The inotropic effects of strophanthidin in Purkinje fibers and the sodium pump

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ABSTRACT  The role of the inhibition of the Na pump in strophanthidin inotropy was studied in canine Purkinje fibers by correlating changes in contractile force with changes in maximum diastolic potential caused by conditions that enhance the electrogenic extrusion of Na. It was found that a brief exposure to a zero-K or to a zero-K, zero-Ca solution (but not to a zero-Ca solution) is followed by an increase in maximum diastolic potential. This hyperpolarization is reduced if NaCl is substituted by LiCl and in the presence of tetrodotoxin. In quiescent fibers exposed to tetrodotoxin, the hyperpolarization is abolished. A low concentration of strophanthidin (5 × 10⁻⁸M) increases contractile force but does not modify the hyperpolarization. Larger strophanthidin concentrations (5 × 10⁻⁷M to 10⁻⁶M) increase and then decrease contractile force and reduce or abolish the hyperpolarization. Metabolic inhibitors also reduce the hyperpolarization. We conclude that the positive inotropic effect of a low (therapeutic) concentration of strophanthidin is due to a mechanism other than Na pump inhibition. Circulation 69, No. 3, 618–631, 1984.

THE MECHANISM by which cardiac steroids increase contractile force is not well understood. It is generally agreed that in the toxic stage cardiac steroids inhibit the Na-K pump. An inhibition of the pump leads to an increase in intracellular Na that in turn increases the cellular Ca through a reduced Na-Ca exchange and hence the increase in force. Such a mechanism has also been invoked for the therapeutic effect, but agreement on this point is not universal. One major problem in resolving this issue is the difficulty in measuring at the same time (and repeatedly as a function of time) the electrical, mechanical, and Na-pump effects of cardiac steroids.

In an effort to gain some insight into this problem, a new approach was adopted. There are several lines of evidence that show that in cardiac tissues the extrusion of Na is electrogenic and therefore is accompanied by hyperpolarization. In recent experiments the activity of the Na pump has been studied by loading Purkinje fibers with Na during the exposure to a zero-K solution; this procedure is followed by an outward (repolarizing) current that is presumably due to the electrogenic extrusion of the Na accumulated during exposure to zero-K solution. This is strongly supported by the fact that the current is abolished by cardiac steroids. Similarly, a brief exposure of quiescent or active preparations to a low-K solution is followed by an increase in the membrane potential, which has been attributed at least in part to a stimulation of the Na pump. If so, strophanthidin should decrease or abolish the hyperpolarization, but only when the pump is inhibited. By measuring at the same time electrical and mechanical effects during and after the zero-K exposure in the presence and in the absence of strophanthidin, it should be possible to determine whether the positive inotropic effect is associated with a decrease in hyperpolarization. Since an increase in intracellular Na during the zero-K exposure should also increase intracellular Ca (which in turn should increase potassium conductance or gK), the effects of a zero-K, zero-Ca solution were also tested in the absence and in the presence of strophanthidin.

The results of these and other procedures show that strophanthidin can induce a positive inotropic effect without modifying the hyperpolarization that follows the exposure to the zero-K, zero-Ca solution. It is only with a toxic concentration of strophanthidin that such hyperpolarization is reduced or abolished. A prelimi-
nary report of this study has appeared in abstract form.18

Methods

Mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg IV). The heart of each was excised through an intercostal incision. Strands of Purkinje fibers (0.5 to 1 mm in diameter) from the right or the left ventricle were superfused in a tissue bath with oxygenated (97% O₂ and 3% CO₂) Tyrode’s solution at 37°C. The Tyrode solution had the following composition (in mM): NaCl 137, KCl 4, NaHCO₃ 11.9, NaH₂PO₄ 0.45, MgCl₂ 0.5, CaCl₂ 2.7, and dextrose 5.5. In some experiments LiCl (Fisher Scientific) was substituted for NaCl. In the zero-K solution, KCl was omitted. In the zero-K, zero-Ca solution both KCl and CaCl₂ were omitted. These solutions were perfused for 30 to 90 sec (usually 60 sec) to avoid depolarization at the plateau and changes in strophanthinid binding (when strophanthinid was present).

Stimulation of the fibers was achieved by means of two steel pins connected to a Grass S4 stimulator via a Grass stimulation-isolation unit (SIU 4678). The rate of stimulation was 60 per minute. The duration of the stimulus was 1.5 to 2.5 msec, and the voltage was about 50% above the threshold. One end of the fiber was kept in place by one of the steel pins used as a stimulating electrode, whereas the other was tied with a short silk thread to a rigid rod attached to a force transducer (Grass FTO 3C). The other steel pin was positioned near the preparation.

The transmembrane potentials were recorded by means of 3M KCl–filled glass microelectrodes connected to an operational amplifier stage. The action potential and the twitch were displayed on a Tektronix model RM565 dual-beam oscilloscope and on a Tektronix model 5111 storage oscilloscope. Electrical and mechanical activity were photographed on film by means of Nihon-Kohden model PC-2C camera. With the preparation driven at 60 stimuli per minute in normal Tyrode’s solution, the length of the fiber was increased in steps until maximal contractile force was developed. The length was then decreased again to a value that resulted in 60% of the maximal contractile force.

A Grass Model 7 polygraph was used to record contractile force at a paper speed of 0.10 mm/sec. At the beginning of the experiment, the preparations were allowed to equilibrate in normal Tyrode’s solution for about 1 hr.

The stock solution of strophanthinid (Sigma Chemical Co.) was prepared the day before the experiment and was stored in a refrigerator. After exposure to strophanthinid, the fibers were allowed to recover fully. The metabolic inhibitors iodoacetic acid (IAA, Sigma Chemical Co.) and NaCN (Fisher Scientific) as well as tetrodotoxin (TTX, Sankyo Co. Ltd., obtained through Calbiochem Behring Corp.) were dissolved in Tyrode’s solution before the test. Changes in action potential were measured under magnification. When in an experiment the same test was repeated under steady-state conditions, the results were averaged for that experiment.

Statistical analysis of the data included the determination of average values ± SD and a paired Student’s t test; a p value less than .05 was taken to indicate a statistically significant difference. The p value was based on the number of experiments (not on the number of tests).

Results

The effect of zero-K on electrical and mechanical events.

In figure 1, top, the action potential and the twitch recorded on film are shown. In figure 1, bottom, the contractile force was recorded at a lower speed on paper to show continuous changes. The perfusion with the zero-K solution brought about a progressive increase in maximum diastolic potential (Eₘ₉₅) and in force. The increase in Eₘ₉₅ subsided during the latter part of zero-K exposure, whereas the contractile force kept increasing during and immediately after the exposure to zero-K. During the recovery Eₘ₉₅ increased once more and then decreased slowly toward the control level. The contractile force also decreased toward control.

![FIGURE 1. The effect of zero-K exposure on electrical and mechanical events. Top. The action potential and the twitch traces. Bottom. Slow-speed recording of contractile force. The zero-K solution was perfused as indicated beneath the top panel and by the cross-hatched rectangle beneath the bottom panel. Voltage, force, and time calibrations are next to the respective traces.](image-url)
Hyperpolarization during the exposure will be referred to as H1 and that after the exposure as H2. In 11 tests in six experiments (table 1, zero-K) during zero-K exposure, H1 was 5.4 mV and the subsequent depolarization was 0.78 mV. During the recovery H2 was 9.0 mV with respect to the value of control E_max. The maximal increase in contractile force (+203%) occurred sooner (76 sec) than the maximal hyperpolarization (118 sec, p < .001) after the end of the zero-K exposure. E_max returned to control levels by 218 sec from the beginning of recovery.

Preliminary and tentative explanations of H1 and H2 are as follows. During exposure to zero-K, the extracellular K concentration should rapidly decrease. This fall in extracellular K has three consequences: (1) the g_k decreases, (2) the K equilibrium potential (E_K) becomes more negative, and (3) the function of the Na pump becomes depressed. The direct effect of a fall in g_k would be a decrease in E_max (as eventually happens), but initially E_max may increase because E_K is more negative and because a fall in g_k reduces the short-circuiting effect of K on whatever current is generated by the pump. During the recovery E_max should increase again as g_k recovers and should become more negative if an electrogenic Na extrusion is stimulated by an increase in intracellular Na. Additional actions are considered below.

The effects of zero-K, zero-Ca solution. An accumulation of Na during the exposure to zero-K should lead to an accumulation of Ca in the cell through an altered Na-Ca exchange8 since contractile force is increased. An accumulation of Ca also increases g_K,17 raising a question about whether a Ca-related increase in g_k contributes to some extent to the observed hyperpolarizations. For this reason, the effect of Tyrode’s solution to which no K and no Ca were added was also tested. In figure 2 (taken from the same preparation as figure 1) the electrical events were similar to those induced by zero-K alone (figure 2, top), whereas the force did not increase (as in zero-K alone), but instead decreased (bottom panels of figures 1 and 2).

In 37 tests in 12 experiments (table 1, zero–K-Ca), H1 was 6.2 mV, the depolarization was 2.2 mV, and H2 was 9.4 mV. The longer the exposure, the more the contractile force decreased. The maximal decrease in force occurred sooner than the maximal hyperpolarization (H2) (p < .005). A comparison of the results with zero-K and those with zero–K-Ca shows that changes in E_max were similar whether the force increased (zero-K) or declined (zero–K-Ca), suggesting that a Ca-induced increase in g_k plays little or no role in H2.

The effects of a zero-Ca solution. Since the effects of zero-K were similar to those of zero–K-Ca, a solution to which no Ca is added should have little effect on E_max, although it should further decrease force. As shown in figure 3, perfusion with (nominally) zero-Ca solution produced neither hyperpolarization nor depolarization, but decreased contractile force markedly (−74%) (figure 3, bottom).

In eight tests in five experiments (table 1, zero-Ca) E_max did not increase either during or after exposure to zero-Ca, whereas the contractile force decreased by 69.2% in 98 sec. The experiments show that a brief exposure to zero-Ca decreases the force of contraction, but does not change the E_max.

**TABLE 1**

<table>
<thead>
<tr>
<th>Solution</th>
<th>n</th>
<th>H1</th>
<th>Depol</th>
<th>H2</th>
<th>%ΔF</th>
<th>MaxΔF (sec)</th>
<th>Max H2 (sec)</th>
<th>Duration H2 (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-K</td>
<td>11/6</td>
<td>5.4 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.78 ± 6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+203.0 ± 15.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.1 ± 6.9</td>
<td>118.2 ± 17.6</td>
<td>218.5 ± 10.3</td>
</tr>
<tr>
<td>Zero–K-Ca</td>
<td>37/12</td>
<td>6.2 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.2 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-54.1 ± 18.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.7 ± 17.9</td>
<td>97.2 ± 24.5</td>
<td>184.4 ± 38.9</td>
</tr>
<tr>
<td>Zero-Ca</td>
<td>8/5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-69.2 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.4 ± 10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTX,</td>
<td>5/4</td>
<td>2.7 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-19.2 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero–K&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(5.6 ± 2.7)</td>
<td>(−4.4 ± 0.7)</td>
<td>(8.6 ± 3.1)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Quiesc. TTX, zero-K</td>
<td>11/4</td>
<td>0</td>
<td>-16.4 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013 ± 0.1</td>
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</table>

The preparations were driven at a constant rate, except for during the last series of experiments (Quiesc., TTX, zero-K) when the preparations were quiescent in the presence of TTX.

n = the number of tests and of experiments, respectively. Δ potential = change in maximum diastolic potential during (H1) and after (H2) exposure to zero-K, zero–Ca, or zero–K-Ca solutions. The decrease in E_max during the exposure is also listed (Depol). %ΔF = percent change in contractile force; MaxΔF = time at which the maximal change occurred with respect to the initiation of the perfusion of the solution; Max H2 = time of occurrence of the maximal hyperpolarization with respect to the beginning of the perfusion of the test solution; Duration H2 = duration of the hyperpolarization in sec.

<sup>a</sup>p < .05 to .001; <sup>b</sup>p > .3.
<sup>c</sup>Numbers in parentheses are control values.
<sup>d</sup>Duration 21.2 ± 0.35 sec.
The action of a zero K-Ca solution on spontaneous activity. If the depolarization with a zero-K or zero–K-Ca solution is due to a fall in $g_K$ and $H_2$ is due at least in part to an enhanced electrogenic Na extrusion, then a spontaneously active fiber should accelerate during and should slow down after exposure to a zero-K solution. This is illustrated in figure 4: on exposure to zero-K in this preparation there was little hyperpolarization and the spontaneous discharge quickly increased to become very fast at a depolarized level, in part facilitated by the voltage-dependent fall in $g_K$. Recovery in Tyrode’s solution was characterized by an increase in $E_{mna}$ above the control level (+11.6 mV) and by a marked slowing of spontaneous discharge. Of interest is the fact that during the latter part of the longest pause, diastolic depolarization did not reach a steady value but actually became more negative (a finding that is compatible with enhanced electrogenic Na extrusion). At the end of the tracing the membrane potential was still negative as compared with control.

In six experiments (three in zero-K, three in zero–K-Ca) $H_1$ was $8.9 \pm 4.0$ mV and $H_2$ was $13.1 \pm 1.1$ mV. The spontaneous rate was measured before exposure ($7.1 \pm 0.8$ beats/min), when it was fastest during

**FIGURE 2.** Effect of a zero-K and zero-Ca exposure on electrical and mechanical events. Same preparation as in figure 1.

**FIGURE 3.** The effect of zero-Ca exposure on the electrical and mechanical events. The zero-Ca solution was perfused as indicated beneath the respective panels.
exposure to low-K solution (97.7 ± 30.8 beats/min), and when it was slowest during recovery (2.9 ± 1.0 beats/min). The rate increased even during H1 since it depends on a fall of $g_K$. The spontaneous rate during recovery was statistically different from that before exposure ($p < .01$).

The substitution of Li for Na and the effects of zero-K-Ca solution. If the Na load is less during the zero-K-Ca exposure, H2 should be less. This was tested with a zero-K-Ca solution in which LiCl was substituted for all NaCl (only NaH$_2$PO$_4$ and NaHCO$_3$ being left in the solution). Li does not exchange with Ca (as Na does), but it can substitute for K in activating the Na pump. Therefore, in a zero-K-Ca solution containing Li, the accumulation of Na inside the fiber should be less and that of Ca should be more. As shown in figure 5 (taken from the same preparation as figures 1 and 2) substitution of LiCl for NaCl did not prevent H1, but markedly diminished H2 both in magnitude and duration (figure 5, top). The slow-speed record in the bottom panel of the figure shows that the contractile force increased during the exposure to zero-K-Ca plus Li as the outward movement of Ca was inhibited and the inward movement of Ca increased (with respect to the zero-K-Ca without Li). The increase in force was not maintained during the exposure to zero-K-Ca-Li solution either because of development of Ca overload or because of the washout of extracellular Ca. As soon as the recovery began there was a marked rebound increase in force, which could have resulted from Ca

![FIGURE 4](http://circ.ahajournals.org/fullarticle-19720422/figures/4.jpg)

**FIGURE 4.** The effects of a zero-K exposure on the electrical and mechanical events of a spontaneously discharging Purkinje fiber. The zero-K was perfused as indicated underneath the panel. No force was developed during the fast spontaneous rhythm at a depolarized level.

![FIGURE 5](http://circ.ahajournals.org/fullarticle-19720422/figures/5.jpg)

**FIGURE 5.** The effect of the substitution of LiCl for NaCl in the zero-K-Ca solution. The zero-K-Ca solution contained 137 mM LiCl and no NaCl. This solution was perfused as indicated underneath the top and the bottom (cross-hatched rectangle) panels. Same preparation as in figures 1 and 2.
overload or from the partial restoration of extracellular Ca when extracellular Na was still below normal. The subsequent transient decline in force is likely the result of the restored Na-Ca exchange.

In 11 tests in five experiments, in the presence of Li H1 was somewhat greater (8.3 ± 2.8 mV) than in the presence of normal Na (6.2 ± 2.3, +33.8%, p < .01). In two of these experiments there was a brief hyperpolarization (figure 5) and in the other three experiments Emax decreased continuously during the recovery without even a transient brief increase. The contractile force increased by +99 ± 15.1% during and by +160 ± 48.4% after the exposure to Li. The experiments show that H2 was sharply reduced or abolished under conditions that markedly decrease Na load and simultaneously increase cellular Ca. This stresses the role of Na (but not that of Ca) in H2.

**TTX and zero-K effects.** TTX is a specific blocker of the fast Na channel and prevents activity in nonstimulated fibers. Therefore, in the presence of TTX, a zero-K solution should result in a smaller Na load and, as a consequence, hyperpolarization should decrease. Figure 6, top, shows the usual features during and after the exposure to zero-K. In the middle panel of the figure the preparation was driven at the same rate as it was in the presence of TTX (1.03 × 10⁻⁹M), but the action potential was smaller, the plateau more negative, and contractile force abolished. On exposure to zero-K, H1 and especially H2 (−56%) were reduced. In figure 6, bottom, the same preparation was quiescent in the presence of TTX: During the exposure to zero-K H1 was no longer present but depolarization was pronounced. On recovery the membrane quickly returned to the original value, but there was no H2.

As shown in table 1, in the presence of TTX in driven fibers H1 decreased by 2.9 mV and H2 by 4.9 mV, while depolarization increased by 14.8 mV. In the quiescent fibers H1 and H2 no longer occurred in the presence of TTX, whereas the depolarization was as large as in the driven fibers. In the absence of TTX, exposure to low-K solution induces activity in previously quiescent fiber. The results show that H1 and

**FIGURE 6.** The effect of TTX on the changes induced by a zero-K solution. Top, Control traces. Middle and bottom, Effects of the zero-K solution in the presence of TTX (10.3 μM). In the middle panel, the preparation was driven as in the control panel and in the bottom panel the preparation was quiescent. No contractile force was developed in the presence of TTX (middle). The middle panel was recorded after 9 min and 10 sec of exposure to TTX. The bottom panel was recorded after recovery in Tyrode’s solution and subsequent exposure to TTX for 7 min.
especially H2 are sensitive to the Na load. In quiescent fibers it would appear that the decrease and recovery of gK are responsible for the depolarization and return to the control potential, respectively.

The effects of zero–K–Ca and low levels of strophanthidin. The results presented above show that H2 especially is sensitive to the Na load and therefore likely to involve the action of the Na pump. On this basis, one might expect that strophanthidin would reduce or abolish H2 only if it at the same time inhibits the Na pump.

Figure 7 illustrates the results when the zero–K–Ca solution was tested in the absence and in the presence of 5 × 10^{-5}M strophanthidin. The test in the control solution gave the usual results. Strophanthidin had increased the force by 55% before the test was repeated: the zero–K–Ca solution induced changes in E_{max} that were similar in amplitude and duration to those seen in the absence of strophanthidin.

In 18 tests in 10 experiments (table 2) 5 × 10^{-6}M strophanthidin increased contractile force substantially (+89.5 ± 49, p < .001) and changed H1 and H2 insignificantly (p > .7 and > .6, respectively).

The effects of zero–Ca in the presence of high levels of strophanthidin. The effects of zero–K–Ca were tested repeatedly during the exposure to high concentrations of strophanthidin (10^{-7}M to 10^{-6}M). It was found that zero–K–Ca decreased or increased force depending on the concentration and the duration of exposure to strophanthidin. The control tracing in figure 8 illustrates that zero–K–Ca had the usual effects in the absence of strophanthidin. In the presence of strophanthidin there was still H2 during the ascending phase of inotropy (second panel and second exposure in the slow-speed recording) and the force decreased as usual during the exposure to zero–K–Ca. The results changed substantially after peak inotropy had been attained. On the third exposure (third trace and slow-speed record) there was a marked depolarization and no H2 and the contractile force increased (instead of decreasing). Similar features were observed in the last two tests (slow-speed trace and last two panels); the force increased during and there was no H2 after the exposure to zero–K–Ca. In fact, there was only depolarization and development of arrhythmias after exposure to zero–K–Ca (bottom panel of figure 8).

When Ca level is lowered in Purkinje fibers overloaded with Ca an increase in force is observed that is a manifestation of toxicity (in agreement with the de-
TABLE 2
Effect of zero–K-Ca in the presence of different concentrations of strophanthidin (stroph), NaCN, and IAA

<table>
<thead>
<tr>
<th>Chemical(s) added</th>
<th>n</th>
<th>%ΔF</th>
<th>H1</th>
<th>Depol</th>
<th>H2</th>
<th>%ΔF</th>
<th>MaxΔF (sec)</th>
<th>Max H2 (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 × 10⁻⁴M stroph</td>
<td>18/10</td>
<td>89.5 ± 49</td>
<td>5.8 ± 2.9</td>
<td>-0.66 ± 4.2</td>
<td>8.8 ± 4.1</td>
<td>49.2 ± 19.7</td>
<td>88.3 ± 19.8</td>
<td>97.6 ± 36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6.1 ± 2.7) a</td>
<td>(9.2 ± 3.0) c</td>
<td>(49.2 ± 19.7) d</td>
<td>(97.6 ± 36.3) d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 5 × 10⁻³M stroph</td>
<td>23/9</td>
<td>208.1 ± 102.3</td>
<td>6.0 ± 4.2</td>
<td>-1.27 ± 6.3</td>
<td>7.4 ± 4.1</td>
<td>43.4 ± 18.0</td>
<td>89.1 ± 18.9</td>
<td>108.2 ± 19.5</td>
</tr>
<tr>
<td>(strop 10⁻³)</td>
<td></td>
<td></td>
<td>(6.6 ± 2.7) f</td>
<td>(2.09 ± 3.4)</td>
<td>(10.1 ± 3.1)</td>
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<tr>
<td>0.5 to 1 × 10⁻⁴M stroph</td>
<td>13/7</td>
<td>338.8 ± 164.7 a</td>
<td>-2.0 ± 1.4 a</td>
<td>-2.43 ± 2.8</td>
<td>-2.4 ± 2.2 a</td>
<td>27.3 ± 3.7 a</td>
<td></td>
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</tr>
<tr>
<td>(strop 10⁻⁴)</td>
<td></td>
<td></td>
<td>(7.3 ± 2.2)</td>
<td>(2.49 ± 3.8)</td>
<td>(10.4 ± 2.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 to 10 × 10⁻³M NaCN and IAA</td>
<td>9/4</td>
<td>-21.16 ± 40.4</td>
<td>3.4 ± 1.6 a</td>
<td>-0.62 ± 5.6</td>
<td>4.0 ± 3.8</td>
<td>(5.8 ± 1.6)</td>
<td>(0.8 ± 2.7)</td>
<td>(8.6 ± 2.1) b</td>
</tr>
<tr>
<td>1 to 8 × 10⁻⁴M NaCN and IAA and</td>
<td>12/4</td>
<td>+128.3 ± 78.8</td>
<td>3.9 ± 2.6</td>
<td>-0.5 ± 4.2</td>
<td>1.8 ± 4.3 a</td>
<td>(6.6 ± 0.8)</td>
<td>(2.4 ± 0.7)</td>
<td>(9.6 ± 2.2)</td>
</tr>
<tr>
<td>5 to 50 × 10⁻⁴M stroph</td>
<td></td>
<td></td>
<td>(met inh)</td>
<td>(met inh)</td>
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</tbody>
</table>

met inh = metabolic inhibition; other abbreviations and explanations are as in table 1.

p < .05 to .001; "p > .7; "p > .6; "p > .1. bDuration of H2 (in sec).

cline strophanthidin inotropy and the onset of arrhythmias). For this reason, the results obtained with 1–5 × 10⁻³M and 0.5–1 × 10⁻⁴M strophanthidin were collected separately depending on whether the contractile force decreased or increased (toxic stage) during the exposure to zero–K-Ca. As shown in table 2, when the force decreased during the exposure to zero–K-Ca, H2 was still present but was smaller (−26.7%, p < .05); in the toxic stage, H2 was substituted by a depolarization (−2.4 mV).

The results show that with higher concentrations there may be a reduction of H2 even during the ascending phase of inotropy. As the exposure to strophanthidin is continued or with a high concentration, H2 is abolished and the mechanical effects of zero–K-Ca are reversed.

The influence of metabolic inhibitors on strophanthidin effects. If an enhanced electrogenic Na extrusion is involved in H2, then H2 should be also affected by metabolic inhibitors. Figure 9, top, shows the usual events with the perfusion of zero–K-Ca solution. The bottom panel shows that NaCN and IAA in small concentrations (4 × 10⁻⁴M) had little effect on electrical and mechanical activity. However, on exposure to zero–K-Ca, H1 was less and H2 was abolished and replaced by a slow decrease in Eₘ₉ₗ₆.

If low concentrations of strophanthidin increase cellular Ca independently of an inhibition of the Na pump, then strophanthidin should increase force even in the presence of metabolic inhibitors. Zero–K-Ca had the usual effects in Tyrode’s solution (H2 = 6.7 mV, not shown). As illustrated in figure 10, on the exposure to 8 × 10⁻⁴M NaCN and IAA, H1 and H2 were markedly depressed (figure 10, top). When strophanthidin (5 × 10⁻⁴M) was present (middle) exposure to zero–K-Ca led to arrhythmias and quiescence. When activity resumed, Eₘ₉₉₆ was less than before exposure to the zero–K-Ca solution. Figure 10, bottom, is a slow-speed record of contractile force and shows that the first zero–K-Ca exposure increased contractile force by a small amount only. A small contracture had begun before strophanthidin administration and the development of contracture became more marked as the exposure to metabolic inhibitors and strophanthidin was continued. Lowering K-Ca to zero did not decrease force but arrhythmias quickly developed. When the procedure was repeated a third time quiescence quickly ensued (end of bottom tracing in figure 10).

As shown in table 2 NaCN and IAA decreased contractile force by 21%, H1 by 2.4 mV, and H2 by 4.6 mV. When strophanthidin (5 to 50 × 10⁻⁴M) was added during the continued exposure to metabolic inhibitors, the contractile force increased by 128.3%, while the decline in H1 and H2 continued (2.7 and 7.8 mV, respectively; table 2). The results suggest that strophanthidin still increases the contractile force in the presence of Na-pump inhibition by metabolic inhibitors but as the inhibition increases with time, H1 and especially H2 continue to decline and contracture develops.
FIGURE 8. Effects of a zero-K-Ca solution in the absence and in the presence of high concentrations of strophanthidin. The first panel shows the lower part of the action potential and the twitch during the control procedure (CONTR). At the end of this panel an action potential and twitch curve are shown. The second, third, fifth, and sixth panels show the lower part of the action potential and the twitch at various times during strophanthidin (3 × 10⁻⁷M) exposure. The fourth panel shows the slow-speed recording of contractile force before (control) and during strophanthidin exposure (STROPH). The times at which the zero-K-Ca solution was introduced are indicated by the rectangles next to each panel and beneath the slow-speed recording.

Discussion

The present experiments show that: (1) a brief exposure to a zero-K solution is followed by a hyperpolarization (H2) that persists in the absence of Ca, is reduced by Li, and is reduced or abolished by TTX in active or quiescent fibers, respectively. (2) small concentrations of strophanthidin (that are of clinical interest) increase contractile force without any significant reduction in H2. (3) larger concentrations of strophanthidin (that eventually cause a decline in force, arrhythmias, and inexcitability) reduce or abolish H2, and (4) metabolic inhibitors act in a manner similar to larger concentrations of strophanthidin. It is concluded that the positive inotropic action of low concentrations of strophanthidin is not related to any measurable inhibition of Na-pump activity and therefore should be related to some other mechanism. The inhibition of the Na pump is present with high concentrations of strophanthidin and should contribute to both the positive and the subsequent negative inotropic effects.

The effects of a zero-K solution: H1. The K concentration outside the cell membrane is unlikely to have fallen to zero during the zero-K perfusion because the exposure was brief and because K leaking out of the cells may accumulate in the intercellular cleft system. Still, the fall in K concentration appears substantial, as indicated by several changes that were noted.

Perfusion with zero-K increases the electrochemical gradient for K and therefore the E_K becomes more negative. As a consequence, E_{max} should become more negative since the internal K does not change in a K-free solution during the first 30 min of exposure. E_{max} becomes more negative when extracellular K is decreased to 2M or 0.54 mM. This mechanism should be involved in the induction of H1. However, when extracellular K is very low, a steady state is not reached and instead E_{max} declines. This decline is not unexpected since g_K and the function of the Na pump decrease as a function of the decrease in extracellular K. Since a decline in g_K and in the function of an electrogentic Na pump
pump have a depolarizing influence. H1 is followed by a depolarization. If the exposure to zero-K is long enough, spontaneous activity and quiescence may follow at a depolarized level, facilitated in this by a voltage-dependent decrease in $g_K$. That the Na pump and the extrusion of Na should be depressed in a zero-K solution is shown by the marked increase in contractile force: An accumulation of Na leads to an accumulation of Ca through a depressed Na-Ca exchange. In fact, intracellular Na activity has been shown to rise at a rate of about 0.24 mM/min in quiescent Purkinje fibers exposed to zero-K. It would seem reasonable to expect that in active Purkinje fibers (60 stimuli per minute in our experiments) the rate of Na accumulation is greater. Furthermore, in zero-K-Ca the increase in intracellular Na should have been enhanced since decreasing extracellular Ca also increases intracellular Na. Even a 1 mM change in intracellular Na activity has a marked influence on the contractile force of canine Purkinje fibers.

If then a shift in $E_K$ and a fall in $g_K$ seem to account for H1 and the subsequent depolarization on exposure to zero-K, our results show that H1 also decreases to some extent under conditions that decrease Na extrusion (and decrease H2). The reason for this is not clear. One possibility is that the fall in $g_K$ occurs faster than the decrease in function of the Na pump. This would decrease the short-circuiting effect of K on the pump current and therefore a degree of hyperpolarization could occur even if the function of the pump decreases. Furthermore, the very accumulation of Na inside the cell may moderate the fall in pump function that would be induced by the falling extracellular K. The net result would be a participation of the pump current in the induction of H1, which is also suggested by the increase in H1 in the presence of Li, since Li can replace K as an activator of the Na pump.

The lack of H1 and H2 in quiescent fibers in the presence of TTX is of interest. When extracellular K is decreased, the resting membrane potential changes little since the decrease in $g_K$ offsets the increase in $E_K$. Instead, if the fiber is active, $E_{max}$ increases as extracellular K is decreased. Therefore, in a quiescent fiber exposed to TTX, the increase in $E_K$ would not lead to H1 but the progressive decrease in $g_K$ would eventually cause depolarization. On restoration of extracellular K, $g_K$ would return to a control level, whereas change in $E_K$ would have little influence on the resting membrane. Furthermore, in a quiescent fiber exposed to TTX, the Na accumulation on exposure to zero-K
would be far less, thus decreasing the effect of this component.

The recovery from zero-K: H2. The hyperpolarization that follows exposure to zero-K probably results from several factors. When extracellular K is restored in the Tyrode solution, $g_K$ will also return to control levels. Since it is unlikely that the restoration in extracellular K at the cell membrane is instantaneous, a gradual return of extracellular K would gradually increase $g_K$ toward its original value. However, this could account only for a H2 similar to (but not greater than) H1.

Another possibility could be that during recovery $g_K$ increases above the control value because of an intracellular Ca accumulation during zero-K exposure. Such an increase in $g_K$ would indeed increase $E_{\text{max}}$ during recovery to a value more negative than that before exposure. Our results show that it is unlikely that this is the mechanism for several reasons. One is that H2 was about the same whether the contractile force increased (normal Ca) or decreased (zero-Ca) during the zero-K exposure. Furthermore, when Li was substituted for most Na (and the cellular Ca was increased as indicated by the increase in force), H2 was much less. Whether the increase in force was due to Ca overload or to a rapid increase in cellular Ca during recovery, H2 was still smaller or absent. In addition in the presence of TTX exposure of quiescent fibers to zero-K did not result in any H2 in spite of the fact that the inhibition of the pump (and some Ca accumulation) may have occurred even then. Finally, at high concentrations of strophanthidin, intracellular Na increased at a maximal rate of about $0.5 \text{ mM/min}^2$ (to the point of causing Ca overload) and yet H2 was reduced or abolished. It would seem that an increase in $g_K$ due to Ca accumulation plays little or no role in H2.

The role of the Na pump in H2. The above findings suggest the need to consider the role of the Na pump in H2, at least in accounting for its value in excess of H1. Ito and Surawicz$^5$ reported that 0.54 mM K causes a hyperpolarization in canine Purkinje fibers during and more so after exposure to low-K solution. From observations on the effects of ouabain, tetraethylammon-
ium, and high-K solution, they concluded that an increase in gK and stimulation of the Na pump were responsible for the extra hyperpolarization after the low-K exposure. Lee and Fozzard decreased K from 5 to 1 mM in quiescent sheep Purkinje fibers for several minutes and found an initial hyperpolarization during the exposure to low-K and a similar hyperpolarization after the exposure. The intracellular K activity remained practically unaltered and based on several findings it was concluded that the hyperpolarization after the low-K exposure was in part due to a delayed return of extracellular K or a slow permeability change and in part to the Na pump, which either induced an electrogenic Na extrusion or maintained a low concentration of K in the intercellular clefts. Similarly, Gadsby and Cranefield have shown that exposure to zero-K is followed by a shortening of the action potential and by the temporary suppression of spontaneous activity; both actions were related to an electrogenic Na extrusion.

In our experiments the increase in contractile force with zero-K is presumably the result of the demonstrated Na accumulation that in turn leads to an accumulation of Ca through a depressed Na-Ca exchange. Since the increase in intracellular Na is the physiologic stimulus for enhanced activity of the pump, once extracellular K is restored and the pump can resume its activity, the extrusion of Na should be enhanced. This theory is supported by the finding that H2 is reduced when the Na load during exposure to zero-K is reduced, as in the cases when Li was substituted for most Na or when TTX reduced Na influx. Also, the reduction or abolition of H2 by high levels of strophanthidin or metabolic inhibitors support a role of the Na pump in H2.

The pump could enhance H2 either by an electrogenic extrusion of Na or by depleting K concentration outside the cell membrane. The available evidence supports electrogenic Na extrusion. In Purkinje fibers, at least under certain conditions, the extrusion of Na creates an outward (hyperpolarizing) current; in fact, it has been shown that exposure to zero-K is followed by an outward current and hyperpolarization that are abolished by cardiac steroids. The suppression of spontaneous activity through hyperpolarization after exposure to zero-K as demonstrated in this and other experiments, strongly supports a role for an electrogenic Na pump. If the pump were to act only by causing a depletion in the intercellular clefts, the spontaneous activity would have increased (rather than decreased).

The Na pump and strophanthidin inotropism. The activation of inhibition of the Na pump by cardiac steroids is well established. However, the concentrations used experimentally are generally much larger (10⁻⁷ to 10⁻³ M) than the plasma levels of free drug found in patients; the therapeutic range is about 1 to 2 × 10⁻⁷ M and toxicity develops usually above a concentration of 3 × 10⁻⁷ M. Concentrations in vitro smaller than 10⁻⁵ M have little inotropic effect in Purkinje fibers, but 5 × 10⁻⁵ M strophanthidin results in a well-defined increase in force that is comparable to the response seen in patients. Higher concentrations also induce toxicity in vitro. For example, in Purkinje fibers 10⁻⁶ M strophanthidin quickly induces an increase and then a decrease in contractile force, aftercontractions, arrhythmias, and inexcitability. In contrast, smaller concentrations have a positive inotropic effect and are not toxic.

This poses the question as to whether, with small concentrations of cardiac steroids, the Na pump is less inhibited or is not inhibited at all. In the former case, the difference with large concentrations would be only quantitative; in the latter case, the mechanism of positive inotropy in the therapeutic range would be different from the inhibition of the Na pump. In fact, there are reports that show that the Na pump is stimulated by low concentrations of cardiac steroids and that this is accompanied by an increase of cellular K and a decrease of cellular Na. This implies that contractile force increases in spite of a stimulation of the Na pump activity and therefore by a different mechanism. Only concentrations above 10⁻⁶ M produce a dose-dependent increase in intracellular Na. Gervais et al. proposed that cardiac steroids modify the configuration of (Na+, K+)-ATPase and thus free more Ca for contraction. Noble has proposed a similar scheme in which the removal of Ca from the transport enzyme would account for the stimulation of pump activity. In our experiments there was no clear or consistent evidence of an increase of H2 above that of control with low levels of strophanthidin. However, the importance of this finding is not clear since between concentrations that excite and concentrations that inhibit the Na pump there must be concentrations (different from preparation to preparation and with different extracellular K) that have little effect either way.

Whether the pump is stimulated or not, the increase in contractile force without evidence of inhibition of the pump supports the concept that the therapeutic effect of low levels of strophanthidin is not dependent on pump inhibition. At high concentrations the pump appears partially inhibited, even during the ascending phase of strophanthidin inotropy, as H2 becomes
smaller. This suggests that with higher concentrations the inhibition of the pump contributes to the inotropic effect.

With regard to whether or not the suppression of electrogenic Na extrusion is an adequately sensitive measure of Na-K pump inhibition, a change in Na pump activity should be reflected in its electrogenic component, as it is when it is known that the pump is inhibited. To maintain an unaltered H2 in spite of an inhibition of the Na pump, it would be necessary for the Na extrusion to become more electrogenic. However, there is no evidence that the coupling ratio of Na and K changes with the degree of inhibition of the Na pump.

Our results are in agreement in more than one respect with those of Hart et al.,34 who found that a positive inotropic effect is obtained at low (< 10^-5M) concentrations of cardiac steroids. At these low concentrations, Na-pump activity (as measured by shifts in the current-voltage relationship at negative potentials) was found to be stimulated. In some fibers the stimulation of the pump resulted in a decreased contractile force. In our experiments, the increase in force induced by strophanthidin (5 x 10^-5M) was consistent and maintained; with higher concentrations there was already a decline in H2. However, Hart et al. used higher concentrations of K.

The decline in strophanthidin inotropy and the pump. The eventual decline in contractile force (mechanical toxicity) is not necessarily related to additional effects of strophanthidin. It is required only that the inhibition of the Na pump becomes greater. Because of this, cellular Ca would increase beyond an optimal level,19, 35 resulting in Ca overload. That this may be so is suggested by following: (1) Decreasing extracellular Ca during the descending phase of strophanthidin inotropy leads to a temporary increase in force19 (as the overload is removed). (2) Increasing extracellular Ca has the opposite effect19 (as the overload is increased). (3) Decreasing extracellular Na increases force in preparations in Tyrode’s solution and decreases it in the presence of high concentrations of strophanthidin.36 (4) Adding local anesthetics37 or increasing K38 decreases force in preparations in Tyrode’s solution and increases it in the presence of high levels of strophanthidin. (5) Increasing extracellular Ca beyond certain values decreases force in Purkinje fibers and the force temporarily increases on returning to low Ca.19 These and several other findings indicate that toxicity may result from a degree of pump inhibition that causes Ca overload. It is at this time that the oscillatory potentials and aftercontractions begin to appear.39

These electrical and mechanical changes are believed to be due to Ca overload40, 41 and to be responsible for the onset of arrhythmias. Significantly, zero–K-Ca decreased force in preparations in Tyrode’s solution but increased it during the declining phase of strophanthidin inotropy.

The action of metabolic inhibitors. The reduction of H2 by metabolic inhibitors also points to the role of electrogenic Na extrusion. At a high concentration of metabolic inhibitor the pump was inhibited to the extent that zero–K-Ca no longer decreased and instead increased force. Under these conditions adding strophanthidin after metabolic inhibitors increased contractile force further so that apparently the increase does not depend on an inhibition of the Na pump. The development of contracture and the rapid onset of toxicity (arrhythmias and inexcitability) should have been facilitated by the strophanthidin-induced enhancement of cellular Ca.

Our results show that with concentrations of cardiac steroids comparable to those used clinically, the contractile force increases considerably but there are no measurable changes in the electrical manifestations of Na-pump activity. The electrical and mechanical manifestations of Na-pump inhibition become apparent at high concentrations; the inhibition of the pump contributes to the larger inotropic effect and to the subsequent decrease in force and the onset of arrhythmias.

References

12. Gadsby DC, Cranefield PF: Direct measurement of changes in sodium pump current in canine cardiac Purkinje fibers. Proc Natl Acad Sci USA 76: 1763, 1979
The inotropic effects of strophanthidin in Purkinje fibers and the sodium pump.
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