Membrane Currents and Tension in Cat Ventricular Muscle Treated with Cardiac Glycosides

By Terence F. McDonald, Hermann Nawrath, and Wolfgang Trautwein

ABSTRACT

The effect of cardiac glycosides on membrane currents and tension in cat ventricular muscle was studied using the single sucrose gap voltage clamp method. Complete tension-voltage and current-voltage relations were obtained in five preparations before and during treatment with dihydro-ouabain (DHO, 1.7 ⋅ 10⁻⁵M). After 1-2 minutes of DHO, the developed tension was 15% greater than control, but there was no change in either the slow inward (calcium) current (I<sub>Ca</sub>) or the level of the outward current flowing at the end of a 300-msec depolarization (I<sub>out</sub>). After 6-8 minutes of DHO, there was a 60% increase in developed tension, a noticeable increase in resting tension, a 20% decrease in I<sub>Ca</sub>, and a smaller increase in I<sub>out</sub>. It seems possible that the reduction of I<sub>Ca</sub> was due to a reduced driving force. In preparations treated with ouabain (5 ⋅ 10⁻⁷M, 3-5 minutes), developed tension was 45-150% greater than control with no change in I<sub>Ca</sub> or I<sub>out</sub> between −45 and +15 mV. We conclude that the inotropic action of these cardiac glycosides is not mediated by an increase in I<sub>Ca</sub>.

During the last 5 years, a growing body of evidence has accumulated suggesting that the slow inward (calcium) current (I<sub>Ca</sub>) is a vital link in the excitation-contraction coupling of mammalian ventricular muscle (1-4). This view has been further strengthened by the demonstration of a tight coupling between the calcium-carrying system and the tension in cat ventricular muscle (5). Thus, changes in I<sub>Ca</sub> must definitely be considered as a mechanism by which drugs exert positive or negative inotropic effects.

The two classes of inotropic agents most widely studied in cardiac muscle are the catecholamines and the cardiac glycosides. Epinephrine increases I<sub>Ca</sub> in Purkinje fibers (6) and frog atrial trabeculae (7). Similarly, norepinephrine increases I<sub>Ca</sub> in mammalian ventricular muscle; moreover, it has been concluded that this increase in calcium influx is of primary importance in the positive inotropic effect of the drug (8). A central question regarding the mechanisms by which the cardiac glycosides exert their inotropic effect on the myocardium is whether they increase I<sub>Ca</sub>. To explain the actions of cardiac glycosides on the cardiac action potentials of different species, Katz (9) has proposed an hypothesis that involves an increase in I<sub>Ca</sub>. Two abstracts have appeared dealing with changes in calcium-mediated action potentials during treatment of mammalian papillary muscles with cardiac glycosides (10, 11). Both of these reports noted decreases in the rate of rise, the overshoot, and the duration of these action potentials during inotropy. The characteristics of the calcium-mediated action potentials (10-13) are thought to be dependent on the amplitude and the time course of I<sub>Ca</sub>. But it should not be forgotten that such responses can only serve as a guide, since changes in outward current can also significantly affect the shape of these action potentials. We are only aware of two published voltage clamp studies on glycoside-treated cardiac muscle. 1

1 The review article by Lee and Klaus (24) attributes an unpublished study to H. Schobe, a reference which has been further recited (40). The original reference appears to have been an error (K. S. Lee and W. Klaus, personal communication).
increases with time, leading to an increase in the flow of current through the extracellular shunt resistance (16). Although the current wave form does not appear to be changed by such increases in shunt current, the recorded current magnitude will reflect more the shunt current amplitude than the true membrane current. For this reason, it is advantageous to minimize the time between control and experimental voltage clamps. With this requirement in mind, we studied the two cardiac glycosides dihydro-ouabain and ouabain, both of which have a rapid onset of action.

**Methods**

Hearts from adult cats of either sex were removed under ether narcosis and placed in warm oxygenated Tyrode's solution. Trabeculae or papillary muscles 0.2-0.7 mm in diameter and 3-5 mm in length were dissected from the right ventricle and mounted in a bath having three compartments. The middle compartment (sucrose) was 1.5-2.3 mm in width and was separated from the right (test) and left (KCl) compartments by rubber sheets. Muscles were drawn from the test compartment to the KCl compartment through holes of appropriate diameter burned through the two rubber sheets.

The test compartment was perfused with Tyrode's solution of the following millimolar composition: NaCl 140.0, KCl 3.0, MgCl$_2$ 1.0, CaCl$_2$ 1.8, NaHCO$_3$ 12.0, NaH$_2$PO$_4$ 0.4, and glucose 5.0. The middle compartment was perfused with sucrose solution containing 304 mm sucrose (enzymatic grade) and 0.01 mM CaCl$_2$ dissolved in deionized water. The KCl compartment was perfused with a solution identical to that of the test compartment except that the NaCl was replaced with KCl. Tyrode's solutions were equilibrated with 95% O$_2$-5% CO$_2$ and activated after 300 msec when the outward current is about 55% activated. If the current record consisted of an $I_{Ca}$ of amplitude 100 on which was superimposed a time-dependent outward current of amplitude 20, then $I_{Ca}$ measured as in Figure 1 would be about 10% too large. From the foregoing discussion, it can be seen that the error in measuring $I_{Ca}$ at 300 msec depends on the time constants of the two currents involved and on their relative amplitudes. Measurements of these parameters at different potentials (McDonald and Trautwein, unpublished observation) lead us to conclude that $I_{Ca}$ estimated as shown in Figure 1, is 5-10% too large at potentials below 0 mv and 10-20% too large at potentials above 0 mv.

The outward current reported in this paper ($I_{out}$) refers to the difference between the current at the holding potential and that flowing after 300 msec at the new potential. As such, this measurement includes an instantaneous, voltage-dependent, time-independent current as well as a component which increases with time (see ref. 17 for outward current analysis in Purkinje fibers). Since the latter component has a rather long activation time constant, the measurement at 300 msec does not estimate the maximum outward current at a given potential. Nevertheless, any large changes in outward current brought about by treatment with cardiac glycosides is detected.
DRUGS

Dihydro-ouabain (DHO) and ouabain were dissolved in distilled water and added to the Tyrode's solution as required.

Results

The calcium necessary for contraction may be released from intracellular stores that can be rapidly filled or depleted. When these stores are overfilled, there may be a reduction in \( I_{Ca} \) amplitude due to a reduction in the calcium equilibrium potential (\( E_{Ca} \)). If cardiac glycosides act to fill these stores, one would expect a reduction in \( I_{Ca} \). However, it is important to know whether this effect occurs pursuant to an earlier increase in the magnitude of \( I_{Ca} \) or whether another mechanism must be sought. For this reason, we thought that it was important to compare control voltage clamp records with those obtained on the first sign of glycoside-induced inotropy as well as with those obtained following longer glycoside exposure.

Time-Dependent Changes in Membrane Currents and Tension during Treatment with DHO

Figure 2 shows voltage clamp records obtained before and 1 minute after treatment with DHO (1.7 \( \times 10^{-5} \)M). The holding potential was -53 mv and the membrane was clamped for 300 msec to potentials between -33 and +27 mv at a rate of 12/min. The drug increased tension by about 15% with negligible effects on the amplitudes of \( I_{Ca} \) and \( I_{out} \).

Longer exposure to DHO produced a greater inotropic action and also, within 10 minutes, an increase in the resting tension. Figure 3 compares voltage clamp records before and after 10 minutes of treatment with DHO (1.7 \( \times 10^{-5} \)M). The holding potential was -55 mv, and 300-msec depolarizing steps were imposed to potentials between -15 and +25 mv at a rate of 12/min. The current and tension records have been photographically superimposed with the lower trace in each case being the control record. In this preparation, there was a marked increase in resting tension on which was superimposed a developed tension whose peak amplitude was nearly twice that of control. The current records indicate that \( I_{Ca} \) was reduced by about 40% and that the amplitude of \( I_{out} \) at 300 msec was slightly greater than control.

The mean results of five complete experiments with DHO are presented in Figure 4. The same protocol was used in each experiment. From a holding potential of -55 mv, the membrane was depolarized for 300 msec to potentials between -45 and +25 mv. Following the control clamp run (solid circles), records were taken 1-2 minutes (open circles) and 6-8 minutes (open triangles) after the addition of DHO (1.7 \( \times 10^{-5} \)M). Treatment with DHO for 1-2 minutes increased the maximum tension by nearly 20%, but no changes were noted in the amplitude of \( I_{Ca} \) or \( I_{out} \). After 6-8 minutes with DHO, muscles produced about 60% more tension than control. Although not shown in the figure, the increase in resting tension at this time was 10-20% of the maximum control twitch tension. Maximum \( I_{Ca} \) was reduced by about 20%, and there was perhaps a 10% increase in \( I_{out} \).

These changes in current-voltage relations can account for the changes in action potential configuration seen with DHO. Typical records obtained during a voltage clamp experiment are shown in Figure 5. After 1 minute of DHO (1.7 \( \times 10^{-5} \)M), the plateau phase of the action potential was unchanged, and the action potential duration was increased by a few percent. The resting tension was...
It is also important to know whether the drug changed the time course of $I_{Ca}$, thereby allowing a smaller or a greater transfer of charge across the membrane. As discussed in Methods, the slow decline of current following the second inward peak consists of both the inactivation of $I_{Ca}$ and the activation of an outward current. Although this situation makes it impossible to get the correct $I_{Ca}$ inactivation time constant from a 300-msec current record, a plot of the current at -10 mV provides a reasonable estimate because $I_{Ca}$ at this potential is large relative to $I_{out}$ and its time course is about five times faster than that of $I_{out}$ (McDonald and Trautwein, unpublished observation). Semilogarithmic plots of the current in the control and the 3-minute ouabain records are also presented in Figure 6, and the values are well fitted in both cases by lines of identical slope ($\tau = 85$ msec).

unchanged, and the developed tension was nearly 25% greater than control. After 11 minutes of DHO, there were significant changes in action potential configuration. The resting potential was reduced by a few millivolts as was the overshoot, and the duration at 25% repolarization was only 60% that of control. The resting tension was increased by 50 mg, and developed tension was approximately twice the control value. These changes are in agreement with earlier studies on DHO: Reiter (18) has reported a small initial increase followed by a decrease in the action potential plateau duration of guinea pig papillary muscle treated with $10^{-5}$ M DHO.

EFFECT OF OUABAIN ON MEMBRANE CURRENTS AND TENSION

The other cardiac glycoside investigated in this study was ouabain. Successful experiments were obtained in three preparations. In one experiment, the muscle was clamped from -50 to -10 mV for 300 msec before and during a 3-minute exposure to ouabain ($5 \times 10^{-5}$ M). The pulsing rate was 12/min. Figure 6 shows clamp records and tension before and after 3 minutes with ouabain. There was no increase in resting tension, developed tension increased by 45%, and there were no detectable changes in $I_{Ca}$ or $I_{out}$.

**Summary of five voltage clamp experiments with dihydro-ouabain (DHO) ($1.7 \times 10^{-5}$ M).** The symbols represent the mean values of $I_{out}$, $I_{Ca}$, and developed tension before (solid circles) and after 1-2 minutes (open circles) or 6-8 minutes (triangles) with the drug. The abscissa refers to the membrane potential during a 300-msec depolarization.
Records from three preparations treated with DHO gave similar results.

Another preparation was clamped to potentials between −45 and +15 mV for 300 msec every 5 seconds before and after 5 minutes of treatment with ouabain (5 × 10⁻⁷ M). The results are depicted in Figure 7. Although there was no change in ICa over the entire voltage range, developed tensions were more than twice the control values. At this time, the increase in resting tension was approximately 5 mg and Iout was marginally smaller than control.

Dependence of tension on ICa before and after treatment with cardiac glycosides

The charge carried by ICa during a typical voltage clamp step does not appear to be great enough to allow a role for ICa in directly activating the contractile machinery (3). For this and other reasons, ICa is thought to influence contractile...
CARDIAC GLYCOSIDES, CALCIUM CURRENT, AND TENSION 679

Discussion

Short exposures to DHO (1.7 x 10^-5M for 1-2 minutes) or ouabain (5 x 10^-7M for 3-5 minutes) increased the contractile force of cat ventricular muscle without changing ICa or the level of Iout at the end of a 300-msec depolarizing clamp. Muscles treated with DHO for 6-10 minutes had an increased resting tension, a reduced ICa, and a slightly increased Iout. The reduction in ICa and the smaller increase in Iout appear to explain the reduced plateau amplitude and duration of the action potential observed after longer exposures to DHO.

There are many possible explanations for the reduction in ICa after 6-10 minutes of exposure to DHO, including (1) a shift in the negative direction of the steady-state inactivation variable (f), (2) a decrease in the maximum calcium conductance (gCa), and (3) a reduction in the calcium equilibrium potential (Eca). Any one or all of these mechanisms could play a role. However, in view of the coincidence between the increase in resting tension and the decrease in ICa, the third explanation, a reduction in Eca, appears to be the strongest candidate.

The results of this study point up a clear difference between the mechanism of action of these cardiac glycosides and that of the catecholamines. In Purkinje fibers (6) and atrial trabeculae (7), epinephrine increases the amplitude of ICa. In

FIGURE 8
Relation between developed tension and ICa amplitude. The data are from 300-msec depolarizing steps to potentials between -35 and +25 mv and are plotted in double-log fashion. A: Data from a single experiment before and after 6 minutes of DHO (1.7 x 10^-5M). B: Maximum amplitude of ICa was given a value 1 and the tension at that potential was also assigned a value 1. Other amplitudes during a control run or a glycoside run were normalized accordingly. Values are means z SE (n = 7 for control, n = 12 for DHO and ouabain runs). All lines fitting the data points have a slope of 1. The arrows in A emphasize values that deviate from a slope of 1, i.e., values that indicate an increase in tension despite a decrease in ICa at +25 mv.

strength by triggering the release of activated calcium from intracellular stores (3-5). The mechanism by which this release occurs is not known, but what is known is that there is a tight coupling between the amplitude of ICa and the accompanying twitch strength (5).

This fact suggests an amplification factor or gain between the magnitude of ICa and the quantity of calcium released from the intracellular stores. DHO and ouabain increased contractile force but not ICa. The foregoing suggests that the glycosides increase the quantity of calcium available for release, change the relation between ICa and release in such a way that smaller increments in ICa result in proportionally larger releases of activator calcium, or both.

By considering ICa as the stimulus and tension as the response, dose-response curves can be constructed from the data. Figure 8A shows a double-log plot of tension versus ICa amplitude before and after 6 minutes of treatment with DHO. The lines drawn through the experimental values have slopes of 1 and the fit suggests a linear relation, which remains unchanged with DHO treatment. Figure 8B summarizes the results of seven experiments, five with DHO and two with ouabain. Data were normalized by assigning a value of 1 to the maximum amplitude of ICa during a voltage clamp run and a value of 1 to the tension at that potential. All other values were then expressed as fractions of these maximums. There is no significant difference between control and glycoside data, and the mean values are well described by a line with a slope of 1.

Although the data appear to indicate a perfectly linear relation between tension and ICa, it must be considered that tension continued to increase slightly despite a decrease in ICa between +15 and +25 mv (arrows on Fig. 8A) and that the amplitude of ICa was probably overestimated by 5-20% within the range of -5 to +25 mv (see Methods). Despite these limitations, the data indicate that glycosides produce a simple shift to the left in the relation between tension and ICa. In the frog heart, ouabain has been reported to shift the (KCl) depolarization-tension curve by about 30 mv toward the resting potential (19). We have no information on whether such a shift occurs in the mammalian ventricle, but it seems certain that the threshold for activation of ICa (and tension) was not shifted to any large degree by ouabain (Fig. 7).
ventricular muscle, the norepinephrine-induced increase in \( I_{Ca} \) has been shown to be due to an increase in the maximum calcium conductance \((g_{Ca})\) (8). These voltage clamp data are consistent with \(^{45}\)Ca flux studies demonstrating that catecholamines increase the influx of \(^{45}\)Ca in contracting guinea pig atriums (20, 21) and rat atriums (22).

Muscles treated with cardiac glycosides for longer periods of time had an increased resting tension and a reduced \( I_{Ca} \) amplitude, suggesting an accumulation of intracellular free calcium. These facts are in agreement with the finding that toxic doses (contracture) of cardiac glycosides increase myocardial calcium content (23, 24). Prior to changes in resting tension but at a time of positive inotropy, there were no changes in the amplitude or the time course of \( I_{Ca} \). This situation indicates an increase in the availability of activator calcium in keeping with \(^{45}\)Ca flux data showing that cardiac glycosides increase the exchangeable myocardial calcium fraction (24, 25). What remains contentious is how this increase in the exchangeable fraction comes about. Some investigators feel that an increase in calcium influx is responsible, but others hold that there is a redistribution of intracellular calcium. Langer and Serena (26) have measured an increase in \(^{45}\)Ca influx and a gain in total muscle calcium of \(10^{-4}\) moles/kg wet weight. They feel that this gain of intracellular calcium is related to an increase in intracellular sodium due to inhibition of the sodium pump. It has been calculated (27) that a \(5\) mM increase in intracellular sodium will increase muscle calcium by \(10^{-5}\)M through the Na-Ca exchange mechanism (28). That the inotropic effect of cardiac glycosides has been shown by many (see ref. 24) to be unaccompanied by changes in intracellular sodium does not completely negate this hypothesis, because a change of 3 mM in total muscle sodium can be difficult to distinguish from control values. Bailey and Sures (29) have also reported an increased calcium influx and a net gain in muscle calcium. However, their net gain is associated with a compartment different from that which has been postulated to support contraction (30). Somewhat analogous is Nayler’s finding (31) that ouabain increases the amount of superficial Ca\(^{2+}\) available for displacement by La\(^{3+}\).

A different view is that the increased fraction of exchangeable calcium during treatment with cardiac glycosides is brought about by a redistribution of intracellular calcium, possibly by mobilization of otherwise tightly bound calcium (24). This mode of action requires neither an increased calcium influx nor an increased myocardial calcium content. Results supporting this concept have been reported in the literature (see ref. 24). In fact, there are many reports suggesting that therapeutic concentrations of cardiac glycosides significantly reduce the myocardial calcium content (23, 32). Considering that the total calcium content is in the millimolar range, that the inotropic effect may require only about \(10^{-4}\)M calcium, and that \(^{45}\)Ca flux data are notoriously difficult to interpret, it is little wonder that a consensus has not yet been reached.

Finally, it should be noted that inotropically active doses of ouabain have no effect on the contracture tension of canine trabecular muscle having highly permeable (EDTA) cell membranes (33). Nor does \(10^{-4}\)M strophanthin increase either cyclic contractions or tonic contraction in skinned rat and rabbit ventricular cells (34). These results argue against, but do not exclude in untreated intact cells, a mechanism of intracellular redistribution of calcium.

The effect of cardiac glycosides on \( I_{out} \) was of interest in another context. Both DHO and ouabain markedly reduce \( I_{out} \) over the entire voltage range in sheep Purkinje fibers (35). Together with further evidence involving changes in temperature (36) and changes in external sodium concentration (37), these findings indicate that a significant fraction of \( I_{out} \) is generated by an electrogenic sodium pump. Within 1 minute of treating Purkinje fibers with DHO (\(2\times10^{-5}\)M), the inhibition of the electrogenic outward current can shift the resting potential from \(-85\) to \(-55\) mV (35). Similar shifts have been seen after 10 minutes with \(10^{-4}\)M ouabain. Large reductions in \( I_{out} \) were not seen in the present work, and this finding is consistent with other evidence which suggests that under normal conditions such a large contribution of an electrogenic sodium current does not exist in ventricular fibers. The decline in the resting potential of ventricular muscle treated with moderately high doses of cardiac glycosides occurs in a slow progressive manner (38–40) which is more consistent with a gradual loss of internal potassium and a reduction in \( E_K \) than it is with the inhibition of an electrogenic current component. Even \(10^{-4}\)M ouabain only reduces the resting potential of guinea pig ventricular muscle by 5–10 mV over a 20-minute period (41), a decline which can be accounted for by the loss of internal potassium (42).

Acknowledgment

We thank Mr. H. Ehrler for skillful electronics support and Ms. R. Quint for excellent technical assistance.
References

1. BEELEY GW JR, REUTER H: Relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. J Physiol (Lond) 207:211-290, 1970

2. OCHI R, TRAUTWEIN W: Dependency of cardiac action potential on depolarization and slow inward current. Pfluegers Arch 323:187-203, 1971


5. TRAUTWEIN W, McDONALD TF, TRIPATHI O: Calcium conductance and tension in mammalian ventricular muscle. Pfluegers Arch 354:55-74, 1975


7. VASSORT G, ROGERS LV, GUITHER D, SAUVIT MP, CORABEUF E, GARGOUIL YM: Effects of adrenaline on membrane inward current during the cardiac action potential. Pfluegers Arch 309:70-81, 1969

8. REUTER H: Localization of alpha adrenergic receptors, and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. J Physiol (Lond) 242:429-451, 1974

9. KATE AM: Increased Ca** entry during the plateau of the action potential: Possible mechanism of cardiac glycoside action. J Mol Cell Cardiol 4:87-89, 1972

10. SCHOLZ H: Influence of adrenaline and digoxigenin on Ca-dependent changes of membrane potential and contraction in mammalian myocardium (abstr). Naunyn Schmiedebergs Arch Pharmac 286:554, 1970


37. ISENBERG G: Cardiac Purkinje fibers: Outward currents in Na**-free solutions. Pfluegers Arch, in press


39. TEN EICK RE, BASSITT AL, OKITA GT: Dissociation of electrophysiological and inotropic actions of strophani...
thidin-3-bromoacetate: Possible role of adenosine triphosphatase in the maintenance of the myocardial transmembrane Na⁺ and K⁺ gradients. J Pharmacol Exp Ther 185:12–23, 1973


41. McDonald TF, Macleod DP: Metabolism and the electrical activity of anoxic ventricular muscle. J Physiol (Lond) 229:559–582, 1973

42. McDonald TF, Macleod DP: Effect of 2,4-dinitrophenol on the electrical and mechanical activity, metabolism and ion movements in guinea-pig ventricular muscle. Br J Pharmacol 44:711–722, 1972
Membrane currents and tension in cat ventricular muscle treated with cardiac glycosides.
T F McDonald, H Nawrath and W Trautwein

Circ Res. 1975;37:674-682
doi: 10.1161/01.RES.37.5.674

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1975 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/37/5/674

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/