The Effects of Membrane Potential, Extracellular Potassium, and Tetrodotoxin on the Intracellular Sodium Ion Activity of Sheep Cardiac Muscle

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SUMMARY. The intracellular sodium ion activity was measured using liquid ion-exchange microelectrodes with rapid response times in sheep Purkinje fibers and ventricular muscle under voltage control. The mean sodium ion activity in quiescent Purkinje fibers was 8.5 mM at a holding potential of —80 mV. With maintained hyperpolarizing (—110 mV) or depolarizing (—40 and 0 mV) voltage steps, sodium ion activity increased or decreased, respectively. At 0 mV, the mean steady state value for the sodium ion activity was 3.8 mM. Following a voltage step to 0 mV, or back to —80 mV, the time course of the sodium ion activity change could be fitted by single exponentials, with similar half-times. Increasing the extracellular potassium ion concentration from 5.4 to 15 mM did not alter the steady state value of the sodium ion activity at clamped voltages of —80 or 0 mV, which suggests that the external potassium ion activating site of the Na-K pump was saturated. With the extracellular potassium concentration 0 mM (holding potential —80 mV), the sodium ion activity increased. When maintained depolarizing steps to 0 mV were applied, the sodium ion activity decreased by up to 20 mM. This large fall in sodium ion activity is assumed to represent partial reactivation of the Na-K pump due to potassium ion accumulation in clefts. We also studied the stimulation-dependent change in sodium ion activity. Trains of action potentials or short duration depolarizing voltage clamp steps caused a frequency dependent rise in sodium ion activity. The magnitude of the rise of sodium ion activity was not altered by lengthening the duration of each voltage clamp step, but was inhibited by tetrodotoxin or by holding the membrane potential at —50 mV between depolarizing steps. These results show that sodium ion activity is a complex function of membrane voltage, depolarization frequency, and time. The rise in sodium ion activity with stimulation appears to depend on sodium ion entry regulated by the sodium channel, and may be important in the modulation of intracellular calcium and tension through the Na+-Ca++ exchange mechanism. (Circ Res 54: 652-665, 1984)
anism. Consequently, variations in $a_{\text{Na}}$ may play an important role in the regulation of cardiac contractility.

Preliminary reports of the work have appeared (January et al., 1981; January and Fozzard, 1982).

Methods

Preparations

Single free-running unbranched Purkinje fibers having diameters of 150–350 $\mu$m were used. In a few experiments, small free-running ventricular trabecular muscle columns, 150–300 $\mu$m in diameter, were substituted. Sheep hearts were obtained from a nearby slaughterhouse and transported in cooled oxygenated Tyrode’s solution.

Electrodes

Membrane potential was measured with glass micropipettes (WPI, 1B150F glass) filled with 3 M KCl, and having resistances of 5–12 M$\Omega$ (for Purkinje fibers) or 10–16 M$\Omega$ (for ventricular muscle) and small tip potentials. Identiﬁcal glass micropipette blanks were used to fabricate the Na$^+$ electrodes. The micropipettes were exposed to the vapors of dichlorodimethylsilane and baked at 200°C for at least 60 minutes. Liquid ion exchange resin ETH 227 (Steiner et al., 1979) was introduced by suction into the micropipette tips for 50–150 $\mu$m, and the electrodes were back-filled with 300 mM NaCl. Electrodes were stored for several hours before calibration. Calibration was performed in the experimental chamber by exposing the Na$^+$-electrode tip to mixed NaCl and KCl solutions of constant concentration (150 mM) and containing 1, 3, 10, 30, or 100 mM of Na$^+$ (for further detail, see Sheu and Fozzard, 1982). Calibration solutions were prepared by diluting appropriate volumes of 1 N NaOH and KOH with 1 N HCl to pH 7.0. Deionized, glass-distilled water was used throughout. The activity coefﬁcient for Na$^+$ in mixed solutions was 0.76 (Sheu and Fozzard, 1982). Calibration curves were performed after, and usually before, each experiment, and for a 10-fold change in [Na$^+$], the curves had slopes greater than 48 mV (10–100 mM) and 35 mV (3–30 mM).

Voltage Control

In these experiments, a major goal was to compare the influence of $V_m$ on $a_{\text{Na}}$ in Purkinje fiber and ventricular muscle preparations. To control $V_m$, we used a single rubber membrane gap voltage clamp system (Fig. 1). Each preparation was ﬂoated into a small-bore polyethylene tube for protection from mechanical damage, and placed through a tightly constricting hole in a single latex rubber membrane which separated the preparation into endpools. The preparation extended into both halves of the chamber. In the test endpool, the distal part of the preparation was crushed in order to create a short segment for voltage clamp. The voltage recording electrode ($V_m$) was impaled approximately one-third the distance from the rubber membrane with the Na$^+$ electrode impaled just distally. The endpool was held at virtual ground potential, with the ground clamp potential-sensing electrode positioned at the surface of the preparation between its middle and end, with the current electrode surrounding the preparation and extending to the rubber membrane. Current for intracellular injection was applied from the left hand endpool.

The voltage electrode was led to the input of a capacitance-compensated high input impedance ampliﬁer before being displayed on a digital voltmeter (Newport, model 201-2). The signal from the Na$^+$ electrode was led to the input of an ultra-high input impedance electrometer (Keithley, model 604) before being displayed on a second digital voltmeter. The shield of the input cable from the Na$^+$ electrode was driven at unity gain to reduce stray capacitance effects and speed the response of the electrode. The electrical time constant for the Na$^+$ electrodes usually was between 0.1 and 1.0 second (see Fig. 2), which permitted rapid measurement of $a_{\text{Na}}$, following voltage clamp steps. All data channels were recorded on a stripchart recorder.

Current flowing between the halves of the chamber must pass through the small interconnecting hole in the rubber membrane and, hence, through the preparation (Suenson, 1979). Advantages of the single rubber membrane gap technique are that both Purkinje fiber and ventricular muscle preparations may be studied in the same experimental apparatus, and a sucrose gap is not used. For ventricular muscle preparations, the use of the single rubber membrane gap technique to control $V_m$ is particularly advantageous because alternate techniques (e.g., current injection using a micropipette or sucrose gap) impose serious limitations (see Beeler and McGuigan, 1978). The preparations were bathed along their entire length in identical media, so that changes in intracellular ion activities that result from exposure to sucrose or solutions of altered ionic composition were avoided. Because intracellular current injection is achieved by a gap technique, use of an additional micropipette for current injection is avoided, as are the problems associated with a point source of current (Eisenberg and Johnson, 1970). The gap method provides imperfect isolation of the current injec-
tion endpool from the test endpool, resulting in a transgap extracellular leak current that interferes with quantitative measurements of transmembrane current. Therefore, analysis of current records was not done. Virtual ground was maintained at the surface of the preparation in the test endpool by a ground clamp circuit (New and Trautwein, 1972) in order to minimize voltage gradients due to current flow to ground.

Figure 2 shows a record from a Purkinje fiber where \( V_m \) was stepped from \(-70 \text{ mV} \) to \(-45 \text{ mV} \) for 500 msec and then returned to \(-70 \text{ mV} \). The signal from the \( \text{Na}^+ \) electrode, \( V_{Na} \), is displayed below the voltage trace, \( V_m \). It shows that the electrical time constant for the \( \text{Na}^+ \) electrode was approximately 100 msec, and that the magnitude of the voltage deflection \( V_{Na} \) was indistinguishable from the voltage step \( V_m \). At the top of Figure 2 is the current record. After the initial capacitance spike, a large amplitude inward sodium current and a small amplitude slow inward current are present.

For the voltage clamp experiments, two basic protocols were employed. The first protocol used voltage clamp steps lasting 7–10 minutes to different \( V_m \) from a holding potential \( (V_h) \) of \(-80 \text{ mV} \). \( V_m \) then was stepped back to \(-80 \text{ mV} \) for a minimum of 10 minutes before another voltage step was initiated. The second protocol used 3-minute-long trains of short-duration depolarizing voltage clamp steps (command pulse rise time < 0.3 msec) 50 mV in amplitude. A minimum of 7 minutes separated each pulse train. In all experiments reported, \( V_m \) and \( a'_{\text{Na}} \) were continuously monitored. If an electrode dislodged, the experiment was terminated.

**Solutions**

Tyrode's solution contained (in mM): 127 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.05 MgCl₂, 2.4 NaH₂PO₄, 22 NaHCO₃ and 5.5 glucose. In a few experiments, KCl was 0 or 15 mM without osmotic correction. Solutions were gassed continuously with a mixture of 95% O₂/5% CO₂ and the pH was 7.4. Each half of the chamber was perfused with identical solution at a rate of 3 ml/min. Tetrodotoxin (TTX; Calbiochem) was dissolved in distilled water to form a stock solution and diluted to a final concentration of \( 4 \times 10^{-6} \text{ M} \) when used. Experiments were performed at 34–37°C with the temperature during an individual experiment constant to ±0.5°C. Statistical analysis was by the Student's t-test with data expressed as mean ± SE.

**Results**

**Measurement of \( a'_{\text{Na}} \) in Voltage-Clamped Purkinje Fibers**

To measure \( a'_{\text{Na}} \), \( V_m \) must be subtracted from the \( \text{Na}^+ \) electrode signal. Since the voltage clamp control electrode and the \( \text{Na}^+ \) electrode were not positioned in the same location, it is possible that apparent changes in \( a'_{\text{Na}} \) could result from voltage differences between the electrodes. Some voltage differences necessarily must exist due to cable properties, and should be greater in ventricular muscle because of its shorter space constant. In addition, it could arise from the transgap leak current of the single-rubber membrane gap voltage clamp we used. In Figure 2, we showed that the magnitude of the voltage step recorded at the control electrode and the \( \text{Na}^+ \) electrode (following the RC response) were nearly identical. Since it is unlikely that \( a'_{\text{Na}} \) changed significantly during a single 0.5-second voltage step, this suggests that the membrane voltage at the \( \text{Na}^+ \) electrode site was well controlled. In order to test further the adequacy of voltage control in our single gap voltage clamp, in several experiments, we replaced the \( \text{Na}^+ \)-electrode with an independent 3 m \( \text{KCl} \) micropipette. The results from one experiment are shown in Figure 3A. \( V_m \) is the signal from the controlling electrode for the voltage clamp. \( V_m' \) is the signal from the independent voltage monitoring electrode (replacing the \( \text{Na}^+ \) electrode) and was imaged 175 \( \mu \text{m} \) distal to \( V_m \). \( V_m - V_m' \) is the signal from the difference channel, where ordinarily the \( \text{Na}^+ \) electrode signal minus the membrane potential signal is displayed. The current trace is shown at the bottom. In this preparation, the resting potential was \(-72 \text{ mV} \), after which the voltage clamp was switched on and \( V_h \) was set at \(-80 \text{ mV} \). The preparation then was depolarized to 0 mV for 10 minutes before being returned to \(-80 \text{ mV} \). Both traces, \( V_m \) and \( V_m' \), are similar throughout, except that the potential change recorded at the distal electrode was slightly less than that recorded at the proximal electrode. As shown in the difference channel, \( V_m' - V_m \), the difference between the electrodes was constant at approximately 1.5 mV, and may represent voltage nonuniformity similar to that found by Eissner et al. (1981a). Using a conventional two-pipette voltage clamp, Fozzard and Hiraoka (1973) showed similar voltage control.

When \( V_m' \) was replaced by a \( \text{Na}^+ \) electrode, similar small nonuniformities in voltage control sometimes were observed during steps in \( V_m \). Results from one experiment are shown in Figure 3B. \( V_{Na} \) is the signal from the controlling microelectrode. \( V_{Na} \) is the signal from the \( \text{Na}^+ \) electrode. \( a'_{\text{Na}} \) is the
difference signal \( (V_{Na} - V_m) \) with the calibration (logarithmic scale) shown to the right, and the voltage gain is the same as that in Figure 3A. The current trace is shown at the bottom. As in panel A, \( V_h \) was -80 mV and a 10-minute depolarizing step to 0 mV was imposed. Upon initiation or termination of the voltage clamp step, and after the brief RC of the high-impedance Na+ electrode, a small difference in the magnitude of the voltage steps between the two electrodes was recorded. If the change in \( a_{Na} \) is extrapolated to time = 0 following the step in \( V_m \), then the magnitude of the voltage nonuniformity in this fiber was <0.5 mV. When compared with previous work, small voltage nonuniformities should be more apparent because of the improved response times of our Na+ electrodes. In most successful experiments, the difference did not exceed 1-2 mV. Some impalements showed larger (5-10 mV) deviations with voltage steps, and these were discarded. As discussed below, when using Na+ electrodes, we are uncertain as to their relative contributions, no attempt has been made to correct records for the small instantaneous voltage jumps when \( V_m \) has been stepped. Voltage nonuniformity effects seem likely to predominate and could lead to a small overestimate of about 0.5 mV in measuring the change in \( a_{Na} \) when \( V_m \) has been altered.

Influence of Long Voltage Steps on \( a_{Na} \)

The effect of \( V_m \) on \( a_{Na} \) initially was studied, using 10-minute-long voltage clamp steps. For the fiber shown in Figure 3B, \( a_{Na} \) was 7.8 mM at \( V_m = -80 \) mV. After depolarization to 0 mV, \( a_{Na} \) declined over several minutes to reach a new steady state value of 5.6 mM. Upon repolarization to \(-80 \) mV, \( a_{Na} \) returned along a similar time course to the level it had before depolarization. The results from this and 12 additional experiments where the steady state \( a_{Na} \) was measured at \( V_m = -80 \) mV and at \( V_m = 0 \) mV are summarized in Table 1. The resting membrane potential for each fiber is shown; in one case (number 7) the fiber repolarized during the experiment. In every fiber studied, \( a_{Na} \) declined to a new steady state level over several minutes following the depolarizing step and returned to the prestep level upon termination of the voltage step, and partially depolarized fibers gave results similar to fibers with resting potentials in the normal range. The average steady state \( a_{Na} \) was 8.5 ± 1.0 mM at \( V_m = -80 \) mV and it decreased to 3.8 ± 0.6 mM at \( V_m = 0 \) mV (P < 0.0001). Recordings from another fiber are shown in Figure 4, demonstrating that \( a_{Na} \) not only decreased with depolarization but also increased with hyperpolarization. In this fiber, the initial steady state \( a_{Na} \) at \( V_m \)
TABLE 1
The Steady State Relationship of $V_m$ to $a_{Na}$

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>RP</th>
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<th>$-80$</th>
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<th>$0$</th>
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<td>12.2</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>6.3</td>
<td>3.9</td>
<td>3.0</td>
<td></td>
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<tr>
<td>3</td>
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<td>5.9</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-75</td>
<td>5.3</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-36</td>
<td>14.0</td>
<td>8.9</td>
<td>7.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
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<td>17.0</td>
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<td>1.9</td>
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<td>(77)</td>
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<tr>
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<tr>
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<tr>
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<td>15.4</td>
<td>11.5</td>
<td>5.6</td>
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</table>

Mean: 13.0 ± 8.5, SE: 4.1 ± 3.8

$RP =$ resting potential (mV), $CP =$ clamp potential (mV). Steady state $a_{Na}$ (mM) are given for different CP. The only statistical test used was Student's paired t-test between measurements at $-80$ and $0$ mV. Even if adjustment were made for possible multiple comparisons using the Bonferroni method, the difference would remain highly significant.

* $P < 0.0001$ (paired t-test).

$V_m = -80$ mV was 4.5 mM. After a voltage step to 0 mV for 10 minutes, $a_{Na}$ had declined to 0.7 mM. With repolarization to $-80$ mV, $a_{Na}$ returned to the initial level. After a depolarizing step to $-40$ mV, $a_{Na}$ declined to approximately 2.0 mM and returned to the initial level after repolarization to $-80$ mV. A hyperpolarizing step to $-110$ mV was then applied for 10 minutes and resulted in an increase in $a_{Na}$ to 17 mM, with it returning to the prestep level after repolarization. $a_{Na}$ was measured in five fibers following 10-minute-long voltage steps to $-40$ and $-110$ mV, and results from these fibers are included in Table 1. In some of the fibers, the change in $a_{Na}$ during the hyperpolarizing step to $-110$ mV appeared to follow a prolonged time course and might have increased slightly more if the voltage step had been maintained beyond 10 minutes. The averaged results from Table 1, showing the influence of $V_m$ on $a_{Na}$, are illustrated in Figure 5.

Because the response times of the $Na^+$ electrodes were rapid, the rate of change of $a_{Na}$ could be studied. This is of interest because the rate of change of $a_{Na}$ may reflect the degree of activation of the Na-K pump. Figure 6 shows results from two experiments where the change in $a_{Na}$ was plotted in semi-log form against time. For each fiber, the rate of change of $a_{Na}$ was plotted for a voltage step to 0 mV (circles), and back to $-80$ mV (squares). The voltage and difference channel traces from the respective fibers are shown in the insets. The rate of change of $a_{Na}$ for the depolarizing step to 0 mV and the repolarizing step back to $-80$ mV could be fitted with a single exponential. Furthermore, individual fibers showed rates of change of $a_{Na}$ following depolarizing or repolarizing voltage steps that were nearly identical. In Figure 6, an example of a fiber with a faster $t_{1/2}$ (50 seconds) is shown, along with a fiber with a slower $t_{1/2}$ (120 seconds). The range of $t_{1/2}$ seen in these experiments was 45 to 140 seconds. In a few Purkinje fibers, after a voltage step, a brief lag could be seen before $a_{Na}$ assumed a rate of change described by a single exponential (see Cohen et al., 1982).

**Effects of Altering $[K]_o$ on $a_{Na}$ during Long Voltage Clamp Steps**

In cardiac tissue, the restricted volume of the extracellular space can lead to the accumulation or depletion of $K^+$ (Noble, 1976; Baumgarten and Isenberg, 1977; Attwell et al., 1979b; Kline and Kupersmith, 1982). Because of possible changes in extracellular $K^+$ during voltage clamp steps, and because this might lead to alteration in the kinetics of the Na-K pump (Eisner and Lederer, 1980; Gadsby, 1980; Cohen et al., 1981b, 1982; Kline and Kupersmith, 1982) we studied the influence of altering membrane potential on $a_{Na}$ at different levels of $[K]_o$ in order to test the hypothesis that the alterations in $a_{Na}$ observed upon changing $V_m$ might be

![Figure 4](image1.png)  
**Figure 4.** Voltage dependence of $a_{Na}$. A continuous recording of $a_{Na}$ during successive 10-minute depolarizing (0 mV and $-40$ mV) and hyperpolarizing ($-110$ mV) voltage steps, showing that $a_{Na}$ can decrease or increase, respectively. $V_m = -80$ mV.

![Figure 5](image2.png)  
**Figure 5.** Relationship of steady state $a_{Na}$ to membrane potential in Purkinje fibers.
the result of alterations of [K]o in the clefts and consequent changes in the Na-K pump activity. In one series of experiments [K]o was increased to 15 mM to minimize any possible effects due to K+ accumulation during depolarizing voltage clamp steps. Results from one fiber are shown in Figure 7 and illustrate the protocol. With [K]o = 5.4 mM, the steady state a'Na was 9.5 mM at Vm = −80 mV. After depolarization to 0 mV for 10 minutes, a'Na declined to 4.2 mM, and returned to the prestep level after repolarization to −80 mV. [K], then was increased to 15 mM and the protocol was repeated. At the holding potential of −80 mV, a'Na was 8.0 mM. Following depolarization to 0 mV for 10 minutes, a'Na declined to a steady state value of 4.1 mM. With repolarization to −80 mV, a'Na returned to 8.1 mM. After [K]o was restored to 5.4 mM, a'Na was 8.7 mM at Vm = −80 mV (not shown). Results from this and three additional fibers are presented in Table 2. With [K]o = 5.4 mM, the mean steady state a'Na was 10.2 mM at Vm = −80 mV, 4.3 mM with Vm = 0 mV (P < 0.002), and returned to 10.3 mM after repolarization to −80 mV. With [K]o = 15 mM, the mean steady state a'Na was 10.3 mM at Vm = −80 mV and decreased to 4.8 mM with Vm = 0 mV (P < 0.002). After repolarization to −80 mV, a'Na was 10.2 mM. After restoring [K]o to 5.4 mM, a'Na was 10.5 mM at Vm = −80 mV. The mean steady state values of a'Na following depolarization to 0 mV with [K]o = 5.4 or 15 mM were not different (P > 0.1).

The mean steady state values for a'Na were not different at Vm = −80 mV with [K]o = 5.4 or 15 mM. Upon switching between solutions, however, small fluctuations in a'Na frequently were observed (see Fig. 7). The change in a'Na (<1 mM) was either a small increase, a small decrease, or it was biphasic. Whether the small variation in a'Na represents a true change in free Na+ or minor voltage inhomogeneity during changes in [K]o is uncertain. It is unlikely to result from the increased tonicity of the 15 mM [K]o bathing solution (a 6% increase in osmolality results from the addition of 9.6 mM KCl), as this is a very small alteration in tonicity, and it does not explain the fall in a'Na sometimes observed when switching to the elevated K+ solution, or the rise in a'Na sometimes observed when switching back to Tyrode's solution with [K]o = 5.4 mM.

![Figure 6](image_url) | Figure 6. Plots of the rate of change of a'Na following long duration voltage steps between −80 and 0 mV in two Purkinje fibers. The voltage and a'Na traces are shown in the insets. Circles and squares show monoexponential rates of change of a'Na following 10-minute steps to 0 mV and repolarization to −80 mV, respectively.

![Figure 7](image_url) | Figure 7. Effect on a'Na of raising [K]o from 5.4 to 15 mM. Long duration depolarizing steps from −80 mV to 0 mV were applied. Following the first 10-minute depolarizing step, [K]o was increased from 5.4 to 15 mM. The protocol was repeated, and shows that the response of a'Na to Vm was similar with both [K]o.
The role of the Na-K pump in the regulation of $a_{Na}$ in cardiac muscle has been studied extensively (Deitmer and Ellis, 1978; Eisner and Lederer, 1980; Gadsby, 1980; Glitsch and Pusch, 1980; Eisner et al., 1981a, 1981b; Eisner et al., 1981a; Sheu and Fozzard, 1982). Exposure of cardiac tissue to K+-free media inhibits the Na-K pump and causes $a_{Na}$ to rise. We studied the effects of $K^+$-free solutions on four Purkinje fibers during long-duration voltage clamp steps between $-80 \text{ mV}$ and $0 \text{ mV}$.

**TABLE 2**

Effect of Different $[K_o]$ (5.4 and 15 mM) on the Relationship of Steady State $a_{Na}$ (mM) to $V_m$ (mV)

<table>
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<tr>
<th>Experiment no.</th>
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</tr>
<tr>
<td>SE</td>
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</tr>
</tbody>
</table>

* * P < 0.002 (paired t-test).
† P > 0.1 (paired t-test).

If adjustment is made for multiple comparisons by the Bonferroni method, the differences remain highly significant.

The protocol was similar to that used in the experiments of Cohen et al. (1982), it is not clear whether the rapid inward current associated with the action potential upstroke, or the prolonged action potential plateau depolarization, or some other mechanism was responsible for the stimulation-induced rise in $a_{Na}$.

In order to study further the mechanism of stimulation-dependent changes in $a_{Na}$ we applied trains of short duration depolarizing suprathreshold voltage clamp steps. In Figure 10 are shown records from one experiment where 20-msec-long voltage steps were depolarized to $0 \text{ mV}$, which caused a nearly 20 mM fall in $a_{Na}$ to $11.1 \text{ mM}$. After repolarization to $-80 \text{ mV}$, $a_{Na}$ again increased to a peak value of $33 \text{ mM}$, from which it returned to baseline with restoration of $[K_o]$ to $5.4 \text{ mM}$. Similar results, with a large fall in $a_{Na}$ following depolarization to $0 \text{ mV}$ with $[K_o] = 0 \text{ mM}$, were found in the other fibers.

**Effects of Short Duration Repetitive Depolarizing Voltage Steps**

Cohen et al. (1982) have shown that $a_{Na}$ is increased in ventricular muscle and Purkinje fibers in response to trains of action potentials. The magnitude of the rise in $a_{Na}$ was dependent on the duration and frequency of action potential stimulation. We have confirmed that a similar rise in $a_{Na}$ is present in the short Purkinje fiber preparations used in our experiments, and results from one experiment are shown in Figure 9. $a_{Na}$ was monitored continuously while 2-minute trains of stimulated action potentials were elicited from a resting potential of $-71 \text{ mV}$. The action potential amplitudes are attenuated due to the frequency response of the strip chart recorder. As the stimulus frequency increased from $0.5 \text{ Hz}$ to $4 \text{ Hz}$, $a_{Na}$ increased from a resting level of $3.2 \text{ mM}$ to a maximal level of $5.2 \text{ mM}$ immediately following the $4 \text{ Hz}$ train. The return of $a_{Na}$ to baseline required several minutes and was associated with a small poststimulation hyperpolarization, presumably representing electrogenic Na$^+$ extrusion. In these and the experiments of Cohen et al. (1982), it is not clear whether the rapid inward current associated with the action potential upstroke, or the prolonged action potential plateau depolarization, or some other mechanism was responsible for the stimulation-induced rise in $a_{Na}$.

In order to study further the mechanism of stimulation-dependent changes in $a_{Na}$ we applied trains of short duration depolarizing suprathreshold voltage clamp steps. In Figure 10 are shown records from one experiment where 20-msec-long voltage

![Figure 8](image)

**Figure 8.** Effect of $a_{Na}$ lowering $[K_o]$ to $0 \text{ mM}$. Ten-minute depolarizing steps from $-80 \text{ mV}$ to $0 \text{ mV}$ were applied initially with $[K_o] = 5.4 \text{ mM}$ and then with $[K_o] = 0 \text{ mM}$. See text for detail.

![Figure 9](image)

**Figure 9.** Recording of $V_m$ and $a_{Na}$ in a shortened Purkinje fiber during 2-minute-long trains of action potentials stimulated at 0.5, 1, 2, and 4 Hz. After each train of action potentials, a frequency-dependent rise in $a_{Na}$ was present. Action potential amplitudes are attenuated by the frequency response of the strip chart recorder.
clamp steps of 50 mV amplitude were applied from a $V_h$ of −80 mV. Three-minute-long trains of voltage clamp steps at 0.5, 1.0, 2.0, and 4.0 Hz caused a frequency dependent rise in $a_{Na}$ similar to that observed with action potentials (see Fig. 9). In the experiment shown in Figure 10, $a_{Na}$ was increased following each pulse train from the baseline value of 3.8 mM and reached a peak value of 5.8 mM immediately after the 4 Hz train. $a_{Na}$ then declined to the resting level with an exponential time course. Similar results were obtained in three additional fibers. In some preparations, the protocol was repeated, using longer duration voltage clamp steps. With a longer duration of depolarization to −30 mV, a further rise in $a_{Na}$ might be expected if significant entry of Na⁺ were to occur during the steady state TTX-sensitive “window” current (Attwell et al., 1979a), or through the slow inward current channel Na⁺ permeability (see Reuter, 1979). In Figure 11 are shown results from the same fiber shown in Figure 10. The protocol was identical to that used in Figure 10, except that for each pulse train frequency, the fiber was depolarized for one-half of the interval (duty-cycle stimulation). The rise in $a_{Na}$ following each pulse train was nearly the same as that observed with the 20-msec-long depolarizing pulse protocol, the difference never exceeding 0.3 mM.

**Effects of TTX and Sodium Channel Inactivation**

In order to investigate further the mechanism of the rise in $a_{Na}$ with repetitive depolarization, we applied interventions that inhibit the Na⁺ channel, and the protocol using repetitive voltage clamp steps was repeated. Results from one fiber are shown in Figure 12 (same fiber as in Figs. 9–11), and only panels from the highest frequency (4 Hz) pulse trains, where the change in $a_{Na}$ was largest, are reproduced. In panel A, the response of $a_{Na}$ to a 2-minute train of action potentials is shown, after which $a_{Na}$ had increased from a resting level of 3.2 to 5.2 mM, and gradually returned to the resting level over several minutes. At the end of panel A, the voltage clamp was switched on and $V_m$ was clamped to −80 mV from a resting potential of −71 mV. After a 10-minute equilibration period (between the end of panel A and the beginning of panel B), $a_{Na}$ increased to a new steady state value of 3.8 mM, consistent with the previously described relationship of $V_m$ to steady state $a_{Na}$. In panel B, a 3-minute train of depolarizing voltage steps, 20 msec in duration and 50 mV in amplitude, was applied. Following this, $a_{Na}$ had increased to 5.8 mM and gradually decreased to the resting level over several minutes. In panel C, a 3-minute train of 125-msec-long depolarizing voltage clamp steps (duty-cycle stimulation) was applied. From a resting value of 3.8 mM, $a_{Na}$ had increased to 5.8 mM at the end of the pulse train and then gradually returned to baseline over a time course similar to that in panels A and B. Between panels C and D, TTX (4 × 10⁻⁶ M) was added to the perfusate, and action potentials no longer could be elicited. The voltage clamp was again switched on ($V_h = −80 mV$), and a 10-minute equilibration period followed. As shown in panel D, the steady state $a_{Na}$ declined in the presence TTX to 3.3 mM. Subsequently, a 3-minute pulse train identical to that used in panel B was applied. Following the pulse train, $a_{Na}$ had increased to 4.1 mM and gradually declined to baseline. Thus, the rise in $a_{Na}$ with repetitive depolarization was reduced by TTX. While adequate to suppress excitability, the TTX dose we used (4 × 10⁻⁶ M) did not block all sodium channels, and a fraction of the sodium current remained in our preparations (see also Cohen et al., 1981a). Following panel D, TTX was washed out and excitability returned. Steady state inactivation of the sodium current is complete at a membrane potential of −50 mV (Weidmann, 1955; Colatsky, 1980), whereas steady state inactivation of the slow inward current is incomplete in this voltage range (Gibbons and Fozzard, 1975; Colatsky and Tsien, 1979; Marban and Tsien, 1982). As shown in panel E, after washout of TTX, $V_m$ was set at −50 mV, and after 10 minutes of equilibration, $a_{Na}$ had declined to 2.9 mM, consistent with previous observations of the relationship of $V_m$ to steady state $a_{Na}$. Subsequently,
Figure 12. Records of $V_m$ and $a_{Na}$ from a Purkinje fiber undergoing repetitive trains of stimulation at 4 Hz. TTX ($4 \times 10^{-7}$ M) and reduced $V_h$ ($-50$ mV) inhibited the depolarization-induced rise in $a_{Na}$. Note that the effect of steady $V_m$ (between pulse trains) on $a_{Na}$ also was present. Dashed line $= 3.0$ mM $a_{Na}$. See text for detail.

Figure 13. Continuous recording of $V_m$ and $a_{Na}$ in ventricular trabecular muscle. The response of $a_{Na}$ to 7-minute-long depolarizing steps to 0 mV and $-40$ mV from a $V_h$ of $-80$ mV is shown. Voltage dependence of $a_{Na}$ is present, with the steady state established in 2 minutes. The response of $a_{Na}$ to action potentials stimulated at 0.5 top 4 Hz is shown in the inset. A frequency-dependent rise in $a_{Na}$ followed each train, and $a_{Na}$ rapidly returned to baseline after the stimulation period. $V_m$ is in mV, and $a_{Na}$ is in mM. See text for detail.

a 3-minute train of depolarizing voltage clamp steps, 20 msec in duration and 50 mV in amplitude, was applied. Following the train, no rise in $a_{Na}$ was observed. In panel E, a protocol similar to that in panel B was used, except 125-msec depolarizing steps (duty-cycle stimulation) were applied so that we might study the effects of lengthened activation of the slow inward current channel. As in panel E, no increase in $a_{Na}$ was observed. In panel G, $V_h$ had been restored to $-80$ mV for 10 minutes and $a_{Na}$ returned to a resting value of 3.9 mM. As in panel B, a 3-minute-long train of depolarizing voltage clamp steps, 20 msec in duration, caused $a_{Na}$ to increase to 6.0 mM and then return to the resting level over several minutes, indicating that the response of $a_{Na}$ to repetitive depolarization had not been lost.

Effect of Membrane Potential on $a_{Na}$ in Trabecular Muscle

Cohen et al. (1982) showed that $a_{Na}$ increased with stimulation in ventricular muscle. We confirmed this finding in shortened trabecular muscles; results from one preparation are shown in the inset in Figure 13. Trains of stimuli, approximately 2 minutes long and from 0.5 to 4.0 Hz, were applied as was done with Purkinje fibers (see Fig. 9). From a resting $a_{Na}$ of approximately 8.2 mM, a frequency-dependent increase in $a_{Na}$ occurred, with a peak value of approximately 10.4 mM following 4.0 Hz stimulation. Recovery from the stimulation-induced rise in $a_{Na}$ was complete within 2 minutes and was associated with poststimulation hyperpolarization of $V_m$.

In three preparations, we were able to study the influence of steady membrane potential on $a_{Na}$ with a protocol similar to that used for Purkinje fibers. Results from one experiment are shown in Figure 13. At $V_h = -80$ mV, the steady state $a_{Na}$ was 6.8 mM. The preparation was then depolarized to 0 mV for 7 minutes. With the initial depolarization, a jump in the voltage recorded by the Na$^+$ electrode was observed, followed by a gradual decline in $a_{Na}$ over about 2 minutes to a new steady state level. After repolarization to $-80$ mV, a second jump in the voltage recorded from the Na$^+$ electrode was observed, followed by a gradual return of $a_{Na}$ to the baseline value. Similar results were obtained in the other two ventricular muscle preparations. The jump in the voltage recorded by the Na$^+$ electrode was larger than that found with Purkinje fiber preparations, and probably reflects greater voltage nonuni-
formity caused by the shorter space constant of ventricular muscle. Without correction for voltage nonuniformity, \( a'_{Na} \) declined in the fiber shown in Figure 13 to a steady state value of 2.8 mM following depolarization of \( V_m \) to 0 mV. If the value of \( a'_{Na} \) obtained at -80 mV is assumed to be correct, and the gradual change in \( a'_{Na} \) following the step in \( V_m \) is extrapolated to \( V_m = 0 \) and the instantaneous jump is subtracted, then \( a'_{Na} \) declined to approximately 4.5 mM following the depolarizing step to 0 mV. The magnitude of the change in \( a'_{Na} \) following a step in \( V_m \) should be considered qualitatively in this experiment because of uncertainty as to the correct method of compensation for instantaneous voltage jumps following steps in \( V_m \). In Figure 13, a depolarizing step to -40 mV also is shown, along with the repolarizing step back to -80 mV. It shows that, following the voltage step, a small decline in \( a'_{Na} \) gradually developed which was reversed after repolarization of the preparation to -80 mV. Thus, steady state voltage dependence of \( a'_{Na} \) similar to that observed in Purkinje fibers seems to be present in sheep ventricular muscle. One difference we noted in ventricular muscle was that the change in \( a'_{Na} \) was completed in 1–2 minutes following a step in \( V_m \) whereas, in Purkinje fibers, several minutes were required.

**Discussion**

**Steady State Level of \( a'_{Na} \)**

The steady state level of \( a'_{Na} \) in Purkinje fibers was 8.5 ± 1.0 mM at \( V_m = -80 \) mV (Table 1). This value compares favorably with mean values reported by others for a variety of conditions and using several fabrication techniques for Na+ electrodes (Ellis, 1977; Glitsch and Pusch, 1980; Lee et al., 1980; Sheu et al., 1980; Eisner et al., 1981a; Glitsch et al., 1981; Bers and Ellis, 1982; Cohen et al., 1982; Sheu and Fozzard, 1982; Lee and Dagostino, 1982; Watson et al., 1982, 1983). Furthermore, this suggests that the Purkinje fibers were not damaged by the voltage clamp technique we used, and that measurement of \( a'_{Na} \) under voltage clamp control is valid. The use of a holding potential slightly more negative than the Purkinje fiber resting potentials \([e.g., -76.4 ± 1.2 \) mV and 8.0 ± 0.6 mM \( a'_{Na} \) (Sheu and Fozzard, 1982)] should cause \( a'_{Na} \) to be slightly increased.

**Influence of Steady Membrane Potential on \( a'_{Na} \)**

Using a voltage clamp to change \( V_m \), Eisner et al. (1981b) first showed that \( a'_{Na} \) was directly sensitive to \( V_m \). Similar findings have been shown by Sheu and Fozzard (1982), using K+ depolarizations with [Na]\( _o \) held constant. We have obtained quantitative confirmation of the dependence of \( a'_{Na} \) on \( V_m \). With maintained depolarization from -80 mV to 0 mV in Purkinje fibers, steady state \( a'_{Na} \) declined from 8.5 ± 1.0 to 3.8 ± 0.6 mM \((P < 0.0001)\). The Na+ resin (ETH 227) used to fill the Na\( ^+ \) electrode tips also is sensitive to Ca\( ^{2+} \) (Steiner et al., 1979; Bers and Ellis, 1982; Sheu and Fozzard, 1982; Dagostino and Lee, 1982), which, under certain conditions, can lead to error in measuring \( a'_{Na} \). With depolarization, \( a'_{Na} \) rises (Sheu and Fozzard, 1982), so that an error introduced by \( a'_{Na} \) would cause an apparent increase in \( a''_{Na} \). In these experiments, \( a'_{Na} \) declined with maintained depolarization, implying a true fall in \( a''_{Na} \) and suggesting a minor contribution from \( a''_{Na} \). A similar conclusion was reached by Sheu and Fozzard (1982).

When 10-minute-long depolarizing and hyperpolarizing voltage steps were studied in Purkinje fibers, it was shown that \( a'_{Na} \) could decrease or increase, respectively. The direction and magnitude of the change in \( a'_{Na} \) were dependent upon the direction and magnitude of the voltage change. As shown in Figure 5, the relationship of \( V_m \) to the steady state \( a'_{Na} \) appeared to be nonlinear and was steeper at more negative membrane potentials, including those near the resting potential. A direct explanation for the dependence of steady state \( a'_{Na} \) on \( V_m \) might be that the Na-K pump acts to maintain the Na\( ^+ \) electrochemical gradient. A change in \( V_m \) would cause \( a'_{Na} \) to seek a new level such that the electrochemical gradient remained constant. The electrochemical gradient, \( \Delta \mu_{Na} \), is the difference between the membrane potential, \( V_m \) and the Na\( ^+ \) equilibrium potential, \( E_{Na} \). \( E_{Na} \) might be calculated from the Nernst equation with \( a'_{Na} \) taken to be 115.1 mM \((0.76 \times [Na]_o)\). In Table 3 are shown values of \( a'_{Na} \), \( E_{Na} \), and \( \Delta \mu_{Na} \) for the four steady membrane potentials studied (Table 1). It shows that, at different \( V_m \), \( a'_{Na} \) did not distribute to a constant electrochemical gradient, and suggests that the rate of Na\( ^+ \) entry into the cells, extrusion from the cells, or both, must not be constant.

**Effect of Varying [K\( \text{\textsuperscript{o}} \)] on \( a'_{Na} \)**

Another possible explanation for the voltage dependence of \( a'_{Na} \) is that the activity of the Na-K pump is sensitive to \( V_m \). This could result if K\( ^+ \) accumulation or depletion in the extracellular clefts of cardiac muscle during voltage clamp steps were to alter the level of activation of the Na-K pump, or if the Na-K pump were directly voltage dependent. Potassium ion accumulation or depletion in cardiac muscle is well described (Noble, 1976; Baumgarten and Isenberg, 1977; Attwell et al., 1979b, Kline and Kupersmith, 1982), and values reported for the K\( _m \) are 60 to 100 mM, corresponding to \( E_{K} \) values of 70 to 90 mV. In Table 3 are shown values of \( a'_{Na} \) and \( a''_{Na} \) for the four steady membrane potentials studied (Table 1). It shows that, at different \( V_m \), \( a'_{Na} \) did not distribute to a constant electrochemical gradient, and suggests that the rate of Na\( ^+ \) entry into the cells, extrusion from the cells, or both, must not be constant.

**Table 3**

<table>
<thead>
<tr>
<th>( V_m ) (mV)</th>
<th>-110</th>
<th>-80</th>
<th>-40</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a'_{Na} ) (mM)</td>
<td>13.0</td>
<td>8.5</td>
<td>4.1</td>
<td>3.8</td>
</tr>
<tr>
<td>( E_{Na} ) (mV)</td>
<td>56.7</td>
<td>67.7</td>
<td>86.7</td>
<td>88.7</td>
</tr>
<tr>
<td>( \Delta \mu_{Na} ) (mV)</td>
<td>166.7</td>
<td>147.7</td>
<td>126.7</td>
<td>88.7</td>
</tr>
</tbody>
</table>

See text for detail.
of the Na-K pump vary from less than 1 mm to 10 mm or more (Deitmer and Ellis, 1978; Eisner and Lederer, 1980; Gadsby, 1980; Eisner et al., 1981b; Glitsch et al., 1981). If the $K_m$ were large, then changes in $[K]$ could lead to changes in $a_{Na}$ by modulating activation of the Na-K pump. This could affect both the steady state $a_{Na}$ and the rate of change of $a_{Na}$ when $V_m$ is changed. We studied the effects of different levels of $[K]_o$ on $a_{Na}$ during long duration voltage steps between -80 mV and 0 mV. As shown in Table 2, the steady state values of $a_{Na}$ at $V_m = -80$ mV or 0 mV were not different ($P > 0.1$) when $[K]_o$ was 5.4 mm or 15 mm. Thus, elevating $[K]$ did not lead to a fall in $a_{Na}$, as would be expected if enhanced activation of the Na-K pump occurred. This is consistent with a value for the $K_m$ of the Na-K pump well under 5.4 mm, and our results using $K^+$ as an external activating cation for the Na-K pump are similar to results obtained using $Rb^+$ (Eisner et al., 1981b; Glitsch et al., 1981).

However, accumulation of $K^+$ during depolarizing voltage clamp steps can be important, as was suggested in the experiments where fibers were exposed to $K^+$-free media. When $V_m$ was held at -80 mV (Fig. 8), inhibition of the Na-K pump with $[K]_o = 0$ mm caused $a_{Na}$ to rise to approximately 30 mm (Ellis, 1977; Deitmer and Ellis, 1978; Eisner et al., 1981b). After depolarization to 0 mV, $a_{Na}$ declined by nearly 20 mm to within a few mm of the baseline value, showing that, in $K^+$-free media, the dependence of $a_{Na}$ on $V_m$ was not blocked—rather, it was accentuated. Eisner et al. (1981b) used a cardiac glycoside to inhibit the Na-K pump, and also showed that $a_{Na}$ remained sensitive to changes in $V_m$. Although the experimental protocols we used differ from those of Eisner et al. (1981b), the decline in $a_{Na}$ we observed following maintained depolarization to 0 mV was large, and seemed accentuated in $K^+$-free media. The simplest explanation for this large depolarization-induced fall in $a_{Na}$ with $[K]_o = 0$ mm is that $K^+$ accumulates within clefts and leads to activation of the Na-K pump and Na$^+$ extrusion. The effect on $a_{Na}$ would be more pronounced if the magnitude of $K^+$ accumulation were large, or if the $K_m$ for Na-K pump were small. We have not excluded the possibility that other mechanisms, such as Na$^+-$Ca$^{2+}$ exchange, may also contribute to the control of $a_{Na}$, but the necessary magnitude of Ca$^{2+}$ movement makes this unlikely. As a consequence of accumulation of $K^+$ in the intercellular clefts during depolarization and the resultant activation of the Na-K pump, it is necessary to interpret cautiously experiments performed in 0 $[K]_o$ (and 0 $[Rb]_o$) solutions (Eisner et al., 1981b).

**Time Course of Change of $a_{Na}$**

A monoexponential time course has been demonstrated for the decline of $a_{Na}$ from elevated levels following reactivation of the Na-K pump (Glitsch and Pusch, 1980; Eisner et al., 1981a, 1981b; Glitsch et al., 1981) and overdrive stimulation (Cohen et al., 1982). In our experiments, $a_{Na}$ was not elevated above baseline, rather it declined in response to maintained depolarization. As shown in Figure 6, the time course of the change in $a_{Na}$ was monoexponential, regardless of whether $a_{Na}$ was falling after depolarization of $V_m$ to 0 mV, or rising after returning $V_m$ to -80 mV. We conclude that the time course of change of $a_{Na}$ is monoexponential over a wide range of $a_{Na}$. If extracellular $K^+$ accumulation occurs during maintained depolarization to 0 mV with $[K]_o = 5.4$ mm, with enhanced Na-K pumping and Na$^+$ extrusion, then a shorter $t_{1/2}$ following the step in $V_m$ to 0 mV would be expected. Furthermore, if Na$^+$ pumping were directly voltage dependent, then a reduction in $V_m$ to 0 mV (reducing the Na$^+$ electrochemical gradient) also might be expected to decrease the $t_{1/2}$. The finding of similar $t_{1/2}$ values for the change in $a_{Na}$ following steps in $V_m$ between 0 and -80 mV suggests that, over this voltage range, the Na-K pump is voltage independent, and that $K^+$ accumulation has little effect, consistent with a $K_m$ for the Na-K pump well under 5.4 mm. We cannot exclude the possibility that small differences in the $t_{1/2}$ values, reflecting very small differences in $a_{Na}$ (0.1 to 0.3 mm), are present, but these differences are near the limit of accuracy of our measurement of $a_{Na}$. Eisner and Lederer (1980) studied the Na-K pump current and also concluded that it was voltage independent over a similar range on $V_m$. Whether depletion of $[K]_o$, or voltage dependence of the Na-K pump could contribute to the shape of the relationship of $a_{Na}$ to $V_m$ in the hyperpolarizing direction is uncertain, since we did not study this region in detail.

**Influence of Repetitive Depolarization on $a_{Na}$**

In sheep Purkinje fibers and ventricular muscles, trains of action potentials lead to an increase in $a_{Na}$ which is dependent on the rate and duration of stimulation (Cohen et al., 1982). A similar dependence of $a_{Na}$ could be demonstrated in our shortened Purkinje fiber (Figs. 9–12) and ventricular muscle (Fig. 13) preparations in response to repetitive action potentials or depolarizing voltage clamp steps. Since trains of depolarizing voltage clamp steps led to a rise in $a_{Na}$, whereas maintained depolarization led to its fall, the stimulation-dependent increase in $a_{Na}$ is likely to result from repetitive activation of a transient inward current. In the experiments we report, precise voltage control during the inward sodium current was not required, and voltage inhomogeneity during the sodium current (lasting only a few milliseconds) should not introduce difficulty in interpreting results. Following termination of stimulation, $a_{Na}$ returned to baseline along a time course usually described by a single exponential and with a $t_{1/2}$ of 1–2 minutes for Purkinje fibers. Coincident with this in non-voltage clamped preparations was the well-known finding of poststimulation...
hyperpolarization of $V_m$ attributed to enhanced electrogenic sodium pumping (Vassalle, 1970; Glitsch, 1973; Gadsby and Cranefield, 1982) resulting from the increased $a_{Na}$ (Eisner et al., 1981a). Sodium entry could occur during the initial surge of Na⁺ current, during the TTX-sensitive "window" current (Attwell et al., 1979a), through the slow inward current channel permeability to Na⁺ (see Reuter, 1979), or by all three mechanisms. If entry of Na⁺ was mediated mainly by the "window" current or by the slow inward current channel, then a further increase in $a_{Na}$ might be expected with lengthening of the duration of the voltage clamp steps. When the duration of each depolarizing voltage step was increased to 50% of the cycle length (duty-cycle stimulation), the frequency-dependent rise in $a_{Na}$ was not changed. This suggests that the "window" current and slow inward current contribute little to the frequency-dependent rise in $a_{Na}$, and that Na⁺ entry leading to the rise in $a_{Na}$ occurred within the first 20 msec of depolarization, probably during the initial surge of sodium current. A difficulty with this interpretation is that with the duty-cycle stimulation technique, the membrane is depolarized for a longer period of time. From the results presented earlier on the influence of maintained changes in $V_m$ on $a_{Na}$, a decline in $a_{Na}$ might be expected because of the lengthened depolarization which could obscure a rise in $a_{Na}$ due to Na⁺ entry via the "window" current or the slow inward current channel.

Interventions that diminish Na⁺ channel permeability were studied. Exposure to TTX caused two effects. A small decline in the steady state level of $a_{Na}$ (3.8 to 3.3 mM, Fig. 12) was found, confirming previous observations by Deitmer and Ellis (1980) and Eisner et al. (1983a), and suggesting that a small TTX-sensitive steady state Na⁺ permeability exists. Also, in the presence of TTX only a small rise in $a_{Na}$ was produced by repetitive depolarization (3.3 to 4.1 mM, Fig. 12). The TTX dose (4 x 10⁻⁶ M) we used was adequate to depress excitability, but did not abolish completely the transient fast inward current. Steady state inactivation of the Na⁺ current is complete at a $V_h$ of −50 mV (Weidmann, 1955; Colatsky, 1980), whereas inactivation of the slow inward current is incomplete (Gibbons and Fozzard, 1975; Colatsky and Tsien, 1979; Marban and Tsien, 1982). When depolarizing trains of pulses, 20 or 125 msec in duration, were applied from a $V_h$ of −50 mV, no rise in $a_{Na}$ was obtained. These findings are consistent with the conclusion that the rise in $a_{Na}$ with repetitive depolarization results from Na⁺ entry through the Na⁺ channel during the Na⁺ current. Cohen et al. (1981b) and Falk and Cohen (1982, 1983) studied the poststimulation increase in the electrogenic Na-K pump current following periods of overdrive in canine Purkinje fibers. They concluded that entry of Na⁺ contributing to stimulation of the electrogenic Na-K pump current occurred through both the fast and slow inward current channels. The reason for the apparent difference in results is uncertain but could reflect technique and species differences.

$a_{Na}$ in Ventricular Trabecular Muscle Columns

Cohen et al. (1982) showed that a frequency-dependent increase in $a_{Na}$ occurred with stimulation in ventricular muscle, an effect we confirmed in shortened trabecular muscles. As with Purkinje fibers, steady state $a_{Na}$ also was dependent on $V_m$ and declined during maintained depolarizing voltage clamp steps. The time required to reach a new steady state $a_{Na}$ usually was between 1 and 2 minutes. This was different from Purkinje fibers, where up to several minutes was required, and could represent differences in the surface to volume ratios of the two tissues (Page, 1978; Cohen et al., 1982). Where we have been able to investigate $a_{Na}$ in ventricular muscle, the responses have been qualitatively similar to those found with Purkinje fiber preparations.

**Mechanism of Control of $a_{Na}$**

In these experiments, we have shown that $a_{Na}$ in sheep cardiac tissue varies as a function of membrane voltage, stimulation frequency, and time. Interventions that alter the permeability of the Na⁺ channel or the Na-K pump rate affect $a_{Na}$. Ellis (1977), Deitmer and Ellis (1978), Sheu and Fozzard (1982), and Eisner et al. (1983a) also have shown that changes in [Na], or [Ca], can affect $a_{Na}$. The simplest explanation for the dependence of steady state $a_{Na}$ on $V_m$ during maintained voltage steps is a pump-leak model. Na⁺ moves into cells proportional to its electromechanical gradient as permitted by the membrane Na⁺ permeability and Na⁺-Ca⁺ exchange. With maintained depolarization, the passive Na⁺ leak is reduced because of diminished electrical driving force, and $a_{Na}$ will decline. Extrusion of Na⁺ against the electrochemical gradient is by the Na-K pump, and can be modified by changes in $a_{Na}$ or [K]. The relationship of $a_{Na}$ to Na-K pumping (measured as pump current) was found to be linear during pump reactivation experiments following Na⁺ loading (Eisner et al., 1981a), although low values of $a_{Na}$ were not investigated. The role of K⁺ accumulation or depletion in modulating the Na-K pump rate is uncertain, as is the role of other possible Na⁺ regulatory mechanisms such as Na⁺-Ca⁺ exchange. A frequency-dependent increase in $a_{Na}$ above the unstimulated level occurred with trains of action potentials or depolarizing voltage clamp steps. We conclude that this results from the large transient increase in Na⁺ channel permeability with each depolarization. Na⁺ entry by other membrane channels cannot be excluded, although it appears to be small.

In addition to its electrophysiological significance, variations in $a_{Na}$ may have important effects on...
tension development in cardiac muscle. A direct correlation between the level of \( a_{\text{Na}} \) and the magnitude of twitch tension (Lee et al., 1980; Eisner et al., 1981a; Daut, 1982; Lee and Dagostino, 1982) and tonic tension (Eisner et al., 1983b), as well as \( a_{\text{Ca}} \) (Lado et al., 1982; Lee and Dagostino, 1982), has been demonstrated. Furthermore, changes in the stimulation rate of cardiac muscle result in complex changes in tension development (Koch-Weser and Blinks, 1963). It is possible that a causal relationship exists between variations in \( a_{\text{Na}} \) and variations in tension development in cardiac muscle, and that it is mediated through the Na\(^+\)-Ca\(^{2+}\) exchange mechanism (Reuter and Seitz, 1968; Langer, 1971; Lee and Dagostino, 1982; Reuter, 1982; Sheu and Fozzard, 1982). It is therefore of special importance for any experimental study of the relation between membrane potential and tension that the effects of \( a_{\text{Na}} \) be considered.

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