Effects of Tonicity on Tension and Intracellular Sodium and Calcium Activities in Sheep Heart

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SUMMARY. We have measured the effects of changing tonicity of the bathing solution on intracellular sodium and calcium activities and tension of sheep cardiac Purkinje strands and ventricular muscle. For Purkinje strands in solutions of normal tonicity, resting membrane potential was $-77.4 \pm 0.4$ mV (mean $\pm$ se), sodium activity was $7.9 \pm 0.4$ nM, and calcium activity was $98 \pm 9$ nM. For ventricular muscle in solutions of normal tonicity, resting membrane potential was $-86.4 \pm 1.2$ mV, sodium activity was $6.9 \pm 0.5$ nM, and calcium activity was $70 \pm 4$ nM. Reduction of tonicity to 75% of normal in both tissues produced depolarization of a few millivolts, and sodium activity fell almost to the level predicted for simple osmotic dilution. In Purkinje strands, calcium activity fell much more than that predicted for simple osmotic dilution. Twitch contraction was reduced in the hypotonic solution. Increase of tonicity to 150% and 200% caused the resting membrane potential to become more negative. In both tissues, sodium activity increased somewhat less than predicted from simple water movement, and calcium activity increased proportionately much more than sodium activity. The much larger change of calcium activity in both hypo- and hypertonic solutions could be explained by water movement plus the effect of sodium-calcium exchange. In hypertonic solutions, tonic tension was increased, along with the rise in calcium activity; however, the twitch tension was reduced. This reduction of twitch tension may be due to a direct effect of hypertonicity on cross-bridge behavior, as has been reported for skeletal muscle. (Circ Res 54: 576-585, 1984)

THE transmembrane Na⁺ gradient appears to be an important factor in a number of membrane transport systems, including Na-Ca exchange (Reuter and Seitz, 1968; Baker et al., 1969). Systematic change in the Na⁺ gradient has been used to investigate the role of the gradient in regulating intracellular Ca²⁺ (Sheu and Fozzard, 1982). In small cells like those in heart muscle, the Na⁺ gradient is most easily influenced by change of extracellular Na⁺, but one would expect that change of gradient by altering intracellular Na⁺ should be equivalent. Several experimental interventions that lead to change of intracellular Na⁺ can cause profound effects on intracellular Ca²⁺, including Na-K pump blockade (Sheu and Fozzard, 1982; Bers and Ellis, 1982; Lee and Dagostino, 1982) and alteration of stimulation rate (Lado et al., 1982a).

Change in tonicity of the solution's bathing tissues causes water movement into or out of cells, thereby diluting or concentrating intracellular ions. We have previously investigated the effects of tonicity on transmembrane potential (Akiyama and Fozzard, 1975) and on intracellular K⁺ activity ($a_{iK}$) (Fozzard and Lee, 1976). Those studies indicated that tonicity changes could alter $a_{iK}$ substantially. We report here, the effects of tonicity changes on intracellular Na⁺ activity ($a_{iNa}$) and intracellular Ca²⁺ activity ($a_{iCa}$). The expectation was that the results might not be simply predicted by water movement, since $a_{iNa}$ is regulated by the Na-K pump, and $a_{iCa}$ is influenced by Na-Ca exchange.

Tonicity is also known to have dramatic effects on tension development in muscle (Hodgkin and Horowicz, 1957; Chapman, 1978). Some of these effects might be related to changes in $a_{iNa}$ and consequent changes in $a_{iCa}$ (Langer, 1968). We monitored twitch and tonic tension effects of the experimental solutions for possible correlation with measurements of $a_{iNa}$. A preliminary report of this work has been made (Lado et al., 1982b).

Methods

Purkinje strands and trabecular muscles were obtained from the left ventricles of sheep hearts, which were transported from the slaughterhouse to the laboratory in cooled oxygenated Tyrode's solution. The strands of muscles were pinned in a tissue chamber and superfused with solutions at a rate of 4 ml/min and at a temperature of 35 ± 1°C. The composition of the normal Tyrode's solution (NT) is shown in Table 1. When gassed with 95% O₂ and 5% CO₂, the pH was 7.3. Sucrose was used to vary the tonicity of the solutions, while keeping the ionic strength constant. We expected that sucrose would enter cells sufficiently slowly that, during that 10- to 15-minute exposure to test solutions, the change in cell volume would be constant. To make solutions that were either hypo- or hypertonic without change in Na⁺, it was necessary to lower the Na⁺ concentration in the control solution (1T) to 116.2 nM, while maintaining the osmolality at 298...
mOsm by addition of sucrose. Table 1 shows the composition of this IT solution, and of the hypo- (0.75T) and hypertonic (1.5T and 2.0T) solutions. The osmolalities of the solutions were measured with an osmometer (G-66, Fiske).

Conventional microelectrodes were made from borosilicate glass (WPI -TW150F), using a vertical puller. Those used for voltage recording were filled with 3 M KCl and had resistances of 5–10 MΩ. The micropipettes used for the Na+-sensitive recording were the same, but those for the Ca++-sensitive recording had a somewhat larger tip size. Pipettes used to make ion-sensitive neutral resin (ETH 1001; Oehme et al., 1976) to selectivity coefficients previously reported from this laboratory (Sheu and Fozzard, 1982). The kNa,K is 0.02, so that an error in estimation of ak as large as 50 mM would result in an error of only 1 mM in aK. Similarly, the kCa,K is 10−3, so that a 50 mM error in aK would cause a 25 mM error in aCa. Effects of the solutions on tip potentials were checked, and were never more than 0.5 mV.

The tension recordings were made with a photoelectric force transducer (TIL 138) similar to that used by Gibbons and Fozzard (1971). The tissues were stimulated at the rate of 0.2 Hz. After equilibration of the tissue in NT solution, it was impaled with the microelectrodes and the solution was switched to IT. It took about 1 minute for the new superfusing solution to reach the tissue after the switch. From this IT solution as control, the changes in tonicity were made, with subsequent return to IT and, finally, to NT solutions. The values reported are steady state values after the initial transients were completed. Because the tissues were not the same size, the control contractions were different. For comparison, we normalized the contraction sizes to those obtained in IT solutions.

### Results

#### Normal Tyrode’s Solution

The values for membrane potential (V_m), aNa, and aCa that were obtained in normal Tyrode’s solution in Purkinje strands and ventricular muscle are shown in Table 2. The average resting value for aNa is somewhat higher in Purkinje strands than in ventricular muscle, as previously reported from this laboratory (Sheu and Fozzard, 1982), and the value for aCa was also higher. The difference in aCa is significant at the 1% level by unpaired t-test, but the difference in aNa does not achieve significance at the 5% level. Sheu and Fozzard (1982) also saw a similar difference in aNa between resting Purkinje fibers and ventricular muscle, but the difference also was not significant at the 5% level. Calculation of the ratio of Ca++ and Na+ electrochemical gradients in normal Tyrode’s solution, using these values and the relationship:

$$n = \frac{2(E_{Ca} - V_m)}{(E_{Na} - V_m)}$$

yielded ratios of 2.60 and 2.55.

#### Low Na+, Isotonic Solution

The IT solution contained 116.2 mM Na+ (90 mM aNa), so we expected that the change from NT to IT solution would hyperpolarize the membrane and lead to somewhat lower aNa and aCa and increased contraction (Sheu and Fozzard, 1982). If aNa should fall linearly with reduction in passive Na+ leak, the fall in aNa would be 22%. We observed, in both Purkinje strands and ventricular muscle, a small hyperpolarization of 1–2 mV (Tables 3 and 4). aNa fell in Purkinje strands from 7.9 ± 0.4 to 6.9 ± 0.3 mM (13% fall), and in ventricular muscle from 6.9 ±

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[Table 1: Solutions]

<table>
<thead>
<tr>
<th>(in mM)</th>
<th>NT</th>
<th>1T</th>
<th>0.75T</th>
<th>1.5T</th>
<th>2.0T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+</td>
<td>152</td>
<td>116.2</td>
<td>116.2</td>
<td>116.2</td>
<td>116.2</td>
</tr>
<tr>
<td>K+</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Ca++</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Mg++</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Cl−</td>
<td>136.2</td>
<td>127.4</td>
<td>127.4</td>
<td>127.4</td>
<td>127.4</td>
</tr>
<tr>
<td>HCO3−</td>
<td>22.0</td>
<td>22.0</td>
<td>22.0</td>
<td>22.0</td>
<td>22.0</td>
</tr>
<tr>
<td>H3PO4</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.0</td>
<td>75.0</td>
<td>0.0</td>
<td>216.0</td>
<td>317.0</td>
</tr>
<tr>
<td>Osmolality</td>
<td>298</td>
<td>298</td>
<td>228</td>
<td>448</td>
<td>610</td>
</tr>
</tbody>
</table>
FIGURE 1. Panel A: Na⁺-sensitive microelectrode calibration curves. The electrode potential is plotted against Na⁺ activity. Various background concentrations in the calibrating solution are used to simulate intracellular K⁺ concentrations at 0.75T, 1T, 1.5T, and 2T. After subtraction of resting membrane potential, the \( \delta_w \) was obtained from curve (O) at 0.75T, curve (Φ) at 1T, curve (V) at 1.5T, and curve (V) at 2T. Panel B: Ca²⁺-sensitive microelectrode calibration curve. The electrode potential is plotted against Ca²⁺ activity. After substitution of resting membrane potential, the \( \delta_w \) was obtained from curve (O) at 0.75T, curve (Φ) at 1T, curve (V) at 1.5T, and curve (V) at 2T.
Effects of Tonicity on ak and at in Heart Cells

**TABLE 2**
Control Measurements in Normal Tyrode's Solution

<table>
<thead>
<tr>
<th></th>
<th>Purkinje strands</th>
<th>Ventricular muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mV)</td>
<td>-77.4 ± 0.4* (26)†</td>
<td>-86.4 ± 1.2 (8)</td>
</tr>
<tr>
<td>ak (mM)</td>
<td>7.9 ± 0.34 (26)</td>
<td>6.9 ± 0.5 (8)</td>
</tr>
<tr>
<td>EK (mV)</td>
<td>+71.5</td>
<td>+75.1</td>
</tr>
<tr>
<td>at (nM)</td>
<td>98 ± 9(25)</td>
<td>70 ± 4(3)</td>
</tr>
<tr>
<td>Ec (mV)</td>
<td>+116</td>
<td>+120.4</td>
</tr>
<tr>
<td>n†</td>
<td>2.60</td>
<td>2.55</td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
‡ See Equation 1 for definition.

0.5 to 5.6 ± 0.6 mM (19% fall). These changes occurred over 2–3 minutes. No significant change in ak could be measured, but contraction strength did increase. For these experiments and those that follow, the changes reported to be significant were tested by paired t-tests, and yielded values of P ≤ 0.05. In addition, all changes were reversible, with return to control levels.

**Hypotonic Solution**

The expected change in ak upon exposure to the solution with reduced osmolarity (0.75T) if the cells behaved as perfect osmometers is a fall of 23% (not 25%, because of adjustment of the activity coefficient to compensate for the lower internal ionic strength). We would also expect a depolarization because of dilution of ak. Figures 2 and 3 illustrate the observed changes in Vm, ak, and at in experiments on Purkinje strands and ventricular muscle. In Purkinje strands, there was the expected depolarization of about 3–4 mV. ak fell to 5.6 ± 0.5 mM (19% fall), and at fell to 67 ± 22 nM (33% fall) (Table 3). Ventricular muscle depolarized 3–4 mV (Table 4) upon exposure to 0.75T solution, and ak fell to 4.3 ± 0.5 mM (23% fall). We were not able to obtain satisfactory measurements of at in ventricular muscle in 0.75T solution. No change in resting tension could be seen, but contraction fell by about 30%. All of the effects of hypotonic solutions were readily reversible.

**Hypertonic Solutions**

The expected result of hypertonic solution is hyperpolarization, related to an increase in at (Fozzard and Lee, 1976), and increase in ak and at. For 1.5T solution the change in ak to be expected from water movement is less than a 50% increase, because of the change in internal ionic strength. Extrapolating

**TABLE 3**
Sheep Purkinje Strands

<table>
<thead>
<tr>
<th></th>
<th>100% (1T)</th>
<th>75% (0.75T)</th>
<th>150% (1.5T)</th>
<th>200% (2T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mV)</td>
<td>-79.0 ± 0.3* (26)†</td>
<td>-75.9 ± 1.0 (8)</td>
<td>-82.2 ± 0.6 (10)</td>
<td>-84.2 ± 0.5 (7)</td>
</tr>
<tr>
<td>ak (mM)</td>
<td>6.9 ± 0.3 (26)</td>
<td>5.6 ± 0.5 (8)</td>
<td>9.5 ± 1.2 (10)</td>
<td>10.8 ± 0.3 (7)</td>
</tr>
<tr>
<td>at (nM)</td>
<td>101 ± 11.2 (25)</td>
<td>67 ± 22 (5)</td>
<td>193 ± 20 (10)</td>
<td>412 ± 43 (6)</td>
</tr>
<tr>
<td>EK (mV)</td>
<td>68.6</td>
<td>74</td>
<td>60.0</td>
<td>56.6</td>
</tr>
<tr>
<td>Ec (mV)</td>
<td>115.5</td>
<td>121.0</td>
<td>106.8</td>
<td>96.7</td>
</tr>
<tr>
<td>n†</td>
<td>2.63</td>
<td>2.58</td>
<td>2.66</td>
<td>2.60</td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
‡ See Equation 1 for definition.

**TABLE 4**
Sheep Ventricular Muscle

<table>
<thead>
<tr>
<th></th>
<th>100% (1T)</th>
<th>75% (0.75T)</th>
<th>150% (1.5T)</th>
<th>200% (2T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mV)</td>
<td>87.5 ± 1.2* (8)†</td>
<td>-84.3 ± 1.4 (6)</td>
<td>-90.8 ± 1.6 (6)</td>
<td>-95.4 ± 3.7 (3)</td>
</tr>
<tr>
<td>ak (mM)</td>
<td>5.6 ± 0.6 (8)</td>
<td>4.3 ± 0.5 (6)</td>
<td>7.1 ± 0.7 (6)</td>
<td>8.1 ± 0.5 (3)</td>
</tr>
<tr>
<td>at (nM)</td>
<td>70 ± 11 (3)</td>
<td>236 ± 28 (3)</td>
<td>67.8</td>
<td>64.3</td>
</tr>
<tr>
<td>EK (mV)</td>
<td>74.2</td>
<td>81.2</td>
<td>104.0</td>
<td></td>
</tr>
<tr>
<td>Ec (mV)</td>
<td>120.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n†</td>
<td>2.58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± se.
† Number of tissues studied.
FIGURE 2. Simultaneous measurement of intracellular Ca++, intracellular Na+, and membrane potential in a cardiac Purkinje strand exposed to 0.75T solution. NT: normal Tyrode’s solution, IT: iso-Na isotonic solution. a trace is the difference between the membrane potential (Vm) and Ca++-electrode (VCa) recordings. a trace is the difference between Vm and Na+-electrode (VNa) recordings.

from our previously measured aK changes, we might expect a 35% change. The change in aCa depends on whether there is simple concentration, as with aK, or if some transport system such as Na-Ca exchange modifies the level of aCa.

Hyperpolarization was seen in Purkinje cells (3–5 mV) (Table 3). aNa in Purkinje cells rose to 9.5 ± 1.2 mM in 1.5T solution (37% increase) and 10.8 ± 0.3 mM in 2.0T solution (57% increase). Examples of these effects are illustrated in Figures 4 and 5. In 1.5T solution, aCa rose to 193 ± 20 nM (91% increase) and in 2.0T solution it rose to 412 ± 43 nM (400% increase). These changes developed rapidly and were completely reversible. Whereas it often appeared that the change in aNa developed faster than the change in aCa, the location of the electrodes in different cells makes direct comparison of time courses unwise. Resting tension rose in 1.5T solution

FIGURE 3. Recordings of intracellular Na+ and membrane potential in ventricular muscle exposed to 0.75T solution. Symbols as in Figure 2.

FIGURE 4. Simultaneous measurement of intracellular Ca++, Na+, and membrane potential in a cardiac Purkinje strand during exposure to 1.5T solution. Symbols as in Figure 2.

FIGURE 5. Simultaneous measurement of intracellular Ca++, Na+, and membrane potential in a cardiac Purkinje strand during exposure to 2.0T solution. Symbols as in Figure 2.
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by 32% and in 2.0T solution by 52%. Contraction size fell to 71% and 26%, respectively (Fig. 6).

Ventricular cells showed somewhat more hyperpolarization (4-8 mV) (Table 4). \( a_N \) rose to 7.1 ± 0.7 mM in 1.5T solution (27% increase) and to 8.1 ± 0.5 mM in 2.0T solution (45% increase). These changes are illustrated in Figures 7 and 8. We were successful in monitoring \( a_{ac} \) only during the change to 1.5T solution, where it rose to 236 ± 28 mM (330% change). Resting tension rose 38% in 1.5T and 45% in 2.0T solutions. On the other hand, contraction size fell to 26% and 6% of control in the two solutions. The contractile changes were also reversible (Fig. 9).

**Na\(^+\) and Ca\(^{++}\) Electrochemical Gradient Ratios**

Since changing tonicity altered \( a_N \) and consequently the \( Na^+ \) electrochemical gradient, the \( Ca^{++} \) electrochemical gradient would be expected to change because of the \( Na-Ca \) exchange process. Our previous studies (Sheu and Fozzard, 1982) have indicated values of 2.5-2.6 for the apparent coupling ratio during change in \( Na_0, Ca_0, V_m, \) and \( Na_i \). The data obtained in these experiments gave the same coupling ratios in solutions of various tonicity (Tables 2-4). Although there was only a small change in \( Na^+ \) electrochemical gradient (about 7 mV at 2.0T), the change in \( Ca^{++} \) electrochemical gradient was much larger (about 27 mV at 2.0T), owing to the nonlinear properties of \( Na-Ca \) exchange.

**Discussion**

There are three interesting and important aspects to the results of these experiments. First, the normal values of \( a_N \) and \( a_{ac} \) add to the small number of measurements of these intracellular ions available, and the responses to tonicity changes reflect intrinsic cell behavior. Second, the alteration in \( a_N \) by water movement provides a test of the hypothesis that the \( Na^+ \) gradient controls \( a_{ac} \) by determining the relation between the \( Na^+ \) electrochemical gradient before

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**Figure 6.** Panel A: the tension development of a cardiac Purkinje strand in 1.5T solution. NT: normal Tyrode; IT: low Na\(^+\), isotonic solution. The upper traces are brief recordings at a faster recording speed, taken at the points indicated. Panel B: the tension development of a cardiac Purkinje strand during exposure to, and recovery from 2.0T solution.
FIGURE 7. Panel A: recordings of intracellular Na\(^+\) and membrane potential in a ventricular muscle during exposure to 1.5T solution. Panel B: recordings of intracellular Ca\(^{++}\) and membrane potential in a ventricular muscle during exposure to 1.5T solution. The symbols are as in figure 2.

and after the interventions. Third, the effects of tonicity (internal ionic strength) on contraction, and the possible relationship to \(a_{\text{Na}}\), offer some information about control of contraction in heart muscle. These three interrelated aspects will be discussed individually.

**Resting Levels of \(a_{\text{Na}}\) and \(a_{\text{Ca}}\)**

Ion-sensitive microelectrodes have only recently become available for measurement of Na\(^+\) and Ca\(^{++}\) in heart muscle. The first measurements of \(a_{\text{Na}}\) in mammalian ventricular muscle were reported by Lee and Fozzard (1975) to be about 6 \(\text{mM}\), and levels subsequently obtained in various cardiac tissues agree rather closely with that value (e.g., Ellis, 1977; Glitsch and Pusch, 1980; Eisner et al., 1981).

In experiments already reported, we (Sheu and Fozzard, 1982) compared ventricular muscle and Purkinje strands and suggested that \(a_{\text{Na}}\) might not be the same in these two tissues. Under conditions of these experiments, \(V_m\) is less negative in Purkinje strands, and it seems likely that resting Purkinje cells are farther away from their K\(^+\) equilibrium potential (Lee and Fozzard, 1975; Sheu et al., 1980). If we assume that this is because resting \(P_{\text{Na}}\) is higher in resting Purkinje cells, then resting inward leak of Na\(^+\) would be greater, and consequently the steady level achieved by the Na-K pump would be higher. The fact that in Purkinje strands \(a_{\text{Na}}\) is reduced by exposure to tetrodotoxin (Deitmer and Ellis, 1980) supports the idea that resting Purkinje strands are relatively depolarized because of a higher \(P_{\text{Na}}\). A difference in \(a_{\text{Na}}\) between the tissues would also predict a difference in \(a_{\text{Ca}}\) if Na-Ca exchange is important in setting the resting level of \(a_{\text{Ca}}\). Our results are consistent with this prediction, but the differences are too small to attribute this with certainty to Na-Ca exchange. We may also speculate that the higher resting \(a_{\text{Ca}}\) may underlie the greater sensitivity of conducting tissue to toxicity by cardiac glycosides (Vassalle et al., 1962), which is thought to be related to elevated \(a_{\text{Ca}}\) levels (Kass et al., 1978; Colquhoun et al., 1981).

The levels of \(a_{\text{Ca}}\) in both Purkinje strands and ventricular muscle are somewhat less than 100 \(\text{nm}\). Using the activity coefficient of 0.32 for solutions of normal ionic strength, this represents [Ca\(^{++}\)] of 0.3 \(\mu\text{M}\). These values agree with our previous measurements (Sheu and Fozzard, 1982; Lado et al., 1982a) and with those of other investigators (for review, see Lee, 1981). The small differences in reported \(a_{\text{Ca}}\) levels are almost entirely explained by use of slightly different apparent stability constants for Ca-EGTA or different temperature for the measurements. All of these measurements are based on the ISE, and use a variation of the resin ETH 1001, so systematic errors fundamental to the ISE method might exist in all of the measurements reported. However, similar values ([Ca\(^{++}\)] = 2.9 \pm 0.3 \times 10^{-7} \text{ M}) have been reported from measurements of the resting aequorin luminescence in cardiac Purkinje fibers (Weir and Hess, 1984).

**Low-Sodium Isotonic Solution**

In order to maintain \(a_{\text{Na}}\) constant through the tonicity changes, it was necessary to lower \(a_{\text{Na}}\) by 23%. Exposing the tissues to the solution of lower Na\(^+\) caused a fall in \(a_{\text{Na}}\) as previously reported (Ellis, 1977; Sheu and Fozzard, 1982). Although contraction strength increased, the measurements in these experiments failed to show a significant change in \(a_{\text{Ca}}\). Sheu and Fozzard (1982) previously demonstra-
ted that lowering Na, does produce a rise in aC. The reduction in Na* gradient in the present experiments was quite small, so that a combination of a decreased slope of the Ca** electrode response, hyperpolarization, and the small decrease of aK may have made a small change in aC difficult to document here.

Hypotonic Solution

Entry into the hypotonic solution was promptly followed by a fall in aNa and aC. The change in aNa in Purkinje strands was 19%, not significantly different from the 23% predicted for simple osmotic dilution. The aNa change in ventricular muscle was exactly 23%. As mentioned earlier, the expected change was not 25%, because osmotic and chemical activity coefficients are not the same. The increase in activity coefficient as a result of lower ionic strength accounts for the difference. In this range, it appears that the cell is a good osmometer and that neither the Na-K pump nor any other factor except dilution affects aNa. It is rather surprising that the fall in aNa produced by dilution was not adjusted to its previous level by action of the Na-K pump. Perhaps the concomitant dilution of aK influenced the inner pump site affinity for aK. Schümpelri et al. (1982) have reported that osmotically induced changes in aK in frog skeletal muscle were also predicted by the water movement, without any apparent secondary compensation by the Na-K pump. Whereas the time of exposure to solutions of different tonicity was only 5-10 minutes, the apparent time constant for action of the Na-K pump is 1.5-2.0 minutes (Ellis, 1977; Eisner et al., 1981; Cohen et al., 1982). Therefore, there should have been sufficient time for response by the Na-K pump.

aC measurements in hypotonic solution could be obtained only in Purkinje strands. The fall exceeded that expected from dilution. The tissues depolarized an average of 3 mV in hypotonic solutions, as expected from dilution of aK (Fozzard and Lee, 1976; Akiyama and Fozzard, 1975).

Hypertonic Solutions

Purkinje strands and ventricular muscle showed similar responses to hypertonic solutions. aNa increased somewhat less than predicted for simple osmotic effect. This modest blunting of the aNa change could result from some stimulation of the Na-K pump, but it is also about the same effect seen previously for aK response to hypertonic solutions (Fozzard and Lee, 1976). In this previous study, we suggested that the cellular ionic strength (and activity coefficients) might not be estimated accurately under these conditions because of the complex polyanion nature of the cytoplasm. We cannot distinguish at this time between these two alternatives. aC rose dramatically in hypertonic solution, far
more than expected from simple osmotic concentration. The Na-Ca exchange hypothesis would predict that the increase in \(a_{\text{Ca}}\) would lead to a secondary rise in \(a_{\text{Ca}}\). Estimation of the apparent coupling ratios under these new conditions showed that \(Ca^{++}\) electrochemical gradients change in proportion to the Na\(^+\) electrochemical gradients, as would be expected if Na-Ca exchange controlled the level of \(a_{\text{Ca}}\). It must be emphasized that the coupling ratio calculation from the two gradients depends on \(V_{\text{m}}\), \(a_{\text{Na}}\) and \(a_{\text{Ca}}\), all of which contain certain measurement error. Consequently, the constant coupling ratios found in these experiments indicate that the dominant role of Na-Ca exchange in setting \(a_{\text{Ca}}\) is plausible, but they do not prove it. We have discussed elsewhere in greater detail the validity of this apparent coupling factor for Na-Ca exchange (Sheu and Fozzard, 1982).

It seems unlikely that the tissues were damaged by the solution changes, since ion activities and contractions returned rapidly to normal after washout of the test solutions. In particular, the tissues survived elevated \(a_{\text{Ca}}\) levels \([Ca^{++}] > 1 \mu\text{M}\) without apparent cellular uncoupling or residual damage.

**Tension Responses to Tonicity Changes**

Resting tension was not altered by the Tyrode's solution with reduced Na\(^+\), or by the hypotonic solution. However, tonic tension developed quickly in hypotonic solutions, with approximately the same time course as change in contraction strength and the change in \(a_{\text{Ca}}\). We have reported that tonic tension often developed when \(a_{\text{Ca}}\) reached 200 nm \([Ca^{++}] > 0.6 \mu\text{M}\), when \(a_{\text{Ca}}\) was raised by lowering \(Na_{\text{a}}\) in unstimulated fibers, and we suggested that this might represent tension threshold for \(Ca^{++}\) (Sheu and Fozzard, 1982). In these experiments, the tonic tension was also associated with elevated \(a_{\text{Ca}}\) of about 200 nm or more. No tonic relaxation was seen in hypotonic solutions, even though \(a_{\text{Ca}}\) fell. If tension threshold is not exceeded under resting conditions, then no relaxation would be expected from the decrease in \(a_{\text{Ca}}\). It is reasonable to suggest that the tonic tension produced by hypotonic solutions in these experiments is the result of these steady \(a_{\text{Ca}}\) levels.

We (Sheu and Fozzard, 1982; Lado et al., 1982a) have reported good correlation between \(a_{\text{Ca}}\) measurements and twitch contraction when the contraction size is modified by low \(Na_{\text{a}}\) solutions or by varying the rate of trains of action potentials. This same relationship can also be seen in hypotonic solution, with a comparable fall in \(a_{\text{Ca}}\) and contractions. However, in hypotonic solution, the contractions become small in spite of elevated \(a_{\text{Ca}}\). This result differs from studies in frog atrial muscle by Chapman (1978), but agrees with the results in other tissues, as reported by Hermsmeyer et al. (1972), Chapman and Leoty (1974), and Kawata and Kawagoe (1975).

In skeletal muscle, hypotonic solution also causes a reduction in twitch (Hodgkin and Horowicz, 1957; Gordon and Godt, 1970). Direct \(Ca^{++}\) injection (April et al., 1968) or exposure of skinned fibers to \(Ca^{++}\) solution (Gordon et al., 1973) also produces less tension. These investigations have suggested that the high ionic strength produced by hypertonic solution directly interferes with contraction. Such an effect would certainly explain our results with twitch contractions. However, we have suggested that the tonic tension produced by hypertonic solutions is the result of the elevated \(a_{\text{Ca}}\). For this to be true, it is necessary that the hypertonic solution still permit \(Ca^{++}\) binding to troponin, but slow the development of force in response to transient \(Ca^{++}\) release. Force-velocity studies in skeletal fiber (Edman and Hwang, 1977) support this idea that exposure to hypertonic solutions interferes with cross-bridge turnover. However, we cannot rule out additional effects of the hypertonic solutions or the high internal ionic strength on \(Ca^{++}\) release from the sarcoplasmic reticulum, such as may occur in tonic skeletal muscle (Godt et al., 1984).

**Na-Ca Exchange**

Examination of the relationship between the Na\(^+\) electrochemical gradient and the \(Ca^{++}\) electrochemical gradient that was begun by Sheu and Fozzard (1982) was extended by these experiments to conditions where \(a_{\text{Ca}}\) was altered by transmembrane water movement. The results of much larger movements of \(a_{\text{Ca}}\) in solutions of various tonicity are consistent with the interpretation that Na-Ca exchange plays an important role in the control of the resting level of \(a_{\text{Ca}}\) in heart muscle under a wide variety of conditions. Alternative transport processes to regulate \(a_{\text{Ca}}\) include an ATP-dependent sarcolemmal \(Ca^{++}\) pump, analogous to the Na-K pump (Caron and Carafoli, 1980; Sulakhe and St. Louis, 1980). The possibility that this alternative transport system has a role in regulating resting \(a_{\text{Ca}}\) cannot be ruled out by experiments such as those reported here. However, if they are responsible for these results seen here, then the alternative transport systems would require a complicated dependence on Na\(^+\).

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