Effects of Epinephrine and Calcium on the Electrogram of the Ouabainized Frog Heart

By WALTER W. BAKER, PH.D., AND JUDITH M. BAKER, B.S.

The effects of bound ouabain on the electrogram of the isolated frog heart are antagonized by epinephrine and potentiated by calcium. Ouabainized hearts also develop increased ventricular automatism (paroxysmal tachycardia, flutter and fibrillation) in the presence of large concentrations of calcium but not of epinephrine. The actions of both epinephrine and calcium are readily reversed by washing the tissue with normal Ringer's solution.

In the perfused frog heart high concentrations of calcium have been shown to cause the release of a sympathin-like substance. Grumbach et al. in a recent study also discussed the possibility that the stimulating role of calcium in enhancing ventricular automatism in the mammalian heart might be linked to the release of epinephrine. Furthermore, certain similarities have been noted between the actions of epinephrine and calcium on the electrical activity of the isolated frog heart. Although clinical and experimental data point to a synergism between calcium and the cardiac glycosides, no comparable relationship of the latter with epinephrine has been established.

In a previous study we developed a simple technic for characterizing electrically the various phases of the fixation and action of ouabain in the isolated frog heart. By the use of this method, a heart can be ouabainized to any desired degree and maintained at a given level for the study of the effects of other agents on the electrogram. Evidence will show that epinephrine and calcium differ markedly in their influence on electrical activity and automatism of the ouabainized ventricle.

METHOD

Hearts were rapidly removed from frogs and perfused with aerated Clark's frog-Ringer solution (NaCl 0.65 per cent, KCl 0.014 per cent, CaCl₂ 0.011 per cent (1mM), and NaHCO₃ 0.02 per cent) until stable. A silver-silver chloride electrode was placed in the sidearm of the cannula containing the perfusion fluid, and another on the surface of the ventricle. Both electrodes were attached to the leads of a Sanborn-Viso-Cardiette so that the upward deflection was negative and the downward one positive. The potential differences between the inner and outer surfaces of the heart were recorded on standard ECG paper moving 25 mm. per second.

Ouabain (U.S.P.) and epinephrine bitartrate solutions, freshly prepared in distilled water for each experiment, were appropriately diluted with Ringer solution. The concentrations of calcium chloride (3.5-10mM) were expressed in terms of the total molar concentration of the calcium chloride in the perfusion fluid. Preliminary tests at various concentrations led to the selection of 0.5 X 10⁻⁴M ouabain for these studies. The course of ouabainization was followed by serial electrograms. Ouabainization occurred progressively in stages: (a) latent period, little change in electrogram; (b) reversible membrane phase, inversion of t-wave; (c) relatively stable ouabain-fixation phase, S-T interval and electrical systole shortened; (d) toxic phase, profoundly shortened electrical systole and large biphasic QRS complexes; (e) terminal phase, complete heart block or cessation of all electrical activity. The perfusion with ouabain was interrupted at various phases and the heart washed for several minutes with Ringer solution to remove the unbound glycoside. The electrical behavior of the ouabain bound heart was stable and not appreciably modified during washing although the effect of epinephrine and calcium was readily reversed.

RESULTS

Epinephrine on Ouabainized Heart. The effects of epinephrine on the ouabainized heart are shown in figure 1. Perfusion with epinephrine during the stable fixation stage of

* Furnished through courtesy of Dr. M. L. Tainter of the Sterling-Winthrop Institute.
ouabainization (A) caused an increased amplitude of the QRS complex, a reappearance of a high negative T wave of long duration, and an acceleration of the heart rate (B-D). These actions antagonized most of the electrical variations produced originally by the ouabain with the exception that the ST segment often still remained depressed. Thus, the overall effect of epinephrine in this case was a lengthening of the electrical systole (Q-T interval). These actions of epinephrine were readily reversed upon washing the drug from the tissues and the electrogram continued to reflect the original ouabainized state of the heart (E).

Ouabainized hearts manifesting elevated biphasic QRS complexes with large negative S-deflections (toxic phase of ouabainization) responded similarly with the action of epinephrine superimposed on the ouabain pattern. Hearts in the terminal phase of ouabainization (cessation of electrical activity) responded to epinephrine by a restoration of electrical activity. At first these electrograms resembled the earlier stages of ouabainization, but later showed a typical epinephrine response. Removal of the epinephrine again resulted in a cessation of electrical activity.

Ouabainized and non-ouabainized hearts were equally sensitive to epinephrine since the same threshold doses, $30 \times 10^{-5}$M, were required with both to obtain a response. In a few preparations, however, arrhythmias developed initially when the ouabainized heart was perfused with epinephrine. In figure 2 D a pre-

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**Fig. 1.** Antagonistic action of epinephrine (Epi) on the ouabainized heart.

**Fig. 2.** Production of transient “electrical alternans” in the ouabainized heart during perfusion with epinephrine (Epi). Tracings also indicate that the actions of epinephrine are reversible.
mature ventricular beat is shown. These irregularities disappeared as the perfusion continued and were not related to the degree of ouabainization. Even at considerably higher concentrations of epinephrine than that used in these experiments ventricular hyper-automatism was not observed.

**Calcium on the ouabainized heart.** The type of electrical changes produced by calcium depended not only on the variability of the individual heart but to a greater extent on the concentration. Relatively low concentrations of calcium (two or three times normal) in the non-ouabainized heart resulted in elevated negative T waves and large QRS complexes which resembled the effects of epinephrine but without the accelerator action of the latter (fig. 3). At higher calcium levels the T wave was in-
verted (fig. 4 B) resembling that seen during the early stages of ouabainization. In a very few preparations the automaticity was markedly increased in an unexplained manner. All of these changes were readily reversed by reducing the calcium concentrations to that normally found in Ringer solution.

In the ouabainized heart small doses of calcium produced large biphasic QRS-waves with elevated S-deflections (fig. 3, E). The ST segment and the duration of the electrical systole was shortened further. Concentrations of calcium (5.5mM) which in the untreated heart only inverted the T wave, increased the automaticity of the ouabainized heart (fig. 4, F-G) as manifested by additional electrical discharges, ventricular paroxysmal tachycardia, flutter and fibrillation of variable duration. The discharges during these episodes were irregular and of large amplitude. There appeared to be no constant coupling of these to the previous beat, but usually they seemed to be initiated by a normal P wave. Such bouts frequently were intermittent and alternated with periods of electrical inactivity or with single large biphasic complexes. Immediately after the pause the heart again resumed its increased automaticity with a few extra electrical discharges lasting a fraction of a second followed by flutter and fibrillation of several minutes duration (fig. 4 F & G). These phenomena were increased by the addition of more calcium. However, concentrations equal to or greater than 10mM calcium chloride (CaCl₂) usually interfered with intraventricular conduction (seen as a widened QRS-complex) and prevented the sustained increase in automatism which was noted at lower concentrations. The concentrations of calcium necessary to augment automaticity was related to the threshold of the preparation. Once this level was exceeded automaticity became even more pronounced with further increases in concentration. This hyper-activity appeared to depend also on the intrinsic properties of the heart; some preparations fibrillated whereas others only developed paroxysmal tachycardia at the same concentration. The fixation of ouabain clearly favored the development of hyper-automaticity. Removal of excessive calcium and perfusion with Ringer restored the normal electrical activity seen previously in the ouabainized heart (fig. 4 H).

Some of the changes in the electrical responses of the non-ouabainized and ouabainized heart to epinephrine and calcium are summarized in table 1. The comparative nature of these responses is indicated by arrows which show the direction of the change, if any, and the number of arrows gives some idea of their magnitude. The data for the response of the non-ouabainized heart to epinephrine which are included for comparison were taken from a previous report.6

**TABLE 1.—Summary of Electrical Responses of Non-Ouabainized and Ouabainized Frog Heart to Epinephrine and Calcium.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Response</th>
<th>Duration of Systole</th>
<th>Negative of T-wave</th>
<th>Duration of ST Interval</th>
<th>Rate</th>
<th>Automatism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>↑</td>
<td>↑</td>
<td>↑ or ↓</td>
<td>↑</td>
<td>↑</td>
<td>or ↓</td>
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<tr>
<td>Calcium</td>
<td>↑</td>
<td>↑</td>
<td>↑ or ↓</td>
<td>↑</td>
<td>↑</td>
<td>or ↓</td>
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</tbody>
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HEART OUABAINIZED

<table>
<thead>
<tr>
<th>Drug</th>
<th>Response</th>
<th>Duration of Systole</th>
<th>Negative of T-wave</th>
<th>Duration of ST Interval</th>
<th>Rate</th>
<th>Automatism</th>
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</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>↑</td>
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<td>↑ or ↓</td>
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<tr>
<td>Calcium</td>
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<table>
<thead>
<tr>
<th>Concentration</th>
<th>Response</th>
<th>Duration of Systole</th>
<th>Negative of T-wave</th>
<th>Duration of ST Interval</th>
<th>Rate</th>
<th>Automatism</th>
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<tr>
<td>3.5mM</td>
<td>↑</td>
<td>↑</td>
<td>↑ or ↓</td>
<td>↑</td>
<td>↑</td>
<td>or ↓</td>
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<tr>
<td>5.5mM</td>
<td>↑</td>
<td>↑ or ↓</td>
<td>↑ or ↓</td>
<td>↑</td>
<td>↑</td>
<td>or ↓</td>
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<tr>
<td>10mM</td>
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<td>↑</td>
<td>↑ or ↓</td>
<td>↑</td>
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<td>or ↓</td>
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</table>

↑ = increased
↓ = decreased
— = no significant change

**DISCUSSION**

Although the actions of epinephrine and calcium on the normal isolated frog heart are somewhat similar, they markedly differ on the ouabainized heart. The changes induced by ouabain in the electrogram are antagonized by epinephrine and potentiated by calcium. Thus, the duration of the electrical systole (absolute refractory period) is markedly increased by...
epinephrine primarily through the reappearance of high negative T wave (late recovery phase) of appreciable duration. In contrast, calcium further shortens the electrical systole at the expense of the ST-interval (early recovery phase) with no indication of a T wave. Whitehorn\textsuperscript{6} likewise reported in frog ventricle strips that the cardiac glycosides shortened the refractory period and were antagonistic to epinephrine which lengthened it. In the digitalized dog heart Smith et al\textsuperscript{7} found no evidence for synergistic or additive effects of calcium; nevertheless, there is considerable evidence supporting such a synergism.\textsuperscript{8,9}

There are some significant similarities between the changes in the QRS complex during the toxic phase of ouabain action\textsuperscript{4} and the response of a less profoundly ouabainized heart to calcium. In both cases the amplitude of the QRS-complex is increased and the S-deflection is very prominent (fig. 3 E). There seems to be little doubt that the fixation of the ouabain potentiates the action of calcium since the response to the latter before ouabainization varies markedly from that after ouabainization. This could well reflect changes due to a shortening or acceleration of the recovery process of the heart produced by ouabainization. No comparable potentiation occurs with epinephrine although the latter itself produces an increased QRS potential in the non-ouabainized heart.\textsuperscript{6}

Mendez and Mendez\textsuperscript{10} concluded that the shortened refractory period resulting from the fixation of cardiac glycosides to the mammalian heart was intimately linked with the promotion of ventricular automatism. Various investigators\textsuperscript{5,11} have shown that increased automatism with the glycosides or other agents is independent of their effects on excitability. Thus, it was reported that epinephrine increased both excitability and automaticity, whereas, high concentrations of calcium decreased excitability but increased automaticity in papillary muscle.\textsuperscript{11}

Our experiments demonstrate that ouabain under the conditions employed here does not of its own accord produce an increased ventricular automatism. On the contrary, it may often actually decrease the heart rate. However, the fixation of ouabain does predispose the heart to automatism in the presence of increased concentrations of calcium, though not in the presence of elevated levels of epinephrine. Some preliminary studies indicate that epinephrine may even antagonize such a predisposition. The concentrations of calcium which increase automatism seem limited within a certain range since very large doses (10mM CaCl\textsubscript{2}) abolish this automatism and simultaneously interfere with intraventricular conduction (widened QRS).

Fibrillation of the isolated frog heart is quite uncommon and differs somewhat from that seen in the mammalian heart. It was observed by DeBoer\textsuperscript{11} under special conditions with single induction shocks. He too reported spontaneous cessation of fibrillation followed by a "post-undulatory pause." It is difficult to draw a parallel between electrically induced fibrillation and that promoted by calcium in the ouabainized heart. In both, a shortened refractory period seems a prerequisite. The fibrillation seen in our electrograms does not appear to be merely an extension of a phase of extrasystoles as suggested by DeBoer\textsuperscript{11} but rather the development of an uncoupled, autorhythmicity of certain cells of the ventricle. It is debatable whether this involves only the specialized conducting tissue.\textsuperscript{12}

**SUMMARY AND CONCLUSIONS**

The electrical responses of the ouabainized, isolated frog heart are markedly altered by epinephrine and calcium. The actions of ouabain as revealed by the electrogram are antagonized by epinephrine and potentiated by calcium. Epinephrine lengthens the absolute refractory period or electrical systole primarily through the reappearance of a high negative T wave. Calcium further shortens the electrical systole at the expense of the ST interval. Calcium also produced large biphasic QRS potentials possessing elevated S-deflections. These actions of epinephrine and calcium are reversible on washing.

Ouabain favors the development of increased ventricular automatism in the presence of large concentrations of calcium. These arrhythmias include uncoupled repetitive electrical systoles, paroxysmal tachycardia, flutter...
or fibrillation. Bouts of these arrhythmias are usually intermittent. Epinephrine under similar conditions produced no increase of automatism, but may induce transient premature systoles followed by the typical epinephrine responses.

The fibrillation produced in the frog heart with ouabain and calcium is reversible and appears to be different from that induced by electrical stimulation.

Acknowledgment

We wish to express our appreciation to Dr. J. M. Coon for his generous assistance in the preparation of the manuscript.

REFERENCES

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Circ Res. 1955;3:274-279
doi: 10.1161/01.RES.3.3.274

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