Relation between Intracellular Sodium and Twitch Tension in Sheep Cardiac Purkinje Strands Exposed to Cardiac Glycosides

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SUMMARY. Changes in intracellular sodium ion activity (\(a_{Na}\)) produced by several cardiac glycosides were correlated with twitch tension in sheep cardiac Purkinje strands. Simultaneous measurements of \(a_{Na}\) and twitch tension were obtained through the use of Na-sensitive intracellular microelectrodes (ETH 227) in Purkinje preparations stimulated at a frequency of 1 Hz. All concentrations of ouabain, acetylstrophanthidin, and actodigin that were tested caused an increase in \(a_{Na}\) immediately before, or coincident with, a positive inotropic effect. No fall in \(a_{Na}\) was observed at any positive inotropic concentration of digitalis in these beating fibers. In all cases, the onset and washout of the positive inotropic effect were paralleled by the rise and fall in \(a_{Na}\), respectively. No dissociation between changes in \(a_{Na}\) and twitch tension occurred at any concentration of any of the agents used. The relation between changes in \(a_{Na}\) and twitch tension was linear with 1 mmol increase in \(a_{Na}\) producing about a 100% increase in the twitch magnitude. Propranolol did not significantly alter this relationship. The increase in \(a_{Na}\) with digitalis was also associated with a reduction in the maximum depolarization rate of the action potential, presumably as a consequence of a reduction in the transmembrane Na electrochemical gradient. These results indicate that the positive inotropic action of digitalis in sheep Purkinje strands is always associated with a rise in \(a_{Na}\) secondary to inhibition of the Na pump. This increase in \(a_{Na}\) could increase calcium available for contraction via the Na-Ca equilibrium exchange process. In addition, the increase in \(a_{Na}\) reduces \(V_m\), as a consequence of decreasing the electrochemical gradient for Na. (Circ Res 52: 697–705, 1983)

The mechanism of action of digitalis has been the topic of much research in recent years. Biochemical and physiological evidence has indicated that the enzyme responsible for Na and K countertransport across the sarcolemma (Na,K-adenosine triphosphatase or Na,K-ATPase) serves as the receptor for cardiac glycosides (for review, see Schwartz et al., 1975). It has been suggested that digitalis binding to this receptor interferes with its normal functioning, thus allowing Na\(^+\) to accumulate in the sarcoplasm, with a concomitant loss in cellular K\(^+\). This increase in cellular Na\(^+\) might then interfere with the normal Ca\(^{++}\) efflux process via Na-Ca equilibrium exchange (Reuter and Seitz, 1968). In this manner the accumulation of Na\(^+\) in the sarcoplasm could cause a rise in the amount of calcium available for contraction (Langer and Serena, 1970). This scheme is popularly invoked to relate the apparently selective binding of digitalis to the Na,K-ATPase to its powerful positive inotropic action.

Recently, however, evidence has been presented that appears to dissociate Na pump inhibition from the therapeutic action of cardiac steroids. Okita and co-workers (1973a, 1973b) found biochemical and electrophysiological indications of a dissociation between these actions of digitalis. Mendez et al. (1974) reported that a semisynthetic glycoside, actodigin, has a greater margin of safety than conventional compounds. This suggests also that pump inhibition and positive inotropy are dissociable. Several investigators have reported a stimulation of Na and K countertransport with therapeutic concentrations of glycosides (Cohen et al., 1976; Godfraind and Ghysel-Burton, 1977; Deitmer and Ellis, 1978, Ghysel-Burton and Godfraind, 1979). These conflicting results may be the consequence of methodological and species differences (Grupp et al., 1982), but, as yet, there has been no comprehensive explanation for the apparent discrepancies in these recent reports. There is some biochemical support for the proposal of digitalis stimulation of the Na,K-ATPase. However, other possible explanations exist; for example, digitalis may cause release of catecholamine in isolated tissues (Seifen, 1974). The catecholamines in turn, stimulate the Na pump and cause the observed fall in \(a_{Na}\) (Wasserstrom et al., 1982).

One of the difficulties in interpreting the above results is that Na pump function has been inferred from the rate of rubidium-86 transport as a substitute for external K\(^+\), or from the Na\(^+\) and/or K\(^+\) content as determined chemically. None of these methods is entirely suited to a direct correlation between changes in intracellular ionic composition and the positive inotropic action of digitalis in intact cardiac tissue. The use of Na-sensitive intracellular microelectrodes (Na-ISE) has recently made these measurements pos-
Lee et al. (1980) demonstrated that intracellular Na ion activity (aNa) increased at the time that dihydro-ouabain had produced a positive inotropic action in dog Purkinje strands. The strength of contraction was measured periodically throughout exposure to and washout of several concentrations of strophanthin. However, Deitmer and Ellis (1978) showed that low concentrations of strophanthin that, presumably, are positively inotropic cause a fall in aNa in quiescent sheep Purkinje fibers. Lee and Dagostino (1982) also found some indications of Na pump stimulation but did not fully examine this phenomenon. The present study was designed to examine the effects of low and high concentrations of several cardiac steroids in regularly stimulated cardiac fibers to determine the exact direction and magnitude of changes in aNa and to relate these changes to the inotropic actions of several agents.

The other aspect of this study was to examine some of the physiologial consequences of changes in the intracellular ionic composition. Eisner et al. (1981) examined the relation between aNa and twitch tension in sheep Purkinje fibers under voltage clamp. Na pump inhibition was accomplished by exposure to 0 K+ solutions. Their conclusion was that the positive inotropic action of 0 K+ solutions was the result of Na+ accumulation and Na-Ca exchange, thus providing a good basis for comparison with digitalis inhibition of the pump. Brown et al. (1981) have related the transmembrane gradient of Na+ to the magnitude of the fast inward Na+ current in internally perfused isolated ventricular myocytes. Our measurements of aNa and the Na+ gradient across the sarcolemma allow us to relate the maximum depolarization rate of the action potential (Vmax) to changes in the Na+ gradient as a result of Na+ pump inhibition.

Methods

Our method for measuring aNa in stimulated preparations has been discussed in detail elsewhere (Cohen et al., 1982; Wasserstrom et al., 1982). Briefly, Purkinje strands were dissected from sheep hearts obtained fresh from the local slaughterhouse. One end of the fiber was pinned to the floor of the beeswax-paraffin tissue chamber through which Tyrode’s solution was flowing (10 ml/min, 37°C). The composition of the solution was (in mM): NaCl, 137; KCl, 5.4; NaHCO3, 22; MgCl2, 1; NaH2PO4 2.4; CaCl2, 2.4; and dextrose, 5.5. The solution was gassed constantly with a mixture of 95% O2, 5% CO2 to maintain oxygenation and pH (7.35). One end of the fiber was pinned to the floor of the test chamber; the other end was attached to a force transducer (based on a Texas Instruments model 138 infrared photodiode) by a hooked stainless steel needle. Extracellular stimulation was delivered through silver wires insulated except at their tips at a frequency of 1 Hz (0.5 msec, 1.5 X threshold). The force transducer was mounted on a micromanipulator so that the fiber could be stretched to about 50% of Lmax (Wasserstrom et al., 1982).

After about 1 hour of stimulation, the fiber was impaled with a conventional and a Na-ISE. The transmembrane potential was recorded with a microelectrode filled with 3 mM KCl (tip resistance of 5-15 MΩ). The Na-ISE were made from the same blank micropipettes (WP instruments, borosilicate capillary tubing), but were siliconized so that their tips would hold the Na-sensitive neutral ligand ion exchanger resin ETH 227 (courtesy of Drs. Simon and Ammann, Zurich). The silanization process has been described elsewhere (Sheu and Fozzard, 1982). Only Na-sensitive electrodes with slopes greater than 48 mV per 10-fold increase in Na+ were used. Each microelectrode was calibrated before and after every experiment, using mixed solutions of NaCl and KCl with a constant total ionic strength of 300 mM. Transmembrane potential was measured with a Pico- metric Instruments electrometer, and the Na-ISE potential was measured with a Keithley model 604C high impedance electrometer. The maximum depolarization rate (Vmax) was measured by an electronic differentiator. Potential and twitch tension measurements were displayed on a Tektronix 5113 storage oscilloscope and Gould Brush 220 chart recorders for data recording. Microelectrode measurements were also displayed on digital panel meters, and these values were used for calculation of aNa.

Once stable measurements were obtained, as indicated by three identical measurements of aNa, one of three cardiac steroids was added to the superfusate. Ouabain, acetylstrophanthin (AS), and actodigin were prepared in concentrated stock solutions of 5% ethanol. Each agent was diluted in Tyrode’s solution to the proper concentration immediately before use in each experiment. The concentrations used were as follows: ouabain, 1-4 X 10⁻⁷ M; AS 0.2-1.3 X 10⁻⁷ M and actodigin 0.6-1.9 X 10⁻⁶ M. Low and high concentrations of each agent were tested so that the time course of changes in aNa and twitch tension could be examined.

The Na-sensitive resin ETH 227 has a selectivity of about 50:1 for Na/K. Because of the high electrode impedance, the noise on these signals is greater than on KCl-filled pipettes, making the digital voltmeter reading a useful one for monitoring the stability and for determining the voltage difference for comparison with calibration curves. Because drift of 1-2 mV can occur over the 6-8 hours of these experiments, we only used data from experiments where continuous recordings were available, usually with after-control periods. Individual measurements are easily made to ±0.5 mV, which corresponds to about ±0.2 mM aNa in the range of values obtained.

Continuous stimulation at a constant frequency is desirable during these experiments in order to obtain reliable and reproducible measurements of twitch tension. In addition, the positive inotropic effect of digitalis is frequency-dependent. Because the electrical response time of these Na-ISE is slow (about 500 msec), it was not possible to measure aNa accurately during the cardiac action potential. Our measurements were obtained during a brief pause (5-15 seconds) every 3 minutes during the control, drug, and washout periods. This procedure allows accurate measurements of aNa during a brief diastolic interval.

Sample means were compared using Student’s t-test, and the level of significance was considered to be P < 0.05.

Results

Relation between aNa and Tension

The time course of the changes in aNa and twitch tension may be important in examining the relation between these two actions of digitalis. Figure 1 shows the effects of ouabain on the force of contraction and
Ouabain thus caused a rise in $a_{Na}$ and contractility with the same time course, but with a very slight lag of twitch tension behind the increase in $a_{Na}$. The effects of this agent do not wash out readily in this preparation. Therefore, we tested the effects of other cardiac steroids whose rapid and reversible actions allow more complete examination of the relation between changes in $a_{Na}$ and the twitch. Acetylstrophanthidin is an ideal agent for this purpose, and it also has properties similar to strophanthidin, which has been reported to cause Na pump stimulation in low positive inotropic concentrations (Deitmer and Ellis, 1978). Figure 2A shows the effects of $1.1 \times 10^{-7} M$ AS on $a_{Na}$ and twitch strength in a sheep Purkinje preparation. This fairly high concentration of the aglycone caused an increase in $a_{Na}$ and twitch within 5 minutes after the addition of the AS to the superfusate. The rapid onset of the inotropic action was paralleled by the rise in $a_{Na}$. Both $a_{Na}$ and twitch reached their maximum values at about the same time, about 35-40 minutes. Drug-free solution then was introduced, and the washout of the drug effects was examined. Within 5 minutes, $a_{Na}$ began to decrease, followed immediately by a fall in twitch tension. Both $a_{Na}$ and twitch fell with nearly the same time course back to their initial values within 30-35 minutes, with $a_{Na}$ returning to control slightly faster than the twitch. This experiment demonstrates that AS produces parallel changes in $Na$ and twitch tension during the onset of the positive inotropic action of digitalis similar to that seen with ouabain. In addition, the washout of these effects of AS also shows a parallel fall in $a_{Na}$ with the change in $a_{Na}$ often slightly leading the fall in twitch tension. These results were obtained in all 19 fibers exposed to moderate concentrations of AS ($0.9-1.3 \times 10^{-7} M$).
The relation between tension and $\Delta Na$ for this experiment is plotted in Figure 2B. The onset curve (solid circles) describes a linear relation between $\Delta Na$ and twitch tension. During washout (triangles), the fall of tension and $\Delta Na$ is also linearly related. However, there is some displacement to the left of the washout curve, indicating a slightly different relation between the twitch and $\Delta Na$ during the two phases of drug action. This hysteresis has also been observed under conditions of inhibition of the Na pump by 0 K$^+$ solutions (Eisner et al., 1981).

The semi-synthetic cardiac glycoside actodigin also has a very rapid action and its effects are easily washed out. If its positive inotropic actions are to some extent independent of Na pump inhibition, as has been suggested by some investigators (Mendez et al., 1974), then we would expect some increase in twitch force before any change in $\Delta Na$ occurred. Figure 3 shows the effects of 1.9 $\times$ 10^{-7} M actodigin on a sheep Purkinje fiber. $\Delta Na$ and twitch tension remained constant until nearly 10 minutes after addition of the drug to the superfusate. After 10 minutes, both twitch tension and $\Delta Na$ began to increase and thereafter both increased rapidly until drug-free solution was super-
fused after 40 minutes. During washout of these effects, akt and twitch tension decreased rapidly and reached their initial values after 20–25 minutes of washout. Neither phase of this experiment demonstrated any dissociation between the changes in akt and twitch tension. These results were obtained in all nine preparations exposed to 1.0–1.9 \times 10^{-6} M actodigin.

We have thus far dealt only with the effects of high concentrations of these three cardiac steroids. All three agents demonstrated the same relationship between akt and twitch tension during both onset and washout of their positive inotropic effects. However, Na pump stimulation and dissociation of pump inhibition from positive inotropy may be demonstrable only at low positive inotropic concentrations of digitals. Figure 4 shows the effects of a threshold positive inotropic concentration of AS on twitch and Na. Propranolol was present in the superfusate starting sixty minutes before AS was added. At time 0, 2.2 \times 10^{-8} M AS was added to the superfusate. There was no measurable change in twitch tension throughout the subsequent 60 minutes of exposure to this agent. No change in akt occurred in either direction during this period. When the concentration was doubled to 4.4 \times 10^{-8} M AS, a doubling of tension occurred that coincided with an increase of about 2 mM akt. Both akt and tension returned to their initial values after removal of the AS from the superfusate. This experiment demonstrates that low concentrations of AS that do not increase twitch tension have no effect on Na. Furthermore, these and slightly higher concentrations that do exert a positive inotropic action were never observed to cause a decrease in Na. These results were obtained in all 17 preparations treated with low concentrations of AS (2.2–6.6 \times 10^{-8} M) in both the presence and the absence of propranolol (10^{-6} M).

Low concentrations of actodigin were also tested for their effects on the relation between contractile force and akt. Figure 5 shows the effects of 7.6 \times 10^{-7} M actodigin. akt and twitch tension both increased after 10 minutes of exposure to this concentration. There was no indication of a fall in akt initially, either before or during the onset of the positive inotropic action of this concentration of actodigin (Cohen et al., 1976). During washout, there was no separation between the recovery of akt and that of twitch tension. These actions were tested in 12 fibers with concentrations of actodigin in the range of 6–8 \times 10^{-7} M, either...
in the presence (four experiments) or absence (eight experiments) of $10^{-6}$ M propranolol.

The characteristic changes of $\Delta a_{Na}$ and tension and the overall relation between twitch tension and $Na^+$ are summarized in Table 1. All results for various concentrations of the three cardiac steroids have been combined in this table because there was no difference between the relation of tension to $a_{Na}$ for these agents (as determined by an analysis of variance). Experiments in which propranolol was added are not included in this table. The relation is more apparent in Figure 6 where these changes in twitch tension are plotted as a function of changes in $a_{Na}$. Standard error bars have been omitted for clarity of the illustration. In this figure, the curve showing the onset of action is denoted by the filled circles. The hollow triangles show this relation during washout. This relation is linear during both phases of drug action. The slope of the onset line is 1.17, which indicates that a 1 mM increase in $a_{Na}$ is associated with approximately a doubling of the initial tension. The washout line has nearly the same slope (1.22), which indicates that the relation between twitch and $a_{Na}$ is nearly the same for the two phases of drug action. There is a very slight shift of the washout curve to the left of the onset curve which is not significantly different. This may reflect a hysteresis that was occasionally observed between the two phases in some experiments (Fig. 2B). However, the overall relation seems to be nearly superimposable for the two phases of the experiments.

### Relation between $a_{Na}$ and Maximal Upstroke Velocity

The changes in $a_{Na}$ induced by positive inotropic concentrations of digoxins might be expected to have additional influences on the normal electrophysiological functioning of the heart. One possible consequence of increasing $a_{Na}$ would be to decrease the electrochemical gradient for $Na^+$ across the sarclemma. The normal $a_{Na}$ for the sheep cardiac Purkinje fibers used in this series of experiments was $7.6 \pm 0.62$ mM ($n = 31$). The doubling of $a_{Na}$ that occurs at these concentrations of cardiac glycosides would markedly reduce the inward driving force for $Na^+$. Consequently, $V_{max}$ would be expected to decrease with the fall in the $Na^+$ electrochemical gradient ($\Delta \alpha_{Na}$) caused by the increase in $a_{Na}$. Figure 7 shows the effects of changes in $\Delta \alpha_{Na}$ on $V_{max}$. Results were obtained only from those fibers that depolarized 2 mV or less during the course of the experiment. $\Delta \alpha_{Na}$ was calculated assuming that $V_m$ was $-16$ mV, which has been experimentally derived as the voltage at which $V_{max}$ is greatest in sheep Purkinje fibers (Walton and Fozzard, 1983). It is assumed that this value did not change with the concentrations of cardiac steroid used in these experiments and with a cell depolarization of 2 mV or less. The initial value of $V_{max}$ for these experiments was $527 \pm 15.9$ V/sec ($n = 17$). The slope of the relation between $V_{max}$ and $\Delta \alpha_{Na}$ is indicated by the solid line. This relation closely approximates the theoretical curve (dotted line) expected if (1) $V_{max}$ is a direct indicator of $Na^+$ inward current ($I_{Na}$; Walton and Fozzard, 1979) and (2) $I_{Na}$ is a function of $\Delta \alpha_{Na}$. This close approximation to that expected for a Nernstian relation between $I_{Na}$ and $\Delta \alpha_{Na}$ also has been recently demonstrated in perfused single heart cell preparations (Brown et al., 1981).

Therefore, one of the important consequences of the change in $a_{Na}$ induced by digitalis aside from the obvious effects on twitch tension is to alter the upstroke velocity of the cardiac action potential. This effect on $Na^+$ current might also explain the shorten-

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**TABLE 1**

<table>
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<tr>
<th></th>
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<th>Onset</th>
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<td>$\Delta %T$</td>
<td>$\Delta a_{Na}$ (mM)</td>
<td>$\Delta %T$</td>
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</tr>
<tr>
<td>31</td>
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<td>0 ± 0</td>
<td>4.50 ± 0.82</td>
<td>512.1 ± 7.9</td>
</tr>
<tr>
<td>29</td>
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<td>3.55 ± 0.15</td>
<td>396.5 ± 4.5</td>
</tr>
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<td>31</td>
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<td>2.67 ± 0.94</td>
<td>299.8 ± 12.5</td>
</tr>
<tr>
<td>25</td>
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<td>196.9 ± 3.5</td>
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<td>197.8 ± 8.5</td>
</tr>
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<td>98.8 ± 4.8</td>
</tr>
<tr>
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<td>0.75 ± 0.35</td>
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</tr>
<tr>
<td>7</td>
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<td>506.3 ± 6.2</td>
<td>0.32 ± 0.36</td>
<td>4.7 ± 3.3</td>
</tr>
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All values represent mean ± SEM.

Values were chosen during onset of action, only, for ouabain, and during both onset and washout for AS and actodigin. Not all values could be obtained for each point, which is why there is such a large variation in $n$, particularly during the rapid washout of the faster-acting agents.
tic concentrations of digitalis actually decrease aka via effects. This is a particularly important point because of the rapidity of the Na-Ca exchange process.

Several investigators have reported that low, therapeutic concentrations of dihydro-ouabain. The positive inotropic effect of this agent was always associated with a rise in aka. Similarly, aka decreased to its initial value as twitch tension returned to baseline during drug washout. Lee and Dagostino (1982) tested several concentrations of strophanthidin and observed a close relation between changes in aka and twitch tension. However, the precise temporal relation between changes in aka and twitch tension could not be derived from these experiments, nor were any other agents tested. Whereas most investigators assume that the effects of cardiac glycosides derive from the same mechanism, there are reports that the therapeutic effects of some cardiac steroids could be separated from their toxic (depolarizing and arrhythmic) effects (Okita et al., 1973; Ten Eick et al., 1973; Mendez et al., 1974).

We have directly investigated these problems in the present series of experiments. We tested the effects of three different cardiac steroids on the relation between changes in aka and twitch tension. The technique of periodically stopping stimulation in order to measure aka with Na-ISE permits a close correlation between aka and the twitch in a regularly beating sheep Purkinje fiber (1Hz). We found that all concentrations of the three agents that produced positive inotropy also increased aka. Ouabain was tested only during the onset of positive inotropic action, and, in each case, aka increased immediately prior to or coincident with an increase in the twitch. The other two agents, acetylstrophanthidin and actodigin, have rapid and reversible actions, and we therefore tested both onset and washout of their positive inotropic effects. Both agents had exactly the same relation between changes in aka and tension as did ouabain during the onset of inotropic action, namely, aka changed in parallel with the increase in the twitch. This parallel between aka and tension was also maintained during washout of positive inotropy. Low concentrations of these agents never caused a fall in aka under all the conditions we used, and when enough drug was used to produce a measurable positive inotropic effect, aka always increased. No Na pump stimulation was seen at positive inotropic concentrations of any of the three agents; neither was there any indication of a separation of the increase in aka from positive inotropy. We cannot rule out a fall in aka under all conditions, but we can affirm the relationship between positive inotropic effect and the rise in aka.

The finding that actodigin has the same effects as the other agents further supports the uniform action for this family of agents. Actodigin is a semi-synthetic cardiac glycoside with its lactone ring attached at the C2 carbon to the steroid nucleus rather than the usual results allowed a direct correlation of changes in aka with positive inotropic effects of low concentrations of cardiac steroids.

Lee et al. (1980) were the first to use Na-ISE in beating fibers in an attempt to provide an answer to this question. These investigators used periodic stimulation to determine the inotropic effects of various concentrations of dihydro-ouabain. The positive inotropic effect of this agent was always associated with a rise in aka. Similarly, aka decreased to its initial value as twitch tension returned to baseline during drug washout. Lee and Dagostino (1982) tested several concentrations of strophanthidin and observed a close relation between changes in aka and twitch tension. However, the precise temporal relation between changes in aka and twitch tension could not be derived from these experiments, nor were any other agents tested. Whereas most investigators assume that the effects of cardiac glycosides derive from the same mechanism, there are reports that the therapeutic effects of some cardiac steroids could be separated from their toxic (depolarizing and arrhythmic) effects (Okita et al., 1973; Ten Eick et al., 1973; Mendez et al., 1974).

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Discussion

The mechanism underlying the therapeutic action of digitalis has been uncertain, despite much research. The most comprehensive explanation relies on the specific action of glycosides on the Na,K-ATPase. That proposal uses the apparently specific binding of cardiac glycosides to this enzyme system, and the subsequent inhibition of its ability to maintain the normal Na and K transmembrane gradients, to explain the subcellular basis for digitalis action. This Na pump inhibition theory relies entirely on the premise that aka increases, and that it is this rise in aka that ultimately is responsible for the increase in Ca** available to the contractile pool. The increase in Ca** presumably is accomplished via the Na*,Ca** equilibrium exchange mechanism in heart (Reuter and Seitz, 1968). This explanation predicts a close relationship between aka and twitch tension throughout the effect of cardiac glycosides, including concentrations that produce only small positive inotropic effects. This is a particularly important point because several investigators have reported that low, therapeutic concentrations of digitalis actually decrease aka via Na pump stimulation (Blood and Noble, 1978; Deitmer and Ellis, 1978). Others have suggested that the anticipated rise in aka might not even be measurable because of the rapidity of the Na-Ca exchange process (Aker and Brody, 1978). Unfortunately, none of these
C₃ attachment as with A5 and ouabain (Deghenghi, 1970). Several investigators have reported that this alteration in configuration imparts a greater margin of safety (therapeutic/toxic) to this type of agent as evaluated in dog heart-lung preparations (Mendez et al., 1974), isolated dog Purkinje fibers (Gliklich et al., 1975), and cat Purkinje-muscle preparations (Wasserman and Kabela, unpublished observations). This would suggest that different cardiac glycosides could indeed have selective potencies for a “therapeutic” receptor which is distinct from a “toxic” receptor, presumably the Na+,K-ATPase. However, actodigin resembles conventional cardiac glycosides in its mechanism of therapeutic action, at least in sheep Purkinje fibers.

The relation between aNa and twitch tension has been reported for individual experiments in which 0 K was used to change aNa and twitch in sheep Purkinje fibers (Eisner et al., 1982) and in dog Purkinje fibers exposed to digitalis (Lee and Dagostino, 1982). We report here the relation between aNa and twitch tension for many experiments (n = 31) using all three cardiac steroids. Our results agree with these previous reports, namely, that this relation is linear at least in the range of relatively small increases in twitch force (up to about six times). The slope of this relation for all three drugs indicates that a 1 mV increase in aNa causes an increase in force of about 100%. We do not yet know why this relation appears to be linear. If the amount of intracellular Ca²⁺ available for contraction is in part determined by the coupling ratio of Na⁺-Ca⁺ exchange and the Na⁺⁺ electrochemical gradient (Sheu and Fozzard, 1982), then contractile strength is a function of aNa as well as aNa. Further speculation at this time is unwarranted, because we do not yet know what other intracellular factors besides aNa modulate contraction, nor do we understand fully the relation between aNa and twitch tension.

Aside from the effects of aNa in twitch tension, changes in aNa have other effects on the electrophysiological functioning of the heart. One of the most prominent of these is the effect of an alteration in the transmembrane Na⁺-electrochemical gradient (ΔINa) on INa. This would explain the fall in Vmax during the action of cardiac glycoside. The fall in Vmax occurs without cell depolarization or any significant changes in other upstroke characteristics. The relation between the decreases in ΔINa and Vmax roughly approximates that expected if Vmax is a function of INa, as has been reported by several investigators (Brown et al., 1981; Walton and Fozzard, in press). Thus a 10% decrease in ΔINa causes nearly a 10% fall in Vmax. Deviation of the changes in Vmax from that predicted simply from the effects of aNa change on the Na⁺⁺ electrochemical gradient is not surprising, since multiple factors influence the action potential upstroke. For example, the increase in aNa may be accompanied by an increase in aCa via Na-Ca exchange, secondarily affecting intercellular resistance.

The fall in ΔINa might also be expected to decrease INa throughout the action potential (Attwell et al., 1979). This would explain why cardiac glycosides characteristically reduce action potential duration without a decrease in membrane responsiveness (Kasebaum, 1963). There is another possibility that might also contribute to the shortening of the action potential. Marban and Tsien (1982) have recently reported that digitalis increases the slow inward current at therapeutic concentrations. The increased Ca⁺ entering by this mechanism could, in turn, increase the Ca²⁺-activated outward current (Isenberg, 1977), causing shortening of the action potential. One might therefore expect an increase in the plateau amplitude or duration associated with this phenomenon. We observed only a decrease in plateau voltage and action potential duration, but others have reported first a lengthening then a shortening of Purkinje fiber action potentials (Kasebaum, 1963). This might indicate an increase in Ca²⁺ current through the slow inward channel which subsequently increases the outward current, thus shortening the action potential.

Several investigators have recently reported a fall in aNa at low therapeutic digitalis concentrations (Godfraind and Ghysel-Burton, 1977; Deitmer and Ellis, 1978; Ghysel-Burton and Godfraind, 1979). Certain methodological constraints may perhaps have been present in those experiments. Godfraind and Ghysel-Burton used guinea pig atria stimulated with field electrodes to demonstrate a fall in Na⁺, and rise in K⁺ in response to nanomolar concentrations of ouabain. However, as Grupp et al. (1982) have recently demonstrated, this effect is apparently dependent on the involvement of autonomic nerve terminals. Needle stimulation of atrial strips rather than whole atria failed to produce these ionic changes. It is known that digitalis can increase catecholamines release (Seifen, 1974). Catecholamines have recently been shown to stimulate the Na pump in cardiac tissues and thus reduce aNa as measured directly by Na-ISE (Wasserman et al., 1982). Perhaps, then, the site of digitalis action to reduce Na⁺ in this case might be to affect Na⁺ and K⁺ indirectly by stimulation of the Na pump via an increased local concentration of catecholamine. In some of our experiments with low digitalis concentrations, propranolol (10⁻⁶ M) was added in order to prevent any possible action of endogenous catecholamines (e.g., Fig. 4). This did not alter the relation between the rise in aNa and twitch tension.

Deitmer and Ellis (1978) demonstrated a fall in aNa in some quiescent sheep Purkinje fibers exposed to low therapeutic concentrations of strophanthidin. It is known that stimulation has prominent effects on rate and magnitude of the inotropic action of digitalis (Ebner and Reiter, 1977). It is therefore difficult to interpret the results of Deitmer and Ellis (1977). In addition, there are possible explanations for a fall in aNa other than Na pump stimulation. If digitalis were to reduce Na⁺ leak in quiescent fibers, in addition to its other actions, this could cause the observed fall in aNa at low concentrations. Alternatively, Ferrier (1980) has suggested that digitalis might reduce the pacemaker current in dog Purkinje fibers. Since this in-
ward current is thought to be carried primarily by Na\(^+\), (Di Francesco, 1981) it is possible that the small decrease in an observed by Deitmer and Ellis (1978) and by Lee and Dagostino (1982) could come from reduction of this inward pacemaker current.

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