Mechanisms of Digitalis Toxicity

Effects of Ouabain on Phase Four of Canine Purkinje Fiber Transmembrane Potentials

By Michael R. Rosen, M.D., Henry Gelband, M.D., Charles Merker, M.D., and Brian F. Hoffman, M.D.

SUMMARY

Microelectrode technics were used to study effects of ouabain (O), $2 \times 10^{-7}$ moles/liter, on phase 4 of Purkinje fiber (PF) transmembrane potentials (TMP). Perfusion for 25–35 min with O caused an increase in phase 4 depolarization which resulted in either increased automaticity or occurrence of low-amplitude potentials (LAP). Increased automaticity was more frequent when PF had been stretched, when $[K^+]_o = 2.5$ mmoles/liter, and when O caused a marked decrease in resting membrane potential (RMP). LAP occurred at all $[K^+]_o$ (2.5, 4.0, and 5.0 mmoles/liter), and were the typical response of unstretched fibers in which RMP had not decreased markedly. The magnitude of the LAP increased at $[K^+]_o = 4.0$ mmoles/liter and at faster stimulus rates. Threshold potential (TP) was decreased to a slightly greater extent than RMP. Although LAP did not result in spontaneous action potentials, superimposition of subthreshold depolarizations on LAP resulted in excitation. Whether O-induced increases in phase 4 slope result in automaticity or LAP depends on interrelationships between RMP, TP, and level of membrane potential reached during phase 4. Although hearts developing LAP due to digitalis toxicity may not demonstrate increased automaticity, the presence of phase 4 depolarization in the form of LAP may cause an impairment of conduction which is dependent on cycle length.

Additional Indexing Words:

Automaticity Microelectrode studies
Stimulus rates Low-amplitude potentials
Resting membrane potential Effects of $K^+$
Threshold potential

Recently we reported the occurrence of slow, graded depolarizations of varying magnitude (low-amplitude potentials) during phase 4 of the transmembrane potential recorded from isolated canine Purkinje fibers exposed to toxic ouabain concentrations. In these experiments the isolated Purkinje fiber preparation was perfused with arterial blood from a donor dog and ouabain was administered intravenously in a dose sufficient to cause ventricular arrhythmias after a period of 25–40 min. The appearance of the low-amplitude potentials in the records of transmembrane potentials was more or less simultaneous with the onset of ouabain-induced junctional or ventricular arrhythmias in the donor. In these experiments the isolated tissues were stimulated at a regular rate approximately equal to donor heart rate, except during brief intervals when the stimulus was discontinued to evaluate the automaticity of the preparation. In eight of 10 experiments, during regular stimulation, ouabain increased the slope of phase 4 depolarization in a manner similar to that reported for isolated Purkinje fiber preparations perfused with physiologic salt solutions and exposed to toxic concentrations of ouabain or other digitalis substances. However, in three experiments on the blood-perfused preparations, when the stimulus was discontinued automatic firing did not occur. Instead the phase 4 depolarization following the last stimulated action potential failed to reach threshold and was followed by repolarization to the maximum diastolic potential.

Similar potentials induced by digitalis and other modalities have been demonstrated by prior investigators. However, the ionic and electrophysiologic
events that may modify their occurrence and magnitude have not been the subject of systematic study. Similarly, the role of digitalis-induced low-amplitude potentials in the initiation and propagation of toxic arrhythmias has not been investigated.

**Methods**

Twenty-seven mongrel dogs weighing 15–25 kg were anesthetized with Na pentobarbital, 30 mg/kg. The hearts were removed through a right lateral thoracotomy and bundles of Purkinje fibers were excised from the right and left ventricles and pinned to the wax bottom of a Lucite chamber perfused with modified Tyrode solution. KCl was maintained at 2.5, 4.0, or 5.0 mmol/liter in different experiments to determine the relative incidence of automaticity and low-amplitude potentials at different extracellular potassium concentrations ([K⁺]o).

The Tyrode solution was maintained at a temperature of 36–37°C with a glass heat exchanger and the tissue chamber was perfused at a rate of 13–15 ml/min. Ouabain was added to a separate reservoir of Tyrode solution to give a final concentration of 2 × 10⁻² moles/liter. This concentration closely duplicated, in terms of magnitude of effect over 25–35 min, the changes in transmembrane potential of Purkinje fibers seen during the blood perfusion studies in which a toxic dose of ouabain (60–90 μg/kg) was injected into the donor dog. Recordings made after 40 min of perfusion showed marked deterioration of action potential properties, to an extent not seen during blood perfusion.

External driving stimuli were delivered to the experimental preparation through bipolar Teflon-coated silver wire electrodes. The basic drive stimulus (S₁) was initiated with waveform and pulse generators (Tektronix models 162 and 161, respectively) and isolated from ground using technics previously described. S₁ was 0.5–1.5 msec in duration and its amplitude was 1.25–1.5 × threshold.

Microelectrodes were machine-pulled from capillary glass and filled by boiling in 3 molar KCl. Electrode tip diameter was less than 1 μm, and resistance was 10–30 MΩ. The electrodes were coupled by a 3-molar KCl interface to an Ag-AgCl₂ bar which led to an amplifier having high input impedance and input capacity neutralization (Bioelectric Instruments NF-1). The tissue bath was connected to ground through a similar junction. Recordings were displayed on a cathode-ray oscilloscope (Tektronix model 564) and photographed on Polaroid film.

For studies of threshold potential (TP) the following technic was used: two glass microelectrodes were impaled intracellularly in a Purkinje fiber bundle within 100 μm of one another. A third microelectrode was positioned in the perfusate adjacent to the surface of the fiber bundle and within 100 μm of the other two microelectrodes. A depolarizing current pulse (S₂), 100 msec in duration and 0.1–1.0 gamp in amplitude, was provided by a constant current source and injected through one of the intracellular microelectrodes. The change in transmembrane potential caused by S₂ was recorded between the intracellular and extracellular microelectrodes. The extracellular electrode also was used to record the zero reference potential. The stimulus current was measured by an operational amplifier between the tissue bath and ground and displayed on the oscilloscope (fig. 1). Threshold current was the minimal current required to initiate an action potential. Threshold voltage was the depolarization induced by a just subthreshold current pulse. Threshold potential was taken as the difference between threshold voltage and 0 potential. In figure 1, cycles 1, 3, and 5 result from the basic drive and 2 and 4 from S₂. In cycle 2, S₂ was just sufficient to initiate an action potential; in cycle 4 only a subthreshold depolarization occurred. In figure 1, maximal diastolic potential (MDP) was –80 mv; threshold voltage, 14 mv; and threshold potential, –66 mv. We recognize that due to the steep spatial decay of electrotonic potentials in the immediate vicinity of a point source of current the difference between membrane potential and threshold potential may be quantitatively underestimated by the methods we used. Hence the numerical values reported here may be inaccurate. However, the method does have validity in terms of demonstrating the direction of change and estimating its magnitude.

To study automaticity and the appearance of low-amplitude potentials the following method was used. For each experiment the preparations were stimulated at cycle lengths of 500–1000 msec and transmembrane potentials recorded continuously. Changes in resting membrane potential (RMP), MDP, and slope of phase 4 depolarization were noted. MDP was the largest negative value of transmembrane potential attained after a driven action potential; RMP was the value of transmembrane potential attained when the stimulus was turned off and the preparation did not develop...
action potentials for at least 2 sec. At 5-min intervals the stimulus was discontinued for 1–2 min to permit observation of the rate and rhythm of any automatic activity. When the preparation did not develop automaticity the driving stimulus was reinstituted and then interrupted for short intervals to observe any low-amplitude potentials which might occur. For study of stretched Purkinje fibers, Purkinje fiber bundles were placed in the tissue chamber and held in position by placing pins in the attached ventricular muscle. Micro-electrodes were impaled in the Purkinje fibers and action potentials were recorded. To stretch the fibers the pins were then moved apart in increments of approximately 1 mm. When action potential amplitude fell to 80–90% of control and remained stable at this level for 1 hour, the experiments were commenced.

Most results reported were recorded from single Purkinje fiber impalements maintained throughout the duration of the experiment. The one exception to this statement is the data reported in table 2B which includes both information from single impalements and results of multiple impalements in an area of the Purkinje fiber bundle approximately 0.5 mm in diameter during control conditions and after 25 min of ouabain perfusion.

Results

Changes in the Voltage-Time Course of Phase 4

Twenty-five to 35 min after the onset of ouabain perfusion, MDP and action potential (AP) amplitude began to decrease. Changes occurred earliest in preparations perfused with Tyrode solution having \( [K^+]_o = 2.5 \) mmoles/liter. Within 1–5 min after the initial decrease in MDP and AP amplitude there was a change in the voltage-time course of phase 4 in 35 of 38 experiments. This change was associated with either an increase in automaticity or the occurrence of low-amplitude potentials, as depicted in figure 2. Panel A shows a record of a Purkinje fiber during perfusion with control Tyrode solution with \( [K^+]_o = 2.5 \) mmoles/liter. When the fiber was driven at a cycle length of 1000 msec there was no phase 4 depolarization. When the drive stimulus was discontinued there was a small decrease in RMP but no spontaneous action potentials. In panel B, 35 min after the onset of ouabain perfusion, potential amplitude and MDP had decreased, the slope of phase 4 depolarization increased, and on discontinuation of the drive a regular spontaneous rhythm occurred after a short interval. Panel C shows a control record from another Purkinje fiber perfused with Tyrode \( [K^+]_o = 4.0 \) mmoles/liter. After discontinuation of the drive there was no spontaneous depolarization. In panel D, following 35 min of ouabain perfusion, action potential amplitude and MDP were somewhat decreased during stimulation and there was a marked increase in the slope of phase 4 depolarization. However, when the drive was discontinued the phase 4 depolarization failed to reach threshold potential and did not result in an action potential. After decreasing slowly by 28 mv membrane potential again returned to a steady level, somewhat less than MDP. No further depolarizations were seen until the drive was reinstituted. The first phase 4 interval following initiation of the drive showed only a small depolarization but with each succeeding cycle the slope of phase 4 depolarization increased. When stimulus rate was increased, the slope of phase 4 and the magnitude of the low-amplitude potentials became greater.

Figure 2

Effects of ouabain on phase 4 of the transmembrane potential. Filled arrows indicate discontinuation of drive stimulus, and clear arrows, its reinstitution. (A and B) Records from a Purkinje fiber impalement recorded during perfusion with control Tyrode solution (A) and after perfusion with ouabain for 35 min (B). Cycle length, 1000 msec; temp, 36–37°C; perfusate \( [K^+]_o = 2.5 \) mmoles/liter. Under control conditions (A), MDP = −89 mv, and discontinuation of the drive is followed by only a slight phase 4 depolarization. After exposure to ouabain (B), MDP = −58 mv, the slope of phase 4 has increased, and discontinuation of the drive is followed by a spontaneous, regular automatic rhythm. (C and D) Records from another preparation under control conditions (C) and after perfusion with ouabain for 35 min (D). Cycle length, 630 msec; temp, 36–37°C; perfusate \( [K^+]_o = 4.0 \) mmoles/liter. Under control conditions (C), MDP = −85 mv, there is no phase 4 depolarization, and discontinuation of the drive is followed by electrical quiescence. After exposure to ouabain (D), MDP = −75 mv, and the slope of phase 4 has increased markedly. Following discontinuation of the drive there is a single low-amplitude potential (of 28 mv magnitude) followed by a gradual increase in membrane potential. After reinstitution of the drive, several cycles are required before the slope of phase 4 increases.
In three of the experiments in which the potassium concentration in the Tyrode solution was 4.0 mmoles/liter, a sequence of 2–3 low-amplitude potentials followed discontinuation of the drive stimulus, as shown in figure 3. Here, after perfusion with ouabain for 32 min, there was marked phase 4 depolarization during regular stimulation. After the drive was discontinued a slow depolarization of 12 mv occurred at an interval approximately equal to the basic cycle length. This was succeeded at slightly shorter intervals by potentials 7 mv and 2 mv in amplitude. Membrane potential then increased gradually until the drive was again started. As previously noted (fig. 2D) several cycles were required before phase 4 depolarization attained its maximum slope. The rapid decrease in the magnitude of low-amplitude potentials in the absence of stimulation and the subsequent gradual increase in membrane potential indicates that under the conditions of these experiments low-amplitude potentials do not recur indefinitely as a self-regenerative phenomenon.

Unlike the phase 4 depolarization associated with increased automaticity, the slope and magnitude of the low-amplitude potentials varied from cycle to cycle and they at times appeared to be depolarizing “out of phase” with the action potential. This behavior also is illustrated in figure 3, where during regular stimulation the peaks of the phase 4 depolarizations clearly occurred prior to phase 0 of the action potential in some cycles and merged with it relatively smoothly in others. This event had the effect of changing the level of membrane potential at which a given action potential was initiated. In the last three cycles in figure 3 the onset of phase 0 depolarization occurred at −63, −62, and −64 mv, respectively, as a result of the phase shift between the peak of the phase 4 depolarization and the timing of the drive stimulus.

**Effect of Stretching Purkinje Fibers on the Voltage-Time Course of Phase 4**

In five experiments we attempted to determine if the likelihood of either an increase in automaticity or the occurrence of low-amplitude potentials was influenced by the condition of the preparation. For these studies, the anterior and posterior divisions of the left bundle branch were separated and placed in the same perfusion chamber. One was then intentionally stretched while the other was not. For two experiments \([K^+]_o\) was 2.5 mmoles/liter, for two \([K^+]_o\) was 4.0 mmoles/liter, and for the other \([K^+]_o\) was 5.0 mmoles/liter. The results of these experiments are summarized in table 1. The range of control action potential amplitudes for the normal fibers was 116–123 mv, and that for the stretched fibers 89–104 mv. In every instance, including the experiment in which Tyrode \([K^+]_o = 5.0\) mmoles/liter after exposure to ouabain an increase in automaticity occurred in the stretched Purkinje fiber bundle. Although the spontaneous rate was quite low during perfusion with ouabain in Tyrode with \([K^+]_o = 5.0\) mmoles/liter, there had been no spontaneous action potentials during perfusion with control Tyrode solution. In comparison, records from the Purkinje fiber bundles that had not been stretched showed low-amplitude potentials and no automaticity. Hence it appears that ouabain increases automaticity of stretched Purkinje fibers while low-amplitude potentials tend to occur when the fiber bundles are not stretched.

**Effect of \([K^+]_o\)**

The effect of \([K^+]_o\) on the relative incidence of automaticity and low-amplitude potentials was studied in 15 experiments, the results of which are
summarized in Table 2A. A ouabain-induced increase in the slope of phase 4 depolarization occurred in 12 of the experiments. When Tyrode \([K^+]_o = 2.5\) mmol/liter the slope of phase 4 depolarization increased in all five experiments within 25-30 min of the start of ouabain perfusion. In three of the experiments the change in phase 4 resulted in an increase in automaticity; in two, low-amplitude potentials occurred. Of note is the fact that those preparations which evinced an increase in automaticity showed a greater decrease in RMP or MDP over the 25-30 min time interval than did the preparations in which low-amplitude potentials occurred.

When Tyrode with \([K^+]_o = 4.0\) mmol/liter was the perfusate, phase 4 depolarization was enhanced by ouabain in five of seven experiments. In the two instances in which phase 4 depolarization was unchanged, no low-amplitude potentials or automaticity were noted. In the five experiments in which phase 4 depolarization increased in slope, low-amplitude potentials of 4-28 mv occurred. Of the three experiments for which the Tyrode solution contained a \(K^+\) concentration of 5.0 mmol/liter increased phase 4 depolarization was seen in two; in both instances this resulted in the appearance of low-amplitude potentials. The magnitude of the low-amplitude potentials tended to be somewhat higher when \([K^+]_0 = 4.0\) and 5.0 mmol/liter than when \([K^+]_0 = 2.5\) mmol/liter.

### Table 1

<table>
<thead>
<tr>
<th>Condition of tissue</th>
<th>No. of preparations</th>
<th>Perfusate ([K^+]_o) (mmol/liter)</th>
<th>Control AP amplitude (mv)</th>
<th>Automaticity</th>
<th>Low-amp potentials</th>
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<tbody>
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<td>5.0</td>
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<td>1</td>
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<td></td>
<td>2</td>
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<tr>
<td></td>
<td>1</td>
<td>5.0</td>
<td>89</td>
<td>1</td>
<td>0</td>
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</table>

*Of 10 Purkinje fiber bundles used, five were stretched and five were not. Ouabain perfusion was not started until the Purkinje fibers had been stable in Tyrode solution for 1 hour.

### Table 2

**Interactions of \(K^+\) and Ouabain**

<table>
<thead>
<tr>
<th>A: Effect of perfusate ([K^+]) on incidence of ouabain-induced increased automaticity and low-amplitude potentials*</th>
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<tbody>
<tr>
<td>Perfusate ([K^+]) (mmol/liter)</td>
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<tr>
<td>PF</td>
</tr>
<tr>
<td>No change in phase 4</td>
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<tr>
<td>Increased automaticity</td>
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<tr>
<td>Low-amplitude potentials</td>
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<tr>
<td>Magnitude of low-amplitude potentials (mv)</td>
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<td>2.5</td>
</tr>
<tr>
<td>4.0</td>
</tr>
<tr>
<td>5.0</td>
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<td>5-10</td>
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<td>4-28</td>
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<td>5-12</td>
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</table>

<table>
<thead>
<tr>
<th>B: Effect of ouabain perfusion on MDP†</th>
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<tr>
<td>Perfusate ([K^+]) (mmol/liter)</td>
</tr>
<tr>
<td>MDP (mv)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>91 ± 3 (18)</td>
</tr>
<tr>
<td>85 ± 1 (12)</td>
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<tr>
<td>82 ± 2 (16)</td>
</tr>
<tr>
<td>Ouabain</td>
</tr>
<tr>
<td>75 ± 4 (10)</td>
</tr>
<tr>
<td>82 ± 2 (12)</td>
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<tr>
<td>79 ± 2 (12)</td>
</tr>
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</table>

*Fifteen PF bundles were studied, none of which was stretched. Magnitude of low-amplitude potentials recorded was the maximum seen in each experiment, regardless of time.
†MDP was measured by performing multiple impalements before and during ouabain perfusion. Number of impalement is in parentheses after each reported value. Mean and standard deviation at each \([K^+]_0\) are listed. Values recorded for ouabain perfusion were observed over a 10-min interval starting 25 min after onset. Abbreviations: MDP = maximal diastolic potential; PF = Purkinje fiber.

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These experiments suggested that in normal Purkinje fiber bundles, ouabain-induced increases in automaticity occurred only at \([K^+]_o = 2.5\) mmoles/liter, and then only in those preparations in which ouabain had induced the largest decreases in RMP.

**Ouabain-Induced Changes in Threshold Potential**

Since the low-amplitude potentials represented a failure of phase 4 depolarization to attain threshold potential, we measured RMP or MDP and threshold potential at various \([K^+]_o\). Table 2B presents the results obtained by multiple microelectrode impalements performed at the three \([K^+]_o\). All results recorded during ouabain perfusion are those which occurred 25–35 min after the onset of exposure to ouabain. The greatest decrease in MDP was seen when \([K^+]_o = 2.5\) mmoles/liter; when \([K^+]_o = 4.0\) or 5.0 mmoles/liter the decreases in MDP were much smaller. It has been demonstrated that the effect of changing \([K^+]_o\) in the range from 2.5–5.0 mmoles does not significantly alter threshold potential.\(^9\) Hence, the decrease in MDP and the increase in phase 4 slope and amplitude should have resulted in an increase in automaticity. Since increased automaticity occurred only during those experiments in which Tyrode \([K^+]_o = 2.5\) mmoles/liter and in which the decreases in RMP or MDP were largest it appeared that a ouabain-induced shift in threshold potential toward 0 might be responsible for the occurrence of low-amplitude potentials.

Changes in threshold potential induced by ouabain, and their relationship to low-amplitude potentials, were studied in nine experiments in which Tyrode \([K^+]_o = 4\) mmoles/liter and two in which Tyrode \([K^+]_o = 2.5\) mmoles/liter. Results of one of these are illustrated in figure 4. During control perfusion with Tyrode solution having \([K^+]_o = 4.0\) mmoles/liter (panel A), the basic drive cycle (depolarizations 1 and 3) was 1000 msec, and an \(S_2\) (depolarizations 2 and 4) was injected 500 msec after each \(S_1\). The first \(S_2\) was just threshold in amplitude and the second just subthreshold. At this time MDP was \(-88\) mv, threshold voltage, 11 mv, and threshold potential, \(-77\) mv. In panel B, 35 min after onset of ouabain perfusion, low-amplitude potentials were present. MDP was now \(-79\) mv, threshold voltage, 12 mv, and threshold potential, \(-67\) mv. Hence, during ouabain perfusion both MDP (and RMP) and threshold potential decreased.

Had there been no change in threshold potential, then with MDP \(-79\) mv, a low-amplitude potential of 6-mv magnitude (as it was in this experiment) would have reached the control threshold potential of \(-77\) mv and resulted in automatic firing. Similar changes in MDP or RMP and threshold were seen in the two experiments on threshold potential in which Tyrode \([K^+]_o = 2.5\) mmoles/liter.

A mechanism by which low-amplitude potentials might initiate premature depolarizations was seen in three of the experiments conducted utilizing Tyrode with \([K^+]_o = 4\) mmoles/liter. One of these is depicted in figure 5. Each arrow indicates the onset of a current pulse injected through the microelectrode. In panel A following a 35-min ouabain perfusion, MDP was \(-76\) mv, and threshold potential, \(-60\) mv. The third \(S_2\) was just subthreshold and the fourth just threshold (marked with asterisks). The three subsequent \(S_2\) were suprathreshold and the resultant increase in rate caused a decrease in MDP and amplitude of alternate action potentials. When the \(S_1\) and \(S_2\) were turned off, a low-amplitude potential of 12-mv magnitude occurred and was followed by a return to RMP. In the sequence depicted in panel B, we attempted to determine whether a subthreshold
EFFECTS OF OUABAIN ON PHASE 4

Figure 5

Effect of subthreshold depolarizations on low-amplitude potentials: temp, 37°C, Tyrode [K+]o = 4.0 mmoles/liter, cycle length, 1000 msec. S2 of 100-msec duration are injected 500 msec after each S1. Oscilloscope sweep speed in A = one half that in B. (A) 35 min after ouabain perfusion. Upper trace, threshold current (measured at *) = 5 x 10^{-7} amp. Lower trace, Purkinje fiber action potential: MDP = -76 mv; TP = -60 mv; threshold voltage = 16 mv. With S2 off (arrow), a low-amplitude potential 12 mv in magnitude is seen. (B) 1 min after (A). Upper trace, S2 (indicated with *). Lower trace, action potential: MDP = -76 mv. The first S2 (cycle 2) is subthreshold, resulting in a 6-mv depolarization. The next two S2 (cycles 4 and 6) are suprathereshold, resulting in action potentials which are followed by low-amplitude potentials. For the fourth S2 (cycle 8) current has been reduced to a subthreshold level. The result is a 3-mv depolarization superimposed on a 12-mv low-amplitude potential. This is followed by a premature depolarization occurring 700 msec after the previous action potential. It is of lesser magnitude than the other action potentials in the series (amplitude = 72 mv, as compared to 96 mv).

depolarization, if superimposed on the low-amplitude potential, would result in a propagated action potential. The first S2 resulted in a subthreshold depolarization; the second and third were suprathereshold and initiated action potentials. With the resultant increase in rate of firing, the slope of phase 4 depolarization increased. For the fourth cycle, the amplitude of S2 was abruptly decreased to slightly below the subthreshold level in cycle 2. The result was the occurrence of a low-amplitude potential of approximately 12-mv amplitude, and superimposed upon it an additional depolarization of 3 mv induced by the current pulse. Rather than a return to RMP, there ensued a further depolarization which resulted in a premature action potential. Hence, it appears that subthreshold stimuli occurring near (in terms of time and distance) the site of low-amplitude potentials may result in the initiation of premature depolarizations.

Discussion

It appears that ouabain may produce two somewhat different changes in the voltage-time course of membrane potential during phase 4. The

primary effect is to increase the slope of slow depolarization but the result of this increased phase 4 depolarization may be either enhanced automaticity or the appearance of low-amplitude potentials. Some of the factors which determine whether the enhanced phase 4 depolarization results in increased automaticity or the occurrence of low-amplitude potentials have been characterized. It seems clear that low-amplitude potentials are more likely if the Purkinje fibers are in good condition i.e., unstretched) and if the [K+]o is somewhat higher (4.0-5.0 mmoles/liter), and that enhanced automaticity is more likely if the fibers have been stretched and/or [K+]o is lower (2.5 mmoles/liter). Also, there seems to be a relationship between the magnitude of the loss in RMP or MDP due to ouabain and the likelihood of low-amplitude potentials; enhanced automaticity was more frequently associated with a larger decrease in RMP and MDP. This, in turn, was seen more often in stretched preparations and at low [K+]o.

The results obtained with stretched, as opposed to normal, Purkinje fiber bundles offer an opportunity to speculate on the mechanisms involved in the production of arrhythmias during clinical digitalis toxicity. It has been noted that in "healthy" hearts, as in young people who ingest large quantities of digitalis, the predominant result is heart block, without signs of increased ventricular automaticity; on the other hand, if patients with cardiac disease develop toxic arrhythmias, ventricular irritability is common.11 In our experiments increased automaticity occurred either in the stretched tissue or at times in normal tissues in the presence of low [K+]o. This finding suggests that one prerequisite for the occurrence of increased automaticity, as seen in the stretched tissues, might be some form of damage to the conducting system. A further suggestion is that healthy Purkinje fibers in situ in the presence of toxic digitalis concentrations may routinely evoce low-amplitude potentials instead of increased automaticity. These events may help to explain the differing spectrum of arrhythmias seen with digitalis toxicity in situations of different plasma potassium concentrations12 and various degrees of cardiac disease.11

The phase shift occurring between the low-amplitude potentials and the action potential (most prominent in figure 3), could potentiate any conduction abnormalities induced by toxic digitalis concentrations. A result of the phase shift is that in successive cycles action potentials originate at different levels of membrane potential. It has been
shown that the maximal slope of phase 0 depolarization and conduction velocity usually increase if the membrane potential at which the action potential originates is increased.\textsuperscript{5, 13} With the variation in membrane potential induced by the phase shift, changes in conduction from cycle to cycle might be expected to occur, an event we have previously noted (Rosen MR, Merker C: Unpublished observations). Although low-amplitude potentials may hence potentiate conduction abnormalities, their importance in the generation of clinical digitalis-induced conduction disturbances is an area that remains to be explored.

The increased slope of phase 4 depolarization which developed at faster stimulus rates during our experiments did not at any time result in automaticity. However, it would be reasonable to assume that under circumstances in which an appropriate balance of rate, external ionic concentrations, and ouabain concentration were attained, and their attendant effects on threshold potential, RMP, and magnitude of low-amplitude potentials occurred, the latter might give rise to an action potential. We have been able to demonstrate this phenomenon when subthreshold depolarizations were superimposed on low-amplitude potentials, as in figure 5. In addition, preliminary reports of other investigators appear to describe spontaneous occurrence of this type of event.\textsuperscript{14} Whether the presence of catecholamines, as in the intact, innervated heart, might alter the magnitude of the low-amplitude potentials, thereby modifying their effect on the occurrence of clinical digitalis-induced arrhythmias, remains to be seen.

The attainment of threshold by low-amplitude potentials could provide a basis for the initiation of repetitive ventricular responses (RVR) in digitalized dogs. Occurrence of RVR is facilitated in situations where digitalis administration is followed by acceleration of stimulus rate.\textsuperscript{15} The stimuli required to elicit RVR are of lesser magnitude than those required to induce ectopic ventricular activity in control situations.\textsuperscript{16} This observation in intact animals may be analogous to the observation that low-amplitude potentials increase in magnitude as cycle length is decreased, and that minimal stimuli (as in figure 5) are necessary for the induction of ectopic beats when low-amplitude potentials are present.

We attempted to determine if the occurrence of low-amplitude potentials resulted from a shift in the threshold potential toward zero, thus preventing phase 4 depolarization caused by ouabain from attaining threshold. The results of others\textsuperscript{17} and our own unpublished findings suggest that, in the absence of ouabain, an increase in $[K^+]_o$ from 2.5 to 5.0 mmoles/liter causes little change in threshold potential even though there is a considerable decrease in RMP or MDP (table 2B). Since MDP and RMP are closer to threshold potential in solutions containing a higher $[K^+]_o$, one might expect that any phase 4 depolarizations present under such conditions would be more likely to reach threshold and initiate an action potential. At the same time an increase in $[K^+]_o$ decreases phase 4 depolarization.\textsuperscript{18} On the other hand, when membrane potential is more markedly reduced, as during the latter part of phase 3 of the action potential, threshold potential is shifted toward zero.\textsuperscript{19} This effect may be the predominant one when Purkinje fibers are exposed to toxic concentrations of ouabain since we have found that, concomitant with the reduction in MDP or RMP, the value of the threshold potential decreases and during the later stages of toxicity the threshold voltage increases markedly. An increase in the difference between MDP and threshold potential would be expected to decrease the probability that phase 4 depolarization would reach the threshold potential because the slow and continuing loss of membrane potential during phase 4 would cause progressively more inactivation of the "sodium-carrying system."\textsuperscript{20} This effect might well be enhanced during the toxic action of ouabain as with other digitalis substances.\textsuperscript{3}

These considerations are sufficient to account for the fact that low-amplitude potentials can occur during ouabain toxicity. Nevertheless, they fail to explain a number of findings. For example, in many instances depolarization resulting from the low-amplitude potentials terminated at a more negative membrane potential than that attained by the phase 4 depolarization in automatic fibers. Also, as stated above, low-amplitude potentials usually were associated with a smaller loss of MDP or RMP than was an increase in automaticity. Whether the low-amplitude potentials result from ouabain-induced changes in potassium flux during phase 4, or whether they are induced by another ionic mechanism, is open to question. It seems clear that a full characterization of the mechanisms responsible for determining whether or not a given preparation will develop low-amplitude potentials can be obtained only through the use of voltage-clamp technics.
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MICHAEL R. ROSEN, HENRY GELBAND, CHARLES MERKER and BRIAN F. HOFFMAN

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