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Research Article

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Ouabain Binding and Cation Transport in Human Erythrocytes

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ABSTRACT In the present studies we have explored the relation between ouabain binding and the inhibition of potassium influx in intact human erythrocytes. The rate at which bound ouabain molecules dissociate from the erythrocyte membrane is not altered by complete replacement of choline with sodium or by partial replacement with potassium. These findings indicate that the effects of these cations on ouabain binding reflect alterations in the rate of association of ouabain molecules with the erythrocyte membrane. Variations in the cation composition of the incubation solution did not alter the relation between the fraction of the glycoside-binding sites occupied by ouabain or the fraction of ouabain-sensitive potassium influx which was inhibited. That is, irrespective of the affinity of the erythrocyte membrane for ouabain molecules and irrespective of the magnitude of glycoside-sensitive potassium influx, occupation of a given fraction of the glycoside-binding sites by ouabain results in the inhibition of an equal fraction of the ouabain-sensitive potassium transport sites.

INTRODUCTION

Using intact human erythrocytes, we have previously explored the first step in the mechanism of action of cardioactive glycosides; namely, the binding of ouabain to the plasma membrane (1). These studies indicated that ouabain binding to the erythrocyte membrane is specific, reversible, involves a single class of binding sites, can be detected at ouabain concentrations as low as 10^{-10} M, and correlates directly with inhibition of erythrocyte potassium influx. Furthermore, ouabain binding appears to involve a combination of glycoside molecules with a membrane "receptor" composed of a glycoside-binding site and a cation site, and the number and type of cations occupying the cation site determine the affinity of the glycoside-binding site for glycoside molecules.

Much of our understanding of membrane cation transport has come from studies of the effects on ion transport of altering the cation composition of the incubation medium (2). These studies frequently involve comparing the effects of a particular alteration to those of the same alteration in the presence of ouabain (3, 4). Since altering the cation composition of the incubation medium also alters the apparent affinity with which ouabain is bound to the cell membrane (1), our understanding of the mechanism by which cardioactive glycosides alter ion transport would be facilitated by distinguishing effects of cations on ion transport from their effects on ouabain binding. In the present study we have explored the events which occur subsequent to ouabain being bound to the cell membrane; namely, the inhibition of potassium influx. In particular, we have examined the relation between the amount of ouabain bound to the erythrocyte membrane and the magnitude of the inhibition of potassium influx. We have also explored the effects of cations on each of these two phenomena as well as on the relation between them.

METHODS

Erythrocytes obtained from normal male and female volunteers (19–34 yr of age) were washed three times in isosmotic choline chloride (pH = 7.4).

Ouabain binding was determined as described previously (1). Erythrocytes were added to incubation solutions (prewarmed to 37°C unless otherwise specified) containing [^{3}H]ouabain. The hematocrit of the incubation mixture was 5–10%. After mixing thoroughly, triplicate 100-μl samples were taken at appropriate times, placed in polyethylene micro test tubes (Beckman Instruments, Inc., Fullerton, Calif.) and washed five times with 300 μl of isosmotic choline chloride by alternate centrifugation and resuspension. Centrifugation was performed using a Microfuge (Beckman Instruments, Inc.) at 10,000 *g* for 15 s. After the final wash, each sample was treated with 100 μl

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of 10% perchloric acid, agitated, and centrifuged for 30 s. The tube and its contents were inverted and placed in a vial containing 20 ml of liquid scintillation solution. When the vial was capped and shaken, the supernate passed from the sample tube into the scintillator and the precipitate remained in the tip of the sample tube. At some time during the incubation, triplicate 100-μl samples of the incubation mixture were added to 100 μl of 10% perchloric acid, agitated, centrifuged, inverted, and placed in a vial containing liquid scintillation solution. The volume of cells counted was calculated from the hemoglobin concentration and hematocrit determined on a separate tube containing the incubation solution and erythrocytes at an hematocrit of approximately 25%. The hematocrit was measured using a Drummond microhematocrit centrifuge (Drummond Scientific Co., Broomall, Pa.) and hemoglobin concentration was measured using the cyanmethemoglobin method (5).

The standard incubation solution had the following composition (millimolars): NaCl, 150; Tris buffer (pH = 7.4), 10; glucose, 11.1. Whenever the cation composition of the incubation solution was altered, isosmotic choline was used as a replacement.

The amount of ouabain bound was calculated from the counts per milliliter of cells and the specific activity of ouabain in the incubation medium. Binding of radioactive impurities or entrapment of [^{3}H]ouabain was determined from the number of counts bound in the presence of 10,000-fold molar excess of nonradioactive ouabain. The concentration of ouabain in the [^{3}H]ouabain supplied by the manufacturer was determined as described previously (1) by measuring the binding of radioactivity in the presence of constant [^{3}H]ouabain and varying concentrations of nonradioactive ouabain. The concentration thus determined was within 11% of the value calculated from the concentration given by the commercial supplier. In previous studies we have demonstrated that all of the cell-associated radioactivity which is detected using this technique represents [^{3}H]ouabain bound to the erythrocyte membrane (1, 6).

Liquid scintillation counting was performed using 20 ml of a solution composed of 15 parts toluene (J. T. Baker Chemical Co., Phillipsburg, N. J.), 5 parts Triton X-100 (New England Nuclear, Boston, Mass.), and 1 part Liquifluor (New England Nuclear). The observed counts were usually such that their standard deviation was less than 2%. Variation in quenching was monitored by using the ratio of counts in two channels produced by an automatic external standard; however, since in all cases the maximum range was less than 3.2%, no quench correction was made.

To explore the relation between ouabain binding and cation transport, potassium influx was measured on cells which had been incubated with or without [^{3}H]ouabain. At appropriate times, triplicate samples were taken for determination of ouabain binding and an additional sample was removed and washed three times with 30 vol of cold (4°C) isosmotic choline chloride. To determine potassium influx, these washed cells were added to incubation solutions (37°C) containing ^{42}K. The hematocrit was 4% or less. After mixing thoroughly, duplicate 100-μl samples were placed in polyethylene micro test tubes and washed four times with 300 μl of cold, isosmotic sodium chloride. After the final wash each sample was treated with 100 μl of 10% perchloric acid, agitated, centrifuged, inverted, and placed in 20 ml of liquid scintillation fluid for counting. At some time during the incubation, triplicate 100-μl samples of the incubation mixture (i.e., cells plus medium) were added to a micro test tube containing 100 μl 10% perchloric acid, agitated, centrifuged, inverted, and placed in liquid scintillation solution. The volume of cells counted was calculated from the hemoglobin concentration in the incubation mixture and the previously measured hemoglobin content per volume of cells. Erythrocyte sodium and potassium concentrations were determined as described previously (7). The incubation solution used to determine potassium influx had the following composition (millimolars): NaCl, 150; Tris buffer (pH = 7.4), 10; glucose, 11.1. The potassium concentration varied depending on the experimental conditions. The sodium and potassium concentrations of the incubation solutions were also determined at the end of the incubation period. Initially the uptake of ^{42}K was determined at 0, 15, 30, and 45 min; however, since the uptake was observed to be constant over this period, potassium influx was calculated from samples taken at 0 and 40 min unless otherwise specified. Potassium influx was calculated using the method described by Sachs and Welt (2) and the average of the potassium concentrations in the incubation solutions at 0 and 40 min. All counts were corrected for decay. Sodium and potassium concentrations were measured with an Instrumentation Laboratory model 143 flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.).

Digoxin was kindly supplied in crystalline form by Dr. Stanley T. Bloomfield, Burroughs Wellcome Co., Research Triangle Park, N. C. All other reagents were of the highest grade of purity obtainable. [^{3}H]Ouabain (Lot no. 184–194, sp act 13 Ci/mmol) was obtained from New England Nuclear, and radiochemical purity was greater than 97% by the supplier's radiochromatographic and reverse isotope dilution criteria. ^{42}K was obtained as the chloride from ICN Corp., Chemical & Radioisotopes Div., Irvine, Calif.

RESULTS

We have previously reported that altering the cation composition of the incubation medium changes both the rate at which ouabain is bound to the erythrocyte membrane and the amount bound at the steady state (1). The data illustrated in Fig. 1 indicate that the rate at which bound ouabain is lost from the cell membrane is not dependent on the cation composition of the incubation medium. Values obtained in the presence of sodium (which increases ouabain binding relative to choline) were not significantly different from values obtained in the presence of potassium (which decreases ouabain binding relative to choline). There was a good correlation between the loss of ouabain molecules from the membrane and the recovery in ouabain-sensitive potassium influx (Fig. 1, insert).

Fig. 2 illustrates ouabain binding and potassium influx in erythrocytes which had been preincubated for 3 h with various concentrations of [^{3}H]ouabain. In these studies, potassium influx was determined using a potassium concentration which was sufficient to produce nearly maximal values for ouabain-sensitive potassium influx (2). As the ouabain concentration in the incubation medium was increased, there was a curvilinear increase in ouabain binding until a ouabain concentration was reached above which further increases produced no fur-

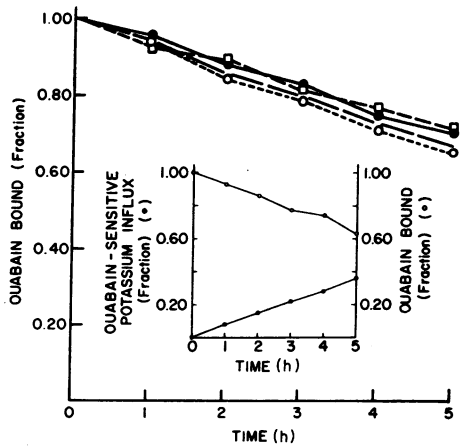

FIGURE 1 Effect of altering the cation composition of the incubation medium on the loss of bound ouabain from the erythrocyte membrane. Erythrocytes were preincubated for 3 h in a solution containing sodium (150 mM) and [^{3}H]-ouabain (1.1×10^{-7} M). The cells were then washed rapidly four times with iced (4°C) isosmotic choline chloride and resuspended in the appropriate incubation solutions at 37°C. At the indicated times, the amount of ouabain bound to the erythrocyte membrane was determined and the values are expressed as the fraction of [^{3}H]ouabain bound to the cells at the beginning of the incubation period. The cation composition of the incubation solutions studied were sodium, 150 mM (closed circles); choline, 150 mM (open circles); potassium, 20 mM, choline 130 mM (open boxes). Each point represents the mean of three experiments. *Insert.* Comparison of the time course for the loss of ouabain from the erythrocyte membrane and that for the recovery of ouabain-sensitive potassium influx. Erythrocytes were preincubated for 3 h in a solution containing sodium (150 mM) and [^{3}H]ouabain (1.21×10^{-7} M). The cells were then washed rapidly four times with iced (4°C) isosmotic choline chloride and resuspended in incubation solution (sodium, 150 mM) at 37°C. At the indicated times the amount of ouabain bound to the erythrocyte membrane was determined and the values we expressed as the fraction of [^{3}H]ouabain bound to the cell membrane at the beginning of the incubation period. At the indicated times samples were also taken, added to tubes containing ^{42}K and potassium influx determined over a 15 min period. Values for potassium influx are expressed as the fraction of glycoside-sensitive potassium influx determined on cells which had been preincubated for 3 h with and without 10^{-3} M ouabain. The potassium concentration used to determine potassium influx was 10.3 mM. Each point represents the mean of two experiments.

ther increase in ouabain binding. A similar relation was observed between ouabain concentration and inhibition of potassium influx. That is, as the ouabain concentration was increased there was a curvilinear decrease in potassium influx until a ouabain concentration was reached above which there was no further decrease in potassium influx. There was good agreement between the ouabain concentration at which ouabain binding was half-maximal and that at which inhibition of potassium influx was half-maximal.

Fig. 3 illustrates the results of an experiment similar to that illustrated in Fig. 2 except that potassium influx was measured at a low extracellular potassium concentration (i.e. one such that ouabain-sensitive potassium influx was 15% of that observed for the experiments illustrated in Fig. 2). Although the values for potassium influx in these experiments were appreciably lower than those illustrated in Fig. 2, a similar relation between ouabain concentration and potassium influx was observed and there was good agreement between the ouabain concentration at which ouabain binding was half-maximal and that at which inhibition of potassium influx was half-maximal. Furthermore, even though the influx values illustrated in Fig. 2 were significantly higher than those illustrated in Fig. 3, there was close agreement between the two experiments for the ouabain concentration at which inhibition of potassium influx was half-maximal.

To further explore this relation between ouabain binding and potassium influx, ouabain binding was measured at five different ouabain concentrations and then potassium influx was measured on cells from each different ouabain concentration using five different external potassium concentrations. At each potassium concentration studied as ouabain binding increased, there was a progressive constant decrease in the values for potassium

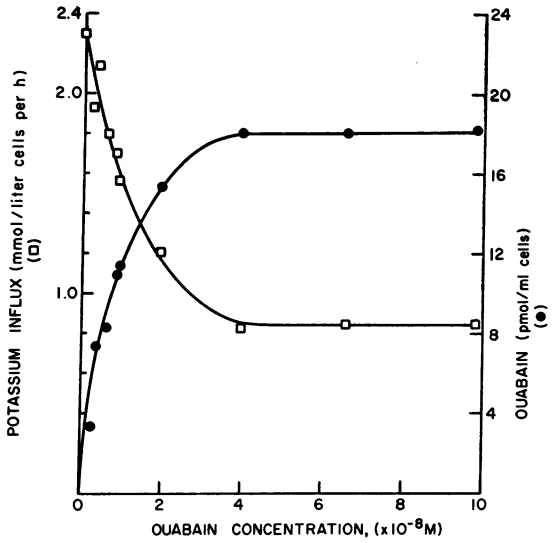

FIGURE 2 Ouabain binding and potassium influx as a function of the ouabain concentration in the incubation medium. Erythrocytes were incubated for 3 h (37°C) in an incubation solution containing sodium, 150 mM and various concentrations of [^{3}H]ouabain. Samples were taken for determination of ouabain binding and then ^{42}K was added to the incubation medium and potassium influx was determined. The potassium concentration in the influx medium was 8.3 mM. Each point represents the mean of two experiments.

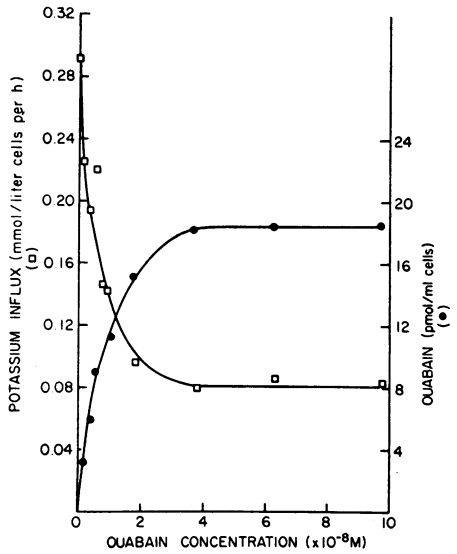

FIGURE 3 Ouabain binding and potassium influx as a function of the ouabain concentration in the incubation medium. The procedure was identical to that given in the legend to Fig. 3 except that the potassium concentration used to measure potassium influx was 0.37 mM. Each point represents the mean of two experiments.

influx and the magnitude of this decrease tended to be less at lower potassium concentrations (Fig. 4).

A more meaningful presentation of the data in Fig. 4 is illustrated by the insert where the data for potassium influx are plotted as the fraction of glycoside-sensitive potassium influx observed at a given potassium concentration. This mode of presentation permits one to compare directly the fraction of ouabain-binding sites which are occupied by ouabain with the fraction of ouabain-sensitive potassium influx which is inhibited. As is indicated, for a given value of ouabain binding, values for the fraction of ouabain-sensitive potassium influx were not significantly different. Furthermore, these data were best described by a single straight line having intercepts on the x- and y-axis of 1.0. That is, occupation of a given fraction of glycoside-binding sites by ouabain inhibits an equal fraction of ouabain-sensitive potassium influx and this relation is independent of the potassium concentration in the incubation solution and of the actual magnitude of glycoside-sensitive potassium influx.

Since each of the studies illustrated in Figs. 2–4 involved determination of ouabain binding in incubation solutions containing 150 mM sodium, we felt it was important to explore further the relation between ouabain binding and potassium influx by measuring ouabain binding in solutions having different cation compositions. Fig. 5 illustrates values for ouabain binding determined on cells which had been incubated in a solution containing choline (150 mM) or choline (130 mM) and potassium (20 mM) and various concentrations of [^{3}H]ouabain. At the end of the incubation, samples were taken for determination of ouabain binding. The remaining cells were washed three times and resuspended in a solution containing 150 mM sodium. ^{42}K was added to the incubation mixture and potassium influx was determined. Under these conditions the values obtained for ouabain binding and for the inhibition of potassium influx were significantly lower than those obtained when the incubation medium contained sodium, 150 mM (see Figs. 2 and 3). However, as was observed when ouabain binding was determined in the presence of sodium, 150 mM, increasing the ouabain concentration produced a progressive increase in ouabain binding and a corresponding decrease in potassium influx. To see if altering the cation composition of the incubation solution altered the relation between ouabain binding and inhibition of potassium influx, the data in Fig. 5 were plotted in the form of fraction of ouabain-sensitive potassium influx vs. the fraction of the total ouabain-binding sites occupied (Fig.

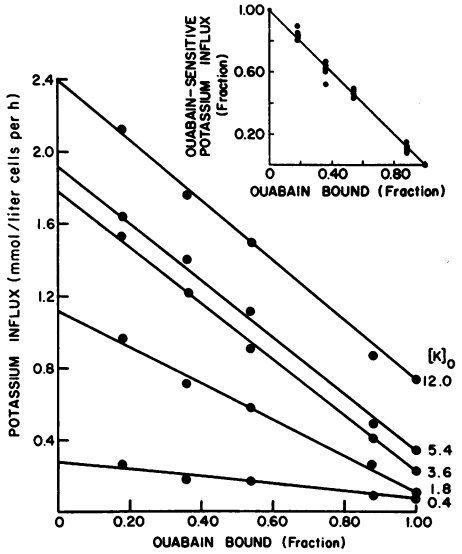

FIGURE 4 Potassium influx measured at different potassium concentrations as a function of ouabain bound to the erythrocyte membrane. Erythrocytes were incubated for 3 h (37°C) in solutions containing sodium, 150 mM and various concentrations of [^{3}H]ouabain. Ouabain binding was determined and then potassium influx was measured on cells which had been incubated at each different ouabain concentration using five different potassium concentrations. The potassium concentrations (millimolars) used to measure potassium influx are indicated at the lower righthand portion of the figure. Ouabain binding is given as the fraction of the maximal amount of ouabain which could be bound. The values represent the mean of three experiments. *Insert* For each different potassium concentration studied, ouabain-sensitive potassium influx was calculated as the fraction of the total ouabain-sensitive potassium influx which was present at that particular potassium concentration and was plotted against the fraction of the maximal amount of ouabain which could be bound.

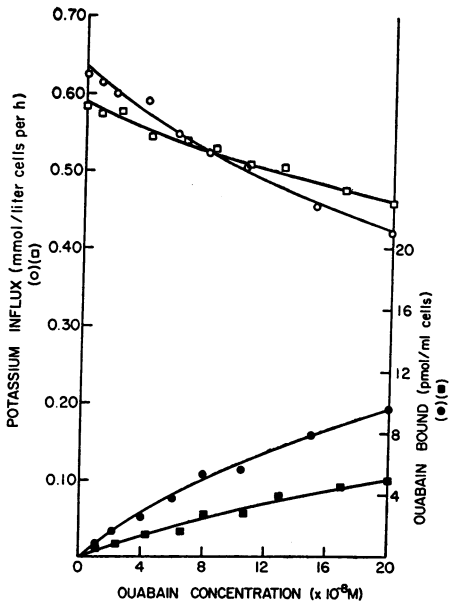

FIGURE 5 Ouabain binding and potassium influx as a function of ouabain concentration for cells incubated with choline or potassium. Erythrocytes were incubated for 3 h (37°C) in solutions containing different concentrations of [^{3}H]ouabain and choline, 150 mM (circles) or choline, 130 mM plus potassium, 20 mM (boxes). Samples were taken for determination of ouabain binding and the cells were washed rapidly four times with iced (4°C) isosmotic choline chloride and resuspended in a solution containing sodium, 150 mM at 37°C. Potassium influx was then determined using ^{42}K and a potassium concentration of 1.30 mM. The values represent the mean of two experiments.

6). The observed linear relation indicates that regardless of the cation composition of the incubation solution used to determine ouabain binding or of the magnitude of ouabain-sensitive potassium influx, when a given fraction of the ouabain-binding sites is occupied, there is a corresponding fractional inhibition of ouabain-sensitive potassum influx.

DISCUSSION

The erythrocyte membrane contains a finite number of glycoside-binding sites whose affinity for cardioactive glycosides is dependent on the cation composition of the incubation medium (1). It is also known that a portion of potassium influx across the erythrocyte membrane can be inhibited by cardioactive glycosides and that this glycoside-sensitive potassium influx is a saturable function of the extracellular potassium concentration and can be inhibited competitively by other monovalent cations (2). The present studies were designed to explore the functional characteristics of the mechanism by which cardioactive glycosides, once bound to the erythrocyte membrane, inhibit erythrocyte potassium influx.

The rate at which bound ouabain dissociates from the cell membrane is not altered by complete replacement of choline with sodium or by partial replacement with potassium (Fig. 1). These findings indicate that the previous observations that sodium increases and that potassium decreases ouabain binding (1) can be attributed to the effects of these cations on the rate of association of ouabain with the erythrocyte membrane. The good correlation observed between the decrease in cell-associated radioactivity and the rise in ouabain-inhibitable potassium influx (Fig. 1, insert) indicates that this decrease in radioactivity represents the loss of pharmacologically active ouabain molecules from the cell membrane and excludes the possibility that the decrease in radioactivity represents the loss of a tritiated contaminant. Furthermore, the relatively slow rate at which ouabain was lost from the membrane indicates that during the incubation period used to measure potassium influx the loss of ouabain from the erythrocyte membrane was negligible.

In agreement with a previous study from this laboratory (1) our value for maximal ouabain binding (18.8± 1.7 pmol/ml cells) indicates that there are approximately 1,100 glycoside-binding sites per erythrocyte. This value is significantly higher than those reported previously by others (8–10). Part of this discrepancy appears to be at-

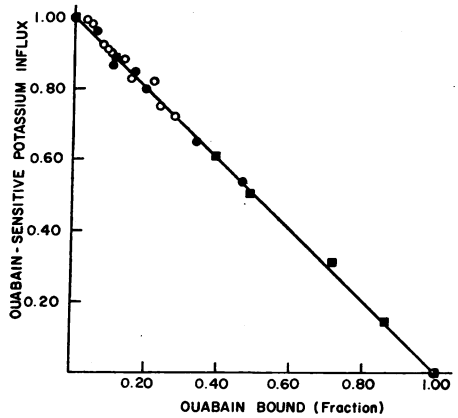

FIGURE 6 Ouabain-sensitive potassium influx as a function of ouabain bound for cells incubated in solutions with different cation compositions. The values for choline (closed circles) and potassium (open circles) were calculated from the values given in Fig. 5. The values for sodium (closed boxes) were obtained using the same procedure as outlined in the legend to Fig. 5 except that erythrocytes were preincubated for 3 h (37°C) in a solution containing sodium, 150 mM and different concentrations of [^{3}H]ouabain. The values for ouabain binding are expressed as the fraction of the value for maximal ouabain binding determined by incubating the cells in the appropriate solutions with a concentration of [^{3}H]ouabain sufficient to produce maximal ouabain binding. Ouabain-sensitive potassium influx was calculated as the fraction of the total ouabain-sensitive potassium influx which was present in cells which had been incubated in each of the three different incubation solutions.

tributable to differences in the method for extracting bound radioactivity from the cells, in the composition of the liquid scintillation mixture and the procedure used to correct for variable counting efficiency (1). Another potential source of this discrepancy may be the different lots of [^{3}H]ouabain used. The specific activity of the [^{3}H]ouabain used in the present experiments was 3–25 times greater than that used by others (8–10). Dunham and Hoffman (11) also noted that they obtained significantly different values for ouabain binding to sheep erythrocytes when they used a different lot of [^{3}H]ouabain from the same commercial supplier and speculated that variation among different lots of [^{3}H]ouabain might account for the differences in values for ouabain binding to sheep erythrocytes obtained by different laboratories.

When the potassium concentration in the incubation solution is 0.4 mM, glycoside-sensitive potassium influx is approximately 15% of what it is when the potassium concentration in the incubation solution is 12 mM (Fig. 4). One question which the present studies were designed to answer was whether more, less, or the same amount of bound ouabain is required to produce 50% inhibition of potassium influx measured at a potassium concentration of 0.4 mM compared with the amount required to produce 50% inhibition of potassium influx measured at a potassium concentration of 12 mM. It seemed that when glycoside-sensitive potassium influx was submaximal, less bound ouabain would be required to produce 50% inhibition than when potassium influx was maximal. However, as is illustrated by Fig. 4 the same amount of bound ouabain is required to produce 50% inhibition of potassium influx measured at 0.4 mM potassium as is required when potassium influx is measured at 12 mM potassium. The relation between ouabain binding and inhibition of potassium influx is that irrespective of the potassium concentration used to measure potassium influx, the fraction of the glycoside-inhibitable potassium influx which was inhibited was equal to the fraction of the glycoside-binding sites which were occupied by ouabain.

We have previously demonstrated that in terms of its effect on ouabain binding, potassium acts at a site which also has an affinity for sodium and which is functionally distinct from the site to which cardiac glycosides bind (1). When this cation site is occupied by potassium, the affinity of the glycoside-binding site for glycosides is decreased. We have also observed that there is a potassium concentration above which no further decrease in ouabain binding was observed and the inhibition of ouabain binding produced by a given concentration of potassium could be overcome by increasing the concentration of ouabain in the incubation medium. Hoffman and co-workers concluded that although ouabain binding could be abolished by raising the external potassium concentration to 30 mM (8, 9), cardiac glycosides were noncompetitive inhibitors of glycoside-sensitive cation transport because cesium could substitute for potassium in activating sodium outflux but cesium was not able to replace potassium in preventing or altering the action of cardiac glycosides on cation transport (4). We have specified previously that the technique used by Hoffman and Ingram was probably not sufficiently sensitive to detect ouabain binding in the presence of 30 mM potassium (1). Glynn concluded that cardiac glycosides competitively inhibited the interaction of potassium ions with the glycoside-sensitive transport mechanism (3). This conclusion was based on the observation that the inhibition of potassium influx by cardiac glycosides was reduced as the concentration of potassium in the incubation medium was increased. These studies, however, did not permit one to distinguish between effects of potassium on glycoside binding and effects of potassium on potassium influx.

The data in Fig. 4 exclude the possibility that binding of ouabain molecules to the glycoside-binding site reduces but does not abolish the apparent affinity of potassium ions for the potassium transport mechanism. If bound glycoside molecules reduced but did not abolish the affinity of the potassium transport sites for potassium, at a given value of ouabain binding, one would expect to find that the fraction of glycoside-inhibitable potassium transport decreased with increasing concentrations of external potassium. Furthermore, this decrease should be more readily observed at lower values for ouabain binding than at higher values. It might be argued that we did not use a sufficiently high concentration of potassium to detect such an effect; however, even at the lowest values of ouabain binding studied raising the external potassium concentration to 30 mM did not alter the fractional inhibition of glycoside-sensitive potassium influx.

Instead, our findings indicate that cardiac glycosides bind to a site which is functionally distinct from the potassium transport site[1] and when the glycoside-binding sites are occupied by ouabain, a proportional number of potassium transport sites are inhibited. That is, ouabain acts to abolish the affinity of the potassium transport sites for potassium and the fraction of potassium transport sites so altered is equal to the fraction of glycoside-binding sites occupied by ouabain. When ouabain molecules are bound to the erythrocyte membrane, the potassium transport sites can be viewed as existing in one of three different functional configurations. A given number of potassium transport sites are unable to combine with potassium ions as a result of their being inhibited by

[1] The use of the term "transport site" should not be taken to indicate that such an entity actually exists. This term is used only to facilitate our expression of certain salient *functional* characteristics of erythrocyte cation transport.

ouabain molecules which are bound to the erythrocyte membrane. The potassium transport sites which are not inhibited may be either active or inactive (in terms of being involved in the translocation of potassium ions across the erythrocyte membrane) depending on the potassium concentration in the incubation medium. Furthermore, for the uninhibited sites, the ratio of active sites to inactive sites is independent of the number of glycoside molecules bound to the erythrocyte membrane.

A second question which the present studies were designed to answer was whether altering the cation composition of the incubation medium altered the relation between ouabain binding and inhibition of potassium influx. Figs. 5 and 6 illustrate that although varying the cation composition of the incubation solution alters the affinity of the glycoside-binding sites for ouabain and alters the magnitude of glycoside-sensitive potassium influx, these variations did not alter the relation between the fraction of glycoside-binding sites occupied by ouabain and the fraction of glycoside-sensitive potassium influx which was inhibited. These observations indicate that irrespective of the affinity of the erythrocyte membrane for ouabain and irrespective of the magnitude of glycoside-sensitive potassium influx, occupation of a given fraction of the glycoside-binding sites by ouabain results in the inhibition of an equal fraction of the ouabain-sensitive potassium transport sites.

The present studies do not indicate whether or not the site at which potassium ions act to alter the affinity of the glycoside-binding site for glycoside molecules also functions as a potassium transport site. The observations that potassium decreases but does not abolish the affinity of the glycoside-binding site for ouabain but that glycoside molecules when bound to their binding sites abolish the affinity of the potassium transport sites for potassium might appear to indicate that the site at which potassium ions act to alter the glycoside site must be functionally distinct from that site at which potassium acts to be transported across the erythrocyte membrane. However, as we have demonstrated previously (1), the observed effects of potassium ions on glycoside binding are compatible with the possibility that when the glycoside-binding site is occupied by ouabain the affinity of the monovalent cation site for potassium is abolished. In other words, potassium ions can combine with the cation site only when the glycoside-binding site is not occupied by glycoside molecules. If this is, in fact, the situation and if the cation site is also a potassium transport site, this effect of cardiac glycosides could account for their ability to inhibit potassium influx.

Sachs and Welt (2) found that the component of potassium influx which was abolished in cells which had been depleted of energy stores and incubated with 10^{-4} M strophanthidin required two potassium ions to be present at some site or sites in the transport mechanism before transport occurred. These authors' data also indicate that the two sites have different affinities for potassium. If the site at which potassium acts to alter the affinity of the glycoside site for ouabain also functions as one of the two potassium transport sites described by Sachs and Welt, our previous studies would indicate that it is the site with the higher affinity for potassium (1). From the data given by Sachs and Welt we have calculated that the value for the dissociation constant describing the interaction between potassium ions and the higher affinity transport site is 0.37 mM. The value which we have previously reported for the dissociation constant describing the interaction between potassium ions and the site at which sodium and potassium act to alter ouabain binding is 0.28 (+ 0.17) mM (mean [±SD]). However, the fact that these two values are not significantly different by no means proves that they reflect the action of potassium at the same site and additional studies are necessary before this question can be resolved.

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