The Effects of Ouabain on the Transmembrane Potentials and Intracellular Potassium Activity of Canine Cardiac Purkinje Fibers

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SUMMARY Open-tip microelectrodes containing a potassium-sensitive liquid ion exchanger (Corning 477317) were used to study the effects of ouabain on the intracellular potassium activity and the transmembrane potentials of beating canine cardiac Purkinje fibers. The preparations were superfused with Tyrode's solution containing ouabain, 2 x 10^-7 M, and potassium, 4 mM, for 30 minutes. At the end of this period, intracellular potassium activity had decreased from the control value of 130.0 mM to 112.2 mM. The resting membrane potential determined through conventional 3 M KCl-filled microelectrodes decreased from -83.6 to -78.8 mV. Comparison of the decrease in the potassium equilibrium potential with the decrease in the resting membrane potential suggests that there was an accumulation of potassium at the exterior surface of the cell membrane. The effect of ouabain on the resting membrane potential, therefore, was due to a change in the transmembrane potassium ion gradient. This, in turn, resulted from a decrease in intracellular potassium activity and, apparently, from an increased potassium activity at the cell surface.

STUDIES OF the effects of digitalis on the electrophysiological properties of mammalian cardiac fibers have revealed a close relationship between digitalis and potassium ion. In 1963, Kassebaum1 reported that G-strophanthin, 1.4 x 10^-6 M, had a biphasic effect on the current-voltage relationship of sheep Purkinje fibers: initially membrane permeability to potassium was decreased; later, it was increased. This result was similar to that in an earlier report by Dudel and Trautwein.2 Kassebaum interpreted the decrease in potassium permeability as explaining the initial digitalis-induced prolongation of the action potential duration (APD) and the later increase in permeability as explaining the associated decrease in APD. The exposure to G-strophanthin that resulted in a decrease in APD also brought about a decrease in resting membrane potential.

Other investigators have used indirect methods to determine the effects of digitalis on the intracellular potassium concentration.3-5 On the basis of these studies, it appears that at low concentrations of digitalis— or following short periods of exposure to digitalis— there is no change (or a slight increase) in intracellular potassium concentration, but at higher concentrations there is a decrease in intracellular potassium concentration. The loss of intracellular potassium and accompanying decrease in membrane potential that occur at high or toxic concentrations of digitalis have been attributed to poisoning of Na^+–K^+–activated ATPase by digitalis.6,7

Despite these studies of the effects of digitalis on membrane permeability, potassium concentration, and membrane potential, direct measurements of the changes in intracellular potassium activity (ak) induced by clinically relevant toxic concentrations of digitalis have not
yet been reported. However, the advent of open-tip microelectrodes containing a potassium-sensitive liquid ion exchanger has made it possible to measure intracellular potassium activity in cardiac fibers. In the present study we used potassium-sensitive microelectrodes to determine (1) the changes in $a_k$ induced by ouabain in toxic concentrations and (2) the association between the magnitude of the change in $a_k$ and the decrease in the transmembrane potential.

**Methods**

**Tissue Preparation**

Twenty mongrel dogs weighing 20-30 kg were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously. The heart was removed rapidly through a right lateral thoracotomy and placed in chilled Tyrode’s solution containing (millimoles per liter): NaCl, 137; NaHCO$_3$, 12; NaH$_2$PO$_4$, 1.8; MgCl$_2$, 0.5; dextrose, 5.5; CaCl$_2$, 2.7; KCl, 4; and equilibrated with 95% O$_2$-5% CO$_2$. Free-running Purkinje fiber bundles and attached small pieces of myocardium were excised from both ventricles, placed in a Lucite tissue bath, and superfused with Tyrode’s solution at 37°C. The volume of the fluid bathing the preparation in the tissue bath was 2.0 ml, and the Tyrode’s solution was pumped into the chamber at a flow rate of 7-8 ml/min.

The preparation was driven at a cycle length of 1000 msec by stimuli isolated from ground (Bioelectric Instruments Isolator ISB 2.5) and delivered through Teflon-coated bipolar silver wire electrodes. Transmembrane potentials were recorded through 3 M KCI-filled microelectrodes. These were coupled by an Ag/AgCl interface to an amplifier (Bioelectric Instruments PAD-1) with high input impedance and input capacity neutralization. The output was displayed on one channel of a cathode ray oscilloscope (Tektronix 564). Records of transmembrane potentials were calibrated with a known 100-mV signal delivered between the tissue chamber and ground.

**Potassium-Sensitive Microelectrode Preparation**

Open-tip microelectrodes were pulled to a tip diameter of <1 μm with a vertical microelectrode puller (model 700 C; David Kopf Instruments). The microelectrodes were made from acid-chrome-cleaved borosilicate glass (Pyrex 7740), 2.0 mm in o.d. and 1.0 mm i.d. Certain of these microelectrodes were used for the routine recording of transmembrane potential. When filled with 3 M KCl, their tip resistances were 10-20 MΩ and tip potentials were <5 mV.

Microelectrodes used to determine intracellular potassium activity were made hydroptic by dipping the tip into a 2% solution of Dow Corning 200 fluid (200 cs) in xylene and then were air dried for 15 minutes. Approximately 200-300 μm of each microelectrode tip were filled with a potassium-sensitive liquid ion exchanger resin (Corning 477317). The potassium-sensitive microelectrode then was back-filled with 1.0 M KCl as the internal reference. An Ag/AgCl wire was inserted into the KCl as the internal reference element.

The potassium-sensitive microelectrode was connected to the input of a Teledyne Philbrick Operational Amplifier which provided an input impedance of $10^{13}$ Ω. Only microelectrodes with resistances from $10^9$ to $10^{10}$ Ω were used. The potassium-sensitive microelectrodes were calibrated in solutions of pure KCl; their response was proportional to the logarithm of the potassium activity and linear from 1 to $1 \times 10^{-4}$ M KCl. The selectivity coefficient was determined by using the method of Walker. If the calibrations for the microelectrode before and after the experiments were not the same, the data obtained with that electrode were discarded. A more complete description of the method for the fabrication and calibration of the potassium-sensitive microelectrodes has been published recently.

**Determination of Intracellular Potassium Activity**

This method has been described in detail in an earlier publication. When the potassium-sensitive microelectrode was advanced into the Purkinje fiber, there was a shift in the measured potential, $\Delta E$. This shift in potential was due to the following factors: (1) the difference between the intracellular and extracellular potassium activities ($a_k$ and $a_k^*$), (2) the effects of extracellular sodium activity ($a_{kNa}$), and (3) the cell transmembrane potential ($E_m$). This can be expressed in the following manner:

$$\Delta E = E_m + RT/F \ln \left( \frac{a_k}{a_k^* + K_{KNa} a_{kNa}} \right)$$

where $RT/F$ has its usual meaning and $K_{KNa}$ is the selectivity coefficient determined during the calibration procedure. To determine the intracellular potassium activity, it is necessary to know the transmembrane action and resting potentials of the cell. Since it was not possible to place a conventional 3 M KCl-filled microelectrode in the same cell as the potassium-sensitive microelectrode, a series of measurements of transmembrane potential was made with the 3 M KCl-filled electrodes in a small area of the fiber bundle and the mean used to determine the term $E_m$ in Equation 1. Another series of impalements was made in the same small area with the potassium-sensitive microelectrode and the mean was used to determine the term, $\Delta E$.

Two separate measurements were obtained with both the 3 M KCl-filled microelectrodes and the potassium-sensitive microelectrodes: (1) at the maximum diastolic potential ($E_D$) during stimulation and (2) at the resting membrane potential ($E_m$) determined 20 seconds after discontinuation of the stimulus (Fig. 1). The value of $\Delta E$ was determined by using the potassium-sensitive microelectrode and Equation 1, where the change ($\Delta E$) was the measured difference in potential from that recorded extracellularly to that recorded intracellularly at the maximum diastolic potential ($\Delta E_D$) and at the resting transmembrane potential ($\Delta E_m$).

**Experimental Protocol**

The method we used to determine $a_k$ requires multiple impalements of Purkinje fibers with 3 M KCl-filled and with potassium-sensitive microelectrodes and then deter-
OUABAIN EFFECTS ON $\alpha_k$/Miura and Rosen

Figure 1 Recording of $E_m$ and $E_d$. Panel A shows an action potential and the maximum diastolic potential, $E_d$. Panel B was recorded at a slower sweep speed. The stimulus was discontinued and the resting transmembrane potential, $E_m$, was determined. For Purkinje fiber preparations showing automaticity with the drive stimulus turned off, the membrane voltage at which spontaneous action potentials were initiated was measured instead of the $E_m$. None of the preparations was automatic while the drive stimulus was on. Vertical calibration, 40 mV; horizontal calibration, 100 msec, panel A; 4 sec, panel B.

minoration of the mean values for membrane potential and $\alpha_k$. For this reason it was necessary to design a protocol that permitted the tissue to equilibrate with ouabain over a period of time until a steady state effect on cellular electrophysiological properties had been attained. During maintenance of such a steady state, it then was possible to make multiple microelectrode impalements without being concerned about variations in membrane potential and $\alpha_k$ over the period of time required for the study. For this reason, we used the following protocol:

The Purkinje fiber preparation was equilibrated in Tyrode’s solution for 60 minutes, and then control records of the transmembrane potential were obtained, using 3 M KCl-filled microelectrodes. Measurements included: action potential (AP) amplitude, $E_d$ (the highest level of membrane potential after repolarization), and $E_m$ determined 20 seconds after discontinuation of the drive stimulus. The value of $\alpha_k$ then was determined. After control records had been obtained, the preparation was superfused with Tyrode’s solution containing ouabain, $2 \times 10^{-7}$ M (G-strophanthin, Eli Lilly), for 30 minutes. A single microelectrode impalement was maintained throughout this 30-minute period.

As soon as the Purkinje fiber preparation had been superfused with ouabain for 20 minutes, multiple impalements again were made with a second 3 M KCl-filled microelectrode, while the first impalement was maintained, and resting and action potentials were recorded.

Thirty minutes after the onset of ouabain superfusion, multiple impalements again were made (the single impalement was maintained), and resting and action potentials were recorded. Ouabain-free Tyrode’s solution then was substituted for that containing ouabain (Fig. 2). In four preliminary experiments we had maintained single impalements throughout the period of ouabain superfusion and a subsequent 60-minute period of superfusion with Tyrode’s solution. We determined that, during the 60-minute period following discontinuation of ouabain superfusion, there were no further changes in the resting and action potentials; that is, this 60-minute period provided us with a “steady state” effect of ouabain on the cellular electrophysiological properties that was required for studies with the potassium-sensitive microelectrodes. Hence, measurements of $\alpha_k$ could be made with the potassium-sensitive microelectrodes during the 60 minutes following the discontinuation of superfusion with ouabain.

Results

Effect of Ouabain on Transmembrane Potential

The control resting and action potentials recorded after the Purkinje fiber bundle had equilibrated for 60 minutes
in Tyrode’s solution are summarized in Table 1. Twenty minutes after the onset of ouabain superfusion, \( E_D \) had decreased by 3.1 mV, \( E_m \) by 3.5 mV, and AP amplitude by 3.6 mV. After 30 minutes of exposure to ouabain, there was a decrease in \( E_D \) of 5.1 mV, a decrease in \( E_m \) of 4.8 mV, and a decrease in AP amplitude of 6.3 mV from control values. There was occasional automatic firing during the determination of the \( E_m \).

During the 60 minutes after discontinuing the ouabain, the transmembrane potentials did not change significantly (\( P > 0.2 \)) from those determined immediately after 30 minutes of ouabain superfusion (Fig. 2 and Table 1).

### Effect of Ouabain on \( a_k \) and the Potassium Equilibrium Potential

Representative records made with the potassium-sensitive microelectrode are shown in Figure 3. For eight Purkinje fiber bundles, \( a_k \) was calculated for \( E_D \) and \( E_m \) using Equation 1. The control value of \( a_k \), using \( E_D \) was 130 ± 2.3 mM. When we used \( E_m \), \( a_k \) was 124.2 ± 1.3 mM. During the 60 minutes after termination of superfusion with ouabain, the value of \( a_k \) determined by using an \( E_m \) of 78.8 mV in Equation 1 was 119.9 ± 0.7 mM. The value of \( a_k \) determined by substituting an \( E_D \) of 84.7 mV in Equation 1 was 112.2 ± 1.3 mM. Values obtained by using \( E_D \) or \( E_m \) differed significantly from control values (\( P < 0.001 \)) but did not differ significantly from one another.

The potassium equilibrium potential (\( E_K \)) was determined, using the Nernst equation, substituting the values calculated for \( a_k \) and the \( a_g \) in the bulk superfusate (assumed to be the \([K^+]_o\) of 4 mM). 

\[
E_K = -\frac{RT}{F} \ln \left( \frac{a_k}{a_g} \right). \tag{2}
\]

The control value of \( E_K \), based on an \( a_k = 130 ± 2.3 \) mM (obtained using \( E_D \)), was -100.6 ± 0.5 mV. After ouabain superfusion, \( E_K \) was -96.7 ± 0.3 mV (\( P < 0.001 \) compared to control). At the control \( E_m \), \( a_k = 124.2 ± 1.3 \) mM and the resultant \( E_K \) was -99.4 ± 0.3 mV. \( E_K \) decreased to -96.7 ± 0.2 mV (\( P < 0.001 \) compared to control) after ouabain superfusion.

It has been shown that the myocardial membrane acts much like a potassium electrode. Hence, it might be expected that the shift in the potassium equilibrium potential would parallel the measured shift in the membrane potential. However in our study, after 30 minutes of superfusion with ouabain, \( E_K \) decreased by 4.8 mV (Table 1) and the calculated value of \( E_K \) decreased by only 2.7 mV (from -99.4 to -96.7 mV). If one uses values obtained with \( E_D \) (Table 1), the ouabain-induced decrease of 5.1 in \( E_K \) was accompanied by a decrease in \( E_K \) of 3.9 mV (from -100.6 to -96.7 mV).

To be sure, the differences between the change in \( E_m \) and the calculated value of \( E_K \) and between the change in \( E_D \) and the calculated value of \( E_K \) are small, and one might question whether they are, in fact, real. The changes were found for every preparation studied, however, and this suggested to us that they were real and required further consideration, as follows: If a cell were functioning as an "ideal" potassium electrode and if \( a_g \) were unchanged, we can assume that the decrease in \( E_K \) should be comparable to the change in \( E_m \). Since it was not, we evaluated whether the measured transmembrane potentials recorded after ouabain superfusion might be the result not only of a decrease in \( a_k \), but also of an increase in \( a_g \), as predicted by Cranefield and Hoffman. To do this, we initially calculated the value of \( a_g \) using a predicted value of \( E_K \).

To determine a predicted \( E_K \), we first assumed that the decrease in \( E_K \) should be comparable to that of the \( E_m \). By subtraction, this gave a predicted value of \( E_K = -94.6 \) mV. When this predicted \( E_K \) was substituted into the equation for the \( K^+ \) equilibrium potential, Equation 2, we obtained a new calculated value of \( a_g \) of 4.3 mM. A similar calculation using the \( E_D \), before and after ouabain predicted an \( a_g \) of 4.2 mM. If the assumptions are valid, the results obtained suggest that in the presence of oua-

### Table 1: Effect of Ouabain on Purkinje Fiber Transmembrane Potentials

<table>
<thead>
<tr>
<th>Control Tyrode's solution</th>
<th>Ouabain (2 x 10^-4 M, 20 minutes)</th>
<th>Ouabain (2 x 10^-4 M, 30 minutes)</th>
<th>Tyrode's solution (60 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_D (-mV) )</td>
<td>89.8 ± 0.4 (24)</td>
<td>86.7 ± 0.4 (24)</td>
<td>84.7 ± 0.5 (24)</td>
</tr>
<tr>
<td>( E_m (-mV) )</td>
<td>83.6 ± 0.4 (24)</td>
<td>80.1 ± 0.7 (24)</td>
<td>78.8 ± 0.4 (24)</td>
</tr>
<tr>
<td>AP (mV)</td>
<td>123.9 ± 0.7 (24)</td>
<td>120.3 ± 0.6 (24)</td>
<td>117.6 ± 0.8 (24)</td>
</tr>
<tr>
<td>APD_{90} (msec)</td>
<td>227.3 ± 6.6 (24)</td>
<td>219.0 ± 6.3 (24)</td>
<td>209.8 ± 5.6 (24)</td>
</tr>
<tr>
<td>APD_{m} (msec)</td>
<td>343.1 ± 8.8 (24)</td>
<td>334.0 ± 8.2 (24)</td>
<td>323.5 ± 7.5 (24)</td>
</tr>
</tbody>
</table>

Control vs. ouabain, 20 minutes (\( P < 0.001 \)). Control vs. ouabain, 30 minutes (\( P < 0.001 \)). Ouabain 30 minutes vs. 60 minutes after discontinuing ouabain (\( P > 0.2 \)).

\( a_k \) is the maximum diastolic potential, \( a_g \) is the resting transmembrane potential, AP is the action potential amplitude, APD_{90} is the action potential duration at 90% repolarization, and APD_{m} is the action potential duration at 100% repolarization. Numbers are expressed as the mean plus or minus the standard error. The number of impalements is in parentheses.
FIGURE 3  Response of the potassium-sensitive microelectrode during determination of intracellular potassium activity. In both panels, the initial part of the trace was recorded with the microelectrode in the bulk superfusate. On penetrating the cell, the microelectrode followed the potential changes inscribed during the action potential. After discontinuation of the stimulus, the fiber gradually depolarized, and this permitted measurement of E_m. The stimulus then was reinitiated and, finally the electrode was removed from the cell. Panel A shows the potential change measured at the maximum diastolic potential (E_D) and the resting membrane potential (E_R). Panel B shows a preparation that developed automatcity when the stimulus was discontinued. E_R was measured at the activation voltage of the spontaneous beats.

bain the extracellular potassium ion activity at the surface of the cell membrane may be higher than that in the bulk superfusing Tyrode's solution. Such an accumulation of extracellular K^+ at the cell surface would result in a decrease in measured transmembrane potential. Since the change in measured transmembrane potential in our studies was smaller than that predicted by the change in E_K alone, one conclusion would be that an extracellular accumulation of potassium did in fact occur and that it depolarized the membrane to the measured value.

Discussion

The toxic effects of ouabain on the Purkinje fiber membrane potentials that occurred in our study are consistent with those observed by other investigators. These include a decrease in E_m, E_D, and AP amplitude, an increase in the slope of phase 4 depolarization and automatcity, and the occurrence of delayed afterdepolarizations. It has been shown that concentrations of ouabain which induce toxic changes in the canine ECG (i.e., the occurrence of ventricular premature depolarizations or tachycardia) alter Purkinje fiber AP characteristics so that E_m is 92% and AP amplitude is 94% of control. Hence, it appears that the changes in AP characteristics reported in the present study (E_m = 94% and AP amplitude = 95% of control) are in a range that is compatible with toxicity in situ.

The present studies demonstrate that there is a decrease in the value of a_k in Purkinje fibers exposed to a toxic concentration of ouabain for 30 minutes. This decrease, from 130.0 to 112.2 mm (13.7%), occurred concomitantly with a decrease in measured transmembrane potentials. If a Purkinje fiber is assumed to be a cylinder 30 μm in diameter and 100 μm in length, the calculated potassium loss over 30 minutes in our study was 7.1 pm/cm² per second. This estimated rate of potassium loss is of the same order of magnitude as previously reported values for potassium loss per beat. For example, for turtle heart muscle, Wilde and O'Brien estimated a net loss of 44.9 pm/cm² per beat while Humphrey and Johnson estimated a net loss of 4.5 pm/cm² per beat for rabbit heart.

Cranefield and Hoffman postulated that digitalis exerts its toxic effects by inhibiting the inward transport of potassium and causing, among other effects, an accumulation of potassium at the external surface of the cell membrane. This increased extracellular potassium would, in turn, increase the cell membrane permeability to potassium and result in a decrease in the AP duration. Further, the loss of intracellular potassium would result in a decrease in resting transmembrane potential. To test further the hypothesis that there may be extracellular potassium accumulation after exposure to ouabain, we performed six experiments in which we measured the potassium activity in the extracellular space with the same potassium-sensitive microelectrode used for the intracellular determinations. In these experiments, the mean value for a_k (using E_D) in beating Purkinje fibers before superfusion with ouabain was 4.6 pm. After 30 minutes of exposure to ouabain, a_k was 5.0 pm. Measurements of E_m were not made. Our calculations and preliminary measurements of a_k adjacent to the cell membrane, as well as the results of direct measurements of extracellular potassium accumulation by Kline and Morad and Cleeman and Morad in frog ventricular muscle, support the hypothesis that potassium can accumulate at the cell surface and reach a concentration in excess of the bulk phase or superfusate.

Potassium may be expected to accumulate in the extracellular cleft spaces in Purkinje fibers during phases 2 and 3 of the transmembrane action potential. Several factors could contribute to the net movement of potassium from these extracellular clefts. First, there could be a net passive diffusion into the bulk phase but there appears to be a physical limitation to the rate of such diffusion. Second, the potassium might be actively transported intracellularly by the Na^+-K^+ exchange pump.
If ouabain inhibits the Na\(^+-\)K\(^+\) exchange pump, clearly this might cause the observed increase in potassium concentration in the boundary layer or clefts. This hypothesis, that potassium accumulation occurs during normal repetitive activity, has been suggested by Baumgarten\(^2\) in experiments using dihydro-ouabain, 2 \(\times 10^{-5}\) M, on sheep Purkinje fibers. He showed that the postulated extracellular potassium accumulation was consistent with a shift in the \(i_{K}\) reversal potential to a more positive value. Further, a late inward current was observed, and this was expected because \(g_{K}\) is a function of \(a_{K}\) at the cell surface.

Observations reported by Cohen et al.,\(^1\) however, showed that the steady state value of \(a_{K}\) at the membrane surface of sheep Purkinje fibers may be reduced when the Na\(^+-\)K\(^+\) exchange pump is maximally active. Their evidence suggested that the \(i_{K}\) reversal potential frequently was negative with respect to the calculated potassium equilibrium potential. This resulted from their observation that the extracellular potassium activity was possibly lower than that of the bulk superfuse, or that the actual value of \(a_{K}\) may have been higher than the values previously assumed (See review and references in Muria et al.\(^3\)). This latter interpretation is unlikely in view of the direct measurement of \(a_{K}\) in the present investigation.

In sum, we have provided direct, quantitative evidence for the decrease in \(a_{K}\) that occurs during the maintenance of clinically relevant toxic concentrations of ouabain. We have provided qualitative evidence of a concurrent increase in \(a_{K}\). Our results are consistent with those of others who suggest that ouabain causes an increase in transient inward current\(^4\) and a decrease in \(i_{K}\). These changes decrease membrane potential, increase the slope of phase 4, and may activate a slow inward current and induce afterdepolarizations.

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