Effect of Calcium on Acetylstrophanthidin-Induced Transient Depolarizations in Canine Purkinje Tissue

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ABSTRACT

The role of calcium ions (Ca\(^{2+}\)) in the generation of transient depolarizations (TDs) by acetylstrophanthidin was examined. Transmembrane activity was recorded from isolated canine false tendons exposed to acetylstrophanthidin; concentrations from \(7.5 \times 10^{-8}\) to \(2 \times 10^{-7}\) g/ml caused TDs coupled to driven action potentials and depressed slow diastolic depolarization. TDs could reach threshold and induce extrasystoles. Elevation of the Ca\(^{2+}\) concentration increased the amplitude of TDs induced by acetylstrophanthidin. High Ca\(^{2+}\) concentration \((12.5 \text{ mM})\) caused TDs and depression of slow diastolic depolarization in the absence of acetylstrophanthidin. Elevation of potassium (K\(^{+}\)) concentration depressed and reduction of K\(^{+}\) concentration potentiated TDs caused by either acetylstrophanthidin or high Ca\(^{2+}\) concentration. The production of TDs and the depression of slow diastolic depolarization by acetylstrophanthidin were reversed by reduction of the Ca\(^{2+}\) concentration or addition of manganese \((2 \text{ mM})\) to the superfusing Tyrode's solution. The results suggest that TDs and arrhythmias produced by acetylstrophanthidin may be caused by a transient Ca\(^{2+}\) influx.

KEY WORDS: digitalis, arrhythmias, manganese, potassium, automaticity, diastolic depolarization, calcium current

Cardiac glycosides are generally believed to cause arrhythmias by enhancing automaticity in ventricular specialized conducting tissue, presumably through inhibition of the sodium-potassium-dependent membrane adenosine triphosphatase (ATPase) associated with sodium (Na\(^{+}\)) and potassium (K\(^{+}\)) transport (1). Inhibition of the ATPase should result in a gradual accumulation of intracellular Na\(^{+}\) and a gradual loss of intracellular K\(^{+}\). Moreover, loss of intracellular K\(^{+}\) in Purkinje tissue is said to mimic the effects of low extracellular K\(^{+}\), thereby enhancing phase-4 depolarization (2-5).

We have recently demonstrated that enhanced automaticity in Purkinje fibers caused by acetylstrophanthidin results not from accelerated phase-4 depolarization but from transient depolarizations coupled to action potentials (6). Transient depolarizations caused in Purkinje tissue by ouabain appear in illustrations of earlier reports but are not discussed (2). In more recent reports, depolarizations of this type have been considered as abortive slow diastolic depolarizations related to "normal" but accelerated pacemaker activity (7-9). However, we found that normal pacemaker activity in isolated false tendons was greatly depressed by acetylstrophanthidin at a time when transient depolarizations and associated automatic activity were demonstrable (6,10).

The two types of automaticity respond differently to periods of repetitive stimulation. The normal pacemaker activity of Purkinje fibers is transiently depressed by periods of pacing. The frequency-dependent escape intervals are greatly prolonged by acetylstrophanthidin. In contrast, pacemaker activity generated by transient depolarizations exhibits postpacing acceleration. This difference suggests that the mechanisms of depolarization might also differ. The data presented in the present paper suggest that transient depolarizations may be due to an influx of calcium ions (Ca\(^{2+}\)).

Methods

Hearts were excised under sodium pentobarbital anesthesia (30 mg/kg, iv) from mongrel dogs (10-25 kg) of either sex. Free-running false tendons from both
ventricles and in some experiments the right anterior papillary muscle with its attached false tendon were removed from the hearts and transferred to a tissue bath. Modified Tyrode's solution equilibrated with 95% O$_2$-5% CO$_2$ flowed continuously through the bath at 10 ml/min. The millimolar composition of the Tyrode's solution was: NaCl 137.0, KCl 4.0, NaH$_2$PO$_4$ 0.9, NaHCO$_3$ 12.0, CaCl$_2$ 2.5, MgSO$_4$ 0.5, and dextrose 5.5. All experiments were performed at 37 ± 0.5°C.

Preparations were driven by rectangular pulses (1–3 msec in duration and twice the threshold voltage) obtained from a Tektronix pulse generator (type 161) triggered by a digital interval counter. After passage through an isolation transformer, the stimuli were delivered to the isolated false tendon or to the apex of the papillary muscle through bipolar silver electrodes. In most experiments the pattern of stimulation consisted of trains of ten stimuli separated by 3-second pauses.

Transmembrane potentials were recorded by glass microelectrodes with resistances of 10–25 megohms. The electrodes were filled with 2.7M KCl by a method described in detail by Tasaki et al. (11). Transmembrane recordings were amplified and displayed on an oscilloscope (Tektronix 565) for photographic records (Grass kymograph camera).

A stock solution of acetylstrophanthidin (10$^{-4}$ g/ml in 4% ethyl alcohol) was diluted tenfold with Tyrode's solution and infused directly into the tissue bath by a Harvard infusion pump. The infusion rate was adjusted to result, after approximately 30 minutes, in a toxic level characterized by distinct transient depolarizations. The final concentration of the drug was most frequently either 7.5 $\times$ 10$^{-8}$ g/ml or 1 $\times$ 10$^{-7}$ g/ml (1.65 $\times$ 10$^{-7}$M or 2.2 $\times$ 10$^{-7}$M) but occasionally was as high as 2 $\times$ 10$^{-7}$ g/ml. The drug was dispersed in the bath by a small stream of gas (95% O$_2$-5% CO$_2$).

When the effects of altering the concentration of a particular ion were studied, that species, if normally present, was omitted from the Tyrode's solution. The ion in question was infused into the tissue bath by a Harvard infusion pump. This method allowed rapid changes in concentration.

**Results**

**EFFECT OF CALCIUM ON ACETYlstROPHANTHIDIN-INDUCED TRANSIENT DEPOLARIZATIONS**

Transient depolarizations (TDs) induced by acetylstrophanthidin, which greatly affected the changes in extracellular Ca$^2+$ concentrations: they were regularly enhanced by increasing the Ca$^2+$ concentration. Figure 1 illustrates one of six such experiments. The preparation was electrically driven by trains of ten stimuli at different basic cycle lengths (BCL). The top record in the figure was recorded from an isolated false tendon exposed to acetylstrophanthidin in Tyrode's solution containing 2.5 mM Ca$^2+$. The last action potential of each train was followed by a relatively low-amplitude subthreshold TD. The middle record demonstrates the effect of elevating the Ca$^2+$ concentration to 5.0 mM. At a BCL of 800 msec, the TD was greatly increased in amplitude but was still subthreshold. The coupling interval of the TD (measured from the upstroke of the last action potential to the peak of the TD) was abbreviated. At a BCL of 250 msec, the increase in the amplitude of the TD was sufficient to bring it to threshold and thereby initiate an extrasystole. After the concentration of Ca$^2+$ was returned to 2.5 mM, the TDs were greatly diminished in amplitude and no longer reached threshold at either BCL (Fig. 1, bottom).

**TRANSIENT DEPOLARIZATIONS INDUCED BY CALCIUM**

Elevation of the external Ca$^2+$ concentration, which enhances phase-4 depolarization in canine Purkinje tissue (12), also caused transient depolar-

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1Generously supplied by Eli Lilly Co., Indianapolis, Indiana.
izations resembling those induced by acetylstrophanthidin. Figure 2 illustrates recordings from an isolated false tendon exposed first to 2.5 mM CaCl$_2$ and then to 10.0 mM CaCl$_2$ at three different BCL. When the concentration of Ca$^{2+}$ was 2.5 mM, the trains of action potentials were followed by slow diastolic depolarization. The slope of the diastolic depolarization was little affected by decreasing the BCL from 400 to 250 msec. When the calcium concentration was 10.0 mM, slow diastolic depolarization was replaced by what appeared to be TDs similar to those induced by acetylstrophanthidin. Decreasing the BCL from 400 to 250 msec resulted in an increase in both the amplitude and the rate of depolarization and a decrease in the coupling interval of the depolarization. Acetylstrophanthidin-induced TDs respond similarly to decreases in BCL (6).

In the presence of 4 mM K$^+$, acetylstrophanthidin depresses normal pacemaker activity in Purkinje fibers and enhances postpacing depression (6). Elevation of the Ca$^{2+}$ concentration from 2.5 mM (normal) to 12.5 mM in the absence of acetylstrophanthidin also depressed the frequency of spontaneous activity (Table 1). In five experiments at a K$^+$ concentration of 4.0 mM, high Ca$^{2+}$ concentration cause either complete suppression of pacemaker activity (no spontaneous discharge in 5 minutes, four experiments) or significant depression of the spontaneous rate and enhancement of the postpacing depression (one experiment).

At lower external K$^+$ concentrations (2.0–2.5 mM), elevation of Ca$^{2+}$ concentration to 12.5 mM resulted in a different pattern of response. Spontaneous activity declined in frequency and eventually ceased for 10–20 seconds, after which spontaneous activity returned for periods of up to 90 seconds. Periods of quiescence lasting for about 60 seconds then alternated regularly with periods of repetitive activity. Alternating periods of quiescence and activity were not observed in the presence of acetylstrophanthidin and low K$^+$ concentration.

**EFFECT OF POTASSIUM ON TRANSIENT DEPOLARIZATIONS**

Whether caused by acetylstrophanthidin or by elevated Ca$^{2+}$ concentration, TDs were depressed by high and enhanced by low K$^+$ concentration. These effects are illustrated in Figure 3 for acetylstrophanthidin and Figure 4 for a Ca$^{2+}$ concentration of 12.5 mM. In the experiment of Figure 3, the effects of K$^+$ concentrations of 2, 4, and 6 mM were studied at BCL of 600 and 250 msec. Perfusion at the normal K$^+$ concentration (4.0 mM) was alternated with perfusion at 2.0 and 6.0 mM. The amplitude of the TD was enhanced at 2.0 mM, and a spontaneous response was generated at the higher driving frequency. In the presence of 6.0 mM K$^+$, TDs were almost totally suppressed. Comparable results were recorded in five experiments.

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Expt. no.</th>
<th>KCl Concentration (mM)</th>
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</thead>
<tbody>
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<td>Enhancement of postpacing depression</td>
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<td>2.0</td>
</tr>
<tr>
<td>and slowing of spontaneous rate</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Quiescence</td>
<td>1</td>
<td>4.0</td>
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</table>
Effect of changing potassium concentration on transient depolarizations caused by acetylstrophanthidin (1 × 10⁻⁷ g/ml). The top trace in each record is a recording from isolated false tendon, and the bottom trace is the stimulus pattern. Spikes were retouched. BCL = basic cycle length.

Similar effects of K⁺ concentration on TDs elicited by high Ca²⁺ concentration were observed in six experiments. Sample records from two of these experiments are shown in Figure 4. TDs developed at a K⁺ concentration of 4.0 mM were greatly enhanced when K⁺ concentration was reduced to 1.0 mM and were suppressed at 6.0 mM.

Transient depolarizations induced by acetylstrophanthidin can be readily brought to threshold, even at 4.0 mM K⁺, by adjustment of the driving rate. Spontaneous discharges were not so easily generated by elevated Ca²⁺ concentration, but in two experiments the calcium-induced TDs reached threshold when the K⁺ concentration was reduced to 1.0 mM and were suppressed at 6.0 mM.

Exposure the TD reached threshold. The spontaneous discharge was followed in turn by a TD at approximately the same coupling interval.

**EFFECT OF CALCIUM-FREE PERFUSATE**

The results of the experiments just described indicate that high Ca²⁺ concentration potentiates and in many respects mimics the effects of acetylstrophanthidin on the electrical properties of Purkinje fibers. Two questions then arise. (1) Is Ca²⁺ necessary for the production of TDs by acetylstrophanthidin? (2) Might an influx of Ca²⁺ be the mechanism responsible for TDs?

That Ca²⁺ is necessary for the production of TDs by acetylstrophanthidin was demonstrated in five experiments, one of which is illustrated in Figure 6.
Decreasing calcium concentration abolishes TDs induced by acetylstrophanthidin (7.5 × 10^{-8} g/ml). Recordings are from isolated false tendon. Spikes were retouched. BCL = basic cycle length.

Decreasing potassium concentration causes TDs induced by high calcium concentration (12.5 mM) to reach threshold. The top trace in each record is a recording from Purkinje tissue, and the bottom trace is the stimulus pattern. Spikes were retouched. BCL = basic cycle length.

After 25 minutes of exposure to acetylstrophanthidin (7.5 × 10^{-8} g/ml) in normal Tyrode's solution (K^+ = 4.0 mM, Ca^{2+} = 2.5 mM), TDs were present at both BCL and reached threshold at the faster frequency (Fig. 6, top). Ten minutes after Ca^{2+} was omitted from the perfusion fluid, TDs were completely absent at all BCL and were replaced by very slow diastolic depolarization. Restoration of Ca^{2+} caused a prompt return to the control situation.

In addition, Ca^{2+} may be necessary for the suppression of normal pacemaker activity, which is characteristic of acetylstrophanthidin intoxication. In the experiment illustrated in Figure 7, TDs were induced by acetylstrophanthidin in normal Tyrode's solution. At the briefest BCL (300 msec), the TD reached threshold, but there was no sign of phase-4 depolarization (Fig. 7A). Ten minutes after depletion of Ca^{2+} the TDs had disappeared, but low-amplitude spontaneous action potentials at a frequency of about 30/min were recorded when stimulation was discontinued (Fig. 7B). When Ca^{2+} was restored to half its initial concentration, TDs reappeared and reached threshold (Fig. 7C). Postspacing depression was also manifest. After 15 minutes of exposure to the low concentration of Ca^{2+}, no phase-4 depolarization was recorded during a 15-second pause. Similar results were recorded in two additional preparations.

**Effect of manganese**

The results of these experiments suggest that Ca^{2+} is necessary for the generation of TDs and perhaps also for the depression of normal pacemaker activity by acetylstrophanthidin. If this is
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A 2.5 mM Ca

B 0 mM Ca 10 min

C 1.25 mM Ca 11 min

FIGURE 7
Decreasing calcium concentration causes return of spontaneous activity subject to postspacing depression in a preparation treated with acetylstrophanthidin (7.5 x 10^-8 g/ml). Gradual replacement of calcium results in progressive depression of this activity and gradual return of transient depolarizations. The top trace in each record is a recording from Purkinje tissue, and the bottom trace is the stimulus pattern. A: Maximum diastolic potential = 87 mv. B: Maximum diastolic potential = 72 mv. C: Maximum diastolic potential = 66 mv. The values indicate a gradual deterioration of the impalement. Immediately following the sequence in C, re-impalement yielded a maximum diastolic potential of 80 mv. Spikes were retouched.

FIGURE 8
Manganese (2 mM) abolishes transient depolarizations caused by acetylstrophanthidin (7.5 x 10^-8 g/ml). Recordings are from Purkinje tissue. Spikes were retouched. BCL = basic cycle length.

true, then manganese (Mn^{2+}), which has been shown to block the influx of Ca^{2+} associated with the cardiac action potential, might be expected to abolish the TDs (13-18).

The effect of MnCl_{2} on TDs induced by acetylstrophanthidin was studied in five experiments, one of which is illustrated in Figure 8. Prominent TDs were recorded after 90 minutes of exposure to acetylstrophanthidin in the presence of normal Ca^{2+} concentration. At the higher driving frequency, the TD reached threshold. Ten minutes after the addition of MnCl_{2} (2.0 mM), TDs were completely abolished. After the Mn^{2+} was washed out, the typical response to acetylstrophanthidin was restored.

Discussion

Transient depolarizations induced in Purkinje tissue by acetylstrophanthidin (and also demonstrated as "low-amplitude potentials" [7, 9] or "enhanced diastolic depolarization" [8] in response to ouabain) could be due to a transient decrease in K^{+} permeability or a transient influx of positively charged ions. The results suggest that TDs may be caused by an influx of Ca^{2+}. The following findings support this hypothesis. (1) TDs induced by acetylstrophanthidin are potentiated by elevation of the extracellular Ca^{2+} concentration. (2) High Ca^{2+} concentration alone, like acetylstrophanthidin, causes TDs and depresses normal automaticity. (3) Extracellular Ca^{2+} is necessary for the production of TDs by acetylstrophanthidin and may also be necessary for the concomitant suppression of normal automaticity. (4) TDs induced by acetylstrophanthidin are abolished by MnCl_{2}, which is known to block the Ca^{2+} current associated with the normal action potential.

The abolition of TDs by MnCl_{2} is probably the strongest evidence for the involvement of Ca^{2+} in the generation of TDs. Mn^{2+} has been shown to block a "slow channel" through which Ca^{2+} and, in some preparations, also Na^{+} may enter the cell.
This slow inward current is an important component in maintaining the plateau of the cardiac action potential (13, 14, 19-21) and in canine ventricular muscle has been shown to be carried entirely by Ca\(^{2+}\) (20, 21). Vitak and Trautwein (17) found that MnCl\(_2\) specifically inhibited only the slow inward current in mammalian Purkinje tissue. Rougier et al. (13) suggested that Mn\(^{2+}\) might cause a slight lowering of the membrane permeability to K\(^+\) in frog auricle. We have found in four preliminary experiments that MnCl\(_2\) (2 mM) does not depress slow diastolic depolarization in otherwise untreated canine Purkinje tissue (unpublished observations). The complete abolition of TDs by Mn\(^{2+}\) therefore strongly suggests that TDs do not represent slow diastolic depolarization related to normal pacemaker activity but are a separate phenomenon generated by an inward Ca\(^{2+}\) current.

If the slow channel conducts both Na\(^+\) and Ca\(^{2+}\), it is possible that TDs represent a temporary influx of Na\(^+\), facilitated somewhat by the presence of Ca\(^{2+}\). Whether mediated by Na\(^+\) or Ca\(^{2+}\), it is possible that the threshold membrane potential at which the slow channel opens is increased by acetylstrophanthidin. Evidence presented by Beeler and Reuter (20) sets the threshold potential for normal activation of the slow inward current in canine ventricular muscle at 35 mv. Similar studies in sheep Purkinje fibers set the threshold at about 55 mv and indicate that the threshold is itself voltage dependent (Fozzard, personal communication). We do not at present know the appropriate threshold value for the dog Purkinje fibers under the conditions of the present study, but the level of membrane potential at which TDs appear can be as high as 85 mv.

The potention of TDs by Ca\(^{2+}\) also suggests that a decrease in K\(^+\) permeability is not possible. Elevation of Ca\(^{2+}\) concentration is known to suppress normal automaticity caused by slow diastolic depolarization (22). However, high Ca\(^{2+}\) enhances the TDs resulting from acetylstrophanthidin and increases the likelihood of automaticity generated by TDs.

Changes in the extracellular concentration of K\(^+\) greatly affect the amplitude of TDs caused by either acetylstrophanthidin or high Ca\(^{2+}\). At present there is no experimental evidence for the mechanism involved, but there is one obvious possibility. If the repolarization phase of TDs is caused by an efflux of K\(^+\), then the antagonistic effect of K\(^+\) on the action of acetylstrophanthidin or high Ca\(^{2+}\) can be readily explained. Elevation of the extracellular concentration of K\(^+\), by increasing the permeability of the membrane to K\(^+\), can speed the repolarization phase of the cardiac action potential (23). Elevation of K\(^+\) concentration might similarly augment the opposing effect that K\(^+\) efflux would have on the generation of TDs.

In three experiments with Purkinje tissue intoxicated by acetylstrophanthidin removal of Ca\(^{2+}\) from the perfusate resulted in the reappearance of pacemaker activity previously suppressed by acetylstrophanthidin. Reduction of the extracellular concentration of Ca\(^{2+}\) is known to cause a profound increase in spontaneous activity of untreated Purkinje fibers (24). The effect of low Ca\(^{2+}\) may therefore represent only physiological antagonism rather than a specific reversal of the mechanism by which acetylstrophanthidin inhibits pacemaker activity.

There is an apparent discrepancy between the results of Temte and Davis (12), who described a direct relationship between external Ca\(^{2+}\) concentration and the slope of phase-4 depolarization, and the earlier conclusions of Weidmann (22). In the records published by Temte and Davis (12), it appears that accelerated diastolic depolarization represents the rising phase of frequency-dependent transient depolarizations rather than acceleration of “true” pacemaker activity.

The evidence suggesting that acetylstrophanthidin-induced TDs are caused by an influx of Ca\(^{2+}\) is relatively strong, but the mechanism must be confirmed by more direct methods such as voltage clamping. If both the inotropic and arrhythmogenic actions of acetylstrophanthidin and other digitaloids are mediated by a Ca\(^{2+}\) mechanism, it may prove difficult to separate the two effects, either by altering the structure of the drug or by attempting to antagonize one or the other effect differentially.

References


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