The Relationship among Intracellular Sodium Activity, Calcium, and Strophanthidin Inotropy in Canine Cardiac Purkinje Fibers

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ABSTRACT The role of sodium and calcium ions in strophanthidin inotropy was studied by measuring simultaneously the electrical, mechanical, and intracellular sodium ion activities in electrically driven cardiac Purkinje fibers under conditions that change the intracellular sodium or calcium level (tetrodotoxin, strophanthidin, high calcium, and norepinephrine). Tetrodotoxin (TTX; 1–5 × 10^{-6} M) shifted the action potential plateau to more negative values, shortened the action potential duration, and decreased the contractile tension and the intracellular sodium ion activity (a_Na). The changes in tension and in a_Na caused by TTX appear to be related since they had similar time courses. Strophanthidin (2–5 × 10^{-7} M) increased tension and a_Na less than the absence of TTX. For any given value of a_Na, tension was less than in the absence of TTX. Increasing extracellular calcium (from 1.8 to 3.5–3.6 mM) or adding norepinephrine (0.5–1 × 10^{-5} M) increased tension and decreased a_Na less in the presence than in the absence of TTX. When two of the above procedures were combined, the results were different. Thus, during the increase in a_Na and tension caused by strophanthidin in the presence of TTX, increasing calcium or adding norepinephrine increased tension markedly but did not increase a_Na further. In a TTX-high calcium or TTX-norepinephrine solution, adding strophanthidin increased both tension and a_Na, and the increase in tension was far greater than in the presence of TTX alone. The results indicate that: (a) the contractile force in Purkinje fibers is affected by a change in a_Na; (b) a decrease in a_Na by TTX markedly reduces the inotropic effect of strophanthidin, possibly as a consequence of depletion of intracellular calcium; (c) increasing calcium influx with norepinephrine or high calcium in the TTX-strophanthidin solution produces a potentiation of tension development, even if a_Na does not increase further; and (d) when the calcium influx is already increased by high calcium or norepinephrine, strophanthidin has its usual inotropic effect even in the presence of TTX. In conclusion, the positive inotropic effect of strophanthidin requires that an increase in a_Na be associated with suitable calcium availability.

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INTRODUCTION

The mechanism by which cardiac steroids increase contractile tension of cardiac muscle is still controversial, but a role of sodium is fairly established. The introduction of Na⁺-selective microelectrodes has conclusively demonstrated that cardiac steroids produce a parallel increase in intracellular sodium ion activity ($a_{Na}^i$) and in contractile tension, and this shows that the positive inotropy is closely related to the increase in $a_{Na}^i$ (Lee et al., 1980; Lee and Dagostino, 1982). The importance of an increase in intracellular Na is stressed by the fact that strophanthidin increases tension but little in the presence of tetrodotoxin (TTX) (Bhattacharyya and Vassalle, 1981), an agent that inhibits the inward movement of Na (see Kao, 1966). TTX is known to reduce $a_{Na}^i$ in quiescent fibers (Deitmer and Ellis, 1980) and conceivably does so in active fibers. A decrease in intracellular Na, in turn, is expected to decrease the intracellular calcium through an enhanced Na-Ca exchange (Reuter and Seitz, 1968; Glitsch et al., 1970). However, any firm conclusion is prevented by the lack of evidence on the behavior of $a_{Na}^i$ in active Purkinje fibers under the conditions mentioned above.

The aims of the present study were to investigate the relationship between the negative inotropic action and a possible decrease in $a_{Na}^i$ in the presence of TTX. Furthermore, we wanted to explore the reasons for the reduced inotropic effect of strophanthidin in the presence of TTX and for the restoration of such inotropy by norepinephrine and high calcium. For example, TTX could have prevented the increase in $a_{Na}^i$ by strophanthidin or alternatively could have allowed $a_{Na}^i$ to increase, but to a lower level. Also, norepinephrine or high calcium could restore strophanthidin inotropy either by modifying $a_{Na}^i$ somehow or by increasing the calcium entering the cell. The procedure adopted involved the continuous and simultaneous measurements of $a_{Na}^i$, contractile tension, and transmembrane potentials in electrically driven Purkinje fibers. The results obtained show that the inotropic effect of strophanthidin is influenced by both intracellular sodium activity and calcium influx and that the role of each can be determined by suitable procedures.

A preliminary report has appeared in abstract form (Lee and Vassalle, 1982).

METHODS

Tissues and Solutions

Mongrel dogs of either sex, weighing 18–25 lb, were anesthetized with sodium pentobarbital (30 mg/kg, administered intravenously). The heart was excised quickly through an intercostal incision and transferred to oxygenated (97% O₂ and 3% CO₂) Tyrode solution. Bundles of Purkinje fibers (diameter 0.5–1.0 mm, length 8–12 mm) were dissected from both ventricles. A fiber bundle was mounted in a narrow channel of a tissue chamber, as described previously (Lee and Dagostino, 1982). One end of the fiber bundle was fixed with an insect pin (diameter 100 μm) to the Sylgard floor of the chamber. The other end of the bundle was connected to a tension transducer by means of a silver wire (diameter 25 μm). Oxygenated Tyrode solution was perfused through the narrow channel of the chamber at a constant rate. Normal Tyrode solution contained (in mM): 137 NaCl, 5.4 KCl, 1.05 MgCl₂, 11.9 NaHCO₃, 0.45 NaH₂PO₄, 1.8 CaCl₂, and 5.0 dextrose. The temperature of the perfused solution was 36–37°C and the pH was 7.3–7.4. The Purkinje
fibers were driven continuously at a constant rate (1 Hz) throughout the experiment by stimulating electrodes connected to a stimulator (301-T; W-P Instruments, Inc., New Haven, CT) through a stimulus isolation unit (model 305-1; W-P Instruments, Inc.). The twitch tension of the fibers was measured by means of a tension transducer (model 405; Cambridge Technology, Cambridge, MA) at a sensitivity of 50 mV/mg.

The following compounds were used: TTX (Sankyo Co. Ltd., obtained through Calbiochem-Behring Corp., La Jolla, CA), strophanthidin (Sigma Chemical Co., St. Louis, MO), and norepinephrine (Levophed bitartrate, Breon Laboratories, New York). TTX (1 mg) was dissolved in 2 ml of distilled water and this stock solution was kept refrigerated. The stock solution of norepinephrine was prepared at the time of the experiment and kept refrigerated until diluted just prior to a test.

Measurements of Intracellular Na Ion Activity

Intracellular Na ion activity ($a_{Na}$) was measured with Na*-selective microelectrodes made with neutral carrier, ETH 227 (Steiner et al., 1979). Construction and calibration of the Na*-selective microelectrodes have been described in detail (Dagostino and Lee, 1982). Purkinje fibers were impaled with a conventional microelectrode and a Na*-selective microelectrode. The distance between the sites impaled with the two microelectrodes was <1 mm. From the potentials measured with the two microelectrodes, the $a_{Na}$ of the fibers was calculated by means of the following equation

$$E_{Na} - V_m = E_o + S \log(a_{Na} + k_{NaK}a_K + k_{NaCa}(a_{Ca})^{1/2})$$

where $E_{Na}$ is the transmembrane potential measured with a Na*-selective microelectrode with respect to a reference electrode in external bathing fluid, $V_m$ is the transmembrane potential measured with a conventional microelectrode, $E_o$ is a constant potential of Na*-selective microelectrode and can be determined routinely (Lee, 1981), and $S$ is the slope of a Na*-selective microelectrode that was determined empirically (Lee, 1981). The $k_{NaK}$ is the selectivity coefficient of a Na*-selective microelectrode for Na* with respect to K*. The $a_K$ is the intracellular K* activity. The $k_{NaCa}$ represents the selectivity coefficient of a Na*-selective microelectrode for Na* with respect to Ca*. $a_{Ca}$ is sarcoplasmic Ca ion activity. The $k_{NaK}$ (0.01-0.02) and $k_{NaCa}$ (1.7-2.5) values were determined as described previously (Lee and Dagostino, 1982; Dagostino and Lee, 1982). In determining control $a_{Na}$, we used 120 mM for $a_K$ and 110 mM for $a_{Ca}$ (Lee and Dagostino, 1982), as well as the selectivity coefficients obtained from the microelectrode calibrations. Interferences by changes in $a_K$ and $a_{Ca}$ were not taken into account because they should not significantly affect measurements of $a_{Na}$ changes. During exposure to $5 \times 10^{-7}$ M strophanthidin, an increase in $a_{Ca}$ does not appear to significantly affect the increase in $a_{Na}$ (Lee and Dagostino, 1982). The $a_{Ca}$ increase induced by strophanthidin caused an interference of 0.1 mM $a_{Na}$ when $a_{Na}$ increased by 1–2 mM. This indicates that the interference by the $a_{Ca}$ increase might be <10% of the $a_{Na}$ change. This relatively small interference should not significantly modify the $a_{Na}$ changes measured in the present experiments. Another argument against a significant interference by $a_{Ca}$ increase is the effect of change in extracellular calcium concentration and norepinephrine on $a_{Na}$. Increasing calcium from 1.8 to 3.6 mM or adding norepinephrine reduces $a_{Na}$ in Purkinje fibers (Lee and Vassalle, 1983). On the other hand, increasing extracellular calcium in the presence of TTX or TTX plus strophanthidin produced little change in $a_{Na}$. Also, norepinephrine decreases $a_{Na}$ but does not change $a_{Na}$ in the presence of strophanthidin (Lee and Vassalle, 1983) or TTX plus strophanthidin (Fig. 4). In both cases, twitch tension was increased considerably. From these results, it is unlikely that Na*-selective microelectrodes are significantly interfered with by changes in $a_{Ca}$ in our experiments.
In order to measure simultaneously and continuously the $a_{Na}^i$ of electrically driven fibers, we used two identical low-pass filters (with a fixed frequency of 0.24 Hz) as described by Lee and Dagostino (1982). Both Na$^+$-selective and conventional microelectrodes actually measure voltage fluctuations caused by the action potential of cardiac Purkinje fibers driven at a constant rate. The amplitude of the voltage fluctuations recorded by the microelectrodes depends on the resistance and capacitance of the microelectrodes used (Lee and Dagostino, 1982). The voltage fluctuations were completely removed with low-pass filters without a ripple before $V_m$ was electrically subtracted from $E_{Na}^i$. In the text, $V_m$ represents the filtered voltage of the action potentials measured with a conventional microelectrode. It has been shown that the use of the filters does not distort the measurements of intracellular ion activities (Lee and Dagostino, 1982).

RESULTS

Effect of TTX on Action Potential, Tension, and $a_{Na}^i$

The effects of TTX on a Purkinje fiber bundle driven at a rate of 1 Hz are illustrated in Fig. 1. Trace A is the filtered membrane potential ($V_m$) of the action potentials measured with a conventional microelectrode. Trace B is the difference between $V_m$ and the filtered potential of $E_{Na}^i$ and represents the intracellular sodium activity ($a_{Na}^i$). Trace C is a continuous slow-speed recording of twitch tension. In D, the action potential and the twitch tension curves were recorded in the absence (a) and in the presence (b) of $10^{-6}$ M TTX at the corresponding points marked over trace C. It is apparent that TTX quickly decreased the duration of the action potential, the intracellular Na ion activity, and twitch tension.

The decrease in twitch tension could be related to several factors. One of these possible factors is the decreased duration of the action potential. However, this appears unlikely because the twitch in Purkinje fibers is considerably shorter than the action potential (Fig. 1D), in contrast to myocardial fibers (see Lin and Vassalle, 1978). Some correlation between the decreases in twitch tension and action potential duration would be expected since both the shortening of the action potential and the fall in $a_{Na}^i$ would result from the same effect, namely a fall in Na influx. However, the relationship between tension and action potential duration breaks down at higher TTX concentrations in that there is no tension developed: this is because tension falls more than the action potential duration decreases (Bhattacharyya and Vassalle, 1982).

Another possibility is that the slow inward current is decreased directly or indirectly by TTX. A direct effect is not supported by voltage-clamp experiments (Rougier et al., 1967) or by the fact that TTX has no effect on slow responses in Purkinje fibers (Carmeliet and Vereecke, 1969; Bhattacharyya and Vassalle, 1982), atrial fibers (Pappano, 1970), and the sinus node dominant pacemaker cells (Yamagishi and Sano, 1966). An indirect effect could be related to the more negative and shorter plateau. In this regard it has to be pointed out that the slow inward current peaks in several milliseconds (Reuter, 1967) and that the calcium transient lasts some 180 ms in dog Purkinje fibers (Wier, 1980). However, the calcium entry during the plateau could be curtailed, and cellular calcium decreases over several beats.
A likely possibility is clearly a reduction of cellular calcium through the Na-Ca exchange as a consequence of a reduction of cellular Na. If this is the major mechanism, then the change in tension should parallel that of $a_{Na}$. This is seen in Fig. 1E. The abscissa shows the $a_{Na}$ in millimolar and the ordinate shows the twitch tension in milligrams. The closed circles show that there is a linear relationship between the decreases in $a_{Na}$ and in tension. Furthermore, the open circles show that this relationship is also maintained during the recovery from TTX.

That the decrease in tension is related to the decrease in intracellular sodium is shown by the fact that with a higher TTX concentration ($5 \times 10^{-6}$ M, Fig. 2), the $a_{Na}$ (B) and tension (C) decreased more, but the relationship between two
parameters remained unchanged (Fig. 2E). It should be noted, however, that in this and other experiments at high concentrations of TTX, the recovery of the contractile tension lagged behind the recovery of $a_{Na}$; the reason for this is not apparent. In five tests (four experiments), $1-1.5 \times 10^{-6}$ M TTX decreased $a_{Na}$ from $9.0 \pm 1.2$ (mean ± SD) to $7.8 \pm 1.0$ mM (−13.3%) and tension by $67.3 \pm 17.1\%$. In seven tests (five experiments), $4-5 \times 10^{-6}$ M TTX decreased $a_{Na}$ from $8.3 \pm 1.0$ to $6.4 \pm 1.0$ mM (−22.8%) and tension by $95.6 \pm 5.4\%$.

**Effects of Strophanthidin and Norepinephrine on Tension and $a_{Na}$**

Cardiac steroids increase tension and $a_{Na}$ (Lee et al., 1980; Lee and Dagostino, 1982), and norepinephrine increases tension but decreases $a_{Na}$ (Wasserstrom et
In Fig. 3, traces A–C show $V_m$, $a_{Na}$, and tension, respectively, during exposure of a Purkinje fiber to strophanthidin and strophanthidin plus norepinephrine. It is apparent that strophanthidin increased tension and $a_{Na}$ (from 8.6 to 12.5 mM). When norepinephrine was added, the force actually declined and arrhythmias developed. This could have been due to strophanthidin, or to the addition of norepinephrine, or to the acceleration of toxic effects by the two agents (see Vassalle and Bhattacharyya, 1981). The $a_{Na}$ continued to increase under the influence of strophanthidin to 17.2 mM, as expected (see Lee and Vassalle, 1983). After changing to the high $[K^+]_o$ (16.2 mM), quiescence ensued and $a_{Na}$ decreased below the control value. In nine tests (six experiments), strophanthidin increased $a_{Na}$ from 8.4 ± 1.4 (mean ± SD) to 12.5 ± 1.4 (+48.8%) in 13–17 min of exposure. Norepinephrine has little effect on the $a_{Na}$ under these conditions whether it decreases force and induces arrhythmias (Fig. 3) or increases force.

**Action of Strophanthidin and Norepinephrine in the Presence of TTX**

The changes in $a_{Na}$, tension, and action potential induced by strophanthidin and norepinephrine in the presence of TTX are illustrated in Fig. 4. Traces A–C show $V_m$, $a_{Na}$, and tension, respectively. In D, the action potential and twitch tension curves recorded during control (a) and in the presence of TTX (b) are shown. In E, the action potential and twitch tension curves in the presence of TTX and strophanthidin (c) and in the presence of TTX, strophanthidin, and norepinephrine (d) are shown. The figure makes it clear that TTX had its usual actions (see B–D). Adding strophanthidin increased $a_{Na}$ more slowly to a new steady state (from 7.7 to 10.2 mM) and increased tension less than in Tyrode
(cf. Fig. 3). When norepinephrine was added (still in the presence of TTX and strophanthidin), \( a_{\text{Na}} \) did not change and the force now increased above the pre-norepinephrine level (see tension curves \( c \) and \( d \) in \( E \)). It should be noted that the increase in tension was accompanied by very small changes in action potential configuration (\( E \)). Of course, high-speed recordings show that norepinephrine shifts the early repolarization to more positive values, presumably because of the

![Graphs and diagrams]

**Figure 4.** Effects of TTX, strophanthidin, and norepinephrine on intracellular Na activity, twitch tension, and action potential of the same experiment as that in Fig. 3. Traces A–C represent the filtered membrane potential (\( V_m \)), intracellular Na activity (\( a_{\text{Na}} \)), and twitch tension (\( T \)). \( D \) shows the superimposed action potentials (\( a, b \)) and twitch tensions (\( a, b \)) recorded at the points \( a \) (control) and \( b \) (exposure to TTX) in trace C. \( E \) shows the superimposed action potentials (\( c, d \)) and twitch tensions (\( c, d \)) recorded at the points \( c \) (exposure to strophanthidin in the presence of TTX) and \( d \) (exposure to norepinephrine in the presence of TTX and strophanthidin) in trace C.

increase in \( I_a \) (Vassalle and Bhattacharyya, 1981). On exposure to high K, the usual effects were present. In seven tests (five experiments) in the presence of TTX, strophanthidin increased \( a_{\text{Na}} \) from 7.2 to 9.5 mM (+32.4%).

When the procedure was reversed and norepinephrine was given first in the presence of TTX, \( a_{\text{Na}} \) decreased and tension increased, but less than in Tyrode. When strophanthidin was added to the TTX-norepinephrine solution, the \( a_{\text{Na}} \)
and tension increased. The increase in tension was far greater than when strophanthidin was given before adding norepinephrine.

The results in Figs. 3 and 4 (obtained from the same fiber) make it clear that strophanthidin was less effective in increasing $a_{Na}$ and tension in the presence of TTX. However, the figures do not clarify whether the smaller increase in tension was due entirely to the smaller increase in $a_{Na}$. To find out whether the slope of

![Graph showing relationships between twitch tension and intracellular Na$^+$ activity ($a_{Na}$). The open circles (O) represent the relationship obtained in the experiment shown in Fig. 3 during exposure to strophanthidin (5 $\times$ 10$^{-7}$ M) in Tyrode solution. The first circle represents control $a_{Na}$ and tension before the drug application. The data during exposure to norepinephrine in the presence of strophanthidin (Fig. 3) were not included. The filled circles (●) represent the relationship obtained in the experiment shown in Fig. 4 during exposure to strophanthidin in the presence of TTX. The relationship during exposure to norepinephrine in the TTX and strophanthidin (Fig. 4) is shown by the ×'s. The relationships in Fig. 5 were obtained from the same fiber.](image)

Figure 5. Relationships between twitch tension and intracellular Na$^+$ activity ($a_{Na}$). The open circles (O) represent the relationship obtained in the experiment shown in Fig. 3 during exposure to strophanthidin (5 $\times$ 10$^{-7}$ M) in Tyrode solution. The first circle represents control $a_{Na}$ and tension before the drug application. The data during exposure to norepinephrine in the presence of strophanthidin (Fig. 3) were not included. The filled circles (●) represent the relationship obtained in the experiment shown in Fig. 4 during exposure to strophanthidin in the presence of TTX. The relationship during exposure to norepinephrine in the TTX and strophanthidin (Fig. 4) is shown by the ×'s. The relationships in Fig. 5 were obtained from the same fiber.

the relationship between $a_{Na}$ and tension is altered by TTX, the results obtained in the absence and presence of TTX were plotted as in Fig. 5. TTX might simply shift the curve downward by reducing the background $a_{Na}$ or might instead shift the curve downward and modify the slope. Fig. 5 shows the relationship between twitch tension and $a_{Na}$ during the exposure to strophanthidin in the absence (open circles) and presence (filled circles) of TTX. In the absence of TTX (Tyrode solution), both twitch tension and $a_{Na}$ increased in an approximately linear fashion, which supports the role of the increased $a_{Na}$ in the
positive inotropy. In the presence of TTX, the relation was shifted downward as strophanthidin increased $a_{\text{Na}}$ from an initially lower level. In addition, the relation apparently shows two slopes as compared with the relation in the absence of TTX. Initially, the twitch tension was small and changed little when the $a_{\text{Na}}$ increased by ~1 mM. As $a_{\text{Na}}$ increased more, the twitch tension also increased. However, the slope (filled circles) between twitch tension and $a_{\text{Na}}$ was less steep as compared with that in the absence of TTX (open circles). It should be stressed that the nonlinearity of the relationship occurs in a range of $a_{\text{Na}}$ below the normal values and therefore it is not clear whether the nonlinearity is caused by TTX or by the low $a_{\text{Na}}$.

Tension and $a_{\text{Na}}$ were measured at fixed intervals, and the closer the points, the smaller the successive increments in $a_{\text{Na}}$, presumably because of a decrease in sodium influx caused by TTX. However, it is obvious that the tension developed at the same $a_{\text{Na}}$ was less in the presence than in the absence of TTX. For example, at an $a_{\text{Na}}$ of 10.0 mM, tension was 0.32 mg in the presence and 0.65 mg in the absence of TTX. Therefore, the tension was half for the same $a_{\text{Na}}$. Reciprocally, for a tension of 0.32 mg, $a_{\text{Na}}$ was 10.0 mM in the presence but 8.8 mM in the absence of TTX. Therefore, the data indicate that the fall in tension by TTX affects the action of strophanthinid through some additional factor. It suggests that at a given $a_{\text{Na}}$ level, there is less calcium released on excitation in the presence of TTX than in the absence of TTX. A clue to this may be given by the relationship (X) between tension and $a_{\text{Na}}$ when norepinephrine (NE) was added at the time that $a_{\text{Na}}$ was increased above the control (Fig. 5). Then the tension increased steeply with practically no change in $a_{\text{Na}}$. This suggests that norepinephrine increased force because it increased cellular calcium in the presence of an increased cellular sodium.

**Effects of Strophanthinid and of High Calcium on Tension and $a_{\text{Na}}$**

If norepinephrine acts by increasing cellular calcium, then similar results should be obtained by increasing extracellular calcium. In Fig. 6, traces A–C show $V_m$, $a_{\text{Na}}$, and tension. Strophanthinid increased $a_{\text{Na}}$ from 8.6 to 12.4 mM (B) and increased tension (C) as expected. Increasing $[\text{Ca}^{2+}]_o$ from 1.8 to 3.6 mM had little effect at the beginning and then produced a small decrease in $a_{\text{Na}}$. The contractile tension did not increase (see Vassalle and Lin, 1979). Exposure to high K (16.2 mM) quickly led to a quiescence and a decline of $a_{\text{Na}}$ below the control value, as seen under the other conditions (Figs. 3 and 4). Increasing $[\text{Ca}^{2+}]_o$ decreases $a_{\text{Na}}$ and increases twitch tension in Tyrode solution in the absence of strophanthinid (see Lee and Vassalle, 1983).

**Action of Strophanthinid and High Calcium in the Presence of TTX**

In Fig. 7, traces A–C show $V_m$, $a_{\text{Na}}$, and tension, respectively. The action potential and twitch curves are shown in the control (D, a), in the presence of TTX (D, b), in the presence of TTX plus strophanthinid (E, c), and in the presence of TTX, strophanthinid, and high calcium (F, d). On exposure to TTX, the action potential duration, $a_{\text{Na}}$, and tension decreased as usual. On exposure to strophanthinid, $a_{\text{Na}}$ and tension slowly increased. On exposure to high calcium there
was no further increase in $a_{\text{Na}}$, but tension increased far more than with TTX and high calcium in the same fiber. As usual, the action potential varied little when strophanthidin increased force in the presence of TTX ($b$ and $c$ in $E$).

The results show that in the presence of TTX, the potentiation between the inotropic effect of strophanthidin and that of high $[\text{Ca}^{2+}]_o$ is unrelated to a further increase in $a_{\text{Na}}$. However, the potentiation should be related to the $a_{\text{Na}}$ already increased by strophanthidin. The tension-$a_{\text{Na}}$ relations in the presence of strophanthidin (open circles) and TTX-strophanthidin (filled circles) are shown in Fig. 8. The relation between twitch tension and $a_{\text{Na}}$ resembles to that shown in Fig. 5 in that the slope in the presence of TTX was somewhat less steep. It can be seen that the tension developed at the same $a_{\text{Na}}$ was less in the presence than in the absence of TTX. When $[\text{Ca}^{2+}]_o$ was increased at the time of the increased $a_{\text{Na}}$ above the control, the twitch tension increased without a change in $a_{\text{Na}}$, as shown in Fig. 8 ($\times$'s).

**Action of High Calcium and Strophanthidin in the Presence of TTX**

In the preceding section, strophanthidin and then calcium were added in the presence of TTX, but it should not matter which of the two agents is perfused
first in order to obtain a potentiation of tension. Also, when high $[Ca^{2+}]_o$ is perfused first in the presence of TTX, its action should be far less than when strophanthidin is also present. In Fig. 9, A and B represent $a_{Na}$ and twitch tension, respectively. The action potentials and twitch tension are shown in C–E: a in the control, b in the presence of TTX, c in the presence of TTX and high $[Ca^{2+}]_o$.

**Figure 7.** Effects of TTX, strophanthidin, and high $[Ca^{2+}]_o$ on intracellular Na$^+$ activity, twitch tension, and action potential of the same experiment as that in Fig. 6. Traces A–C represent the filtered membrane potential ($V_m$), intracellular Na$^+$ activity ($a_{Na}$), and twitch tension ($T$). D shows the superimposed action potentials (a, b) and twitch tensions (a, b) recorded at points a (control) and b (exposure to TTX) in trace C. E shows the superimposed action potentials (b, c) and twitch tensions (b, c) recorded at points b (exposure to TTX) and c (exposure to strophanthidin in the presence of TTX) in trace C. F shows the superimposed action potentials (c, d) and twitch tension (c, d) recorded at points c (exposure to strophanthidin in the presence of TTX) and d (exposure to high $[Ca^{2+}]_o$) in the presence of TTX and strophanthidin) in trace C.

and d in the presence of TTX, high $[Ca^{2+}]_o$, and strophanthidin. TTX had usual actions, reducing $a_{Na}$ and twitch tension. High $[Ca^{2+}]_o$ increased twitch tension and shifted the action potential plateau to a more positive value and prolonged it (Bhattacharyya and Vassalle, 1982). The $a_{Na}$ was not increased by the high $[Ca^{2+}]_o$. When strophanthidin was added, $a_{Na}$ and twitch tension increased quite substantially. Fig. 9F shows the relationship between twitch tension and $a_{Na}$.
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when the fiber was exposed to high [Ca²⁺]₀ (triangles) and high-[Ca²⁺]₀ strophanthidin (circles) in the presence of TTX. When the high [Ca²⁺]₀ was added, twitch tension increased but aNa did not. When the fiber was exposed to the high calcium and strophanthidin, however, both twitch tension and aNa increased with a steep slope. In other words, even in the presence of TTX, the inotropic action of strophanthidin is much more effective when intracellular Ca is made available by increasing [Ca²⁺]₀. The results indicate that the positive inotropic action of strophanthidin occurs as usual in the high [Ca²⁺]₀ even when inward Na movements during action potentials were reduced by TTX.

**DISCUSSION**

In the present study, the following results were obtained: (a) TTX decreases the intracellular sodium ion activity (aNa) and twitch tension in cardiac Purkinje fibers. (b) Strophanthidin produces a parallel increase of aNa and tension in normal Tyrode solution. In the presence of TTX, however, during an initial
Figure 9. Effects of TTX, high [Ca\textsuperscript{2+}]\textsubscript{o}, and strophanthidin on intracellular Na\textsuperscript{+} activity, twitch tension, and action potential of a Purkinje fiber. Traces A and B represent the intracellular Na\textsuperscript{+} activity (a\textsubscript{Na}) and twitch tension (T). In C, control action potential (a) and twitch tension (a) recorded at point a in trace B are shown. D shows the superimposed action potentials (b, c) and twitch tensions (b, c) recorded at points b (exposure to TTX) and c (exposure to the high [Ca\textsuperscript{2+}]\textsubscript{o} in the presence of TTX) in trace B. E shows the superimposed action potentials (c, d) and twitch tensions (c, d) recorded at points c and d (exposure to strophanthidin in the presence of TTX and high [Ca\textsuperscript{2+}]\textsubscript{o}) in trace B. F shows relationships between twitch tension and a\textsubscript{Na}. The open triangles represent the relationship during the exposure to the high [Ca\textsuperscript{2+}]\textsubscript{o} (3.6 mM) in the presence of TTX (3.7 × 10\textsuperscript{-6} M). Note that the a\textsubscript{Na} practically did not change. The open circles represent the relationship during the exposure to strophanthidin (5 × 10\textsuperscript{-7} M) in the presence of TTX and high [Ca\textsuperscript{2+}]\textsubscript{o}. 

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**FIGURE 9.** Effects of TTX, high [Ca\textsuperscript{2+}]\textsubscript{o}, and strophanthidin on intracellular Na\textsuperscript{+} activity, twitch tension, and action potential of a Purkinje fiber. Traces A and B represent the intracellular Na\textsuperscript{+} activity (a\textsubscript{Na}) and twitch tension (T). In C, control action potential (a) and twitch tension (a) recorded at point a in trace B are shown. D shows the superimposed action potentials (b, c) and twitch tensions (b, c) recorded at points b (exposure to TTX) and c (exposure to the high [Ca\textsuperscript{2+}]\textsubscript{o} in the presence of TTX) in trace B. E shows the superimposed action potentials (c, d) and twitch tensions (c, d) recorded at points c and d (exposure to strophanthidin in the presence of TTX and high [Ca\textsuperscript{2+}]\textsubscript{o}) in trace B. F shows relationships between twitch tension and a\textsubscript{Na}. The open triangles represent the relationship during the exposure to the high [Ca\textsuperscript{2+}]\textsubscript{o} (3.6 mM) in the presence of TTX (3.7 × 10\textsuperscript{-6} M). Note that the a\textsubscript{Na} practically did not change. The open circles represent the relationship during the exposure to strophanthidin (5 × 10\textsuperscript{-7} M) in the presence of TTX and high [Ca\textsuperscript{2+}]\textsubscript{o}. 

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Intracellular Na⁺ Activity and Contractile Force

Phase, strophanthidin increases \( a_{Na} \) but has little effect on tension. During a latter phase, tension and \( a_{Na} \) increase slowly but the slope in the relation between tension and \( a_{Na} \) remains less steep than in Tyrode solution. (c) Adding norepinephrine or high calcium to the TTX-strophanthidin solution increases twitch tension substantially, but does not increase \( a_{Na} \). (d) Adding strophanthidin in the presence of TTX plus high calcium increases both tension and \( a_{Na} \). The slope in the relation between tension and \( a_{Na} \) becomes similar to that in normal Tyrode solution as calcium influx is increased by high \([Ca^{2+}]_o\) and \( a_{Na} \) is increased by strophanthidin.

It appears that (a) tension is sensitive to relatively small changes in \( a_{Na} \) since an increase in \( a_{Na} \) of 1 mM approximately doubles twitch tension; (b) the modulating influence of \( a_{Na} \) on tension is indirect, as it appears to be mediated through calcium; (c) strophanthidin increases tension less in the presence of TTX because the inotropic effect requires an increase in \( a_{Na} \) and a suitable amount of calcium on activation; (d) in the presence of TTX, the potentiation of tension when strophanthidin and norepinephrine (or high calcium) are given together is due to the fact that strophanthidin provides the increase in \( a_{Na} \) and norepinephrine (or high calcium) causes the increased calcium influx.

Negative Inotropic Effect of TTX

There are several ways in which TTX could decrease twitch tension. One of these ways is through a decrease in Na influx. Several reports show that TTX indeed decreases Na influx. In canine Purkinje fibers, TTX decreases the rate of rise of the upstroke of the action potential (Coraboeuf et al., 1979), the amplitude of the upstroke, and the duration of the action potential (Coraboeuf et al., 1979; Bhattacharyya and Vassalle, 1982). A partial block of the fast Na channel (Cohen et al., 1981) would account not only for the reduction in the upstroke but also for the shortening of the action potential since the inactivation of the fast channel during the plateau is incomplete. This residual sodium current (the "window current") is abolished by TTX (Attwell et al., 1979), which would result in a more negative plateau and a shorter action potential. In fact, TTX repolarizes Purkinje fibers depolarized at plateau level (Gadsby and Cranefield, 1977; Deitmer and Ellis, 1980). A decrease in Na influx by TTX should in turn result in a decrease in \( a_{Na} \) and this has been found in quiescent (Deitmer and Ellis, 1980) as well as in active Purkinje fibers (present results). The fall in \( a_{Na} \) would in turn decrease cellular calcium through the Na-Ca exchange mechanism (see Mullins, 1981). The present results provide direct evidence for a role of \( a_{Na} \) decline in the negative inotropic effect of TTX, since they show that TTX decreases \( a_{Na} \) and tension simultaneously and that there is a close relationship between the two changes. Changes in both \( a_{Na} \) and tension are related to the concentration of TTX.

An alternative explanation is that the decrease in tension on exposure to TTX is due to a shortening of the action potential. Not surprisingly, both the action potential and tension decrease as the TTX concentration is increased (present results; Bhattacharyya and Vassalle, 1982), since both depend on a reduction of Na influx. However, a direct role of the action potential duration on the
magnitude of the twitch is doubtful, since in Purkinje fibers (in contrast to myocardial fibers) twitch tension is much shorter than the action potential (see Lin and Vassalle, 1978) and the calcium transient lasts for ~180 ms after the beginning of the action potential (Wier, 1980). Furthermore, when tension is increased by strophanthidin, high Ca, or norepinephrine in the presence of TTX, the changes in action potential are minimal, which shows its small role in the tension changes. Finally, at high TTX concentrations, the tension (but not the action potential) is abolished (Bhattacharyya and Vassalle, 1982). For these reasons, it would appear that the shortening of the action potential is not a major direct factor in the decline in tension.

The shortening of the action potential could, however, influence force indirectly. For the reasons already discussed, it is unlikely that TTX would decrease tension by decreasing the slow inward current that flows at the beginning of the plateau. However, the incomplete inactivation of the slow channel during the plateau leaves a residual slow inward current (see Reuter, 1979). This residual current could be reduced by TTX because of the shortening of the action potential and the negative shift of the plateau (Figs. 1 and 2). This, in turn, may lead to a decrease in intracellular calcium over several beats. While this is a possible contributory factor, the demonstrated decrease in $a_{Na}$ should certainly contribute to the fall in contractile tension. In fact, a similar fall in tension and in $a_{Na}$ is seen with the inactivation of fast Na channels because of high $[K^+]_o$ (Lee and Dagostino, 1982).

Because TTX decreased tension substantially, the implication is that a relatively small change in $a_{Na}$ would decrease cellular calcium markedly.

**Effects of Strophanthidin in the Absence and Presence of TTX**

In the absence of TTX, strophanthidin increased tension and $a_{Na}$ simultaneously, as previously reported (Lee and Dagostino, 1982). This suggests that the two events are causally related, as one would expect from the known inotropic effects of an increased $a_{Na}$. It should be mentioned that at the concentrations used (2–5 x 10^{-7} M), strophanthidin did not cause any detectable decrease in $a_{Na}$ or any negative inotropic effect.

In the presence of TTX, strophanthidin increased $a_{Na}$ and tension less. There could be several reasons for the markedly reduced inotropic action of strophanthidin. It could be due to the fact that TTX had decreased intracellular calcium through an enhanced Na-Ca exchange, as a consequence of the fall of $a_{Na}$. This would account for the fact that strophanthidin initially increases sodium toward the normal value but increases tension little (the intracellular calcium being subnormal). Several other factors could contribute to the reduced inotropic response. One possibility is that calcium influx may depend on the level of intracellular calcium (see Marban and Tsien, 1982). In this case, a low level of intracellular calcium produced by TTX may reduce calcium influx and tension would increase more slowly as $a_{Na}$ increases. Another possibility is that calcium release from intracellular stores may depend on the calcium level in the sarcoplasm. Therefore, a lower calcium level may reduce the calcium released on activation. Finally, intracellular calcium could be regulated also by other mechanisms, such as an ATP-driven pump (Caroni and Carafoli, 1980).
The relationship between tension and $a_{Na}^+$ becomes steeper once $a_{Na}^+$ increases above the normal value (Figs. 5 and 8). The $a_{Na}^+$ increases more slowly in the presence of TTX and strophanthidin (as shown by the closeness of the filled circles in Figs. 5 and 8) and this would be expected for a reduced Na influx. Still, the tension increased less for the same increase in $a_{Na}^+$. The explanation for this finding is not obvious, but the curtailment of the residual slow inward current during the shorter and more negative plateau could play a role. Whatever the explanation may be, the importance of calcium is stressed by the fact that the slope returns to normal even in the presence of TTX, if strophanthidin is given in the presence of higher $[Ca^{2+}]_o$ (Fig. 9).

Effects of Norepinephrine or High Calcium Given Singly in the Presence of TTX

The concept that TTX decreases tension because it decreases $a_{Na}^+$ and therefore cellular calcium is supported by the results with norepinephrine and high calcium. Norepinephrine increases the slow inward current ($I_\alpha$) through an increase in $g_{Ca}$ (Reuter and Scholz, 1977) and high calcium increases it by increasing the driving force (Reuter, 1967); the same actions occur in the presence of TTX as tension increases. The fact that norepinephrine and high calcium increase tension in the presence of TTX but do not restore Na shows that the inotropic effect is related only to an enhanced release of calcium on activation. Presumably, a larger $I_\alpha$ would lead to a larger uptake of Ca into the sarcoplasmic reticulum during the subsequent diastole, and more calcium would be released during the subsequent activation. The gradual increase in tension over several beats support this view. Because of increased magnitude of the signal ($I_\alpha$) and the stores, more calcium would be released during the action potential (Fabiato and Fabiato, 1977; Allen and Blinks, 1978); hence, the increase in tension. Of course, because of the lower $a_{Na}^+$, the absolute value of the calcium released on activation would be lower than at normal $a_{Na}^+$.

Potentiation of the Tension Developed by Strophanthidin and High Calcium or Norepinephrine in the Presence of TTX

When strophanthidin was administered in the presence of TTX, the absolute tension was small (Fig. 7). When extracellular calcium was increased in the presence of TTX, the absolute increase in tension was also small. However, when strophanthidin and high calcium were perfused together in the presence of TTX, twitch tension increased to a value greater than the sum of the tension developed by strophanthidin alone or by high calcium alone. Similar results were obtained when (instead of increasing calcium) norepinephrine was added. Therefore, the experiments show that the simultaneous perfusion of strophanthidin and high calcium (or norepinephrine) leads to a potentiation in the development of tension.

As for the mechanism, the results make it clear that the potentiation did not result from a further increase in $a_{Na}^+$ by norepinephrine or high calcium in the presence of TTX; in fact, $a_{Na}^+$ showed no change or even a decrease at the time the tension development was potentiated. This is not too surprising since high calcium decreases $a_{Na}^+$ in active Purkinje fibers (Lee and Vassalle, 1983), as does norepinephrine (Wasserstrom et al., 1982; Lee and Vassalle, 1988). Instead, the potentiation is certainly related to the fact that norepinephrine and high calcium
increase $I_w$ since removal of high calcium or norepinephrine leads to a quick decrease in tension (Bhattacharyya and Vassalle, 1981). The implication of the present findings is that the potentiation results from the fact that strophanthidin increases $a_{Na}$, whereas high calcium (or norepinephrine) increases calcium during activation. The increase in calcium released on activation should result from the increase in calcium influx induced by high $[Ca^{2+}]_o$ or norepinephrine.

Therefore, the two major factors in potentiation appear to be the increase in $a_{Na}$, which in turn modulates the level of cellular calcium (Reuter and Seitz, 1968; Baker et al., 1969; Mullins, 1981; Sheu and Fozzard, 1982), and the increase in $I_w$. In the presence of TTX, high calcium or norepinephrine would increase calcium on activation, but not to the usual levels since $a_{Na}$ would have been lowered by TTX and is not restored to normal values by either high calcium or norepinephrine. Because of the increased Na gradient, the cellular calcium would remain less than normal. In the presence of TTX and strophanthidin, high calcium and norepinephrine would provide the extra calcium at a higher $a_{Na}$. Because of the diminished Na-Ca exchange, the cellular calcium would increase above the level that would have been present with high calcium alone; hence the far more marked inotropic effect.

**Inotropic Action of Strophanthidin under Different Conditions**

The mechanism of action of cardiac steroids is not generally agreed upon (Noble, 1980; Hougen et al., 1981; Adams et al., 1982; Marban and Tsien, 1982). Recent experiments employing Na$^+$-selective microelectrodes leave no doubt that the inotropic effect of cardiac steroids is closely related to the intracellular Na ion level (Lee et al., 1980; Lee and Dagostino, 1982).

In agreement with that, the present results show that if TTX decreases $a_{Na}$ (and therefore the cellular calcium), the inotropic effect of strophanthidin is reduced. In addition, the present results indicate that optimal levels of both intracellular sodium and calcium are necessary for the full inotropic effect of strophanthidin. The results also suggest that (in contrast to norepinephrine or high calcium) strophanthidin does not increase calcium influx during the action potential.

It has been proposed that an enhanced increase in $[Na^+]_i$ during action potential is responsible for the cardiac steroid inotropy (Brody and Akera, 1977). The results with TTX could be interpreted in that way because TTX is known to inhibit inward Na current during action potential. However, since strophanthidin markedly increased the twitch tension in the presence of TTX and high calcium, and neither norepinephrine nor high $[Ca^{2+}]_o$ is known to increase sodium influx, it appears unlikely that the action of cardiac steroids is related to a localized and transient increase in Na near the cell membrane. The experiments also rule out the possibility that norepinephrine or high $[Ca^{2+}]_o$ may potentiate strophanthidin through a change in action potential configuration. As for the proposed increase in calcium exchangeability (see K. S. Lee and Klaus, 1971; Gervais et al., 1977), it could well be that the increment in cellular sodium results in an increased release of calcium on activation. For example, Carafoli and Crompton (1978) have proposed a mechanism by which an increase in cytosolic Na induced by...
cardiac glycosides would release calcium from mitochondria. This mechanism is not demonstrated, but the possibility should be entertained that an increase in $a_{Na}$ may somehow facilitate the role of calcium in excitation-contraction coupling.

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