Effect of quinidine on the digoxin receptor in vitro.

W J Ball Jr, … , A Schwartz, V P Butler Jr

J Clin Invest. 1981;68(4):1065-1074. https://doi.org/10.1172/JCI110329.

Research Article

To investigate the basis for a clinically important digitalis-quinidine interaction that is characterized by increases in serums digoxin concentrations when quinidine is administered to digoxin-treated patients, we have studied in vitro the interaction of quinidine with the digoxin receptor. Evidence has been obtained that quinidine is capable of decreasing the affinity for digoxin of cardiac glycoside receptor sites on purified Na,K-ATPase and on intact human erythrocyte membranes. As others have shown, quinidine is capable of inhibiting Na,K-ATPase activity, and evidence has been obtained in the current study that, while quinidine can reduce the affinity of the enzyme for digoxin, it is also capable of acting together with digoxin in inhibiting enzyme activity to a degree greater than the inhibitory effect of digoxin alone. The concentrations of digoxin and quinidine used in this study were considerably greater than their therapeutic serum concentrations. Nevertheless, these observations are consistent with the hypothesis that the increases in serum digoxin concentrations and the decreases in volumes of digoxin distribution observed clinically when quinidine is administered to digoxin-treated patients may reflect, at least in part, a decrease in the affinity of tissue receptors for digoxin. The possibility must also be considered that enhanced cardiac effects of digoxin may occur clinically as the result of an augmentation, by quinidine, of digoxin effects, which more than compensates for […]

Effect of Quinidine on the Digoxin Receptor In Vitro

WILLIAM J. BALL, Jr., DORIS TSE-ENG, EARL T. WALLICK, JOHN P. BILEZIKIAN, ARNOLD SCHWARTZ, and VINCENT P. BUTLER, Jr., *Department of Medicine, Columbia University, College of Physicians & Surgeons, New York 10032; Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

ABSTRACT To investigate the basis for a clinically important digitalis-quinidine interaction that is characterized by increases in serum digoxin concentrations when quinidine is administered to digoxin-treated patients, we have studied in vitro the interaction of quinidine with the digoxin receptor. Evidence has been obtained that quinidine is capable of decreasing the affinity for digoxin of cardiac glycoside receptor sites on purified Na,K-ATPase and on intact human erythrocyte membranes. As others have shown, quinidine is capable of inhibiting Na,K-ATPase activity, and evidence has been obtained in the current study that, while quinidine can reduce the affinity of the enzyme for digoxin, it is also capable of acting together with digoxin in inhibiting enzyme activity to a degree greater than the inhibitory effect of digoxin alone. The concentrations of digoxin and quinidine used in this study were considerably greater than their therapeutic serum concentrations. Nevertheless, these observations are consistent with the hypothesis that the increases in serum digoxin concentrations and the decreases in volumes of digoxin distribution observed clinically when quinidine is administered to digoxin-treated patients may reflect, at least in part, a decrease in the affinity of tissue receptors for digoxin. The possibility must also be considered that enhanced cardiac effects of digoxin may occur clinically as the result of an augmentation, by quinidine, of digoxin effects, which more than compensates for the modest reduction in digoxin binding.

This work was presented in part at the Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics at Dallas, Texas, 7 April 1979 (1979. *Fed. Proc.* 38: 1042).

Dr. Ball was a postdoctoral trainee supported by a National Research Service Award in Immunology from the National Institute of Allergy and Infectious Diseases (AI 07132). Dr. Bilezikian is the recipient of a National Institutes of Health Research Career Development Award (HL 00383).

Received for publication 21 April 1980 and in revised form 18 June 1981.

INTRODUCTION

The cinchona alkaloid quinidine is used in the treatment of certain cardiac arrhythmias, notably atrial fibrillation, multiple premature ventricular depolarizations and ventricular tachycardia. Quinidine is frequently administered to patients who are also being treated with digitalis glycosides. Recently, Ejvinsson (1), Leahey et al. (2), and others (3–5) have reported that serum concentrations of the digitalis glycoside digoxin almost invariably rise when quinidine is administered to digoxin-treated patients. Leahey et al. suggested that the observed increases in serum digoxin levels might result from the displacement of digoxin from tissue binding sites (2). Several subsequent studies have suggested that the volume of distribution of digoxin in man (6–8) and in the dog (9) is decreased during quinidine administration, a finding consistent with the hypothesis of Leahey et al.; however, there has been considerable interindividual variation (8) and, in one study, no change in the volume of distribution was observed (10). A decrease in renal clearance of digoxin has also been noted (5–8, 10–15), but the relationship of this observation to the increase in serum digoxin is not clear because rises in serum digoxin levels may occur without additional digoxin administration following the institution of quinidine therapy (2, 13) and because the prolongation of the $t_{1/2}$ of digoxin elimination observed during quinidine administration (7, 8, 10) has not always been striking (6, 8, 10).

Several experimental and clinical observations have suggested that interactions between digitalis and quinidine are accompanied by an augmented effect of one or both of these drugs. The addition of quinidine to cultured beating embryonic heart cells together with digoxin resulted in a significant increase in the proportion of arrhythmic cells over that observed with either drug alone (16). Kwit and Gold (17) have reported that ventricular tachycardia was induced in digitoxin-treated dogs by doses of quinidine that did

not produce this effect without digitalis. It has also been noted that quinidine, in otherwise nontoxic doses, may cause lethal rhythm disturbances in patients (18, 19) and in experimental animals (20) with digitalis intoxication. Finally, it would appear that the incidence of adverse reactions to digitalis is greater in patients receiving quinidine than in patients not receiving this drug (21) and, conversely, that adverse reactions to quinidine are more common in digitalis-treated patients than in patients who are not receiving cardiac glycoside therapy (22).

The basis for these effects of the two drugs has never been firmly established, but several in vitro studies suggest that cardiac glycosides and cinchona alkaloids may interact at a glycoside receptor site at the cellular level. Quinidine, like digitalis glycosides, is capable of inhibiting Na,K-ATPase activity (23–26) and dihydroquinidine, an active quinidine derivative, binds to the Na,K-ATPase of dog heart (26). It has also been reported that another cinchona alkaloid, quinine, decreases the initial rate of uptake of the cardiac glycoside ouabain by isolated rat hepatocytes (27).

These observations have suggested the possibility that the administration of quinidine to patients receiving digitalis may result in decreased binding of digitalis to tissue receptors but that, despite such decreased digitalis binding, certain effects of digitalis at the cellular level are augmented or potentiated by the action of quinidine on these receptors. We have carried out experiments that provide evidence that quinidine is capable of decreasing the affinity of purified sheep kidney Na,K-ATPase and of human erythrocyte membrane receptors for digoxin and that, under certain conditions, quinidine may augment the action of digoxin on Na,K-ATPase. The present report describes these experiments and discusses their possible clinical implications.

METHODS

Materials. Crystalline digoxin was a generous gift from the Burroughs Wellcome Co., Research Triangle Park, N. C.; [12α-^{3}H]digoxin (13.9 Ci/mmol) was purchased from New England Nuclear, Boston, Mass., and was found to contain <5% radiochemical impurities by thin-layer chromatography on silica gel G in a solvent system consisting of cyclohexane/acetone/glacial acetic acid in a ratio of 49:49:2 (28). Quinidine hydrochloride monohydrate was purchased from Sigma Chemical Co., St. Louis, Mo. Human serum albumin was obtained as fraction V powder from Miles Research Laboratories, Kankakee, Ill. ^{86}Rb (2.5–8.7 mCi/mg in 0.5 N HCl) was purchased from New England Nuclear, Boston, Mass. and was diluted before use in a solution with the following final concentrations: 0.67 mCi/ml; 150 mM KCl; 11.1 mM glucose; 45.3 mM Tris-Cl, pH 7.5.

Na,K-ATPase was purified from frozen lamb kidney outer medulla by the procedure of Lane et al. (29) and was generously supplied by Dr. Lane. The enzyme preparations used had specific activities of 834–1363 μmol ATP hydrolyzed/mg protein per h at 37°C, bound 1.8–2.2 nmol of ouabain or digoxin/mg protein, and had <0.5% ouabain-insensitive ATPase activity.

Measurement of Na,K-ATPase activity. Na,K-ATPase activity was measured by a spectrophotometric coupled enzyme assay (30) in a medium containing 30 mM histidine, 2.5 mM Na_2ATP (P-L Biochemicals, Inc., Milwaukee, Wis.), 5.0 mM $MgCl_2$, 10 mM KCl, 95 mM NaCl, 1.0 mM EGTA-Tris, 0.36 mM NADH, 2 mM phosphoenol pyruvate, and 10 μl pyruvate kinase/lactate dehydrogenase (Sigma Chemical Co.), pH 7.2. Na,K-ATPase (1.2 μg/ml) and quinidine and/or digoxin were incubated in the reaction mixture, without ATP, for 10 min at 30°C and then the reaction was started by adding ATP. The reaction was allowed to proceed until a stable rate was obtained (4–5 min) and then the rate was determined over a subsequent 4-min period.

[^{3}H]Digoxin binding to Na,K-ATPase. [^{3}H]digoxin was diluted with nonradioactive digoxin to specific activities of 1,000, 125, or 100 mCi/mmol. The digoxin binding was carried out at 30°C, essentially as described by Wallick and Schwartz (31) using five different standard binding conditions: (*a*) Mg = 2.5 mM $MgCl_2$; (*b*) MgATP = 2.5 mM $MgCl_2$ and 2.5 mM Tris-ATP; (*c*) MgP_i = 2.5 mM $MgCl_2$, and 2.5 mM Tris-phosphate; (*d*) NaMgATP = 100 mM NaCl, 2.5 mM $MgCl_2$, and 2.5 mM Tris-ATP; (*e*) NaKMgATP = 100 mM NaCl, 1 mM KCl, 2.5 mM $MgCl_2$, and 2.5 mM Tris-ATP. The medium also contained 50 mM Tris-Cl, pH 7.4, and varying concentrations of [^{3}H]digoxin and quinidine. In most experiments, the binding reaction was initiated by adding Na,K-ATPase (5–40 μg) and stopped by adding 1 mM unlabeled ouabain, cooling the solution on ice and then filtering it through nitrocellulose filters (0.22 μm, Millipore Corp., Bedford, Mass.). In some experiments, Na,K-ATPase was incubated in medium with quinidine for 15 min at 30°C and the binding reaction started by adding [^{3}H]digoxin. Nonspecific binding of [^{3}H]digoxin to the enzyme and/or filter was determined by preincubating complete or enzyme-free medium with 1 mM ouabain before adding [^{3}H]digoxin. All values have been corrected for nonspecific binding.

Human erythrocyte binding of [^{3}H]digoxin and uptake of ^{86}Rb. Human erythrocyte binding of [^{3}H]digoxin and uptake of ^{86}Rb were determined by methods based on those described by Gardner et al. (32). Erythrocytes, obtained from heparinized blood from four healthy volunteer subjects who were receiving no drugs at the time of study, were washed three times in isosmotic choline chloride solution, pH 7.4. [^{3}H]digoxin was diluted in incubation medium (150 mM NaCl; 11.1 mM glucose; 10 mM Tris-Cl, pH 7.5) containing 0.35% human serum albumin; the presence of albumin decreased the amount of nonspecific binding of [^{3}H]digoxin. In kinetic experiments, 0.75 ml packed, washed erythrocytes were suspended in 6.25 ml incubation medium with or without quinidine and incubated for 2 h at 37°C in a shaking water bath, after which 0.5 ml [^{3}H]digoxin was added, and the incubation continued. In experiments designed to assess the possible displacement of [^{3}H]digoxin from erythrocytes, 0.75 ml packed, washed erythrocytes were added to 6.75 ml incubation medium containing 0.21 μM [^{3}H]digoxin. After 2 h at 37°C, the cells were washed three times with 50 ml chilled incubation medium; the washed pellet was resuspended in 7 ml incubation medium with or without quinidine, and again incubated at 37°C. Samples were removed at appropriate times for the measurement of membrane binding of [^{3}H]-digoxin and for measurement of ^{86}Rb uptake.

For the measurement of membrane binding of [^{3}H]-digoxin, triplicate 100-μl samples were removed and added to tubes containing 8 ml of a chilled aqueous solution of 0.05% human serum albumin. After thorough mixing, the resulting hemolysates were then passed over nitrocellulose

filters (1.2 μm, Millipore Corp.). The filters were washed three times with 10 ml chilled, deionized water and added to 15 ml scintillation fluid (Liquiscint; National Diagnostics Inc., Somerville, N. J.). The membrane-bound radioactivity was determined in a liquid scintillation spectrometer. To determine specific binding of [^{3}H]digoxin, all values were corrected for nonspecific binding of [^{3}H]digoxin by subtracting the amount of bound [^{3}H]digoxin observed in tubes subjected to identical incubation conditions in the presence of 1 mM unlabeled ouabain. Results were expressed as picomoles of [^{3}H]digoxin bound per milliliter cells.

For ^{86}Rb uptake measurements, 700-μl samples of each erythrocyte suspension were removed and added to 50 μl (33 μCi) of $^{86}Rb^+$. After 1 h at 37°C, triplicate 100-μl aliquots were removed to microfuge centrifuge tubes (Beckman Instruments, Inc., Fullerton, Calif.). The cells were washed four times with 300 μl of chilled isosmotic choline chloride and their radioactivity was determined in a gamma scintillation spectrometer. Results were expressed as the percentage inhibition of ^{86}Rb taken up per milliliter test cells in comparison with the amount of ^{86}Rb taken up per milliliter control cells, which had been incubated for identical time periods in the absence of digoxin and quinidine.

Erythrocyte volumes were determined as previously described (28), from erythrocyte hemoglobin concentrations, measured by the cyanmethemoglobin method (33).

Analysis of data. The binding rate data are presented as pseudo first-order rate plots according to the equation:

$$\ln \frac{(Ae - A)}{Ae} = -(kI + k')t \qquad (1)$$

as described by Wallick et al. (34), where A and Ae represent the amount of cardiac glycoside bound to the enzyme at time t and at equilibrium, respectively, k is the apparent second order forward rate constant, k' is the dissociation rate constant and I, the concentration of the cardiac glycoside. The $t_{1/2}$ values and the apparent binding rate constants are obtained by plotting ln (Ae-A)/Ae vs. t. The I_{50} values, the concentrations of digoxin and quinidine which are required to produce 50% inhibition, and the *n* values, the apparent number of binding sites for the inhibitor or the degree of binding cooperativeness, were calculated using the following equation:

$$\frac{v_i}{v} = \frac{1}{1 + (I_{50}/I)^n} \qquad (2)$$

where v and v_i are enzyme activities in the absence and presence of inhibitor, I (35).

RESULTS

Effects of digoxin and quinidine on Na,K-ATPase activity. The effects of varying concentrations of digoxin and quinidine on the activity of purified Na,K-ATPase are shown in Fig. 1. Analysis of the dose-response curve by Eq. 2 (35) yielded an I_{50} value, or concentration of digoxin required to produce 50% inhibition, of 1.2 μM, with *n*, the number of calculated interacting binding sites, equal to 1. This I_{50} value is similar to that reported by Caldwell and Nash (36) for swine brain Na,K-ATPase, 1.6 μM. Quinidine was found to be an effective inhibitor of Na,K-ATPase activity, but its I_{50} value of 92 μM is ~80 times higher than that of digoxin, while its *n* value equaled 2. Quinidine was also found to affect the dose response of the enzyme to digoxin. A noninhibitory concentration of quinidine (10 μM) appeared to cause a slight reduction in the effectiveness of lower (<1 μM) concentrations of digoxin. Inhibitory levels of quinidine increased the extent of inhibition beyond that of digoxin alone, but the inhibitory effect appeared to be less than additive.

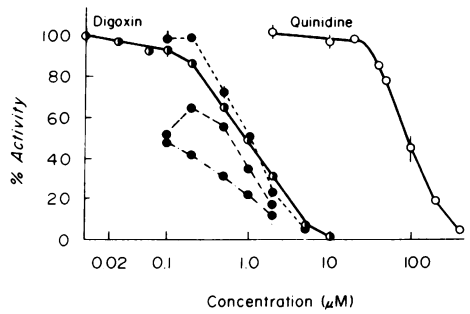

FIGURE 1 Effects of quinidine and of digoxin on Na,K-ATPase activity. Enzyme activities are expressed as percentages of control Na,K-ATPase activity observed in the absence of quinidine and digoxin. The solid lines depict the responses to various concentrations of quinidine alone (○) and of digoxin alone (◑). The ● represent the responses to various concentrations of digoxin in the presence of quinidine, 10 μM (– – –), 50 μM (— —), and 100 μM (·—·—·), respectively. The reactions were started by the addition of ATP following a 10-min preincubation period. Each point represents the average of two to six determinations. Representative error bars are given as ±SEM.

If the assumption is made that digoxin and quinidine are acting independently at separate sites, the predicted inhibition that would occur in the presence of both compounds can be calculated using the following equation:

$$\text{fraction inhibited} = \frac{v_i}{v} = \frac{1}{1 + (D/K_{Dig} + Q^2/K_{Dig}^2 + DQ^2/K_{Dig}K_Q^2)^{-1}}, \qquad (3)$$

where v and v_i are enzyme activities in the absence and in the presence of inhibitor and where K_{Dig} and K_Q are the I_{50} values obtained under the assay conditions, and D and Q are the concentrations of digoxin and quinidine. Table I gives the values for the actual observed inhibition at different quinidine and digoxin concentrations and the predicted, or calculated values. The experimental data corresponded well with the calculated values. The largest deviations occurred at noninhibitory levels of quinidine. These data are consistent with the concept that digoxin and quinidine act independently on Na,K-ATPase activity.

Effect of quinidine on digoxin binding to Na,K-ATPase. The effect of quinidine on [^{3}H]digoxin binding to the enzyme was determined under different binding conditions, each of which alters the affinity of

TABLE I
Inhibition of Na, K-ATPase Activity by Digoxin and Quinidine

[Quinidine]	[Digoxin] 0 Obs.	0 Calcu.	0.2 μM Obs.	0.2 μM Calcu.	0.5 μM Obs.	0.5 μM Calcu.	1 μM Obs.	1 μM Calcu.
	Percent inhibition of activity							
0	0	0	13	14	33	29	51	45
10 μM	3	1	0	15	29	30	49	45
50 μM	21	23	32	33	44	45	65	57
100 μM	54	54	59	60	69	67	78	75

Observed (Obs.) inhibition values represent the averages of two to six determinations, with an average SEM of 4%. The calculated (Calcu.) values were obtained using Eq. 3 as discussed in the text, and used dissociation constants that were derived from Fig. 1 and had values for digoxin of 1.2 μM and for quinidine of 92 μM.

the enzyme for digoxin. In the presence of Mg (Fig. 2A) and MgATP (data not shown), quinidine (0.2 mM) reduced the amount of [^{3}H]digoxin that was bound at 1 h, but had no effect on binding in the presence of NaKMgATP or NaMgATP or MgP_i. At lower concentrations of digoxin, a slight effect of quinidine (0.4 mM) could be detected at 90 min in the presence of NaMgATP (Fig. 2B). There was no effect under these conditions in the presence of MgP_i. Binding in the presence of NaKMgATP was not studied since the digoxin concentrations were too low to prevent depletion of ATP during the binding period. These studies suggest that the ability of the quinidine to decrease digoxin binding is ligand dependent. The binding of digoxin is very slow (Table II) and at the lower concentrations of digoxin, the experiments depicted in Fig. 2 are not at equilibrium. The data therefore were not evaluated by Scatchard analysis (37). The data of Fig. 2 were fit, however, to a form of Eq. 2. Quinidine caused a modest shift in apparent EC_{50} from 0.19 to 0.27 μM in the presence of Mg and from 19 to 24 nM in the presence of NaMgATP. There was no difference in the slope factor. The calculated maximal binding levels in the presence of Mg were slightly reduced by quinidine from 1,628±52 to 1,483±72 pmol digoxin/mg protein. In the presence of MgP_i, quinidine caused a slight increase from 1,772±27 to 1,842±34 pmol digoxin/mg protein. These apparent slight changes in the number of digoxin binding sites were not significant, and it seemed more likely that quinidine was affecting the dissociation constant (K_D) and not the number of sites.

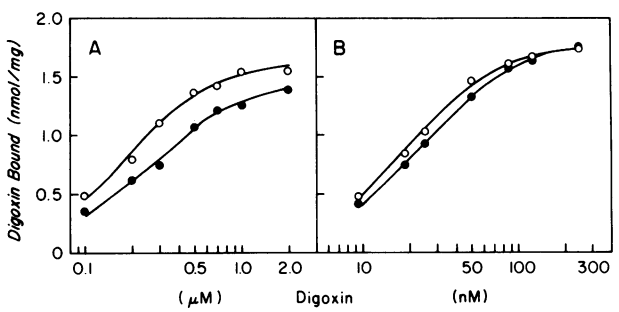

FIGURE 2 Effect of quinidine on equilibrium binding of [^{3}H]digoxin to Na,K-ATPase under different binding conditions. Digoxin binding at each concentration is recorded as nanomoles of [^{3}H]digoxin bound per milligram protein at each digoxin concentration. Each point represents the average of at least two separate experiments done in duplicate; the curves were obtained by direct fitting of results to a form of Eq. 2, $B = B_{max}/[1 + (EC_{50}/I)]^n$, where B is the amount of digoxin bound, B_{max} is the number of digoxin-binding sites, EC_{50} is the concentration of digoxin that causes occupation of 50% of the binding sites, I is the digoxin concentration, and n is the slope factor. (A) Binding of [^{3}H]digoxin in the presence of 2.5 mM Mg^{++}, and in the presence (●) and absence (○) of 0.2 mM quinidine. The reaction was started by adding [^{3}H]digoxin to enzyme (40 μg/ml) and stopped after 60 min. (B) Binding of [^{3}H]digoxin using NaMgATP binding conditions, in the presence (●) and absence (○) of 0.4 mM quinidine. The enzyme was incubated in binding medium with quinidine for 15 min and the binding reaction started by adding [^{3}H]digoxin. The reaction was stopped after 90 min.

Effect of pre-incubation of Na,K-ATPase with quinidine on digoxin binding. The incubation of the enzyme with quinidine for 15 min before starting the binding reaction with the addition of either 0.2 or 1 μM [^{3}H]digoxin was found to give the same results as non-preincubated controls when using MgATP, NaMgATP, and MgP_i binding conditions. Experiments using 25 nM digoxin showed that in the presence of NaMgATP, but not MgP_i, preincubation of the enzyme with quinidine (0.12 mM) did increase its inhibitory effects. The maximum effect (twofold over that observed for nonincubated controls) was observed to occur within 15 min. Therefore, all further studies using NaMgATP conditions were done with a 15-min enzyme-quinidine preincubation before adding [^{3}H]-

TABLE II

Kinetic Parameters for Binding of [^{3}H]Digoxin to Na, K-ATPase

Binding condition	Quinidine	k_{obs} (min^{-1})	k' (min^{-1}) × 10^3	K_D
	mM			*nM*
Mg*	0	0.034±0.004 (5)	2.0±0.1 (2)	62
	0.2	0.024±0.005 (5)	2.2 (1)	100
Mg	0	0.067±0.016 (5)	1.9±0.1 (4)	29
	0.2	0.051±0.009 (5)	1.8±0.2 (3)	37
MgATP	0	0.11±0.03 (5)	1.9±0.1 (3)	17
	0.2	0.082±0.021 (5)	2.1±0.1 (2)	26
MgP_i	0	0.95±0.09 (5)	1.8±0.3 (2)	1.9
	0.2	0.92±0.04 (5)	2.0±0.1 (3)	2.2
NaMgATP‡	0	0.30±0.03 (3)		
	0.2	0.29±0.04 (3)		
NaMgATP	0	0.68±0.05 (4)	1.6±0.2 (4)	2.4
	0.2	0.64±0.04 (4)	1.7±0.1 (3)	2.7
NaKMgATP	0	0.27±0.07 (5)	1.3±0.1 (3)	4.8
	0.2	0.30±0.06 (5)	1.14±0.02 (2)	3.8

* Mg = 0.25 mM.

‡ Na = 15 mM.

Unless otherwise indicated, Mg = ATP = P_i = 2.5 mM, Na = 100 mM, K = 1 mM and [^{3}H]digoxin = 1 μM. k_{obs}, the first-order rate constant for approach to equilibrium, was obtained from experiments as shown in Fig. 2 by direct fitting, using a non-linear least squares program, to Eq. 1, where k_{obs} equals kI + k'. The dissociation rate constant was obtained by least squares fit to slopes as shown in Fig. 5. The equilibrium dissociation constant, K_D, was calculated as k'-k. Averages±SD are shown. A paired *t* test gave *P* values of 0.0006, 0.028, and 0.009 for the k_{obs} values for 0.25 mM Mg, 2.5 mM Mg, and MgATP conditions. Number of separate determinations of rates are shown in parentheses.

digoxin. These data suggest that under some conditions the effect of quinidine is time dependent.

Effects of quinidine on the rate of digoxin binding to Na,K-ATPase. If quinidine is altering the K_D of digoxin for Na,K-ATPase, it could do so by altering the association rate constant, the dissociation rate constant, or both. In the presence of Mg (Fig. 3A) and MgATP (Table II), the rate of binding was reduced by ~25%. At this concentration of digoxin (1 μM), no effect on the rate of association could be detected in the presence of MgP_i, NaMgATP, or NaKMgATP (Table II). To determine if quinidine was affecting the affinity of the enzyme for magnesium or sodium, binding was carried out at suboptimal concentrations of these ions. Lowering the concentration of magnesium from 2.5 to 0.25 mM or sodium from 100 to 15 mM caused approximately a 50% reduction in rate in the presence or absence of quinidine (Table II), suggesting that quinidine has a direct effect on the intrinsic rate constant rather than on the binding of these ions. Although, in the presence of NaMgATP, we were unable to detect a change in the rate of binding at a digoxin concentration of 1 μM, we did detect a reduction in the rate at a lower concentration (25 nM) of digoxin (Fig. 3B). Fig. 4 shows that, in the presence of Mg or MgATP or NaMgATP, the decrease in rate of digoxin binding

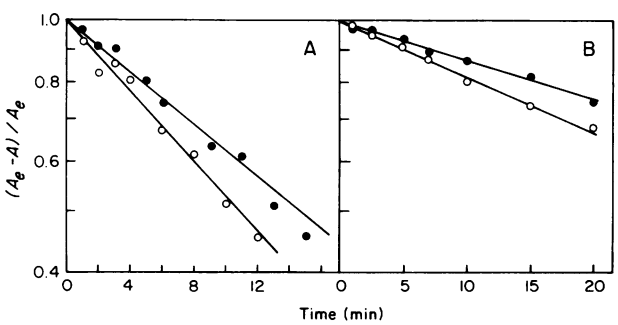

FIGURE 3 Pseudo first-order binding of [^{3}H]digoxin to Na,K-ATPase in the presence (●) and absence (○) of 200 μM quinidine: (A) in the presence of 2.5 mM Mg^{++} and 1 μM [^{3}H]digoxin; and, (B) in the presence of 100 mM Na^+ and 2.5 mM MgATP and 25 nM [^{3}H]digoxin. The data from binding rate curves are plotted using Eq. 1 as described in Methods.

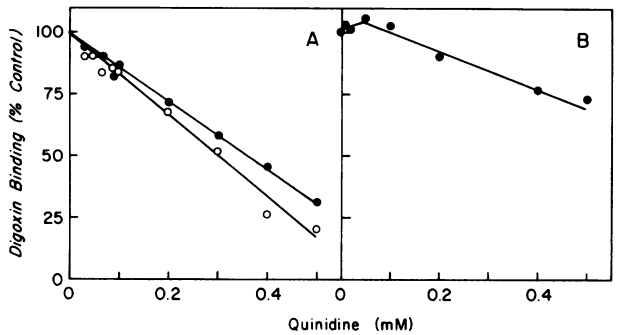

FIGURE 4 Effect of quinidine concentration on binding of [^{3}H]digoxin to Na,K-ATPase in the presence: (A) of 2.5 mM Mg^{++} (○) and 2.5 mM Mg^{++} and ATP (●) and 2.5 μM [^{3}H]-digoxin; and, (B) of NaMgATP and 25 nM [^{3}H]digoxin. The data are from two experiments, each done in duplicate, and the results are expressed as percentages relative to the binding of digoxin observed in the absence of added quinidine for a 20-min (A) or a 5-min (B) binding interval at 30°C.

is dependent upon the quinidine concentration with reductions of 65, 55, and 25%, respectively, in digoxin binding at a quinidine concentration of 0.4 mM.

Effect of quinidine on rate of dissociation of digoxin from Na,K-ATPase. The effect of quinidine on the rate of dissociation of enzyme-bound digoxin was tested by binding [^{3}H]digoxin (1 μM) to Na,K-ATPase for 30 min and then adding an excess of unlabeled ouabain (1 mM) to "chase" the labeled compound. The decrease in bound [^{3}H]digoxin over several hours was observed in the presence and absence of added quinidine (0.2 mM). Fig. 5 shows that the rates of dissociation (Mg only conditions, $t_{1/2} \cong 6.0$ h and NaKMgATP conditions, $t_{1/2} \cong 9.5$ h) were not significantly influenced by quinidine. In these experiments, Mg, MgATP, MgP_i and NaMgATP binding conditions gave similar dissociation rates ($t_{1/2} \cong 6.0$ h) while the rates observed for NaKMgATP were slower. Quinidine did not alter the dissociation rates of digoxin, but the slower dissociation rate observed under turnover conditions contrasts with the work of Wallick and Schwartz (31) using ouabain in which similar rates ($t_{1/2} \cong 3.0$ h) were observed for all binding conditions.

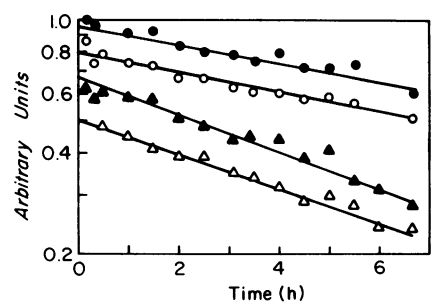

FIGURE 5 Rate of dissociation of enzyme-digoxin complex. Equilibrium binding of [^{3}H]digoxin (1 μM) to Na,K-ATPase (10 μg/ml) was achieved: in the presence of 2.5 mM Mg^{++} in the presence (▲) or absence (△) of 0.2 mM quinidine; and, in NaKMgATP conditions in the presence (●) or absence (○) of quinidine. At time zero, 1.0 mM unlabeled ouabain was added; aliquots were taken at the times indicated, and the decrease in enzyme-bound [^{3}H]digoxin determined. Values are given as fractions (log scale) relative to maximum or equilibrium digoxin binding. The ordinate has been arbitrarily scaled in order to present both sets of data.

The effects of quinidine on the rate of digoxin binding to human erythrocytes. The effect of quinidine on digoxin binding to the intact membrane system of human erythrocytes was also investigated. The binding of digoxin to erythrocyte membranes exhibited linear first order rates (Fig. 6) and, as shown in Table III, 1 mM quinidine caused 70% increases in the $t_{1/2}$ values for the binding of [^{3}H]digoxin (19.5 and 95.6 nM). The maximal extent of [^{3}H]digoxin binding as calculated using an unweighted nonlinear curve-fit analysis of the rate data was found to be unaltered by the presence of quinidine; thus the maximum number of binding sites was not affected by quinidine.

Effect of quinidine on the rate of digoxin-induced inhibition of $^{86}Rb^+$ uptake by erythrocytes. Utilizing the fact that Rb^+ has been demonstrated to be taken up by the erythrocyte through the same process that is involved in the active transport of K^+ (38, 39), we used $^{86}Rb^+$ to study the effect of quinidine upon the action of digoxin on monovalent cation transport in human erythrocytes. Other workers have reported both increases (40, 41) and decreases (42, 43) in $^{42}K^+$ flux into mammalian cells in the presence of quinidine. As shown in Table IV, quinidine exerted minimal effects on $^{86}Rb^+$ uptake under the conditions of this study. As

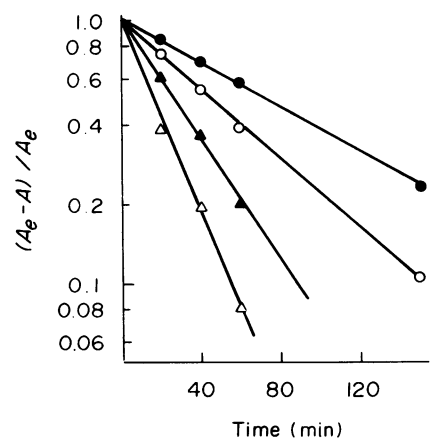

FIGURE 6 Pseudo first-order binding of [^{3}H]digoxin to erythrocyte membranes in the presence and absence of 1 mM quinidine. The data from the binding rate curves are plotted using Eq. 1, as described in Methods. Each point represents the average of six to nine separate determinations. ○, 19.5 nM digoxin; ●, 19.5 nM digoxin plus 1 mM quinidine; △, 95.6 nM digoxin; ▲, 95.6 nM digoxin plus 1 mM quinidine.

TABLE III
Rates of Inhibition of Digoxin Binding and of Inhibition of $^{86}Rb^+$ Influx in Human Erythrocytes

[Digoxin]	$t_{1/2}$ (min)	
	Control	+ Quinidine
nM		
	[^{3}H]Digoxin binding	
19.5	45.9	77.6
95.6	15.6	27.2
	Inhibition of $^{86}Rb^+$ Influx	
19.5	46	132
95.6	12.1	23.2

The $t_{1/2}$ values are obtained from first-order rate plots of binding or inhibition data treated in a manner similar to that for Table II results. Each data point of plot was average of six to nine determinations. Quinidine concentration was 1 mM.

shown in Fig. 7, when digoxin was added to erythrocytes that had been incubated in 1 mM quinidine for 2 h, the rate of inhibition was decreased, but quinidine did not alter the final effectiveness of digoxin. The $t_{1/2}$ values for the inhibition rates are given in Table III and they show that quinidine can cause 186 and 92% increases in the $t_{1/2}$ values for the rates of inhibition of 19.5 and 95.6 nM digoxin. Less marked increases in $t_{1/2}$ were observed when 0.1 mM quinidine was used, and no effect was noted in the presence of 10 μM quinidine.

Lack of effect of quinidine on rate of dissociation of digoxin from receptor sites. Although the data obtained using the purified Na,ATPase enzyme did not support the hypothesis that quinidine displaces receptor-bound digoxin, the possibility remained that such displacement could occur with native membrane systems. Therefore, intact erythrocytes were incubated for 2 h with 0.19 μM [^{3}H]digoxin, washed, and resuspended in the presence or absence of 1 mM quinidine. In agreement with the experiments with the purified enzyme, quinidine had no effect, although ouabain caused a slight displacement of [^{3}H]digoxin (Table V). Similarly, the extent to which digoxin inhibited $^{86}Rb^+$ influx in intact erythrocytes, 4 h after washing them free of unbound digoxin, was not altered by the presence of quinidine (Table V).

TABLE IV
$^{86}Rb^+$ Uptake by Human Erythrocytes after 2 h Incubation with Quinidine

Quinidine concentration	$^{86}Rb^+$ Uptake*
	±*SD*
10 μM	102±5%
0.1 mM	106±3%
1 mM	112±7%

* Values compared with $^{86}Rb^+$ uptake by erythrocytes incubated for 2 h in medium without quinidine.

DISCUSSION

These studies provide evidence that quinidine is capable of decreasing the affinity of receptor sites on purified Na,K-ATPase and on intact erythrocyte membranes for digoxin. Thus, the decreases in volumes of digoxin distribution observed clinically when quinidine is administered to digoxin-treated patients (6–8) may reflect, at least in part, a decrease in the affinity of tissue receptors for digoxin.

The decrease in affinity of receptors for digoxin noted in this study was slight and no displacement of digoxin could be detected when quinidine was added to Na,K-ATPase preparations or to erythrocyte membranes that had previously been incubated with digoxin. This decrease in the affinity of the receptor for digoxin under nonsaturating conditions should increase serum digoxin concentration as long as quinidine is present. Although the extent to which in vivo bound digoxin levels would decrease is unclear, it should be pointed out that, because <1% of the total body glycoside stores are present in the serum of digoxin-treated patients (44), a loss of 1% of tissue digoxin stores could result in a twofold increase in serum digoxin concentration, and would approximate the increase frequently noted clinically following quinidine administration (1, 2, 4, 7, 8, 11–15). The precision of our experiments on purified Na,K-ATPase

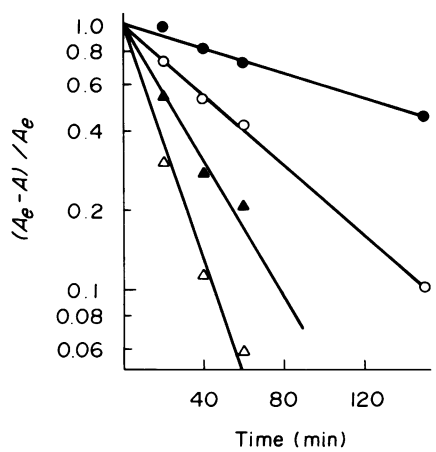

FIGURE 7 Effect of quinidine on digoxin-inhibited $^{86}Rb^+$ influx. The rates of inhibition of $^{86}Rb^+$ influx into erythrocytes are plotted as pseudo first-order rates. ○, 19.5 nM digoxin; ●, 19.5 nM digoxin plus 1 mM quinidine; △, 95.6 nM digoxin; ▲, 95.6 nM digoxin plus 1 mM quinidine.

TABLE V

Effects of Addition of Quinidine to Erythrocytes That Had Previously Been Incubated with 0.19 μM Digoxin

Quinidine concentration	15 min		4 h	
	Membrane bound digoxin	Inhibition of $^{86}Rb^+$ uptake	Membrane bound digoxin	Inhibition of $^{86}Rb^+$ uptake
	pmol/ml RBC		*pmol/ml RBC*	
None	12.3±1.1	70±5.2%	11.4±0.9	68±6.9%
1 mM	11.9±1.2	72±8.3%	11.2±1.3	70±6.3%
0.1 mM	11.8±1.1	73±7.5%	11.1±1.0	70±7.4%
10 μM	12.4±1.4	63±3.3%	11.3±1.2	64±2.5%
None (1 mM ouabain)	12.0±1.4	79±7.7%	10.2±1.3	78±6.4%

Erythrocytes (RBC) were incubated with digoxin for 2 h, washed, and incubated with quinidine. Rubidium uptake is expressed as percent inhibition of $^{86}Rb^+$ uptake by control cells which had not been exposed to digoxin or quinidine. All values are the mean (±SD) of three experiments.

and erythrocytes does not allow us to detect such a small change; one might anticipate that displacement of digoxin might be particularly difficult to demonstrate in human erythrocytes (the only human tissue readily available for these studies) since the dissociation rate of digoxin from erythrocyte receptors as observed here (Table V) and in a previous study (32), is significantly slower than its dissociation rate from receptors in skeletal and cardiac muscle (45), tissues containing the major pools of glycoside binding sites in man (44). Therefore, the inability to demonstrate displacement of digoxin from receptor sites in the present study does not constitute incontrovertible evidence against the hypothesis that a displacement of digoxin from tissue receptors by quinidine may contribute to the increased serum digoxin levels observed clinically after quinidine administration.

Although the renal excretion of digoxin in man and in the dog predominantly reflects glomerular filtration of the drug (44, 46), evidence has been presented that tubular secretion may also play a role in man (47). Quinidine is capable of decreasing the renal clearance of digoxin (5–8, 10–15), and it has been suggested that quinidine may exert this effect by interfering with the tubular secretion of the cardiac glycoside (6, 7, 10–12, 14, 15). For reasons stated in the introduction, it seems unlikely that decreased renal excretion of digoxin is the sole explanation for an increase in serum digoxin concentration after quinidine administration. However, it is possible that both a decrease in the affinity of tissue digoxin receptors and a decrease in the renal clearance of digoxin may play roles in the increase in serum digoxin concentration observed after quinidine administration. In this connection, the possibility can be considered that the decreased clearance of digoxin may merely be a reflection of a quinidine-induced decrease in affinity of digoxin receptors in the renal tubular cells responsible for digoxin secretion.

The present studies indicate that quinidine decreases the rate of [^{3}H]digoxin binding to both the purified sheep kidney enzyme and to erythrocyte receptors by decreasing their affinity for digoxin and not by decreasing the total number of binding sites. We have used the sheep kidney enzyme rather than the cardiac enzyme because its highly purified state makes it suitable for studying these drug-receptor interactions. Cardiac enzyme preparations clearly contain sarcoplasmic and mitochondrial fragments and have been shown to have a large nonspecific quinidine binding component (26). In addition, studies by Wallick et al. (45) have shown that the rates of association and dissociation, for ouabain, and the I_{50} values, for both ouabain and ouabagenin, of kidney and cardiac preparations are similar. In a recent abstract, Straub et al. (48) have also reported that quinidine decreases the binding of ouabain to a beef heart membrane Na,K-ATPase preparation, but these workers have suggested that the decreased binding reflects a decreased number of glycoside binding sites. Except for this difference in interpretation, their results and ours are consistent with the hypothesis that quinidine can act to decrease binding of digoxin to glycoside receptor sites.

The effects of quinidine in the present study were noted to be dependent upon the ligand conditions present during [^{3}H]digoxin binding. Quinidine exerted its greatest effect on digoxin binding under conditions (Mg or MgATP) that favor the dephosphorylated form of the enzyme (Table II) while, on the other hand, quinidine had little effect under conditions that favor the phosphorylated form of Na,-

K-ATPase (MgP_i or NaMgATP). In the absence of direct studies of quinidine binding, our data do not allow us to determine whether Na^+ (in the presence of MgATP) or P_i (in the presence of Mg) decreases the affinity of the enzyme for quinidine or directly inhibits the effect of quinidine on the affinity of the enzyme for digoxin. Lowry et al. (25) have reported that inhibitory effects of quinidine on both the Na,K-ATPase activity and the K^+ dependent *p*-nitrophenylphosphatase activity of rat brain Na,K-ATPase can be reduced in the presence of high K^+, thus suggesting that quinidine binding may be ligand dependent. The decreased affinity for digoxin caused by quinidine in the presence of Mg and MgATP also suggests either that binding of quinidine occurs at the digoxin binding site or that quinidine affects the Mg^{++} binding sites of the enzyme.

Although quinidine at noninhibitory levels (10 μM) decreased the action of digoxin, inhibitory concentrations of quinidine augmented the action of digoxin in a manner suggesting independent sites. Moreover, augmentation by quinidine of the effect of digoxin on active monovalent cation transport was not observed in the present study. These findings together with the observations on the importance of ligand conditions for quinidine inhibition of digoxin binding to Na,K-ATPase suggest that the interaction between digoxin, Na,K-ATPase and quinidine is a complex one. Nevertheless, it is clear from the present study, that despite reducing the affinity of receptors for digoxin, quinidine is capable, at concentrations above 50 μM, of augmenting the inhibitory effects of digoxin on Na,-K-ATPase activity in vitro. Caution must be exercised in extrapolating this observation to clinical situations because the concentrations of the two drugs which exerted this combined effect (50 μM quinidine and 0.1 μM digoxin) were considerably in excess of their therapeutic serum concentrations in man (5–15 μM quinidine and 1–3 nM digoxin) (49, 50) and because *in vivo* tissue-to-serum drug concentration ratios (44, 51, 52) may differ from the ratios of receptor to medium quinidine and digoxin concentrations under the various conditions used in the current study. The relative ratios of quinidine to digoxin concentrations that were used in these studies are, however, comparable to those observed clinically. We would therefore propose the hypothesis that some of the enhanced cardiac effects of digoxin which have been reported clinically during quinidine treatment (1–3, 13, 18, 53, 54) may result from an augmentation, by quinidine, of digoxin effects that more than compensates for a modest reduction in digoxin binding. In proposing this hypothesis, however, we would not exclude the possibility that impaired digoxin elimination (7, 8, 10) may also play a role in the reported enhancement, by quinidine, of the effects of digoxin.

ACKNOWLEDGMENTS

We are grateful to Dr. J. Thomas Bigger, Jr., and to Dr. Edward B. Leahey, Jr., for helpful discussions during the course of these experiments. We wish to thank Kathleen Donnelly for secretarial assistance.

This work was supported by grants from the New York Heart Association and from the National Institutes of Health (HL 10608, HL 20859, HL 22039, and HL 22619).

REFERENCES

1. Ejvinsson, G. 1978. Effect of quinidine on plasma concentrations of digoxin. *Br. Med. J.* **1:** 279–280.
2. Leahey, E. B., Jr., J. A. Reiffel, R. E. Drusin, R. H. Heissenbuttel, W. P. Lovejoy, and J. T. Bigger, Jr. 1978. Interaction between quinidine and digoxin. *JAMA (J. Am. Med. Assoc.).* **240:** 533–534.
3. Kaufmann, G. 1978. Is there a need for new cardiac glycosides? For more blood level determinations? *In* Cardiac Glycosides. G. Bodem and H. J. Dengler, editors. Springer-Verlag, Berlin. 410–421.
4. Doering, W., and E. König. 1978. Anstieg der Digoxinkonzentration im Serum unter Chinidinmedikation. *Med. Klin.* **73:** 1085–1088.
5. Hooymans, P. M., and F. W. H. M. Merkus. 1978. Effect of quinidine on plasma concentration of digoxin. *Br. Med. J.* **2:** 1022.
6. Hager, W. D., P. Fenster, M. Mayersohn, D. Perrier, P. Graves, F. I. Marcus, and S. Goldman. 1979. Digoxin-quinidine interaction: pharmacokinetic evaluation. *N. Engl. J. Med.* **300:** 1238–1241.
7. Schenck-Gustafsson, K., and R. Dahlqvist. 1981. Pharmacokinetics of digoxin in patients subjected to the quinidine-digoxin interaction. *Br. J. Clin. Pharmacol.* **11:** 181–186.
8. Leahey, E. B., Jr., J. T. Bigger, Jr., V. P. Butler, Jr., J. A. Reiffel, G. C. O'Connell, L. E. Scaffidi, and J. N. Rottman. 1981. Quinidine-digoxin interaction: time course and pharmacokinetics. *Am. J. Cardiol.* In press.
9. Gibson, T. P., and H. A. Nelson. 1980. Digoxin alters quinidine and quinidine alters digoxin pharmacokinetics in dogs. *J. Lab. Clin. Med.* **95:** 417–428.
10. Steiness, E., S. Waldorff, P. B. Hansen, H. Kjaergård, J. Buch, and H. Egeblad. 1980. Reduction of digoxin-induced inotropism during quinidine administration. *Clin. Pharmacol. Ther.* **27:** 791–795.
11. Doering, W. 1979. Quinidine-digoxin interaction: pharmacokinetics, underlying mechanism and clinical implications. *N. Engl. J. Med.* **301:** 400–404.
12. Risler, T., U. Peters, B. Grabensee, and L. Seipel. 1979. Untersuchungen zur Interaktion von Chindin und Digoxin beim Menschen. *Dtsch. Med. Wochenschr.* **104:** 1523–1526.
13. Dahlqvist, R., G. Ejvinsson, and K. Schenck-Gustafsson. 1980. Effect of quinidine on plasma concentration and renal clearance of digoxin. A clinically important drug interaction. *Br. J. Clin. Pharmacol.* **9:** 413–418.
14. Mungall, D. R., R. P. Robichaux, W. Perry, J. W. Scott, A. Robinson, T. Burelle, and D. Hurst. 1980. Effects of quinidine on serum digoxin concentration: a prospective study. *Ann. Intern. Med.* **93:** 689–693.
15. Pedersen, K. E., J. Hastrup, and S. Hvidt. 1980. The effect of quinidine on digoxin kinetics in cardiac patients. *Acta Med. Scand.* **207:** 291–295.
16. Mercer, E. N., and G. E. Dower. 1966. Normal and arrhythmic beating in isolated cultured heart cells and the

effects of digoxin, quinidine and procaine amide. *J. Pharmacol. Exp. Ther.* **153:** 203–210.
17. Kwit, N. T., and H. Gold. 1934. Further experimental observations on the combined effects of digitalis and quinidine on the heart. *J. Pharmacol. Exp. Ther.* **50:** 180–197.
18. Gold, H. 1950. Quinidine in Disorders of the Heart. Paul B. Hoeber, Inc., New York. 85–92.
19. Acierno, L. J., and R. Gubner. 1951. Utility and limitations of intravenous quinidine in arrhythmias. *Am. Heart J.* **41:** 733–741.
20. Rodensky, P. L., J. H. Kathe, and F. Wasserman. 1960. The effect of quinidine on tolerance to digoxin. *Am. J. Cardiol.* **5:** 498–505.
21. Storstein, O., V. Hansteen, L. Hatle, L. Hillestad, and L. Storstein. 1977. Studies on digitalis. XIII. A prospective study of 649 patients on maintenance treatment with digitoxin. *Am. Heart J.* **93:** 434–443.
22. Selzer, A., and H. W. Wray. 1964. Quinidine syncope: paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. *Circulation.* **30:** 17–26.
23. Kennedy, K. G., and W. G. Nayler. 1965. The effect of quinidine on the activity of a sodium-potassium activated, magnesium-dependent ATPase enzyme isolated from toad cardiac muscle. *Biochim. Biophys. Acta.* **110:** 174–180.
24. Song, S. Y., and J. Scheuer. 1968. The effects of pharmacologic agents on myocardial sodium (Na^+) and potassium (K^+) stimulated ATPase. *Pharmacology.* **1:** 209–217.
25. Lowry, K., S. N. Rao, B. J. R. Pitts, and A. Askari. 1973. Effects of quinidine on some reactions and ion translocations catalyzed by the Na^+,K^+-ATPase complex. *Biochem. Pharmacol.* **22:** 1369–1377.
26. Besch, H. R., Jr., and A. M. Watanabe. 1977. Binding and effect of tritiated quinidine on cardiac subcellular enzyme systems: sarcoplasmic reticulum vesicles, mitochondria and Na^+,K^+-adenosine triphosphatase. *J. Pharmacol. Exp. Ther.* **202:** 354–364.
27. Eaton, D. L., and C. D. Klaassen. 1978. Carrier-mediated transport of ouabain in isolated hepatocytes. *J. Pharmacol. Exp. Ther.* **205:** 480–488.
28. Watson, J. F., and V. P. Butler, Jr. 1972. Biologic activity of digoxin-specific antisera. *J. Clin. Invest.* **51:** 638–648.
29. Lane, L. K., B. M. Anner, E. T. Wallick, M. V. Ray, and A. Schwartz. 1978. Effect of phospholipase A treatment on the partial reactions of and ouabain binding to a purified sodium and potassium activated adenosine triphosphatase. *Biochem. Pharmacol.* **27:** 225–231.
30. Schwartz, A., J. C. Allen, and S. Harigaya. 1969. Possible involvement of cardiac Na^+,K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. *J. Pharmacol. Exp. Ther.* **168:** 31–41.
31. Wallick, E. T., and A. Schwartz. 1974. Thermodynamics of the rate of binding of ouabain to the sodium, potassium-adenosine triphosphatase. *J. Biol. Chem.* **249:** 5141–5147.
32. Gardner, J. D., D. R. Kiino, T. J. Swartz, and V. P. Butler, Jr. 1973. Effects of digoxin-specific antibodies on accumulation and binding of digoxin by human erythrocytes. *J. Clin. Invest.* **52:** 1820–1833.
33. Crosby, W. H., J. I. Munn, and F. W. Furth. 1954. Standardizing a method for clinical hemoglobinometry. *U. S. Armed Forces Med. J.* **5:** 693–703.
34. Wallick, E. T., G. E. Lindenmayer, L. K. Lane, J. C. Allen, B. J. R. Pitts, and A. Schwartz. 1977. Recent advances in cardiac glycoside-Na^+,K^+-ATPase interaction. *Fed. Proc.* **36:** 2214–2218.
35. Parker, R. B., and D. R. Waud. 1971. Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J. Pharmacol. Exp. Ther.* **177:** 1–12.
36. Caldwell, R. W., and C. B. Nash. 1978. Comparison of the effects of aminosugar cardiac glycosides with ouabain and digoxin on Na^+,K^+-adenosine triphosphatase and cardiac contractile force. *J. Pharmacol. Exp. Ther.* **204:** 141–148.
37. Scatchard, G., J. S. Coleman, and A. L. Shen. 1957. Physical chemistry of protein solutions. VII. The binding of some small anions to serum albumin. *J. Am. Chem. Soc.* **79:** 12–20.
38. Kahn, J. B., Jr. 1962. The entry of rubidium into human erythrocytes. *J. Pharmacol. Exp. Ther.* **136:** 197–204.
39. Bernstein, J. C., and Y. Israel. 1970. Active transport of Rb^{86} in human red cells and rat brain slices. *J. Pharmacol. Exp. Ther.* **174:** 323–329.
40. Holland, W. C., and R. L. Klein. 1958. Effects of temperature, Na and K concentration and quinidine on transmembrane flux of K^{42} and incidence of atrial fibrillation. *Circ. Res.* **6:** 516–521.
41. Choi, S. J., J. Roberts, and G. J. Kelliher. 1972. The effect of propranolol and quinidine on ^{22}Na- and ^{42}K-exchange in the cat papillary muscle. *Eur. J. Pharmacol.* **20:** 10–21.
42. Kärki, N., G. P. Burn, and J. H. Burn. 1957. The effect of quinidine on potassium flux. *Lancet.* **I:** 565.
43. McCall, D. 1976. Effect of quinidine on cation exchange in cultured cells. *J. Pharmacol. Exp. Ther.* **197:** 605–614.
44. Doherty, J. E. 1968. The clinical pharmacology of digitalis glycosides: a review. *Am. J. Med. Sci.* **255:** 382–414.
45. Wallick, E. T., B. J. R. Pitts, L. K. Lane, and A. Schwartz. 1980. A kinetic comparison of cardiac glycoside interactions with Na^+,K^+-ATPases from skeletal and cardiac muscle and from kidney. *Arch. Biochem. Biophys.* **202:** 442–449.
46. Risler, T., J. C. Somberg, R. D. Blute, Jr., and T. W. Smith. 1980. The effect of altered renal perfusion pressure on clearance of digoxin. *Circulation.* **61:** 521–525.
47. Steiness, E. 1974. Renal tubular secretion of digoxin. *Circulation.* **50:** 103–107.
48. Straub, K. D., J. J. Kane, J. K. Bissett, and J. E. Doherty. 1978. Alteration of digitalis binding by quinidine: A mechanism of digitalis-quinidine interaction. *Circulation.* **58:** II-58 (Abstr.).
49. Koch-Weser, J. 1972. Serum drug concentrations as therapeutic guides. *N. Engl. J. Med.* **287:** 227–231.
50. Smith, T. W., V. P. Butler, Jr., and E. Haber. 1969. Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *N. Engl. J. Med.* **281:** 1212–1216.
51. Scherlis, L., L. F. Gonzalez, and S. P. Bessman. 1961. Quinidine: arterial, venous, coronary sinus and myocardial concentrations. *J. Clin. Invest.* **40:** 60–65.
52. Cho, Y. W. 1973. Quantitative correlation of plasma and myocardial quinidine concentrations with biochemical and electrocardiographic changes. *Am. Heart J.* **85:** 648–654.
53. Leahey, E. B., Jr., J. A. Reiffel, R. H. Heissenbuttel, R. E. Drusin, W. P. Lovejoy, and J. T. Bigger, Jr. 1979. Enhanced cardiac effect of digoxin during quinidine treatment. *Arch. Intern. Med.* **139:** 519–521.
54. Holt, D. W., A. M. Hayler, M. E. Edmonds, and R. F. Ashford. 1979. Clinically significant interaction between digoxin and quinidine. *Br. Med. J.* **2:** 1401.