RELATION BETWEEN INTRACELLULAR Na ION ACTIVITY AND TENSION OF SHEEP CARDIAC PURKINJE FIBERS EXPOSED TO DIHYDRO-OUABAIN

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ABSTRACT The intracellular Na ion activity (a^i_{Na}) and the contractile tension (T) of sheep cardiac Purkinje fibers were simultaneously measured employing recessed-tip Na^+-selective glass microelectrodes and a mechano-electric transducer. The a^i_{Na} of 6.4 ± 1.6 mM (mean ± SD, n = 56) was obtained in fibers perfused with normal Tyrode's solution. The changes in a^i_{Na} and T were measured during and after the exposure of fibers to a cardiac glycoside, dihydro-ouabain (DHO) in concentrations between 5×10^{-8} M and 10^{-5} M. The exposure time to DHO was 15 min. Both a^i_{Na} and T did not change in fibers exposed to 5×10^{-8} M DHO, and the threshold concentration for the effect of DHO appeared to be around 10^{-7} M. In DHO concentrations greater than the threshold, the increases in a^i_{Na} and T strongly correlated during the onset of DHO effects. The recoveries of a^i_{Na} and T were variable and slow, being dependent on the DHO concentration. In those fibers which recovered from the effects of DHO, the time-course of a^i_{Na} recovery was similar to that of T recovery. In fibers exposed to DHO of 5×10^{-6} M or greater, the apparent toxic effects were observed in both action potential and contraction after an initial increase in T. The fibers manifesting the apparent toxic effects had a a^i_{Na} of ~30 mM or greater. The results of this study indicate that the increase in a^i_{Na} is associated with the positive inotropic action of the cardiac glycoside.

INTRODUCTION

Many studies have suggested that changes in intracellular Na ion activity play an important role in the positive inotropic action of cardiac glycosides. It has been postulated that the positive inotropic action may be related to changes of sodium and potassium transport across the cell membrane (Hajdu and Leonard, 1959; Glynn, 1964; Hougen and Smith, 1978). Further, it has been shown that there is a correlation between the inhibition of Na^+, K^+-ATPase by cardiac glycosides and the positive inotropic action (Akera et al., 1973; Schwartz et al., 1974). It has been proposed that the primary action of the cardiac glycosides is to produce an elevation of the intracellular Na concentration secondary to inhibition of the cellular Na-K pump (Langer, 1977). Such an increase in intracellular Na ion concentration may augment cellular Ca ion concentration (Glitsch et al., 1970; Allen and Blinks, 1978). Thus, the increased intracellular Ca^{++} is responsible for the positive inotropic action of the cardiac glycosides. However, there are some observations that do not support the above proposal (Okita, 1977). It was reported that ouabain exerts its positive inotropic effect without affecting the intracellular Na and K concentrations of atrial muscle of the guinea-pig (Bentfeld et al., 1977). In addition, it has been proposed that the inhibition of the Na pump by

cardiac glycosides results in an enhancement of the intracellular Na transient (Brody and Akera, 1977). Schwartz (1976) has suggested that cardiac glycosides may cause a conformational change in the Na, K-ATPase, which alters the affinity for calcium.

The question is still whether or not the intracellular accumulation of Na^+ by cardiac glycosides is essential for the positive inotropic action of cardiac glycoside. More specifically, is there a causal correlation between the change in intracellular Na ion activity and that in contractility that may be manifested in the time-course of changes of these parameters? To answer this question, we measured the changes in intracellular Na ion activity and contractile tension of sheep cardiac Purkinje fibers during and after the exposure to a cardiac glycoside, dihydro-ouabain. The glass microelectrodes with a recessed tip (Thomas, 1970) are suitable for the measurement of changes in intracellular Na ion activities of small cells. We made recessed-tip Na^+-slective glass microelectrodes and directly measured intracellular Na ion activities and contractile tensions in cardiac Purkinje fibers during exposure to the dihydro-ouabain. Some preliminary results of this study have been reported previously (Lee et al., 1979).

METHODS

Preparation and Solutions

Sheep hearts were obtained at a slaughterhouse and brought to the laboratory in cool oxygenated Tyrode's solution. Bundles of Purkinje fibers were dissected from the left ventricle and kept in oxygenated Tyrode's solution until needed. A bundle was mounted in a narrow channel (Fig. 1) of the muscle chamber described previously (Fozzard and Lee, 1976). The bundles used in this study had diameters ranging from 0.4 to 0.9 mm and lengths from 7 to 12 mm. Tyrode's solution was continuously perfused through the narrow channel shown in Fig. 1. One solution in the region occupied by a bundle was changed to another solution within ~1 s. Tyrode's solution contained (in millimoles/liter): NaCl,

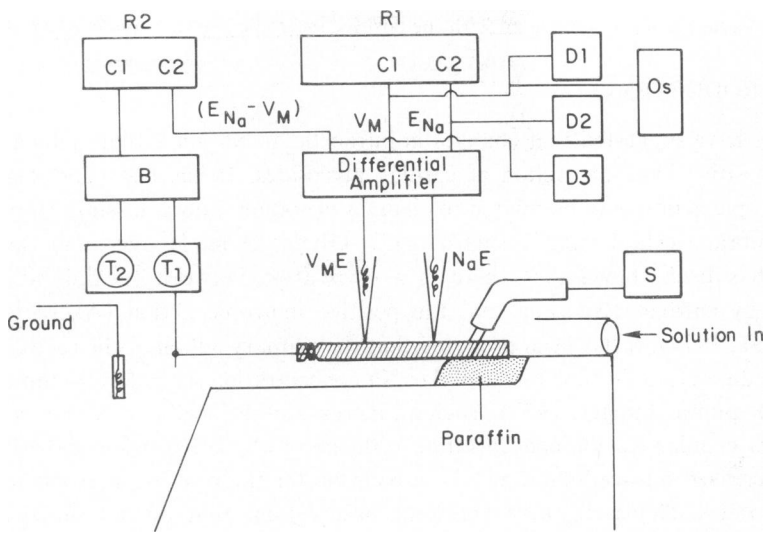

FIGURE 1 Schematic diagram of experimental system for the measurements of intracellular Na ion activity and contractile tension of sheep Purkinje fibers. A part of the muscle chamber is shown. Hatching represents Purkinje fiber bundle. See text for abbreviations.

137; KCl, 5.4; $MgCl_2$, 1.05; $NaHCO_3$, 13.5; NaH_2PO_4, 2.4; $CaCl_2$, 1.8; dextrose, 11.1. Tyrode's solution was saturated with a gas mixture of 95% O_2 and 5% CO_2 to achieve a pH of 7.2–7.4. Temperature of the solution was kept at 36°C throughout the experiments.

Tension Recording

One end of the fiber bundles was held by stimulating electrodes on paraffin in the narrow channel as shown in Fig. 1. The muscle fibers were stimulated with square pulses delivered to the electrodes from a stimulator (S, WPI model 301-T, W-P Instruments, Inc., New Haven, Conn.) through a stimulus isolation unit. The stimulus voltage was generally 20–40% above threshold and the stimulus rate was 60/min throughout this study. The other end of the bundle was tied with one end of fine surgical silk. The other end of the silk was connected to a pin of a mechano-electric transducer by means of a small ring. The fibers were stretched to ~140% of slack length throughout the experiments (Gibbons and Fozzard, 1971). The transducer was composed of two RCA 5734 tubes (T_1 and T_2 in Fig. 1; RCA Solid State, Somerville, N.J.) in a heat sink. One tube (T_2) was used to prevent potential drift caused by heat. The tubes were connected to a modified Wheatstone bridge (B). The output of the bridge was connected to the channel 1 (C1) of the recorder 2 (R2, Brush model 280, Gould Inc., Instruments Div., Cleveland, Ohio). The transducer was calibrated with several weights. The transducer was linear, with a sensitivity of 1.1 mV/mg. In this study, the changes in tension were expressed as the ratio of peak contractile tension during exposure to dihydro-ouabain to control contractile tension.

Measurement of Intracellular Na Ion Activities

Recessed-tip Na^+-selective glass microelectrodes were made from Eisenman's sodium glass (NAS 11-18, Microelectrodes, Inc., Londonderry, N.H.) as described by Thomas (1976). The construction and characteristics of the microelectrodes were described previously (Lee, 1979). The behavior of the Na^+-selective microelectrode can be described according to the following equation:

$$E_{Na} = E_0 + S \log (a_{Na} + k_{NaK} a_K), \quad (1)$$

where E_{Na} is a microelectrode potential; E_0 is a constant of a microelectrode; S is an empirical slope obtained from the electrode calibration ($\Delta E/\Delta \log a_{Na}$); a_{Na} and a_K are Na and K ion activities, respectively; k_{NaK} is the selectivity coefficient of a microelectrode. The microelectrode response was linear in the range of 1–100 mM NaCl solutions, and the slopes (S) were between 60 and 66 mV per 10-fold change in Na^+ concentration at 36°. E_0 was obtained from the linear calibration curve. However, the potential in KCl solutions was not linear and the slope ($\Delta E/\Delta \log a_K$) is less than the corresponding slope in NaCl solutions. This indicates that the selectivity of the Na^+-selective glass microelectrode depends on the concentrations of calibration solutions. For this reason, it was suggested that the mixture solutions of NaCl and KCl in addition to the pure solutions be used for the microelectrode calibrations (Lee, 1979). In this study, we used a mixture solution of 10 mM NaCl + 150 mM KCl. Fig. 2 *A* (right panel) shows a typical potential recording of a Na^+-selective microelectrode in pure solutions and the mixture solution. The response time of the microelectrodes to a stable potential was normally between 60 and 120 s in the mixture solution. Note that the microelectrode potential in 10 mM NaCl is very close to that in 10 mN NaCl + 150 mM KCl. The selectivity coefficient (k_{NaK}) in Eq. 1 was determined from the measured potentials as shown in Fig. 2 *A* (right panel). The ionic activities of the calibration solutions are known and can be determined. The selectivity coefficients of the microelectrodes were between 0.015 and 0.05. These selectivity coefficients are satisfactory to determine intracellular Na ion activity, although they are substantially higher than those (about 0.005) of the electrodes with long exposed-tip length (Lee, 1979). When the Na^+-selective microelectrode penetrates a cell, cell membrane potential (V_M) measured with a conventional microelectrode should be subtracted from E_{Na}. Thus, eq. 1 gives the following equation:

$$E_{Na} - V_M = E_0 + S \log (a^i_{Na} + k_{NaK} a^i_K), \quad (2)$$

where a^i_{Na} and a^i_K are intracellular Na and K ion activities, respectively. Fig. 1 shows the arrangement for

measurements of E_{Na}, V_M, and ($E_{Na} - V_M$). A Na^+-selective microelectrode (NaE) and a conventional microelectrode (V_ME) were connected to a differential amplifier (model 604, Keithley Instruments, Inc., Cleveland, Ohio). The outputs (E_{Na} and V_M) of the amplifier were connected to channels 1 and 2 (C1 and C2) of R1 (Brush model 2400, Gould, Inc.). They also were connected to the digital voltmeters (D1 and D2) to read precise voltages. The output of V_M also was connected to an oscilloscope (Os, model 5113, Tektronix, Inc., Beaverton, Oreg.) to record action potentials. The differential output ($E_{Na} - V_M$) of the amplifier was connected to the channel 2 (C2) of the recorder R2 and a digital voltmeter (D3). With this arrangement (Fig. 1), E_{Na}, V_M, ($E_{Na} - V_M$), and tension were simultaneously measured. Thus, intracellular Na ion activities (a^i_{Na}) were calculated by Eq. 2. 120 mM was used for the intracellular K ion activities, a^i_K (Lee and Fozzard, 1979). The relative contribution by the intracellular K ion activity was small because of the low k_{NaK}. The relative contribution by changes in a^i_K was almost negligible.

The cardiac glycoside used was dihydro-ouabain (ICN International Chemical and Nuclear Corp., City of Industry, Calif.). A stock solution of 10^{-4}M dihydro-ouabain was prepared. It was further diluted with Tyrode's solution before application to Purkinje fibers. Dihydro-ouabain (DHO) concentrations used in this study were 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6}, 5×10^{-6}, and 10^{-5} M. Before measurement of intracellular Na ion activities, the fibers were stimulated for ~20 min to measure contractile tension of normal fibers (control tension, T_c). Then, without stimulation, fibers were impaled with a Na^+-selective microelectrode and a conventional microelectrode to measure intracellular Na ion activities. The distance between the sites impaled by the two microelectrodes was ~1 mm. After the microelectrode potentials reached stable values, Tyrode's solution containing DHO was applied for 15 min. When the potentials changed and reached new levels, the fibers were stimulated to measure changes of contractile tension. In some experiments, the fibers were stimulated during the DHO application so that changes in intracellular Na ion activity and contractile tension could be measured during onset of the cardiac glycoside effect.

RESULTS

Normal intracellular Na Ion Activity

Fig. 2 illustrates the method of measurement of intracellular Na ion activity. Fig. 2 *A* represents the potential recording measured with a Na^+-selective microelectrode with respect to a grounded reference electrode. The Na^+-selective microelectrode was immersed in bathing Tyrode's solution and its recessed space was filled with the solution. This filling could be easily checked by measurement of the microelectrode resistance and stability of the microelectrode potential in the solution. The first potential level (~−83 mV) from the zero potential represents the Na ion activity in Tyrode's solution. Fig. 2 *B* represents the potential of a conventional microelectrode with respect to the grounded reference electrode. The potential recorded in Fig. 2 *C* represents the difference between the Na^+-selective microelectrode potential (Fig. 2 *A*) and the conventional microelectrode potential (Fig. 2 *B*). After the Na^+-selective microelectrode potential reached a stable level in bathing solution, a cell was impaled. The impalement is indicated by an arrow in Fig. 2 *A* and *C*. The impalement resulted in a large change of the Na^+-selective microelectrode potential. There were two components in the large potential change; (*a*) the initial rapid potential change corresponding to the cell membrane potential and (*b*) the following slow potential change corresponding to the potential change caused by equilibration of the ions in the recessed space with those in myoplasm. Then, a cell was impaled with a conventional microelectrode to measure cell membrane potential (V_M). This impalement is indicated by an arrow in Fig. 2 *B*. On the impalement of the conventional microelectrode, the Na^+-selective microelectrode potential (E_{Na}) registered in Fig. 2 *C* was rapidly changed by the same magnitude as the membrane

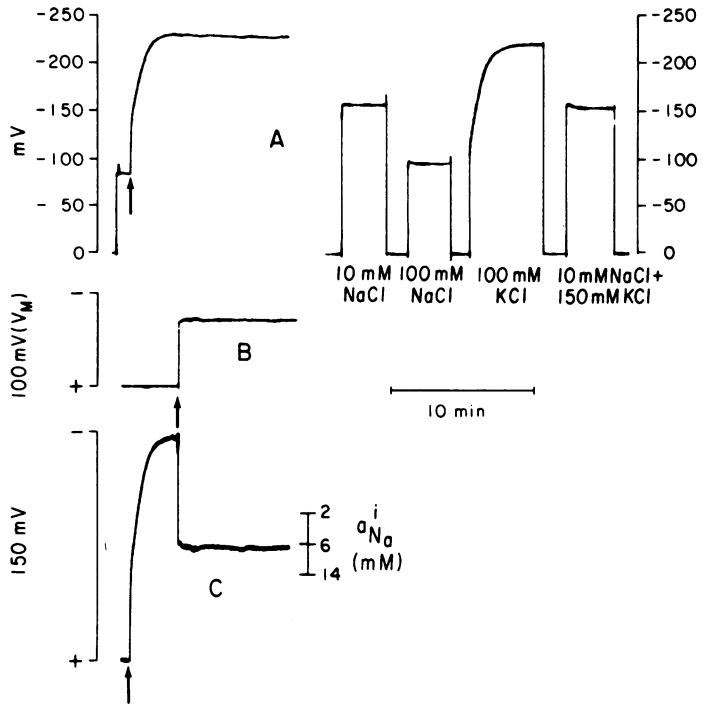

FIGURE 2 (*A*) Na^+-selective microelectrode potentials measured with respect to a grounded reference electrode. The arrow indicates an impalement of a cardiac Purkinje fiber with a Na^+-selective microelectrode filled with 1.5 M NaCl solution. The right panel of *A* shows calibration of the microelectrode used. (*B*) Transmembrane potential measured with respect to the grounded reference electrode with a conventional microelectrode. The arrow indicates an impalement. (*C*) The difference between the potential measured in *A* and that measured in *B*.

potential and reached another stable potential level. This potential level represents the difference ($E_{Na} - V_M$) between the Na^+-selective microelectrode potential and the membrane potential. The Na^+-selective microelectrode then was calibrated as shown in the right panel of Fig. 2 *A*. With the data obtained in Fig. 2, the intracellular Na ion activity was calculated according to Eq. 2. The relative scale of the intracellular Na ion activity (a^i_{Na}) is shown in Fig. 2 *C*. With the simultaneous measurements of E_{Na} and V_M (Fig. 2), the change in intracellular Na ion activity could be continuously monitored.

In certain conditions, the intracellular Na ion activity was determined by individual nonsimultaneous measurements of a Na^+-selective microelectrode potential and a conventional microelectrode potential. Two to four E_{Na} were measured at a certain part of the Purkinje fiber bundle. Then the Na^+-selective microelectrode was calibrated. V_M was measured four to seven times in the same area as that impaled with the Na^+-selective microelectrode. The mean values of the measured E_{Na} and V_M were used to determine the intracellular Na ion activity.

The intracellular Na ion activity of 6.4 ± 1.6 mM (mean ± SD) was obtained from 56 measurements in the resting fibers. The mean value of the resting membrane potentials was −76.6 ± 4.2 mV (SD, n = 118). In these measurements, the K^+ concentration of bathing solution was 5.4 mM.

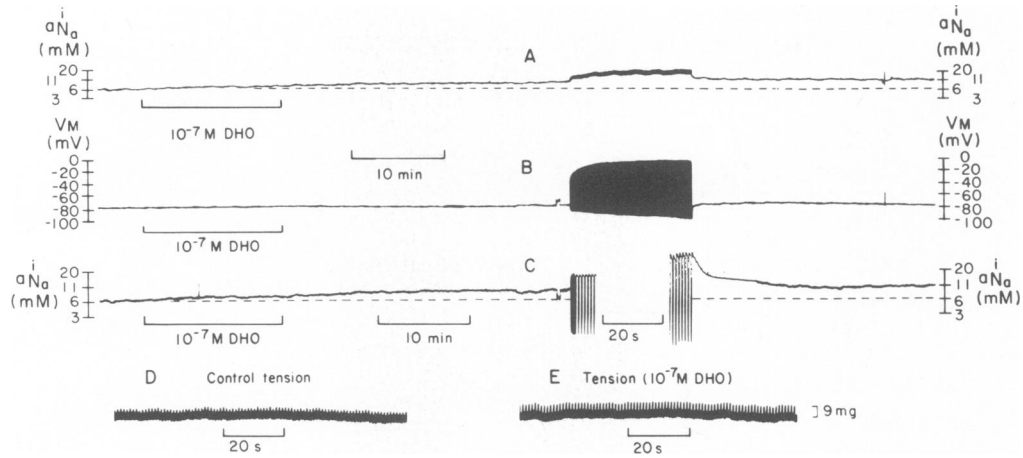

FIGURE 3 *A* and *C* show change of a^i_{Na} after the exposure to 10^{-7} M DHO for 15 min. After the increase in a^i_{Na}, the fibers were stimulated for ~13 min to measure contractile tension. During the stimulation in *C*, the chart speed of recorder 2 was increased for a better resolution of the tension measurement. The potential changes of recordings *A* and *C* during stimulation are due to the potential fluctuations by action potentials and not related to changes in a^i_{Na}. (*B*) Recording of the transmembrane potential of a cardiac Purkinje fiber. (*D*) Control tension measured before the exposure to 10^{-7} M DHO. (*E*) Steady-state tension measured during the stimulation after the increase in a^i_{Na}.

Effects of DHO on Intracellular Na Ion activity and Contractility

Intracellular Na ion activity and contractile tension of the fibers were measured before, during exposure to, and after washout of DHO. The concentrations of the cardiac glycoside applied were 5×10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6}, 5×10^{-6}, and 10^{-5} M. The changes in intracellular Na ion activity and contractile tension are illustrated with two examples: one for relatively low dose, 10^{-7} M (Fig. 3), and the other for relatively high dose, 5×10^{-6} M (Fig. 4).

Fig. 3 *A* and *B* represent the Na^+-selective microelectrode potential (E_{Na}) and membrane potential (V_M), respectively. The difference ($E_{Na} - V_M$) between the E_{Na} and the V_M is shown in Fig. 3 *C*. The equivalent scales of the intracellular Na ion activity (a^i_{Na}) are shown in both ends of Fig. 3 *A* and *C* recordings. After exposure to 10^{-7} M DHO, there was no substantial change in resting membrane potential (Fig. 3 *B*). In those cases without change of membrane potential, the a^i_{Na} change registered in recording *A* is the same as that in recording *C* since the constant membrane potential is not related to changes in a^i_{Na} (see Eq. 2). After the exposure, the intracellular Na ion activity increased from ~6 to ~11 mM (Fig. 3 *A* and *C*). In this experiment, the time taken for the a^i_{Na} change was ~30 min. After the increase in a^i_{Na}, the fibers were stimulated to measure contractile tension. Fig. 3 *D* and *E* represent the tensions measured before and after the increase in a^i_{Na}, respectively. The contractile tension increased by about two times.

Fig. 4 shows the changes in intracellular Na ion activity and contractile tension of fibers exposed to 5×10^{-6} M DHO. Before the exposure to 5×10^{-6} M DHO, the fibers had been exposed to 10^{-6} M DHO. Fig. 4 *A* represents the control tension (T_c) of the fibers before the exposure to 10^{-6} M DHO. When the fibers were exposed to 10^{-6} M DHO, intracellular Na

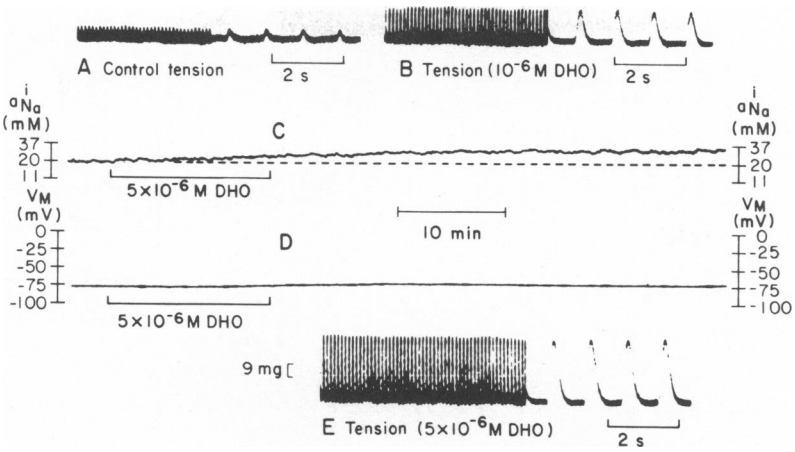

FIGURE 4 (*A*) Control tension measured before the exposure to 10^{-6} M DHO. (*B*) Steady-state tension after the exposure to 10^{-6} M DHO for 15 min. (*C*) Change of a^i_{Na} after the exposure to 5×10^{-6} M DHO for 15 min. (*D*) Change of transmembrane potential after the exposure to $5 \times f10^{-6}$ M DHO for 15 min. (*E*) Steady-state tension measured after the a^i_{Na} measurement in recording *C*.

ion activity increased from 7 to 19 mM and contractile tension increased by about five times ($T/T_c = 5$). The initial level of the recording C (Fig. 4) represents the increased a^i_{Na} (~19 mM). Fig. 4 *B* shows the tension measured from the fibers of the increased a^i_{Na}. Fig. 4 *C* and *D* show the changes in intracellular Na ion activity and membrane potential after the fibers were exposed to 5×10^{-6} M DHO. The intracellular Na ion activity increased from 19 to 30 mM. This increased a^i_{Na} was 23 mM greater than the control a^i_{Na} (7 mM). The cell membrane depolarized by ~5 mV. This potential change may be due to the loss of the intracellular K ion. Fig. 4 *E* represents the contractile tension measured from the fibers of the increased a^i_{Na}, ~30 mM. The tension was about eight times greater than the control tension ($T/T_c = 8$). After exposure to DHO, it was difficult to determine whether resting tension changed or not. With time we usually observed small changes of the base line of tension recording which might be due to the thermal drift of the tension transducer.

Fig. 5 *A* and *B* illustrate the effect of DHO concentration on contractile tension and intracellular Na ion activity, respectively. As DHO concentration was increased, tension and intracellular Na ion activity increased in similar fashion. Fig. 6 summarizes the results of changes in intracellular Na ion activities and contractile tensions of the fibers exposed to various concentrations of DHO. The fibers exposed to 5×10^{-8} M DHO did not show detectable changes in intracellular Na ion activity and contractile tension. It appeared that DHO 10^{-7} M produced a small increase in intracellular Na ion activity and contractile tension. As DHO concentration was raised, intracellular Na ion activity and contractile tension increased. The results indicate that the relationship between the a^i_{Na} increase and T/T_c (the ratio of the increased tension and the control tension) is linear. However, the changes in a^i_{Na} and T varied together among the fiber bundles used (i.e., large standard deviations).

In the fibers exposed to 5×10^{-6} and 10^{-5} M DHO, initial increase in contractile tension was followed by apparent toxic effects. In the fibers exposed to 5×10^{-6} M DHO, there was an initial increase in contractile tension without apparent toxic effects. With time, apparent

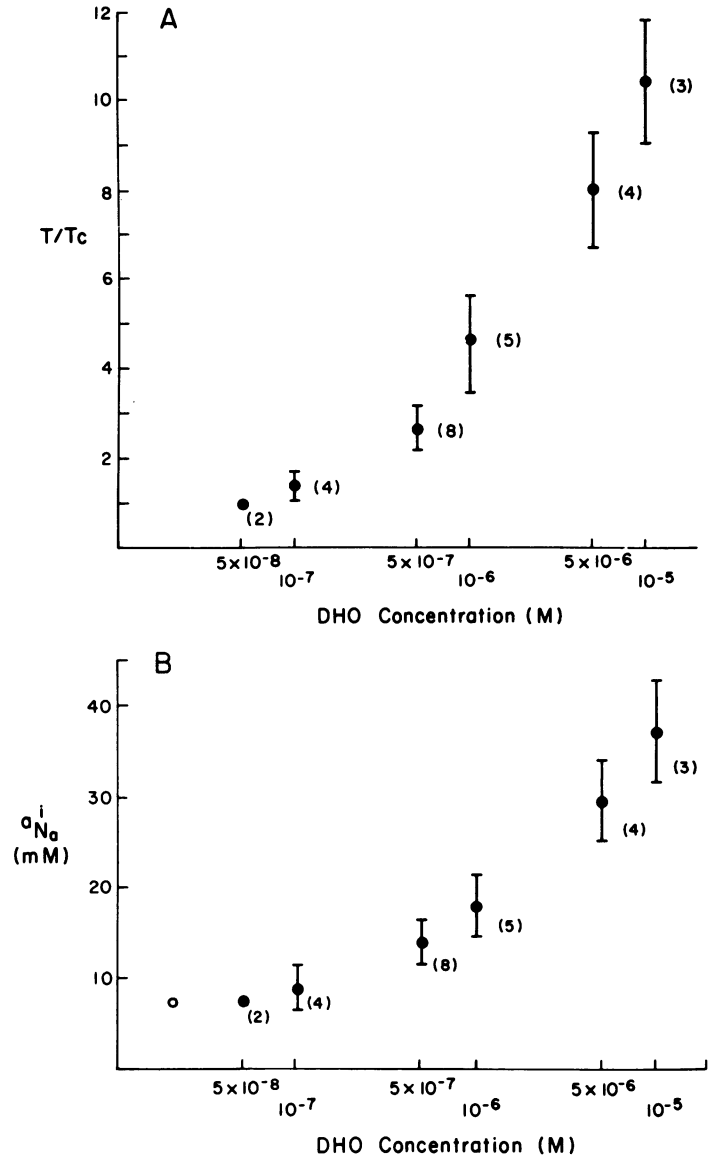

FIGURE 5 (*A*) Relation between relative contractile tension (T/T_c) and concentration of DHO exposed. (*B*) Relation between a^i_{Na} and concentration of DHO exposed. The open circle represents the control a^i_{Na}.

toxic effects were observed on both action potential and contraction of the fibers. The resting membrane potentials were reduced (more positive) and the peak of the action potentials decreased (more negative). The upstroke velocity of the action potentials was markedly reduced so that phase 1 of the action potentials could not be seen. Moreover, transient depolarizations were observed in the membrane potential during the diastolic period. A transient increase in tension occurred during the transient depolarization. The time-course of

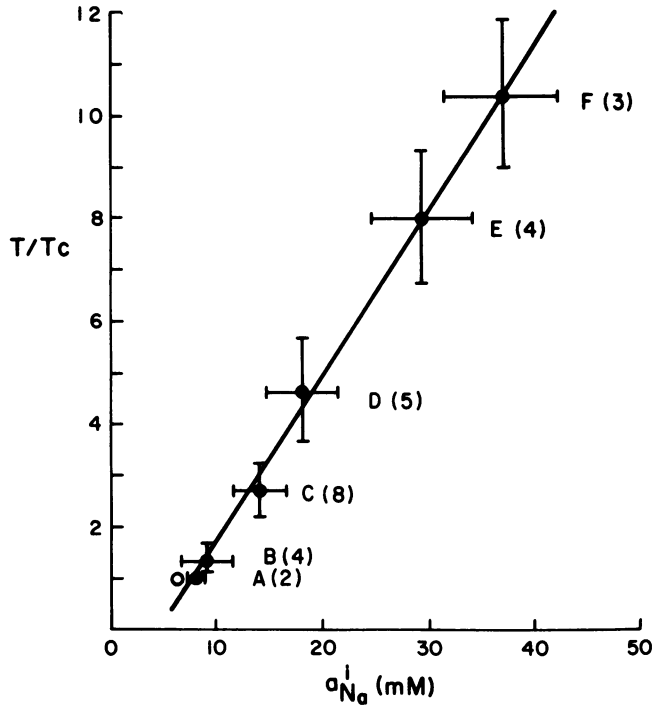

FIGURE 6 Relation between relative tension, T/T_c and intracellular Na activity, a^i_{Na} at the DHO concentrations of A (5×10^{-8} M), B (10^{-7} M), C (5×10^{-7} M), D (10^{-6} M), E (5×10^{-6} M), and F (10^{-5} M). The number in each parenthesis represents the number of measurements. The open circle represents the mean a^i_{Na} before the exposure to DHO.

the transient potential and tension changes suggests that the transient increase of the tension is associated with the transient depolarization.

Changes in a^i_{Na} and T during Onset and Washout of DHO Effects

We have shown the increase of the fiber tension when the intracellular Na ion activity increased after the exposure to DHO. In order to observe the time-courses of the changes during the onset of the cardiac glycoside effect, both intracellular Na ion activity and tension were simultaneously measured before and after the exposure to DHO. Fig. 7 *A* and *C* show the changes in intracellular Na ion activity and contractile tension, respectively, before and after the exposure to 10^{-6} M DHO. The simultaneous measurement of the membrane potential is shown in Fig. 7 *B*. After the exposure, the fibers were stimulated with certain intervals to measure contractile tension. It can be seen in Fig. 7 *A* that the intracellular Na ion activity increased to a new level after each stimulus during the onset. The potential change during each stimulus (recording A) is due to potential fluctuations by action potentials and may not be related to changes in a^i_{Na}. Fig. 7 *C* shows some of the tensions measured during each stimulus; they increase with time. For about a 50-min period after the exposure, the resting membrane potential did not change (Fig. 7 *B*). In Fig. 7 *D* and *E*, the changes in contractile tension and intracellular Na ion activity (Fig. 7 *C* and *A*) are plotted as a function

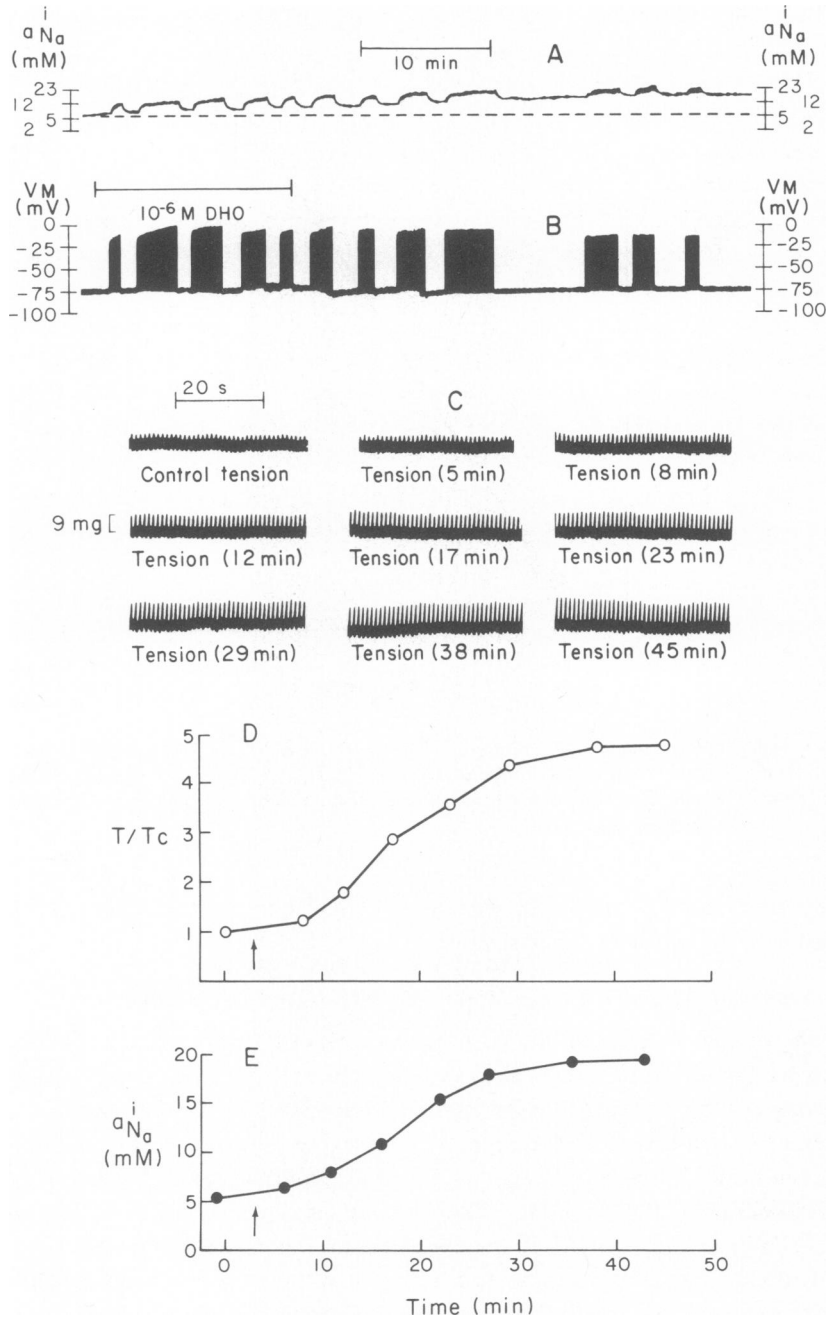

FIGURE 7 (*A*) Change in a^i_{Na} during and after the exposure to 10^{-6} M DHO. Note the potential drift at each stimulation. (*B*) Transmembrane potential during and after the exposure to 10^{-6} M DHO. (*C*) Control tension and steady-state tension measured at the time (indicated in each parenthesis) after the exposure to 10^{-6} M DHO. Note that the chart speed in *C* was much faster than that in *A* and *B*. (*D*) Relative contractile tensions (T/T_c) were plotted as a function of time. (*E*) Intracellular Na ion activities (a^i_{Na}) were plotted as a function of time. Arrow indicates the beginning of 15 min exposure of DHO (10^{-6} M).

of time. The initial T/T_c and a^i_{Na} were the control values of the fibers before the exposure. The T/T_c and a^i_{Na} increased with time and reached relatively stable levels ~30 min after the exposure. The time taken to reach the relatively stable levels varied from 20 to 30 min. The time may be influenced by some factors including the diffusion time of the cardiac glycoside through extracellular space (see Discussion). In any case, the time-course of the increase in T/T_c is similar to that of the increase in a^i_{Na} as shown in Fig. 7 *D* and *E*.

Recovery of intracellular Na ion activity and contractile tension varied considerably among the fibers. In the fibers exposed to low concentrations of DHO, the intracellular Na ion activity and contractile tension usually recovered within 40–60 min after washout of DHO. Fibers exposed to high concentrations of DHO recovered slowly or incompletely within ~2 h after washout. Fig. 8 shows the results obtained from a fiber that completely recovered intracellular Na ion activity and contractile tension. After the exposure to 5×10^{-7} M DHO, the intracellular Na ion activity of the fiber increased from 8 to 14 mM and then completely recovered (Fig. 8 *A*). During the change in a^i_{Na}, the resting membrane potential of a fiber did not change. After the recovery of a^i_{Na}, contractile tension of the fibers was measured (Fig. 8 *B*) and was similar to that before the exposure to 5×10^{-7} M DHO. Then the fibers were exposed to 10^{-6} M DHO (Fig. 8 *C*). After the exposure, the intracellular Na ion activity increased from 8 to 19 mM. After the increase of a^i_{Na}, the fiber tension was measured and is shown in Fig. 8 *D*. The tension was about four times greater than that (Fig. 8 *B*) before the exposure to 10^{-6} M DHO. Fig. 8 *E* shows that after washout of DHO, the intracellular Na ion activity recovered to the initial level. After the recovery of a^i_{Na}, the tension measured is shown in Fig.

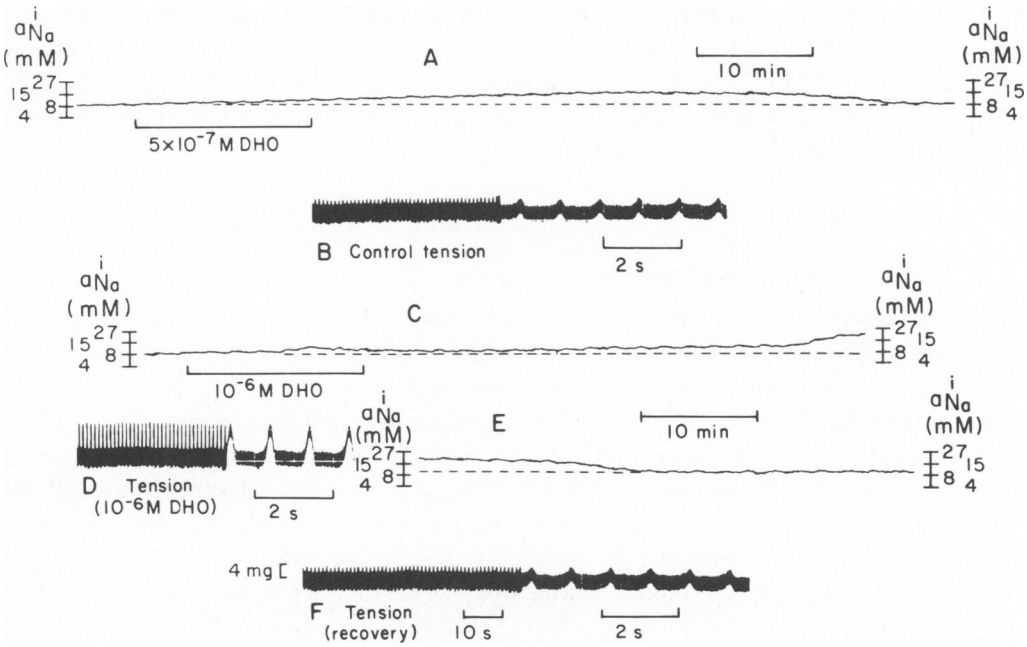

FIGURE 8 (*A*) Increase and recovery of a^i_{Na} after the exposure to 5×10^{-7} M DHO for 15 min. (*B*) Control tension measured after the recovery of a^i_{Na}. (*C*) Increase of a^i_{Na} after the exposure to 10^{-6} M DHO for 15 min. (*D*) Steady-state tension measured after the increase of a^i_{Na}. (*E*) Recovery of a^i_{Na} after washout of 10^{-6} M DHO. (*F*) Tension measured after the recovery of a^i_{Na}.

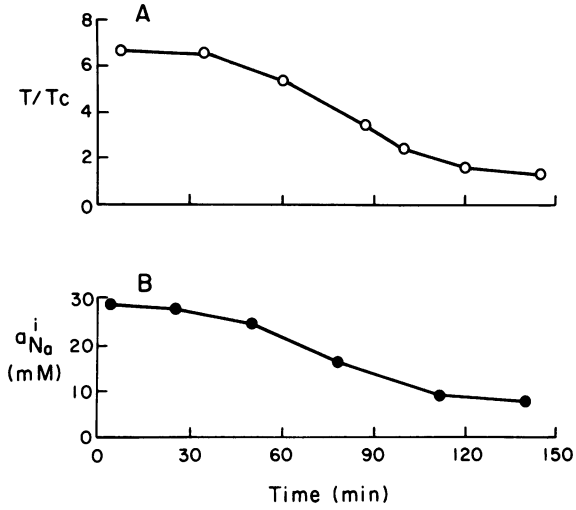

FIGURE 9 Time-course of fall in contractile tension (*A*) and intracellular Na ion activity (*B*) during washout of 5×10^{-6} M DHO. This fiber had been exposed to DHO for 15 min.

8 *F*. The tension also recovered and was similar to the initial tension (Fig. 8 *B*) before the exposure to 10^{-6} M DHO. Thus, this experiment shows that the recovery of a^i_{Na} was accompanied by the recovery of contractile tension. Fig. 9 shows the results of an experiment in which after the exposure to a relatively high dose of DHO the recoveries of contractile tension and a^i_{Na} were slow and took about 2 h. The initial a^i_{Na} of the fibers was 7 mM before the exposure to 5×10^{-6} M DHO. After the exposure, the a^i_{Na} increased to ~28 mM and the T/T_c was ~7. As shown in Fig. 9 *A* and *B*, the time-course of the fall in contractile tension was similar to that of the return to control levels of a^i_{Na}.

DISCUSSION

Intracellular Na Ion Activity

The measurement of ion activities rather than ion concentrations is important in the study of physiological functions such as membrane potential, action potential, ion transport, and muscle contraction. This is because the processes respond to the ion activities in the strict sense. Intracellular Na ion activities have been measured in various tissues since Hinke (1959) introduced cation-selective glass microelectrodes made from Eisenman's glasses (NAS 11-18 and NAS 27-4). The total cellular Na concentrations also have been measured by using flame photometry. There appears to be general agreement that the cellular Na concentration is much greater than the intracellular Na ion activity in excitable cells such as barnacle muscle (Hinke, 1959), giant axons (Hinke, 1961), frog skeletal muscle (Lev, 1964), and rabbit ventricular muscle (Lee and Fozzard, 1975). The ratio of the cellular Na ion activity and concentration (an apparent activity coefficient) has been found to be ~0.3, a much lower value than that of ~0.75 in the extracellular bathing medium. The possible reasons for the difference have been described (Lee and Armstrong, 1974; Lee and Fozzard, 1975; Ellis, 1977). Ellis (1977) measured the intracellular Na ion activity of sheep cardiac Purkinje fibers

and found it to be 7.2 mM. In the present study, the intracellular Na ion activity was 6.4 mM. We did not measure the cellular Na concentration of the fibers. Bosteels and Carmeliet (1972) reported a value of 25 mM for the cellular Na concentration of cardiac Purkinje fibers. If this value is used, the apparent Na ion activity coefficient is ~0.3. This agrees with the observations that the intracellular Na ion activity was much lower than the cellular Na concentration.

The recessed space of Na^+-selective microelectrodes is filled with Tyrode solution containing 150 mM Na ion before each impalement. Therefore, it is expected that the high Na concentration in the recessed space may influence measurements of intracellular Na ion activity and the microelectrode response time. Calculation showed that the Na ion concentration in the recessed space of a typical microelectrode causes an error of only ~1% of the intracellular Na ion activity in Purkinje fibers. The recessed space may slow response time of the microelectrodes (Lee, 1979). Another problem of the microelectrodes may be the clogging of the tip, which occurs rarely and may cause a very slow response. However, the typical response time of 1–2 min should not influence the results in this study. In intracellular application of ion-selective microelectrodes, the ion-selective microelectrodes must measure the same membrane potential as the conventional microelectrodes. Membrane potentials of Purkinje fibers were measured with the insulating micropipettes filled with 3 M KCl and were compared with the membrane potentials measured with conventional microelectrodes. There was no substantial difference between the two membrane potentials. This indicates that subtraction of conventional microelectrode potential from Na^+-selective microelectrode potential may not produce a significant error.

Intracellular Na Ion Activity and Cardiac Glycoside Action

It is well known that Na^+, K^+-ATPase is responsible for the active transport of Na and K ions across cell membrane (Skou, 1965). Thus, it is expected that inhibition of Na^+, K^+-ATPase results in a net gain of Na and a net loss of K by the cells. The specific inhibitory action of cardiac glycosides on Na^+, K^+-ATPase has been established and well documented (Lee and Klaus, 1971; Schwartz et al., 1975). It has been shown that inhibition of Na^+, K^+-ATPase by cardiac glycosides is related to the cardiac glycoside inotropy action (Akera et al., 1973; Schwartz et al., 1974). Recently it has been shown, by direct measurements with Na^+-selective glass microelectrodes, that the intracellular Na activity increases after the exposure to various concentrations of cardioactive steroids (Ellis, 1977; Deitmer and Ellis, 1978*b*). However, it has not been reported whether or not the positive inotropic action of the cardiac glycosides is related to the intracellular Na ion activity. We have measured the changes in intracellular Na ion activity and tension of cardiac Purkinje fibers before and after exposure to various concentrations of DHO. After exposure to 5×10^{-8} M DHO for 15 min, no detectable changes in intracellular Na ion activity and contractile tension were observed. Deitmer and Ellis (1978*b*) also showed that DHO at concentrations between 10^{-8} and 10^{-7} M did not produce a decrease or an increase of a^i_{Na}. The intracellular Na ion activity and contractile tension changed after the fibers were exposed to 10^{-7} M DHO. Thus, it appears that the threshold for the changes is around 10^{-7} M for the cardiac glycoside under the present experimental conditions. This observation is similar to that by by Deitmer and Ellis (1978*b*). The increase in DHO concentration above the threshold resulted in progressively greater increases in a^i_{Na} and tension. The progressive increase in a^i_{Na} is similar to that observed

by Dietmer and Ellis (1978*a*). In a given concentration of DHO, however, the a^i_{Na} increase in this study was somewhat greater than that observed in their study.

The time taken to reach the relatively stable intracellular Na ion activity and tension after the exposure was usually in the range of 20–30 min. This onset time may be influenced by certain factors, such as diffusion and the time constant of the binding of cardiac glycoside to Na^+, K^+-ATPase. At low concentrations of cardiac glycosides, the inhibition of Na^+, K^+-ATPase is time dependent, and a variety of physiological ligands affect the rate of binding of cardiac glycosides to Na^+, K^+-ATPase (Wallick et al., 1977). Other possible factors may include the fiber condition, potency, and concentration of cardiac glycosides and, in addition, the effective time for the solution change in the muscle chamber. In any case, the most important observation in this study is the intimate correlation existing between the intracellular Na ion activity and the positive inotropic action of the cardiac glycoside during both exposure to and washout of the drug.

The apparent toxic effects have not been observed after the fibers were exposed to DHO of 10^{-6} M or less for 15 min. During exposure to 5×10^{-6} or 10^{-5} M DHO, however, after an initial positive inotropic effect, toxic effects were observed on both action potential and contraction. The resting membrane potential decreased to less negative values (Fig. 4). This may be due to the fall in the intracellular K ion activity (Miura and Rosen, 1978). At the same time, the amplitude and upstroke velocity of action potential were reduced. This may be due to the reduction of the driving force of Na ion because of the increase in intracellular Na ion activity, and an inactivation of the fast Na channels, which is due to the fall in resting membrane potential. Another effect is the transient depolarization in the membrane potential during the diastolic period, which has been documented by others (Ferrier et al., 1973; Rosen et al., 1973; Lederer and Tsien, 1976; Kass et al., 1978). The apparent toxic effects usually occurred when the intracellular Na ion activity was ~30 mM or greater. Therefore, such alterations in action potential and contractile tension are probably secondary to the changes in the intracellular ionic environment brought about by the drug as suggested by Beeler (1977).

The time-courses of recoveries in intracellular Na ion activity and contractile tension are usually slower than those of the onsets. After the exposures to 5×10^{-6} or 10^{-5} M DHO and the subsequent toxic effects, the increased intracellular Na ion activity and tension do not usually return to the control level for ~2-h periods after washout. In the fibers exposed to 10^{-6} M or less, the intracellular Na ion activity and tension recovered within about 1 h. Thus, the recovery time may be dependent on the concentration of the cardiac glycoside. This is similar to the observations reported by Dietmer and Ellis (1978*b*). In those fibers which recovered, the time-course of the recovery in intracellular Na ion activity is similar to that of the recovery in contractile tension. Therefore, the results in this study lead to the conclusion that during exposure to cardiac glycosides, an increase in intracellular Na ion activity is intimately associated with the augmentation in contractile force. Since the intracellular free Ca ion is directly responsible for the increase in cardiac contractility, the increased intracellular Na ion activity may bring about an enhancement of the intracellular free Ca ion concentration. This may result from a reduction of Ca ion extrusion or enhanced Ca entry via Na-Ca exchange, and/or a Ca ion release from intracellular binding sites such as mitochondria. Na-Ca exchange has been shown in cardiac tissues (Reuter and Seitz, 1968). These processes may increase the base level of the intracellular Ca ion concentration that leads to the positive

inotropic effect of cardiac glycosides. Recently, it has been reported that the monovalent cation ionphores monensin and nigericin produce positive inotropic responses in cardiac muscle (Sutko et al., 1977; Shlafer et al., 1978). These results support the Na-Ca exchange mechanism as an important modulator of contractility.

In low doses ($<10^{-7}$ M) of ouabain, however, intracellular Na ion activity does not increase (Ellis, 1977), and the Na pump is stimulated (Cohen et al., 1976) while positive inotropic effect is observed (Blood, 1975; Blood and Noble, 1977). These observations suggest that at the low doses the correlation between Na pump inhibition and inotropism is questionable. At the low doses, ouabain and ouabagenin induce a stimulatory effect on the pump while DHO exerts only an inhibitory effect (Ghysel-Burton and Godfraind, 1977). This result suggests that in the low doses the action of DHO may be different from that of ouabain.

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