Correlation between Effects of Ouabain on the Canine Electrocardiogram and Transmembrane Potentials of Isolated Purkinje Fibers

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

SUMMARY

Isolated canine Purkinje fiber bundles (PF) were perfused with the blood of intact donor dogs to correlate changes induced by ouabain (O) in the PF transmembrane potential (TP) and the donor ECG. O was administered intravenously to the donor, and standard microelectrode techniques were used to record the TP. The only significant O effect prior to occurrence of toxic arrhythmias was induction of ST-T wave changes in the ECG and prolongation of action potential duration (APD). Early O toxicity was defined as the onset of junctional or ventricular premature contractions or junctional tachycardia. Simultaneous with early toxicity there were decreases in AP amplitude, resting membrane potential, maximal slope of phase 0 depolarization, APD, and plateau, and slowing of conduction, which often varied in extent from cycle to cycle. With increased duration of early toxicity or onset of late toxicity (ventricular tachycardia), TP changes were accentuated. Increased automaticity occurred at the time of early toxicity when plasma potassium concentration ([K + I₀]₀ < 4 mEq/liter. In three of seven instances in which [K + I₀] > 4.0 mEq/liter, low amplitude potentials were recorded during phase 4. The possible role of these potentials in relation to the generation of toxic arrhythmias is discussed.

Additional Indexing Words:
Automaticity Microelectrode recording techinics Blood perfusion Phase 4 low amplitude potentials Digitalis toxicity Plasma potassium concentration Junctional arrhythmias Ventricular arrhythmias

The effects of digitalis glycosides on electrophysiologic properties of cardiac tissues have been the subject of a large number of studies conducted both in vivo and in vitro. Electrophysiologic monitoring has demonstrated that digitalis therapy induces a shortened Q-T interval and changes in the S-T segment and T wave, and that digitalis toxicity is associated with atrial and ventricular arrhythmias, most commonly ventricular premature contractions. The mechanisms implicated in the induction of digitalis-induced ventricular arrhythmias are excessive automaticity and/or reentry. The extent to which either predominates is uncertain. However, there is evidence to suggest that the likelihood of digitalis-induced automaticity is increased in diseased hearts, when cardiac rate prior to toxicity has been rapid, and in the presence of hypokalemia.

Microelectrode studies on the electrophysiologic properties of single cardiac cells have frequently shown an increase in action potential duration at low rates of stimulation with lower drug concentrations or short periods of exposure. With higher concentrations or longer periods of exposure decreases in action potential amplitude, resting membrane potential, plateau, and action potential duration have also been described. Automaticity of cells in the specialized conducting system may be increased by high concentrations of digitalis glycosides especially in the presence of lower extracellular potassium concentrations or faster stimulus rates.

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In an attempt to correlate the effects of digitalis on the electrocardiogram of intact dogs with changes in the electrophysiologic properties of single cells of the ventricular conducting system, we conducted a series of experiments in which isolated preparations of cardiac Purkinje fibers were perfused with arterial blood from a donor dog. The ECG was recorded from the donor, transmembrane potentials were recorded from the isolated fibers, and digitalis was administered to the donor by intravenous injection. Through such a study we hoped to improve our understanding of the cellular electrophysiologic events which correlate with certain of the electrocardiographic changes induced by digitalis.

**Methods**

The technic employed for blood perfusion has been described previously. In 10 experiments bundles of cardiac Purkinje fibers were excised from the hearts of pentobarbital-anesthetized (30 mg/kg, i.v.) mongrel dogs and mounted in a Lucite chamber perfused with modified Tyrode’s solution at 37°C. The Purkinje fiber preparations were stimulated externally, using bipolar silver wire electrodes. Three or four intracellular microelectrodes were used to record transmembrane potentials from different cells. In any given experiment at least two of the microelectrode impalements were maintained from start to finish. The additional impalements were performed to insure that even if one or two of the microelectrodes came out during the experiment those remaining would be sufficient to provide data concerning any changes in the transmembrane potential and impulse conduction. In five of the experiments in which resting membrane potential was studied, one of the four microelectrodes was kept in the perfusate immediately adjacent to an intracellular electrode, permitting constant monitoring of the level of zero potential in the tissue chamber. Previously described technics were employed to stimulate the preparation at a constant rate and to measure action potential duration, conduction time, and maximal slope of phase 0 depolarization (Vmax). The rate at which the preparation was stimulated was approximately equal to the spontaneous heart rate of the donor prior to development of arrhythmias. The range of cycle lengths for the series was 500–800 msec. During each experiment, at any time prior to the onset of a ouabain-induced arrhythmia, the variation in donor heart rate was less than ±10 beats/min. For studies on automaticity the drive stimulus was discontinued for 1 min and, if automatic firing ensued, the slope of phase 4 and the interval between spontaneous action potentials were recorded. Records of automaticity were analyzed only when obtained prior to the occurrence of significant changes in arterial pressure (±10 mm systolic or 5 mm diastolic pressure) in an effort to minimize the effects on the isolated tissue that might be induced by endogenous catecholamine release in the donor.

To perfuse the isolated tissue with arterial blood a second dog was anesthetized (Na pentobarbital, 30 mg/kg) and heparinized (Na heparin, 3 mg/kg) and the femoral artery and vein cannulated. Arterial blood (12–15 ml/min) was used to perfuse the chamber in place of Tyrode’s solution, and effluent from the chamber was channeled through an air trap to the femoral vein of the donor. Donor electrocardiogram and arterial pressure were monitored continuously. Oxygen saturation (Astrup) and pH of donor arterial blood were measured prior to ouabain injection. Arterial O₂ saturation was 90–96% and pH, 7.38–7.41. All cellular electrophysiologic data reported are from impalements maintained throughout an experiment.

The digitalis preparation used was ouabain (Eli Lilly) administered intravenously to the donor in a total dose of 60–90 μg/kg. We have previously demonstrated that with this technic ouabain uptake by Purkinje fibers isolated in the tissue chamber and those in situ in the donor heart is comparable. Following injection of ouabain, on-line observations of donor electrocardiogram and Purkinje fiber transmembrane potentials were made, utilizing Tektronix 565 and 564 oscilloscopes, and a digital timer (Mansanto, model 101B counter-timer) and voltmeter (United Systems Digitec). Data were recorded on Polaroid film. Plasma potassium concentration of donor arterial blood was determined at intervals during each experiment, utilizing a flame photometer.

**Results**

**Effect of Ouabain on the Donor Electrocardiogram**

In six experiments an initial dose of ouabain, 30–50 μg/kg, was given as a single intravenous injection. During the first hour, cardiac arrhythmias did not develop in any instance although S-T segment depression and/or T-wave inversion occurred in three of the six animals. Within 30 min of a subsequent intravenous dose of ouabain, 20–40 μg/kg, there was electrocardiographic evidence of toxicity in the form of junctional or ventricular arrhythmias in all six dogs. For the four remaining donor dogs the initial injection of ouabain was 60–90 μg/kg. All developed ventricular or junctional arrhythmias within 30–40 min. Regardless of the drug administration schedule used, the initial arrhythmias seen were either ventricular or junctional premature beats or junctional tachycardia. Ventricular tachycardia always occurred later, following one of the three arrhythmias mentioned.

**Effects of Ouabain on Transmembrane Potentials of Isolated Tissues**

The effects of ouabain on action potential (AP) amplitude, Vmax, and action potential duration (APD) were studied in 10 experiments. Changes in these variables were recorded before the onset of toxicity (20 min after drug administration in the six dogs that initially received the lower ouabain dose,
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15 min after drug administration in the other four dogs), during early toxicity (onset of junctional tachycardia or junctional or ventricular premature contractions), and late toxicity (occurrence of ventricular tachycardia). The results are summarized in table 1.

Prior to drug toxicity in seven of 10 experiments there was a decrease in action potential amplitude of 1 to 4 mv and a concomitant decrease in V\textsubscript{max}. However, for the series, these changes were not significant (P > 0.05) and in three instances AP amplitude increased by 2 to 3 mv. In the five experiments in which resting membrane potential (RMP) was measured; changes in RMP were equal to those in AP amplitude. APD was prolonged in every experiment; this resulted from increases in the duration of phases 2 and 3. The change in APD, although quite small, was statistically significant for the series (P < 0.01). During early toxicity there was a decrease in AP amplitude and V\textsubscript{max}. Simultaneously, APD decreased and the plateau shortened. These changes were progressive: the more prolonged the duration of toxicity the greater the change in these variables.

The results of a representative experiment illustrating the effect of nontoxic concentrations of ouabain on the Purkinje fiber action potential (i.e., changes seen within 15-20 min of the initial injection) are shown in figure 1. AP amplitude was 123 mv and RMP was -89 mv under control conditions (fig 1A), and these values were the same 20 min after ouabain injection. V\textsubscript{max} did not change significantly. However, both the plateau and the overall APD were prolonged, the latter from a control of 340 msec (fig 1A) to 372 msec (fig. 1B). Similar prolongation of the voltage-time course of repolarization was noted in every experiment.

Figure 2 shows the early and late toxic effects of ouabain on the donor electrocardiogram and Purkinje fiber action potential. At the time of onset of an ectopic tachycardia (fig 2B) AP amplitude, RMP, and V\textsubscript{max} had decreased, and APD and plateau shortened. By the time ventricular tachycardia supervened (fig. 2C) these changes all were accentuated. It is important to note that in this particular experiment, following the onset of the ectopic tachycardia in figure 2B, the donor heart rate was faster than the drive stimulus for the isolated tissue. Because the stimulus rate for the Purkinje fiber bundle was not changed, the alterations in

Table 1

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<th>Effect of Ouabain on AP Characteristics*</th>
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<tr>
<td>Parameter</td>
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<td>AP amplitude (mv)</td>
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<td>V\textsubscript{max} (v/sec)</td>
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<td>APD (msec)</td>
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*Results expressed as mean ± sd for 10 impalements (one in each experiment, maintained through the duration of that study). Control in each instance is 100%, and all subsequent values are recorded as percent of control. "Before toxicity" indicates readings made 15-20 min after ouabain injection; "early toxicity," readings at onset of junctional arrhythmias or ventricular premature contractions; "late toxicity," readings at onset of ventricular tachycardia.

†Maximal slope of phase 0 depolarization.

‡Action potential duration measured to full repolarization.

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Figure 2

Effect of toxic ouabain concentration on ECG and Purkinje fiber action potential: temperature, 36–37°C; cycle length, 500 msec. (A) Control. ECG shows sinus rhythm, rate 140 beats/min, AP amplitude, 118 mc; resting membrane potential (RMP), —96 mc; V_{max}, 457 v/sec; APD, 310 msec. (B) Forty minutes after injection of ouabain, 60 μg/kg, ECG shows an ectopic tachycardia; AP amplitude, 110 mc; RMP, —90 mc; V_{max}, 314 v/sec; APD, 280 msec. (C) Sixty minutes after ouabain injection. ECG shows ventricular tachycardia. AP amplitude, 100 mc; RMP, —83 mc; V_{max}, 252 v/sec; APD, 265 msec.

AP characteristics reflect ouabain effect alone, and not the effect of an altered stimulus rate.

Effect of Ouabain on Conduction Time

Conduction time was measured between two microelectrodes impaled in the same Purkinje fiber bundle, separated by a distance of 3–4 mm. There were no significant changes in conduction time prior to the onset of ouabain toxicity in the donor. Within 3 min of the onset of early ouabain toxicity changes in conduction time in the Purkinje fiber bundle were present in every experiment. Conduction slowed by 5–20% during early toxicity and by 8–30% in late toxicity. Figure 3 depicts the results from one experiment on conduction time. In figure 3A, the control electrocardiogram showed sinus rhythm, and interelectrode conduction time was 1.2 msec. In figure 3B, recorded just prior to the onset of ventricular fibrillation, conduction time increased to 1.6 msec.

The changes in conduction time were not consistent in all experiments. Conduction time varied from beat to beat during both early and late toxicity in four of the studies. The effect was such that during one cycle conduction time might approximate the control, while in the subsequent cycle it was increased, the degree of increase varying from cycle to cycle.

Effects of Ouabain on Phase 4

In three experiments in which plasma potassium concentration was less than 4 mEq/liter, electrocardiographic evidence of early ouabain toxicity was accompanied by increases in slope of phase 4 depolarization and spontaneous rate. The spontaneous rate and slope of phase 4 increased in proportion to the duration of toxicity. The results of one such experiment (plasma potassium = 3.2 mEq/liter) are shown in figure 4. In figure 4, recorded under control conditions, the donor heart was in sinus rhythm, and the spontaneous Purkinje fiber rate was 16 depolarizations/min. In figure 4B, recorded at the onset of ventricular tachycardia, the spontaneous Purkinje fiber rate increased to 26 depolarizations/min. The records suggest that this increased rate really reflects an increase in automaticity. As this change in automaticity occurred at

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Figure 4
Effect of ouabain on automaticity. Temperature, 37°C. Drive discontinued for 1 min prior to each recording. (A) Control. AP amplitude, measured from the peak of phase 0 to the end of phase 3, 100 mv. Spontaneous rate 16 depolarizations/min. (B) Forty minutes after 70 µg/kg ouabain injection, at onset of ventricular tachycardia; AP amplitude, 84 mc; spontaneous rate, 26 depolarizations/min. Donor plasma potassium = 3.2 mEq/liter.

The onset of ventricular tachycardia, and prior to any decrease in arterial pressure, it is probable that the increase in automaticity was induced by ouabain rather than by catecholamines released in the donor.

In the seven other experiments, plasma potassium was equal to or greater than 4 mEq/liter (range 4.0-5.2 mEq/liter). In one experiment there was an increase in slope of phase 4 depolarization during regular stimulation and an increase in spontaneous rate on cessation of stimulation. No changes in phase 4 depolarization had increased. However, seen in three experiments. In the remaining three experiments there was no automaticity after the drive was discontinued for 1 min. Instead events like those depicted in figure 5 occurred. In figure 5A, recorded under control conditions, phase 4 depolarization was seen during regular stimulation. There was no spontaneous activity when the drive was transiently discontinued. In figure 5B, recorded at the onset of ventricular tachycardia, the slope of phase 4 depolarization had increased. However, when the drive was discontinued there was a low amplitude depolarization but no automatic firing. In figure 5C, recorded 15 min later, there was a marked decrease in AP amplitude and a further increase in the slope of phase 4. Again, discontinuation of the drive was followed by a low amplitude depolarization and then by slow hyperpolarization which continued until the drive was resumed.

In the three instances in which these low amplitude potentials were recorded, stimulus cycle length was 500 msec. When stimulus rate was transiently increased, the low amplitude potentials became larger; when rate was slowed, their amplitude decreased. In the other four experiments in this group in which low amplitude potentials did not appear, cycle length was longer (630-800 msec).

Discussion

The results of these studies permit several observations to be made concerning the interrelationship between ouabain-induced changes in transmembrane potentials of single Purkinje fibers and changes in the electrocardiogram caused by digitals. In the absence of ouabain toxicity in the donor, when the electrocardiogram was unchanged or showed only ST-T wave changes, there was a consistent prolongation of the Purkinje fiber action potential and plateau. Under the same conditions there was no significant change in AP amplitude, Vmax, or conduction time. Electrocardiographic evidence of early toxicity was associated with a shortened APD and plateau, and decreases in AP amplitude, RMP, and Vmax. As duration of any ouabain-induced arrhythmia increased, or as there was progression from early to late electrocardiographic evidence of toxicity, there was a concomitant deterioration of transmembrane potentials of the isolated Purkinje fibers.

Our observations of the changes in Purkinje fiber conduction time and electrocardiographic QRS...
duration and configuration permit the following conclusions: The onset of slowed or variable conduction in the Purkinje system correlates well with the onset of junctional or ventricular arrhythmias in the electrocardiogram. With this change in Purkinje fiber conduction, there may be a change in QRS duration or configuration (as in fig. 2B). However, the pathway for initiation and propagation of ectopic beats during a ventricular arrhythmia may differ from the normal, an event which in itself alters the QRS complex. Hence, it is impossible to casually relate the changes in conduction time recorded during our experiments with the changes observed in QRS duration and configuration. However, it is tempting to speculate that the frequent presence of some degree of abnormality in the configuration and duration of the QRS complex during A-V junctional rhythms caused by digitalis may result from changes in conduction similar to those we have demonstrated in the isolated Purkinje fiber preparations. Variability in QRS configuration from cycle to cycle during such rhythms may result from the variable depression of conduction we have shown for the isolated preparations.

No correlation of changes in the action potential and the electrocardiographic Q-T interval was attempted despite the fact that ouabain initially prolonged APD. The reason for this is the fact that the Q-T interval correlates not with the Purkinje fiber action potential per se, but rather with that of ventricular muscle. Since no recordings of electrophysiologic events in ventricular myocardial cells were made during these experiments, and since the sensitivity of the latter cells to the effects of ouabain apparently differs from that of Purkinje fibers, it was felt that an attempt to correlate alterations in the voltage-time course of action potentials recorded from Purkinje fibers with changes in the Q-T interval was not justified.

Whereas there was a consistent association between electrocardiographic evidence of toxicity and deterioration of the action potential, this association was not as evident when electrophysiologic events were correlated with changes in automaticity of the isolated preparation. Depending in large part on plasma potassium concentration, either increased automaticity or low amplitude potentials were noted. The former appeared at $[K^+]_o = 3.2-4.0$ mEq/liter and the latter at $[K^+]_o > 4.0$ mEq/liter. These observations of low amplitude potentials occurring during phase 4 were unexpected on the basis of published reports of several prior studies. However, in recent abstracts similar phenomena in Tyrode-perfused tissues exposed to acetylstraphanolid have been reported. Further, in two earlier studies what appear to be low amplitude potentials are present in several illustrations, but are not mentioned in the text.

For the most part, prior investigations into the effects of digitalis on phase 4 of the transmembrane potential have stressed the occurrence of increased automaticity, an event which we were able to demonstrate only when plasma potassium concentration was less than 4.0 mEq/liter. (In most prior studies $[K^+]_o$ was less than this.) When the effects of higher extracellular potassium concentrations in the physiologic range on digitalis intoxication have been reported, it has been shown that transmembrane potentials deteriorate less rapidly, and increased automaticity is less prominent at higher than at lower potassium levels. This information is consistent with our observations on the relative incidence of automaticity and low amplitude potentials at different $[K^+]_o$.

The mechanisms responsible for the induction of low amplitude potentials are not known. When these potentials were recorded their magnitude was greater at faster rates of stimulation. Although the effect of heart rate on the frequency of escape rhythms has been described both for the normal2 and digitalis-intoxicated heart, and although it has been shown that an increase in rate increases the slope of phase 4 depolarization in Purkinje fibers poisoned with digitalis,7 the relationship between rate and the amplitude and voltage-time course of the low amplitude potentials requires further study. Similarly, more information is needed concerning the mechanism for their initiation. A possible mechanism is the following: The increase in slope of phase 4 depolarization induced by ouabain probably results from a decreased potassium conductance during this phase of the transmembrane potential. If, simultaneous with the ouabain-induced increase in slope of phase 4, there was a decrease in threshold potential (i.e., a shift toward 0 potential) the appearance of low amplitude potentials could be explained by the failure of phase 4 depolarization to reach threshold levels. Although this thought is plausible, further information concerning the incidence of low amplitude potentials and factors which modify their appearance is needed before the mechanisms responsible for their initiation can be fully understood.

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The role of low amplitude potentials in the generation and maintenance of ventricular arrhythmias is uncertain: however, in relation to figure 5C, some speculation is possible. In this panel, the first three action potentials were initiated at or near the peak of low amplitude potentials and as a result were of significantly lesser magnitude than the last two action potentials which commenced when phase 4 was flat. As a general rule, action potentials which arise at lower levels of membrane potential have a lower amplitude and \( V_{\text{max}} \) and conduct more slowly than those which arise at a higher membrane potential and consequently have a greater amplitude and \( V_{\text{max}} \). Hence, it is conceivable that low amplitude potentials might contribute to the slowing of conduction which was caused by ouabain toxicity at all levels of [K⁺]. Further, the variability in conduction time from cycle to cycle described in four of our experiments could be explained if low amplitude potentials of differing magnitudes were occurring concomitantly. This variability in conduction was seen in the three experiments in which low amplitude potentials were described, and in the other experiment there was some variation in the slope of phase 4 depolarization. Hence, changes in the level of membrane potential at which successive action potentials were initiated may offer an explanation for the observations we have made concerning conduction.

The ouabain-induced changes in transmembrane potentials of Purkinje fibers shown in these experiments were similar in many ways to those observed during studies on Tyrode-perfused fibers. The decreases in action potential amplitude, RMP, and \( V_{\text{max}} \), which were associated with ventricular or junctional arrhythmias in the donor electrocardiogram, were predictable in light of the previously reported effects of high concentrations of ouabain on isolated, Tyrode-perfused tissues. Digitalis-induced prolongations of APD and plateau in Purkinje fiber and ventricular muscle have also been reported, but mainly when these tissues were driven at long cycle lengths (approximately 2000 msec). The basis for this prolongation has been attributed to a ouabain-induced decrease in potassium conductance. The fact that in our experiments prolongation of APD was observed at cycle lengths as short as 500 msec provides evidence for this same mechanism occurring over a wide range of stimulus rates.

In summary, these studies serve to underscore the complex situation that exists when digitalis toxicity occurs in a clinical setting. While it is apparent that previously made associations between lower potassium concentrations and increased automaticity are supported by these experiments, they also illustrate the marked effect of ouabain on conduction which occurs regardless of potassium concentration. Further, the occurrence of low amplitude potentials at higher physiologic potassium concentrations and in the presence of faster stimulus rates may provide an additional mechanism for the induction of aberrant conduction and toxic arrhythmias.

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References

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Correction

Rosen MR, Gelband H, Hoffman BF: Circulation 47: 65, 1973. On page 69, line 10 should read: "No changes in phase 4 depolarization or spontaneous rate were seen in three experiments." On page 70, line 12, "casually" should read, "causally."