) showed results similar to those of Fig. 13. The results indicate that the filtering of the electrical signals changed slowly did not affect the measurements of a^i_{Na} changes.

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