Ouabain Effects on Cardiac Contraction, Action Potential, and Cellular Potassium

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The mechanisms by which cardiac glycosides increase the strength of the heartbeat are far from clear. Digitalis glycosides have no major influence on cell metabolism, nor on the contractile proteins. At sufficiently high concentrations they interfere with the movement of ions across cell membranes and it is tempting to assume that the inotropic action of glycosides is a consequence thereof. A close relationship between potassium transport and the inotropic effect is suggested by four types of results: 1. At an increased extracellular potassium concentration, \([K]_o\), the glycosides are less effective both in slowing down potassium uptake and in increasing contractile strength. 2. At reduced \([K]_o\) the action of glycosides is more pronounced in depressing K influx and in augmenting the strength of contraction. 3. During hypothermia potassium exchange is reduced, in particular potassium uptake. Reduction of temperature by itself results in stronger contractions; glycosides then have no additional effect, either on the potassium flux or on the mechanical events. 4. With a high cardiac rate, when the turnover of potassium is expected to be large, smaller concentrations of glycosides are effective in producing a given inotropic effect.

Numerous experiments have been performed with the aim of correlating the intracellular potassium concentration, \([K]_i\), with the contractile strength. As a result of exposure to cardiac glycosides \([K]_i\) has been reported unchanged, reduced or increased. Contradictory results also were reported for the changes of K influx; K influx was found decreased, unchanged or increased. Recent findings seem to support the view that only toxic glycoside concentrations lower \([K]_i\), whereas "therapeutic" doses leave \([K]_i\) unchanged or increased.

The changes in electrical behavior of myocardial fibers during glycoside exposure have been explained successfully on the basis of an increased potassium conductance of the cell membrane. Since the inotropic action of glycosides develops when the shape of the action potential is still normal the increase of the "passive" membrane conductance for K ions is not likely to be responsible for the digitalis effect on contraction amplitude. Several authors describe a rise in the rate of K efflux during glycoside exposure which would agree with the finding of a higher membrane conductance. Others, however, have found K efflux unchanged or even decreased.

In view of these conflicting experimental results it seemed worth while to reinvestigate the problem using methods which would give simultaneous data for a) isometric tension, b) transmembrane potential, and c) intracellular K content.

Methods

Ungulate hearts (sheep, calf) were obtained at the slaughterhouse and transported to the laboratory in Tyrode solution at 4°C. Trabeculae carnaeae, 5 to 10 mm long and about 0.5 mm in diameter, were removed from the right ventricle. These are muscle bundles that run free alongside the ventricular wall and need cutting only at their ends. Using silk threads, one end was tied to the wall of the specimen chamber, the other end of the bundle was fixed to the protruding anode pin of a mechno-electrical transducer tube, RCA 5734 (fig. 1). By moving the transducer forward or backward, parallel to the axis of the muscle strip, resting tension was set at 250 to 500 mg. The tissue bath had a capacity of 0.3
Perspex bath for simultaneous measurements of membrane potential, contraction, and K\(^{2+}\) content of a muscle strip. The tissue bath of 0.3 ml volume is embedded in a Perspex case through which water at 37°C flows with the aid of a pump (not shown). Inflow from pump upper right; outflow to pump lower left. The muscle strip is fixed to the wall of the specimen bath and to the anode pin of the electro-mechanical transducer with two silk threads. The intracellular action potentials are recorded by impaling single fibers from above. The light of a bulb, placed outside the water case, is directed to the tissue bath by a Perspex bar. The scintillation detector is placed underneath the preparation, within a cylindrical opening in the water case. Two Perspex foils 0.3 mm thick and an air channel 5 mm long separate the muscle strip from the \(\beta\)-crystal of the scintillation detector. Further explanations are in the text.

The modified Tyrode solution had the following composition (mM/L): Na 149.8, K 4.05, Ca 1.8, Mg 0.5, Cl 145, HCO\(_3\) 11.9, H\(_2\)PO\(_4\) 0.32, and glucose: 5.6. It was equilibrated with 95% O\(_2\) and 5% CO\(_2\). The pH of the solution was 7.1. The concentrations of ouabain were 1 to 5 \(\times\) 10\(^{-5}\) M/L.

The preparations were stimulated by means of external electrodes from a Grass S4 stimulator at a rate of 1 per second. Membrane potentials were measured through intracellular microelectrodes. No attempt was made to determine an average value of the resting and action potentials. Impalements were considered satisfactory when a stable resting potential of not less than 80 mV was recorded during several beats. Stimulation was not interrupted for the purpose of introducing electrodes. A dual beam oscilloscope (Tektronix 502) was used to display membrane potentials and isometric tensions.

K\(^{38}\)Cl solutions were obtained from K\(^{13}\)CO\(_3\) (Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland) by titrating to pH 7.0 with 0.1 M HCl. Specific activity was 80 mc per g K\(^{13}\)CO\(_3\). The radioactive test solution with a K concentration of 4.05 mM/L was prepared by adding K\(^{13}\)Cl to a K free Tyrode solution. Radioactivity was measured as \(\beta\)-radiation using a Tracerlab scintillation detector (P 20C) which was mounted underneath the tissue bath (fig. 1). To reach the beta crystal (SPB-1S) the rays had to penetrate the Perspex bottom of the bath (0.3 mm), an air channel (5 mm), and a Perspex foil (0.3 mm) separating the scintillation detector from the water case. Radiation from both ends of the preparation was absorbed by the water layer between the tissue bath and \(\beta\)-crystal. The background reached 3 to 4 counts per minute (cpm).

**Results**

**EFFECTS OF PROLONGED PERFUSION ON THE VENTRICULAR MUSCLE**

It was noticed in preliminary experiments that the low potassium concentration of the normal Tyrode solution (2.7 mM/L KCl) often led to a slow deterioration of the preparation after incubation periods of some 12
to 24 hours. Parallel with a progressive depolarization of the cell membrane both the excitability and the contractility of the muscle strips were lost. When potassium concentration of the bath was increased to 4.05 mM/L the membrane resting potentials remained stable and the duration of the action potentials was found unchanged or only slightly decreased for a period of more than 24 hours. The contraction amplitude of untreated preparations followed various time courses. As a rule the contraction strength decreased during the first 3 to 6 hours, thereafter reaching a new steady level. In other experiments the contraction amplitude decreased slightly throughout the experiment. These findings suggest that, under in vitro conditions, the contraction mechanism is more sensitive than the electrical behavior.

In order to detect possible net changes in the intracellular potassium content \([K_i]\) of the trabecular muscle preparations occurring during the prolonged stay in the tissue bath, the following tracer experiments were performed: The cellular potassium of the muscle strips was equilibrated with radio-potassium in a 4.05 mM/L \(^{42}\text{K}\)Tyrode solution. When a steady state was reached at the end of 4 to 5 hours, \(^{42}\text{K}\) influx equal to \(^{42}\text{K}\) efflux, the radioactivity of the preparation could be taken as representative of the cellular potassium content. Any gain or loss in radioactivity of the preparation thus indicated a net change in \([K]\).

With an extracellular potassium concentration of 4.05 mM/L and in the absence of ouabain, \([K]\) remained at a constant level for up to 24 hours (2 experiments). Other investigators working with even thicker bundles under comparable conditions found that the potassium concentration in the fiber water remained constant and relatively high (170 mM/L) over 2 hours.\(^{17}\)

**Dose-effect relationship**

It has been shown previously\(^{45}\) that relatively high ouabain concentrations (10\(^{-7}\) to 10\(^{-6}\) M/L) lead to progressive and irreversible changes of the resting and action potentials. For instance, with a concentration of 10\(^{-6}\) M/L excitability was irreversibly lost at the end of about 60 minutes.\(^{46}\) In most of the in vitro experiments reported in the literature, the glycosides were used at concentrations which do not permit the system to approach a new steady state. Figure 2 shows the time course of the corresponding loss of intracellular potassium. A decrease from 100% to 80% took 35 minutes at a high ouabain concentration and a relatively low extracellular K concentration (10\(^{-6}\) M/L; 2.7 mM/L); a comparable loss took 5 to 8 hours when conditions were chosen which corresponded more closely to those of the experiments reported in the present paper (10\(^{-8}\) M/L; 4.05 mM/L).

**Figure 2**

Effect of ouabain on potassium loss. At zero time the trabecular muscles had been equilibrated with \(^{42}\text{K}\)Tyrode solution. Ouabain was then added, \([K_i]^{15}\) being kept as before. Each point on the graph represents the radioactivity of the muscle after a five-minute washout of the extracellular space. The experimental values are fitted by exponentials. Their dotted parts give a rough idea of \([K]\) to be expected during a prolonged exposure to ouabain.
The sensitivity of the muscle strips (using low ouabain concentrations, $10^{-8}$ M/L) compared rather well with that of human erythrocytes, the embryonic duck heart, chick cardiac cells in tissue culture, isolated rabbit atrium and transport ATPase of both human erythrocytes and guinea pig hearts.

**Time Course of the Action Potential and of Isometric Tension**

The addition of ouabain ($1 \times 10^{-8}$ M/L) to the tissue bath had the expected positive inotropic effect on the trabecular muscle preparation. The mechanical changes observed in the first 3 to 5 hours were not associated with any marked drug effects on the size or duration of the action potentials (fig. 3A). Long perfusion times of 7 to 12 hours, however, shortened the plateau of the action potential (phase 2) appreciably and decreased the slope of the repolarization (phase 3), but produced little change in the amplitude of the action potential (fig. 3B). The membrane resting potentials were unchanged. The observation that continuous ouabain perfusion for up to 12 hours resulted only in a shortening of the electric systole of the muscle strip seems to justify the assumption that nontoxic inotropic ouabain concentrations were reached.

The time from the triggering of the contractile process to attainment of peak isometric tension ($t_p$) and the duration of the over-all contraction either remained constant or shortened during an increase of contraction strength. The developed control tension of the trabecular muscle preparations ranged from 5 to 15 g/cm² cross section. Although the contraction amplitude increased by as much as 400% during the first 3 to 5 hours of the ouabain action, $t_p$ and the over-all contraction time did not change (fig. 3A). These two parameters decreased only after a prolonged glycoside exposure (7 to 12 hours) at a time when the action potentials showed marked shortening (fig. 3B). The increase in contraction strength, together with an unchanged electrical behavior of the preparation in the first hours of ouabain action, reveals a certain independence between the electrical events and the contractile process, as is known from the positive inotropic effect caused by increasing the resting fiber length of the myocardium. The shortening of both the action potential and the mechanogram, on the other hand, demonstrates that the action potential duration is at least one factor determining $t_p$ and the over-all contraction time.

**Temporal Parallelism Between Inotropic Glycoside Action and Cellular Potassium Content**

When the radioactivity of a trabecular muscle was followed as a function of time (fig. 4) a constant counting rate was reached a few hours after replacement of an inactive Tyrode solution by K¹² Tyrode. A constant counting rate indicates that K¹² influx is equal to K¹² efflux, and that the intracellular K content stays constant. The admixture of ouabain, $10^{-8}$ M/L, resulted (fig. 4) in a net potassium loss down to about 80% of the initial value, over a period of 5 to 8 hours. Simultaneously, the contractile strength increased. A net loss of potassium may result from a lower rate of uptake, a higher rate of loss, or a combination thereof. From earlier experiments with higher ouabain concentrations ($10^{-6}$ M/L), it can be concluded that
Effects of ouabain on cardiac contraction, action potential, and cellular potassium. Above: transmembrane action potentials and isometric contractions, measured simultaneously. The time of recording may be identified by referring the number in the upper left hand corner to the number of the vertical bar in the lower row. Time calibration for potential and tension records: 200 msec. Below: K⁺ content of a trabecular muscle (counts per minute) and contraction amplitude, both as a function of time. At the end of an equilibration period of seven hours, the addition of ouabain resulted in a loss of potassium. A new steady level, some 20% below the initial one, was reached at the end of six hours. In parallel to the potassium loss there was an increase in contraction strength to a new plateau. The membrane resting and action potentials were not appreciably changed throughout the experiment.

FIGURE 4

Increase of contraction strength plotted against decrease in [K]ᵢ. Control values at the moment of drug addition were taken as 100%. Data from 10 experiments, obtained at different times after exposure to ouabain, 10⁻⁸ to 10⁻⁷ M/L. Regression line fitted according to the method of least squares.

the net loss was the result of a decreased uptake. An exponential drop to a new steady level would be expected if a given glycoside concentration inhibited K uptake by a limited amount and if the degree of inhibition were constant during the whole period of drug exposure.

A temporal parallelism between the loss of [K]ᵢ and the increase of contraction strength was evident in each of 10 experiments. In figure 5 the results were plotted in a different way: increase of contractile strength against decrease in [K]ᵢ. As might be predicted, there was a pronounced tendency for contractile strength to rise when [K]ᵢ fell. It seems important to stress that an inotropic effect never corresponded to a gain of intracellular potassium.

A [K]ᵢ loss of more than 30% during perfusion with high concentrations of ouabain (10⁻⁷ to 10⁻⁶ M/L) was followed regularly by deterioration of the electrical and mechanical behavior of the trabecular muscle preparations. In the course of perfusions with 2.7 mM/L KCl Tyrode solutions the addition of even low ouabain concentrations (1 to 5 × 10⁻⁸ M/L) was usually accompa-
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The reversibility of the glycoside effect was necessary to take measurements over a considerable length of time. It is for this reason that the evidence for recovery after the ouabain exposure rests on the results of no more than 3 experiments.

Discussion

The radioactivity of the preparation is a measure of K\textsuperscript{i2} content. A decrease of the counting rate at a time when the specific activity is constant may signify shrinking of the cells at the same K concentration, or it may signify a decrease of intracellular K concentration at the same cell volume. It has been assumed throughout this paper, although there is no direct evidence, that the radioactivity is a measure not only of K\textsuperscript{i2} content but also of K\textsuperscript{<2} concentration.

With high concentrations of ouabain (10\textsuperscript{-6} M/L) and low extracellular potassium concentrations (2.7 mM/L) a cellular potassium loss of 20% occurs in approximately 35 min-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Borderline concentration of ouabain and reversibility of the glycoside effect. Legend as in figure 4, except for the time calibration mark in the upper row: 500 msec. At the end of the equilibration period the addition of ouabain (10\textsuperscript{-8} mM/L) was followed neither by a change in cellular potassium content nor by an increase in contraction strength. An increase in the ouabain concentration by a factor of 5 brought about, simultaneously, a potassium loss and an increase of the contraction amplitude. The initial cellular potassium content was regained in a glycoside-free radioactive Tyrode solution after eight hours. A second exposure to ouabain had essentially the same effects as the first one. However, the shapes of the contraction and the action potential were not completely normalized after the second drug exposure.}
\end{figure}
utes. With lower concentrations of ouabain (10^{-8} M/L) and higher potassium concentrations (4.05 mM/L) a 20% loss takes 5 to 8 hours. This difference in magnitude of effect may be explained by a dose dependent inhibition of potassium uptake.

When in the absence of ouabain [K]_o is varied over a fairly wide range (1 to 50 mM/L), the intracellular K concentration stays essentially constant near 170 mM/L of fiber water. The rate of K influx and that of K efflux, however, increase with rising [K]_o values. A possible hypothesis to account for the role of [K]_o in setting the sensitivity of the tissue to ouabain is as follows: at a given concentration, the drug may decrease K influx by a given amount rather than by a given percentage, while initially leaving K efflux unaltered. At low values of [K]_o, the influx inhibition would thus be relatively important, in terms of percentage; and a new flux equilibrium (K efflux = K influx) would require a relatively low [K]_i.

Any attempt to explain the interaction between ouabain and potassium encounters a number of difficulties. Thus, a) Quantitative information concerning the contribution of 'active' influx to the total K influx into cardiac tissue is missing. b) Studies of ATP splitting by cell fragments demonstrate a competition between ouabain and potassium, suggesting that the absolute amount of inhibition of 'active' K uptake (in pM cm^{-2} sec^{-1}) caused by a given ouabain concentration may even be reduced when [K]_o is increased. c) Lowering [K]_o, as well as adding ouabain, depresses the rate of 'active' sodium extrusion, pointing to intracellular sodium concentration as an additional factor in setting [K]_i. d) Several reports claim that ouabain initially decreases the 'passive' K exchange, the experimental basis being a drop of K efflux or a fall of the membrane conductance.

The time course of the change in [K]_i during the period of drug exposure and after the omission of the drug from the bathing solution may be compared to the time course of uptake or release of radio-K in a ouabain-free solution (figs. 4 and 6). Upon addition or removal of ouabain, [K]_i starts to drop or rise with no measurable lag. The rate constants by which the new [K]_i levels are approached correspond roughly to the rate constants for K efflux or release in a ouabain-free medium. Therefore, the time course of [K]_i can be adequately explained by saying that the ouabain effect on changing K uptake is instantaneous and that a new steady state is reached at the end of a few hours, when K efflux equals the new rate of K influx. To fulfill this condition [K]_i will drop along a roughly exponential curve if K influx is decreased instantaneously; it will rise slowly when K influx returns to normal. The reversibility of the effects of cardiac glycosides so far reported for the embryonic duck heart and cultured embryonic chick cells also holds true for the mammalian heart muscle strip according to previous and present results.

The inotropic effect of ouabain during the first hours of exposure to low concentrations occurs without an alteration in the duration of the action potential and without any pronounced change in the time to peak tension (t_p). These observations are consistent with the assumption that the duration of the so-called 'active state' is not affected. After about 10 hours of ouabain exposure the action potential usually shortens while t_p decreases. Using relatively high ouabain concentrations a 'diphasic effect' has been reported for the duration of the action potential (initial lengthening, late shortening). This has been successfully correlated with an initial decrease and a later fall of K conductance as demonstrated for Purkinje fibers, but not as yet for myocardium. While it is conceivable that K conductance keeps changing all through the administration of ouabain and does so in a diphasic way, the present findings, obtained with low concentrations of the drug, give no indication of a diphasic process. In particular, there was no initial lengthening of the action potential, or an initial increase of [K]_i. The explanation mentioned above, namely an instantaneous drop
of K uptake, seems to cover all the present observations. A possible reason for the apparent discrepancy may be the difference in drug concentration. Theoretically, a drop of [K]i should result in a lower rate of potassium efflux during the action potential and a prolongation of electrical activity. It may be argued, however, that Na ions are most probably replacing the potassium ions that are lost from the cells. With a lower concentration gradient from outside to in, the rate of influx of Na ions would decrease also, and the second effect might be even more important than the first, thus leading to a shortening of the action potential.

It is agreed almost unanimously that the cardiac glycosides are capable of reducing the K uptake in various tissues and that this event leads to a cellular potassium deprivation. The controversy begins with the question of whether the potassium loss from myocardium and the increase of contraction strength should be considered related or separate. To those who look upon the phenomena as being completely independent, an important argument has been the lack of temporal parallelism between loss of K and of contractility, a finding which is contrary to that reported in the present paper. The common denominators of the work cited are: the high digitalis concentrations employed, the lack of steady state conditions in the control preparations; and, closely related with the first factor, a relatively short glycoside exposure. If preparations are not in a steady state with respect to potassium balance, the effects of digitalis are obviously difficult to interpret. The so-called 'therapeutic' concentrations of the different glycosides, ranging from 10^-1 to 10^-5 M/L, lead regularly to a deterioration of the membrane resting and action potential, both of the conduction system and the muscle. It seems, therefore, hardly justified to call these concentrations 'therapeutic,' even taking into account the difference in the experimental arrangements (type of drug, tissue, [K]o). A further objection to the aforementioned experiments is that the [K]i and contraction measurements were not made simultaneously. The mechanical events were recorded together with a one-way flux; net changes of intracellular potassium concentration were predicted either on the basis of earlier chemically determined [K]i values or by measurement of the missing opposite flux at another time.

The finding of a temporal parallelism between cellular potassium loss and the positive inotropic effect of ouabain strengthens the view that the two phenomena are somehow related. However, it would not be permissible to conclude from the present results that the potassium loss is the direct cause of the increased contraction strength.

Summary

Transmembrane potential, isometric tension, and K content of sheep or calf myocardial bundles were recorded simultaneously during periods up to 30 hours. Ouabain at low concentrations (1-5 X 10^-8 M/L) decreased intracellular K to a new steady level which was reached after several hours. Its value depended on drug concentration. The effect was reversible. There was a striking temporal parallelism between the potassium loss and the inotropic effect which was suggestive of a close relationship between the two phenomena. Action potentials during these experiments did not change in a consistent way.

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