Role of Oscillatory Potential and Pacemaker Shifts in Digitalis Intoxication of the Sinoatrial Node

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Background. Digitalis intoxication causes tachycardia, pacemaker shifts, and conduction disturbances in the sinoatrial (SA) node, but the mechanisms underlying these changes have not been clarified. We studied the role played by oscillatory potentials, electrical inhomogeneity, and calcium overload in cardiac steroid intoxication of the SA node.

Methods and Results. Guinea pig SA nodes (isolated from atrial tissue) were perfused in vitro. Transmembrane potentials and force were recorded. Strophanthidin (1 μmol/L) induced minor changes, although it was perfused for more than 30 minutes. In contrast, ouabain (0.5 μmol/L) and digoxin (1 μmol/L) intoxicated the SA node in 10–20 minutes. Ouabain and digoxin increased spontaneous rate and slope of diastolic depolarization, shifted the plateau to more negative values, and decreased the maximum diastolic potential. These cardiac steroids increased and then decreased contractile force and eventually caused the action potential and twitch to become irregular in amplitude and rhythm. In the presence of acetylcholine (ACh, 0.01–1 μmol/L), cardiac steroids decreased the resting potential, caused spontaneous activity, and increased force and, eventually, oscillatory potentials (Vₒ) and aftercontractions as well as overdrive excitation. To make the SA node electrically homogeneous (only slow responses), the SA node was perfused with high extracellular potassium concentration (with and without norepinephrine), tetrodotoxin (2.61 μmol/L), or lidocaine (50 μmol/L). Adding ouabain or digoxin to these solutions increased the rate but far less than in Tyrode’s solution. Recovery in Tyrode’s solution initially caused fast and irregular rhythms, which then subsided. Low extracellular calcium concentration ([Ca]ₒ) (0.54 mmol/L) decreased force; adding ouabain markedly increased force and induced Vₒ. High [Ca]ₒ (8.1 mmol/L) increased force; adding ouabain decreased force and made action potentials as well as contractions quite irregular.

Conclusions. Ouabain and digoxin quickly intoxicate the SA node by inducing calcium overload and its manifestations (Vₒ, decrease in contractile force and aftercontractions), whereas strophanthidin does not, possibly because of the lack of a sugar moiety. The intoxication is less pronounced when sodium influx is decreased (slow responses), and this accounts for the shifts from dominant to subsidiary pacemakers. Marked conduction disturbances result from calcium overload, leading to the fractionation of SA node potentials. (Circulation 1993;87:1705–1714)

Key Words • sinoatrial node • digitalis • pacemakers • calcium • sodium • steroids

Cardiac steroids ("digitalis") intoxicate the sinoatrial (SA) node, causing tachyarrhythmias and depression of conduction. Multiple pacemaker sites develop, and the dominant pacemaker shifts toward the crista terminalis. These changes are presumably the result of the inhibition of the sodium-potassium pump ("sodium pump") as in other cardiac tissues (see References 6 and 7). The consequent increase in intracellular calcium concentration ([Ca]ᵢ) via the sodium–calcium exchange may be responsible for the increase in intercellular resistance. In turn, uncoupling induced by digitalis interferes with the electrotonically mediated interactions between SA node pacemakers and with their mutual entrainment; these changes play an important role in arrhythmias caused by digitalis intoxication of the SA node.

Still, several major questions about mechanisms underlying the patterns of digitalis intoxication of the SA node remain unanswered. One is whether digitalis increases the SA node rate by inducing an oscillatory potential (Vₒ), as it does in other cardiac tissues (see References 14 and 15). To find out whether this is indeed the case, in the present experiments the sinus rate was decreased by means of acetylcholine (ACh) administration. A slower rate allows us to unmask Vₒ, since Vₒ peaks and decays during a long diastole, in contrast to diastolic depolarization (DD). Contractile force ("force") of the SA node (separated from surrounding atrial tissue) also was recorded, and this facilitated the distinction between an electrotonic depolarization caused by conduction block and Vₒ (which is associated with an aftercontraction and not with a full twitch). In addition, the recording of force allows the identification of increasing and decreasing inotropy, the
latter being associated with the development of calcium overload and toxicity.

The second question is related to the fact that the SA node is not electrically homogeneous. Thus, dominant pacemaker cells are activated only by the slow inward current.18 Instead, subsidiary pacemaker cells show an initial fast component (caused by inward sodium current \([I_{Na}]\)) followed by a slow component (caused by inward calcium current \([I_{Ca}]\)) (see References 17–20). If digitalis intoxicates the SA node by inhibiting the sodium pump, then the intoxication could be more severe in those cells in which sodium load is greater (because of the sodium influx through the fast channel).

We exploited the fact that subsidiary pacemaker cells are activated through the slow channel when the fast sodium channel is blocked18 to study digitalis intoxication in the SA node uniformly activated by slow responses. Tetrodotoxin (TTX, a blocker of the fast sodium channel) or high extracellular potassium concentration \([K_e]\) gradually decreases the fast component of the upstroke until DD initiates only a slow response.18 In the present experiments, the fast component of the upstroke was eliminated either by depolarizing the cells with high \([K_e]\) (with or without norepinephrine [NE]) or by blockers of the fast channel (TTX, lidocaine). Thus, it becomes possible to analyze digitalis intoxication of dominant and subsidiary pacemakers separately. Such an analysis may provide the key to understanding digitalis-induced shifts in pacemaker sites.

The third question addressed is whether modifying calcium load alters digitalis toxicity patterns, in that increasing calcium may result in fractionation of pacemaker activity caused by predominant disturbances of conduction. To this aim, digitalis was administered in the presence of low or high extracellular calcium concentration \([Ca_e]\). Severe disturbances in conduction would be expected to result in a chaotic mechanical activity showing little relation to the electrical activity recorded in any given cell. The cardiac steroids used were strophanthidin (which has often been tested in other cardiac tissues4–7), ouabain, and digoxin.

A preliminary report has appeared in abstract form.21

**Methods**

Guinea pigs of either sex weighing 350–1,250 g were anesthetized with sodium pentobarbital (40 mg/kg i.p.). The excised heart was placed in a Petri dish filled with oxygenated Tyrode’s solution. The SA node was identified as a translucent whitish area and was cut from the surrounding red-brown atrial tissue. The SA node of the guinea pig is thin (and therefore well perfused in vitro) and is made up of SA node cells throughout its thickness.20,22

The SA node was perfused at 37°C with oxygenated (97% O₂/3% CO₂) Tyrode’s solution of the following composition (mmol/L): NaCl 136.9, KCl 4, CaCl₂ 2.7, NaHCO₃ 11.9, NaH₂PO₄ 0.45, MgCl₂ 1.05, and glucose 5.5. [Ca] and [K] were varied in several experiments, as will be specified.

The SA node was spontaneously active; when overdriven, electrical stimuli were delivered by a Grass stimulator through a Grass SIU 5A stimulus isolation unit. The stimuli were 8–15 V in amplitude and 1–2 msec in duration. Overdrive was carried out at rates varying from 120 to 300 beats per minute for 5–60 seconds in different tests. The SA node was held in a tissue bath at one end by a double-tipped stainless steel electrode (used also as one of the stimulating electrodes) and at the other end by a short silk thread attached to the rigid rod of a force transducer (Grass Model FT03C). The force transducer was connected to a Grass model 7D polygraph. Transmembrane potentials were recorded by means of microelectrodes filled with 3 mol/L KCl and coupled to a Dagan probe and to a Dagan model 8500 operational amplifier. In some of the experiments, the rate of rise of the action potential (AP) was measured by differentiating the signal. The traces were displayed on a Tektronix model 5111 storage oscilloscope and recorded on paper on a three-channel chart recorder (Gould Brush 2400) and, in some experiments, also on an FM tape recorder (Store 4, Lockheed Electronics, Inc.) at 15 inches per second.

Three types of AP are recorded in the SA node (e.g., see Reference 17): slow responses in dominant pacemaker cells, APs with an upstroke made up of a fast and a slow component in subsidiary pacemaker cells, and APs similar to those in atrial fibers but followed by a DD in transitional cells. The experiments were conducted in each of these types of cells.

The following chemicals were used: strophanthidin, ouabain, digoxin, ACh chloride, lidocaine (Sigma Chemical Co.), tetrodotoxin (TTX, Sankyo, obtained through Calbiochem), and NE bitartrate (Levophed, Winthrop Pharmaceuticals). Ouabain, digoxin, and lidocaine were dissolved in 50% ethyl alcohol, and these stock solutions were diluted 1,000–2,000 times before testing. The different cardiac steroids tested will be collectively referred to as “digitalis.”

The results are expressed as mean±SEM. A Student’s t test was used, and a value of p<0.05 was considered significant.

**Results**

**Minimal Effects of Strophanthidin in the SA Node**

Strophanthidin had little effect on APs and contractile force in the SA node. In Table 1, the average results show that strophanthidin increased the rate and force minimally and nonsignificantly, despite its concentration (1 μmol/L) and long exposure (36 minutes).

**Ouabain Toxicity in the SA Node**

In contrast, ouabain quickly intoxicated the SA node at a smaller concentration than strophanthidin. The pattern of ouabain intoxication in a subsidiary pacemaker is shown in Figure 1.

In Figure 1A, ouabain decreased the maximum diastolic potential \(E_{max}\) and AP amplitude, shortening the plateau and prolonging the final phase 3 repolarization (11 minutes). Ouabain increased force by +84% at 5 minutes and by +265% at 11 minutes of perfusion. At 13 minutes, irregularities appeared in rhythm as well as in configuration of APs and of twitch curves. In Figure 1B, at 20 minutes of ouabain perfusion, force was decreased and there was an irregular dissociation between electrical and mechanical activities. During diastole, oscillations of membrane potential appeared that could be caused by either block of conduction (the first oscillation was associated with a large twitch) or with
the failure of a $V_{th}$ to attain the threshold potential (arrows). As the arrows indicate, $V_{th}$ was associated with an aftercontraction. The effects of ouabain were reversible (Figure 1B, second tracing).

The average values (Table 1) show that ouabain increased the rate (+48.8%) and increased (+398%) and subsequently decreased (−69.3%) force, although force was still significantly higher than in control solution (asterisk in Table 1). Arrhythmias appeared in eight of nine experiments after 22.1±3.2 minutes of exposure.

**Digoxin Toxicity in the SA Node**

Since strophanthidin and ouabain had different effects on the SA node, digoxin was also tested. In Figure 2A, the first tracing shows the control APs of a subsidiary pacemaker cell and twitch curves. Digoxin initially decreased force (−21%, 5-minute tracing), but shortly thereafter substantially increased rate (+20%) and force (+214%, 8-minute tracing). AP amplitude and $E_{\text{max}}$ gradually decreased, and eventually the preparation suddenly stopped (14-minute tracing). The last twitch curve was followed by an aftercontraction (arrowhead). In panel B, at the beginning of recovery in

**FIGURE 1.** Tracings showing ouabain toxicity in the sinoatrial node. In panel A, the first tracing shows control (cont.) action potentials and twitch curves. Ouabain administration (0.5 μmol/L) was initiated before the second tracing and continued through panel B. The last tracing (Rec.) shows recovery in Tyrode’s solution. Arrows point to oscillatory potentials ($V_{\text{os}}$) and aftercontractions, and dashed lines make the change in resting force with time more evident. Numbers before tracings indicate time in minutes of cardiac steroid perfusion.

**FIGURE 2.** Tracings showing digoxin (Digox.) toxicity in the sinoatrial node. In each panel, top tracing shows action potentials and bottom tracing the twitch curves. In panel A, first tracing was recorded in Tyrode’s solution (Tyr.) and subsequent tracings during 1-μmol/L digoxin exposure. Beginning of digoxin was recorded at a lower speed to show initial force decrease. Arrowhead at the end of last tracing in panel A points to an aftercontraction. Tracings in panel B are at beginning and end of recovery. In panel C (recorded from another preparation during the administration of 1 μmol/L digoxin), arrows point to oscillatory potentials ($V_{\text{os}}$) (upper tracing) and aftercontractions (lower tracing). In panel D (recorded from another preparation during the administration of 0.5 μmol/L ouabain), a 4-second 300-beat per minute overdrive was carried out in the middle of the tracings shown. Numbers before tracings indicate time in minutes of cardiac steroid perfusion.
Tyrode’s solution, spontaneous activity resumed, showing irregularities in AP amplitude and in cycle length as well as in twitch curve amplitude (first tracing). The second tracing in panel B shows APs and twitch curves recorded later during recovery.

In panel C (recorded from another preparation during digoxin intoxication), the first AP was followed by $V_{\infty}$ and aftercontraction (arrows). After the second AP, $V_{\infty}$ attained the threshold potential and initiated another AP (arrow pointing downward and to the right). This AP was apparently propagated, since the associated contraction was larger than the aftercontraction. The AP thus elicited was followed by damped $V_{\infty}$.

In panel D (recorded from another preparation exposed to ouabain), a short overdrive increased the subsequent spontaneous discharge (from 165 to 210 beats per minute, +27.2%) (“overdrive excitation”).

The average results (Table 1) show that digoxin (1 $\mu$mol/L) initially decreased force (~49%) in three of five preparations. Subsequently, in all experiments, digoxin increased rate (+46%) and force (+256%). Later, force decreased by ~44% ($n=3$). Irregularities of the rhythm appeared in each experiment after 9.2±1.2 minutes.

**Actions of Ouabain in the Presence of ACh**

To distinguish $V_{\infty}$ from a steepened DD, ACh was used to decrease the rate and prolong diastole.

In the absence of ACh, ouabain increased rate and force until both became irregular (not shown). In Figure 3, after full recovery, ACh hyperpolarized the membrane potential (3.6 mV) and decreased rate (~19%) and force (~57%); DD was absent (1-minute tracing), and quiescence eventually followed. Adding ouabain to the ACh solution decreased the resting potential, and the preparation became spontaneous again. The AP was followed by an undershoot and a subsequent DD; at the same time, force increased (5-minute tracing). Later on, DD became more pronounced and the rate faster. As shown in Figure 3B, driving the preparation for 8.5–10 seconds at 120, 150, and 200 beats per minute was followed by overdrive excitation (increases in rate of +64%, +68%, and +76%, respectively).

In Figure 3C, the AP was followed by $V_{\infty}$ (arrows) and a shallow aftercontraction (22-minute tracing). Shortly thereafter (23-minute tracing), the aftercontraction increased in size (as made more evident by the dashed lines). Also, $V_{\infty}$ (downward arrowheads) apparently elicited an occasional extra beat in other cells (bigeminy), since $V_{\infty}$ coincided with a second contraction (upward arrowheads). The second contraction was followed by an aftercontraction, as seen in the 24-minute tracing, recorded at a faster time base. Therefore, at least in the late stage of ouabain administration, the steepening of DD is a result of a superimposed $V_{\infty}$. Recovery in ACh allowed the preparation to become quiescent again, but a period of drive still induced overdrive excitation through $V_{\infty}$ (not shown).

The average results show that ACh decreased the rate by ~77.4% in four experiments (Table 2) and caused quiescence in three additional experiments (not reported in the table). Ouabain or digoxin increased the rate ($p=NS$) in the active fibers and induced spontaneous activity in two of three quiescent fibers. In the absence of ACh, the same concentrations of digitalis increased the rate (237.1±9.8 beats per minute) significantly more (asterisks in Table 2). Administration of digitalis in the presence of ACh caused $V_{\infty}$ in seven of seven and aftercontractions in six of seven experiments. Overdrive was applied in four experiments: In two quiescent preparations, it induced repetitive activity at a mean rate of 120 beats per minute, and in the other two, it increased the rate by +143% (six tests).

**Digitalis Intoxication of Cells Activated by Slow Responses**

Ouabain intoxication of a dominant pacemaker cell is shown in Figure 4, where the impalement of the same cell was maintained throughout the exposure to the drug. From the 28th minute of exposure, ouabain gradually decreased $E_{\text{max}}$, rate of rise of the upstroke, and AP amplitude and increased but then decreased force. Later on (40 minutes), the AP became irregular (although there was a pattern in the irregularity), and the rate of rise was very low. The twitch was small and irregular in amplitude. The rate was similar to that in control Tyrode’s solution (~2.4%). As usual, toxicity was reversible in Tyrode’s solution, although by the time the last panel was recorded, force had not yet recovered.
A comparison of Figures 1 and 4 suggests that digitalis may intoxicate the subsidiary and dominant pacemaker cells in the SA node differently. For this reason, the role of sodium entering through the fast sodium channel was investigated either by inactivating the channel through depolarization in high [K]o, or by blocking it by means of the fast channel blockers.

**Actions of Digitalis in the Presence of High [K]o.**

In high [K]o plus NE, the depolarization inactivates the fast sodium channel in all sinus cells, and only the slow responses induced by NE activate the SA node. In Figure 5A, 20 mmol/L [K]o progressively reduced AP amplitude, E\textsubscript{max} (top tracing), and rate of rise (middle tracing) until only oscillations of decreasing amplitude were present at a depolarized level and no force was developed (bottom tracing).

In Figure 5B, adding NE induced slow responses at 88 beats per minute (first tracing). Ouabain quickly increased the rate (+50%, 2-minute tracing), but then the rhythm became slower and irregular (11-minute tracing). APs of variable amplitude were followed by large V\textsubscript{m} and contractions were larger as well as variable in size. In panel C (18 minutes of recovery), the rate had returned to control rate, but force was still greater.

In Table 2, in the presence of high potassium plus NE, ouabain increased rate (+17%, \( p=NS \)) and force (+179%). The increase in force was consistent but not statistically significant because of the large variations in the increase. V\textsubscript{m} and aftercontractions were apparent in three of four experiments. No overdrive excitation occurred in the two experiments in which overdrive was tested.

To avoid the possible effects of NE on digitalis toxicity, [K]o was increased but to a level that did not suppress spontaneous discharge.

In Figure 6, in 8 mmol/L [K]o, the preparation was still active and developed force (first tracing). The upstroke was relatively slow, but it slowed even further toward the peak of the AP (see arrowheads). Digoxin initially decreased rate (−17%) and force (−40%, 5-minute tracing) and subsequently increased force (+151%, 8-minute tracing). As digoxin decreased E\textsubscript{max}, the boundary between the first and second slow com-

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**TABLE 2. Effects of Different Interventions on Digitalis Toxicity in SA Node**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetylcholine (1–0.01 μmol/L)</th>
<th>Digoxin (1 μmol/L) or ouabain (0.5 μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n=4 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate (bpm)</td>
<td>184.7±6.0</td>
<td>41.7±9.6*</td>
<td>84.3±33.6 (NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>(K) (_{0}) (50 μmol/L L) or tetrodotoxin (2.61 μmol/L)</strong></td>
<td><strong>(K) (_{0}) (1 μmol/L) or ouabain (0.5 μmol/L)</strong></td>
</tr>
<tr>
<td>( n=5 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate (bpm)</td>
<td>175.3±9.5</td>
<td>125.4±13.3*</td>
<td>145.8±14.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>([K]o, 16–20 mmol/L)</strong> plus norepinephrine (0.1–0.01 μmol/L)**</td>
<td><strong>([K]o, 2.61 mmol/L)</strong> ouabain (0.5 μmol/L) **</td>
</tr>
<tr>
<td>( n=4 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate (bpm)</td>
<td>154.2±14.1</td>
<td>161.7±18.5</td>
<td>177.0±13.1</td>
</tr>
<tr>
<td>Force (mg)</td>
<td>2.02±0.58</td>
<td>0.44±0.19*</td>
<td>4.41±1.86</td>
</tr>
<tr>
<td>( n=4 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate (bpm)</td>
<td>154.0±10.1</td>
<td>141.2±10.1</td>
<td>150.5±13.0</td>
</tr>
<tr>
<td>Force (mg)</td>
<td>1.89±0.59</td>
<td>9.39±3.28*</td>
<td>8.54±2.59†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.71±2.09†</td>
</tr>
</tbody>
</table>

Control, values obtained in Tyrode’s solution; \( n \), number of experiments; rate, SA node rate; bpm, beats per minute; force, contractile force; [K]o, extracellular potassium and calcium concentrations, respectively.

\( ^{*} p<0.05 \) vs. previous value.

†2–7 minutes exposure to ouabain.

‡Results obtained at end of exposure.

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**FIGURE 4. Tracings showing ouabain toxicity in dominant pacemaker cell. In each panel, top tracing shows action potentials (APs), middle tracing the rate of rise of upstroke, and bottom tracing the twitch curves. APs were recorded in the same cell in control solution (contr.) and during 0.5 μmol/L ouabain exposure and from another cell during recovery. Numbers before tracings indicate time in minutes of cardiac steroid perfusion.**
components shifted periodically to more negative values (8-minute tracing). Eventually, the initial component failed to attain the threshold every other beat, but this electrical alternance was not associated with mechanical alternance (11-minute tracing). The last tracing shows the recovery in Tyrode’s solution at the time when force was still much increased.

In Table 2, during exposure to ouabain or digoxin, the rate decreased (−14%, p=NS) and force increased (+247%, p=NS). Combining the results in high [K], and in high [K], plus NE (seven experiments) shows that digitalis did not change the rate (123.5±17.1 versus 123.1±27.2 beats per minute), but it increased force significantly (0.37±0.07 to 1.15±0.38 mg, +210.8%; p<0.05).

**Digitalis Intoxication in the Presence of Blockers of the Fast Sodium Channel**

High [K] can act not only by depolarizing and therefore inactivating the fast sodium channel but also by counteracting the effects of digitalis. In another approach, the contribution of sodium entry through the fast channel to digitalis toxicity was reduced by testing digitalis in the presence of lidocaine or tetrodotoxin.

In Figure 7A, lidocaine decreased the rate (−21%) as well as force (−78%) (second tracing). In panel B, digoxin initially decreased both rate (−24%) and force (−33%) with respect to lidocaine alone (4-minute tracing). Subsequently, digoxin progressively decreased AP amplitude and increased the rate (+27%) as well as force (+1,264%) (10-minute panel). That the steeping of DD was caused by a superimposed V_{off} is supported by the fact that during diastole, the resting tension gradually increased (see dotted lines in the bottom tracings), apparently as a result of aftercontractions that gradually continued into the following twitch. In panel C, the APs were smaller and their amplitude changed in a cyclic pattern, as emphasized by the arrows. The twitch was also markedly reduced and was dissociated from the APs.

Since TTX selectively blocks the fast sodium channel, ouabain was tested in the presence of TTX. In Figure 8A, TTX decreased rate of rise (−75%), AP amplitude (−17%), rate (−19%), and force (−89%) (7-minute tracing). Ouabain decreased E_{max}, rate of rise, and AP amplitude and increased the rate (+29%) as well as force (+290%) (7-minute tracing).

In panel B, the electrical activity consisted of subthreshold oscillations that waxed and waned in a cyclical pattern. In panel C, during recovery in Tyrode’s solution, the oscillations reached the threshold potential and initiated APs; the AP amplitude also waxed and waned, apparently as a result of the changes in the underlying oscillations. The 7-minute tracing shows APs, rate of rise, and twitch curves at a faster speed: the APs preceded by the most negative E_{max} had the fastest rate of rise and the largest amplitude. Twitch amplitude varied far less and contractions were more frequent than APs. The full recovery is shown in the 68-minute tracing.

In Table 2, lidocaine (n=2) and TTX (n=3) decreased the rate by −28.4%; adding digitalis increased the rate by +16.2%. In one additional experiment, TTX induced quiescence, and adding ouabain again initiated spontaneous discharge at 108 beats per minute. Thus, digitalis increases the rate far less when the fast sodium channel is blocked, and the SA node is activated only by slow responses.

To facilitate the recovery of the SA node from digitalis intoxication, the preparations were generally
perfused for a length of time in high [K+]o. However, in those experiments in which the SA node (activated by slow responses) was perfused in Tyrode’s solution during recovery, the return of the fast component initially was associated with an increase in rate, in force, and in marked signs of toxicity.

The Influence of [Ca]o on the Toxicity Patterns

High [Ca]o increases intracellular resistance,11,12 which in turn may account for conduction disturbances in digitalis toxicity of the SA node. This was studied by changing [Ca]o.

In cardiac tissues, lowering [Ca]o decreases calcium influx and increases33,24 intracellular sodium activity (a’na). How these changes influence digitalis intoxication of the SA node is not clear. In Figure 9A, low [Ca]o decreased the resting potential and force (−91%, second tracing). Ouabain decreased AP amplitude and Emax and markedly increased force (fifth tracing). Adding ACh almost abolished the twitch and caused SA node arrest; during quiescence, the membrane potential slowly repolarized. In panel B, in the presence of ouabain and ACh, the spontaneous activity resumed, and the rate as well as force increased. Also, in spite of the presence of low [Ca]o and ACh, the twitch amplitude increased substantially, and the resting force between twitch curves increased gradually emphasized by the black dotted lines, as expected from an aftercontraction (see arrowheads). In the inset, recorded shortly thereafter during slower discharge, the contraction was followed by a clear aftercontraction.

In Table 2, low [Ca]o did not change the rate (+4.8%, p=NS) and decreased force (−78.2%). Adding ouabain caused a small increase in rate (+9.4%, p=NS) and a marked increase in force (+9.022%).

High [Ca]o increases calcium influx and decreases33,24 a’na; therefore, it should act differently from low [Ca]o.

In Figure 10A, high [Ca]o increased the AP amplitude (+13%) and force (+339%) (8-minute tracing). The rate actually decreased somewhat (−9.2%), whereas the plateau shortened and the final phase of repolarization lengthened. Ouabain quickly reduced force, and the twitch amplitude became irregular at the time when APs were still regular (12-minute tracing). Force was decreased also when the twitch was temporarily regular at a similar rate (not shown). In the 18-minute tracing, the rate was faster and irregular, and contractile activity was completely irregular. In panel B, the electrical activity was temporarily regular, but the mechanical
activity continued to be irregular and dissociated from the electrical activity (21-minute tracing). The rate slowed again, and the APs once more became irregular, DD much steepened, the plateau short, and the final repolarization still slowed (22-minute tracing).

In Table 2, 8.1 mmol/L [Ca], slightly decreased rate (−8.3%, \( p = \text{NS} \)) and increased force (+397%). Adding ouabain to the high-calcium solution increased the rate by +6.2% (\( p = \text{NS} \)) but decreased force by −9% in 2–7 minutes and −49.8% by the end of the exposure. In three of four experiments, both \( V_m \) and aftercontractions were present.

**Discussion**

The present results show that the SA node is not equally sensitive to different cardiac steroids, since strophanthin had little effect. The increase in rate induced by digitalis intoxication is apparently caused by calcium overload and its manifestations. This is demonstrated by the association of digitalis toxicity with a decrease in inotropy (typically found in calcium overload), the appearance of \( V_m \) and of aftercontractions, and the induction of overdrive excitation. The pacemaker shifts induced by digitalis may be related to the different ionic mechanisms of excitation in dominant and latent pacemakers. Thus, in subsidiary pacemakers, a larger sodium influx would enhance (via the sodium–calcium exchange) the calcium overload associated with the digitalis-induced inhibition of the sodium–potassium pump. The larger calcium overload would induce the oscillatory potentials sooner, which would increase the rate of subsidiary pacemakers above that of the dominant pacemakers. In addition, in high [Ca], digitalis disorganizes electrical and mechanical activities, which is presumably a result of conduction disturbances.

**Minimal Effects of Strophanthin**

Strophanthin and acetylstrophanthin have frequently been used in the study of digitalis toxicity because of quick onset of action and fast recovery.\(^4\)\(^-\)\(^7\)

Several years ago, we observed that strophanthin had little effect on the electrical activity of the guinea pig SA node and therefore abandoned its use (S.L. Lipsius and M. Vassalle, unpublished observations). The present experiments show that, indeed, strophanthin has little effect on both electrical and mechanical activities of the guinea pig SA node. Although quite interesting, the finding remains unexplained. One major difference between strophanthin and ouabain or digoxin is that strophanthin lacks the sugar moiety. Since the sugar increases the potency of cardiac glycosides (see Reference 4), one possibility is that the sugar moiety is very important for the action of strophanthin in the guinea pig SA node.

**Initial Decrease in Contractile Force During Digoxin Exposure**

The initial decrease in force by digoxin may be related to the fact that small concentrations of digitalis decrease force,\(^2\)\(^5\)\(^26\) possibly through a stimulation of the sodium pump.\(^3\)\(^25\)\(^26\) The concentration tested here was far greater than those causing a negative inotropic effect, but at the beginning of digoxin perfusion, the concentration of digoxin at the plasmalemma may have been sufficiently low (before it increased to a steady level). This would account for the fact that force decreased transiently and then increased above control as the perfusion of digoxin was continued.

An alternative explanation might be that the initial negative inotropic effect was caused by a release of ACh. In fact, sometimes digitalis also causes an initial slowing of the rate,\(^2\) as confirmed by the present results. Arguing against this explanation is the fact that the slowing also occurs after atropine.\(^2\) However, the slowing has not been consistently observed.\(^1\)

**Steepening of DD and the Oscillatory Potential**

In the presence of ACh, digitalis initially caused DD (before it caused \( V_m \)), probably by depolarizing the resting membrane through the sodium pump inhibition; on excitation, the AP temporarily undershot the diminished resting potential. In Purkinje fibers also, cardiac steroids initially increase DD amplitude and only subsequently induce \( V_m \) (when force approaches its peak).\(^2\)\(^7\)

In the toxic stage, digitalis steepened DD through a superimposed \( V_m \), since the slope of DD was similar to that of \( V_m \) when the threshold was missed and an aftercontraction appeared. Also, when the rate was slow in the presence of ACh, each AP was followed by \( V_m \), and a fast drive induced overdrive excitation (instead of overdrive suppression as in the absence of calcium overload).\(^7\)

ACh hyperpolarizes in the presence of ouabain and decreases subthreshold oscillations.\(^1\) This apparent contradiction is because ACh was administered for about 1 second during ouabain administration.\(^1\) In contrast, in the present results, digitalis caused intoxication in the presence of ACh.

The occurrence of calcium overload and its manifestations (\( V_m \), aftercontractions, and overdrive excitation) in the SA node are found also under other conditions of calcium overload, and the cessation of excitation is associated with both \( V_m \) and aftercontraction.\(^2\)\(^8\)\(^9\) The
Digitalis Intoxication in the Sinoatrial Node

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The present results show that calcium overload induced by digitalis also can bring about overdrive excitation, which is facilitated by the fact that overdrive suppression is hindered by the inhibition of the sodium pump by digitalis.7

Digitalis might increase the rate either by increasing the effects of NE or by decreasing those of ACh. This is ruled out by the demonstration7 that ouabain increased the SA rate to a similar extent before and after the administration of a dose of propranolol (0.2–0.5 mg/L) that abolished the increase in rate caused by epinephrine. Similarly, ouabain increased the rate after a dose of atropine (0.1–0.2 mg/L) that almost abolished the negative chronotropic action of carbamylcholine.2 In addition, the present results show that digitalis intoxication (Vₐ, aftercontraction, overdrive excitation) was not prevented by ACh and was not enhanced by NE in high [K]₀. The concentration of TTX was sufficient to block the APs of cardiac nerves, but this did not prevent digitalis toxicity.

Shifts in Pacemaker Sites

Digitalis has been reported to shift the pacemaker site either to areas poorly innervated by the vagus but sensitive to ACh1 or to the crista terminalis (because digitalis does not increase DD in pacemaker cells as much as in subsidiary pacemakers5,23). The latter conclusion is supported by the present findings, since digitalis increased the sinus rate less when the SA node was activated only by slow responses.

The reason why Vₐ should become larger in subsidiary than in dominant pacemakers is that different currents are involved in their excitation. In dominant pacemakers, activation is caused by the slow inward current, and TTX has little effect.16 In subsidiary pacemakers, the fast component that precedes the slow component during the upstroke results from the activation of the fast sodium channel. Thus, both TTX and the depolarization brought about by high [K]₀ lead to the gradual substitution of the fast component by the slow component.18

The sodium pump would be expected to be present in the dominant pacemaker cells, since the slow channel can also be sensitive to ACh and there would be a sodium leak anyway. However, even if the sodium pump inhibition is the same in dominant and subsidiary pacemakers, the sodium load would still be greater, and a sodium would increase more rapidly and to a larger extent in subsidiary pacemakers. A larger a sodium (by decreasing the transmembrane sodium gradient) would increase the sodium more via sodium–calcium exchange. The consequence larger Vₐ would steepen DD more in subsidiary than in dominant pacemakers, and the pacemaker site would shift to the former cells. This concept is supported by the present findings that digitalis increased the rate less when the activation of the whole SA node was caused by slow responses (however induced). The two-component APs are recorded near the crista terminalis20; this is the area where digitalis shifts the pacemaker site.23

During digitalis exposure, dominant pacemaker cells would not necessarily become follower cells, since the slow component apparently recovers slowly during diastole, in contrast to the fast component.30 In addition, as [Ca]₀ increases, so does intercellular resistance,13 and conduction within the SA node is impaired.3 The original dominant pacemaker is depolarized by digitalis; therefore, its activation is more than ever dependent on the slow channel. These reasons may prevent subsidiary pacemakers from driving dominant pacemakers and also account for fractionation of pacemaker activity in the SA node.

Therefore, digitalis shifts pacemaker activity toward the crista terminalis, and this might become the site that activates the atria but not necessarily the rest of the SA node. As a consequence, digitalis causes not only a shift in pacemaker site but also the onset of multiple pacemaker activity within the SA node. This is strongly supported by the present findings that the dominant SA node cells and those activated by a slow response may discharge at a relatively slow rate and that mechanical activity of the SA node becomes irregular and dissociated from electrical activity.

Influence of [Ca]₀ on Digitalis Intoxication

The experiments with low [Ca]₀ show that digitalis intoxication is not prevented by reducing calcium influx. The reason for this is that low [Ca]₀ decreases I Na but increases a sodium,24 which in turn may increase [Ca] more than in the absence of this factor. In addition, a decrease in [Ca]₀ may enhance sodium influx through the slow channel in the SA node.18 And the sodium pump inhibition by digitalis would increase a sodium from the already higher level caused by low [Ca]₀. Indeed, both Vₐ and aftercontraction also develop in low [Ca]₀ (Figure 9). Similarly, in ventricular Purkinje fibers, strophanthidin intoxication may occur sooner in low than in high [Ca]₀.31

In the present experiments, high [Ca]₀ loaded the cells with calcium to the point that digitalis had only a negative inotropic action by quickly causing calcium overload. The finding also supports the conclusion that at normal [Ca]₀, the late fall in force induced by digitalis is caused by calcium overload. Thus, in Purkinje fibers, lowering [Ca]₀ decreases force in the control solution but increases it during the decreasing phase of strophanthidin inotropy.31

The experiments make it clear that it matters little whether [Ca]₀ is increased or decreased as far as digitalis toxicity is involved. Thus, low [Ca]₀ increases a sodium as digitalis does (and therefore [Ca]₀), and high [Ca]₀ decreases a sodium but increases [Ca] at any given a sodium level. In either case, digitalis is expected to increase a sodium, and the stage is reached when calcium overload develops. However, digitalis increases calcium from a low level in low [Ca]₀ (so the percent increase in force is far greater than in Tyrode's solution) and from a high level in high [Ca]₀ (so that calcium overload and a decrease in force are quickly induced).

Role of Intercellular Uncoupling in Digitalis Intoxication

The present experiments also provide evidence for electrical and mechanical uncoupling between SA node cells and its dependence on calcium. Thus, occasionally there was no AP but still a strong twitch, and, later on, APs were completely dissociated from contractions. In high [Ca]₀, fractionation of mechanical activity is indicated by the completely irregular development of force.
In high [Ca], plus ouabain, contractions were still markedly irregular even when APs were regular. This shows that the cell from which the recording was made was uninfluenced by the activity of other cells. This nonuniform spread of activation might result from an increase in intercellular resistance by an increased [Ca], a phenomenon that in the SA node may be facilitated by the sparsity of intercellular connections. The result would be multiple pacemaker activity, although the atria may be activated from the pacemaker(s) shifted to the periphery of the SA node.

References

Role of oscillatory potential and pacemaker shifts in digitalis intoxication of the sinoatrial node.
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