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The Effect of Quinidine on the Positive Inotropic Actions of Digoxin in Isolated Cardiac Muscle

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Joshua R. Berlin

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Tai Akera

Major professor

Tai Akera

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THE EFFECT OF QUINIDINE ON THE POSITIVE INOTROPIC ACTIONS OF DIGOXIN IN ISOLATED CARDIAC MUSCLE

By

Joshua R. Berlin

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ABSTRACT

The Effect of Quinidine on the Positive Inotropic Actions of Digoxin in Isolated Cardiac Muscle

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The effect of quinidine-induced elevations in plasma digoxin concentration on the pharmacologic effects of the glycoside are unclear. One possibility was that quinidine could decrease the specific binding of the glycoside to the putative receptor for digoxin (specific binding), the sarcolemmal sodium pump, because quinidine reduces sodium influx into the cardiac muscle fibers. The present study was undertaken to determine whether quinidine does decrease the amount of sodium available to the sodium pump, thereby reducing the specific binding of digoxin and decreasing the positive inotropic effect of the glycoside in left atrial muscle preparations of guinea-pig heart.

Quinidine decreased the rate of sodium influx in atrial muscle as indicated by a frequency-dependent reduction in maximal upstroke velocity (dV/dt_{max}) of the action potential and in steady-state ouabain-sensitive $^{86}Rb^{+}$ uptake in beating heart muscle ($^{86}Rb^{+}$ uptake). In preparations stimulated at 3 Hz, 20 μM quinidine decreased $^{86}Rb^{+}$ uptake by 30±3% and dV/dt_{max} by 50%. Under these conditions, quinidine failed to reduce either the rate of onset or magnitude of the positive inotropic effect of 0.6 μM digoxin or the specific binding of

the glycoside. Effects of quinidine were compared with those of benzocaine which decreased dV/dt_{max} at concentrations $\geq$300 μM. Benzocaine (300 μM) decreased $^{86}Rb^{+}$ uptake by 32±11% and delayed the onset of the positive inotropic effect of digoxin; however, specific binding of digoxin was not significantly reduced by benzocaine. These results indicate that decreases in sodium influx did not reduce glycoside binding to the sodium pump.

The binding of [^{3}H]ouabain to Na^{+},K^{+}-ATPase, the biochemical correlate of the sodium pump, was inhibited by quinidine only in concentrations $\geq$300 μM (in the presence of Na^{+}, Mg^{2+} and ATP with or without K^{+}). Ouabain binding was stimulated by Na^{+} in concentrations of $\geq$5 mM, and quinidine failed to influence Na^{+}-induced stimulation of ouabain binding. The curve representing Na^{+}-induced stimulation of glycoside binding indicates that decreases in sodium influx caused by pharmacologic concentrations of quinidine are likely to reduce glycoside binding by approximately 5-10%. This small decrease in digoxin binding is not likely to be pharmacologically significant. Thus, quinidine does not have a significant pharmacodynamic interaction with digoxin.

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

INTRODUCTION

A. General Background

Digitalis glycosides and quinidine have been administered concomitantly for the treatment of cardiac rhythm disorders. As early as 1932, however, Gold et al. (1932) cautioned against the hazards of this drug combination. Using the database collected by the Boston Collaborative Drug Surveillance Program, retrospective studies have shown that the incidence of adverse drug reactions in patients on digoxin maintenance therapy was increased after the inclusion of quinidine in the patient drug regimen (Cody et al., 1980) and that the majority of patients with apparent quinidine-induced ventricular dysrhythmias were also receiving digitalis glycosides (Cohen et al., 1977). These findings gave suggestive evidence for a drug interaction between quinidine and digoxin. The incidence of untoward effects for quinidine alone was almost 30% (Bigger and Hoffman, 1980), so that it was not clear if the increase in the number of adverse drug reactions was simply a result of an additive drug effect. In 1978, three laboratories (Doering and Konig, 1978; Ejvinsson, 1978; Leahey et al., 1978) independently reported that quinidine increased serum digoxin concentration when the antiarrhythmic agent was administered to patients on digoxin maintenance therapy. These reports have stimulated a great deal of research designed to investigate the mechanism by which quinidine increases

serum digoxin levels and the clinical implications of the increased digitalis levels on cardiac function.

B. Effects of Quinidine on Digoxin Pharmacokinetics

Reports subsequent to those in 1978 have shown that quinidine, in therapeutic doses, produces an approximately 100% increase in digoxin levels in the serum of patients and healthy volunteers receiving digoxin (Bigger, 1979; Fichtl and Doering, 1983). The increase in serum digoxin levels, though quite variable between individuals, appears to be proportional to the dose of quinidine administered (Doering and Konig, 1978; Powell et al., 1980). Serum digoxin concentration begins to increase within 24 hr of initiation of quinidine, reaches a plateau in 4 to 5 days and remains elevated for the duration of quinidine administration (Leahey et al., 1978; Doering, 1979; Doering and Konig, 1981; Leahey et al., 1981).

Quinidine could increase serum digoxin concentration by several mechanisms. Alterations in digoxin absorption, distribution and/or elimination might be responsible for the elevation in digitalis levels. As will become apparent, the actions of quinidine on digoxin pharmacokinetics are quite complex and are, as yet, not completely understood.

Chen and Friedman (1980) have shown that a single dose of quinidine enhanced absorption of digoxin from the gastrointestinal tract. Digoxin, however, is well absorbed after oral administration (>70%; Fichtl and Doering, 1983) so that even a marked increase in the adsorption of the administered dose would not account for the magnitude of the change in serum digoxin concentration seen in the presence of quinidine. Furthermore, β-methyldigoxin, a lipophilic analogue of

digoxin which is absorbed with almost 100% efficiency from the gut, exhibits similar increases in serum concentration as digoxin with concomitant quinidine administration (Doering, 1979). Therefore, changes in absorption of digoxin do not appear to explain the marked increase in serum digoxin observed with combined drug therapy.

A decrease in the elimination rate of digoxin from the body could account for the sustained rise in serum glycoside concentration. The rate of disappearance of digoxin from plasma was slowed by quinidine in volunteers administered an i.v. bolus dose of digoxin (Steiness et al., 1980). Similar results were reported in patients on combined digoxin/quinidine therapy (Doering et al., 1981; Pedersen et al., 1983). Conflicting data, though, has been reported by Hager et al. (1979) who found no consistent effect of quinidine on the half-time of the disappearance of digoxin from plasma. Even so, serum elimination half-times increased in 3 of 6 individuals. Quinidine appeared to decrease the rate of elimination of digoxin from the body.

Digoxin is eliminated from the body primarily by the kidney (Hoffman and Bigger, 1980), and several studies have shown that renal clearance of digoxin is decreased by quinidine (Doering, 1979; Hager et al., 1979; Steiness et al., 1980; Leahey et al., 1981). Creatinine clearance, an index of glomerular filtration rate (Doering, 1979; Hager et al., 1979; Steiness et al., 1980), and plasma protein binding (Fichtl and Doering, 1983) of digoxin are unchanged by quinidine. Taken together these data show decreased renal clearance of digoxin observed during concomitant quinidine administration is due to a direct effect of quinidine on the renal handling of digoxin. Leahey et al. (1981) found that the magnitude of the decrease of renal digoxin clearance

correlated well with the plasma quinidine concentration, even at levels below therapeutic quinidine concentrations. These results suggest that altered renal elimination of digoxin may, in part, be responsible for elevated serum digoxin levels seen in patients on combined quinidine/digoxin therapy.

Extrarenal clearance which accounts for approximately one-third of total elimination of digoxin (Hoffman and Bigger, 1980), is decreased by quinidine to a similar extent as renal clearance (Steiness et al., 1980; Pedersen et al., 1983). Quinidine also elevates plasma digoxin concentration in anuric patients on chronic digoxin maintenance therapy to a similar extent as seen in patients with normal kidney function. Serum digoxin in these patients reached a new steady-state concentration 8 days after initiating quinidine administration, whereas serum quinidine concentration remained constant after 3 days (Fichtl et al., 1983). That serum digoxin continued to rise after serum quinidine concentration had plateaued indicates that the increase in the concentration of digoxin was not simply due to a competitive displacement of digoxin from tissue binding sites by quinidine. Fichtl et al. (1983) concluded from these findings that extrarenal clearance of digoxin was slowed although values for digoxin clearance were not reported. Quinidine decreases the rate of digoxin elimination from the body both by renal and extrarenal pathways, and a recent overview of published reports (Fichtl and Doering, 1983) points out that the magnitude of the observed decrease in digoxin clearance (>40% reduction) produced by quinidine may be sufficient to account for the doubling of serum digoxin concentration reported in patients simultaneously administered quinidine and digoxin.

Alterations in digoxin clearance from the body may not be the sole mechanism by which quinidine increases serum glycoside concentration. Leahey et al. (1978) noted that serum digoxin concentration was increased by quinidine even when digoxin administration was discontinued prior to the initiation of quinidine therapy. Risler et al. (1980) reported, in contrast to Leahey et al. (1981), that although the increase in serum digoxin concentration depended on the administered dose of quinidine, renal clearance of digoxin was decreased to a similar extent whether quinidine was given in a low (500 mg/day) or high (1000 mg/day) dose. These results suggested that decreased renal clearance was not the major cause for elevated serum digoxin concentration. Displacement of tissue stores of digoxin was suggested by the authors as the underlying cause for these effects. Numerous studies, have since been undertaken to examine the influence of quinidine on digoxin distribution within the body.

An increase in plasma digoxin concentration may result from a decline in the apparent volume of distribution of the drug. Published reports dealing with the influence of quinidine on the distribution volume of digoxin after intravenous administration of the glycoside have yielded conflicting results. No change (Steiness et al., 1980; Leahey et al., 1981) or a decrease (Hager et al., 1979) in the volume of digoxin distribution has been noted. Aside from differences in methodology, the conflicting result may be due to differences in the administered dose of quinidine. Both Steiness et al. (1980) and Leahey et al. (1981) administered quinidine which resulted in mean serum quinidine concentrations of less than 2 μg/ml while the mean serum concentration achieved in the study of Hager et al. (1979) was 2.5

μg/ml. Indeed, when Leahey et al. (1981) segregated the subjects into low (less than 1.9 μg/ml) and high (greater than 1.9 μg/ml) serum quinidine concentration, a significant decrease in apparent volume of distribution was observed in those subjects with "high" serum quinidine concentrations. A recent study by Pedersen et al. (1983) in patients on chronic quinidine/digoxin therapy verifies the results of the earlier single dose studies. The volume of distribution of digoxin was reduced in patients on quinidine as compared to when quinidine therapy was withdrawn. The magnitude of the decrease was correlated with the plasma quinidine concentration. These data suggest that quinidine decreases the volume of digoxin distribution in patients on combined quinidine/digoxin therapy.

A quinidine-induced mobilization of tissue stores of digoxin may explain the decreased volume of digoxin distribution. As already noted, the presence of quinidine does not influence the binding of digoxin to plasma proteins. Schenck-Gustafsson et al. (1981) reported that, although skeletal muscle digoxin content increased 50% as compared to before quinidine administration, the ratio of skeletal muscle to serum digoxin concentrations decreased by 23% in patients treated for atrial fibrillation after 4 days of concomitant quinidine administration. This was taken as evidence for reduced binding of digoxin to skeletal muscle. Similar evidence, in dogs, showed that tissue/serum ratios of digoxin were reduced during quinidine administration in many organs including skeletal and heart muscle, kidney and liver (Doherty et al., 1980). The reported decrease in the apparent volume of digoxin distribution may be the result of decreased tissue binding by digoxin in the presence of quinidine.

Changes in digoxin volume of distribution may account for a transient increase in serum digoxin concentration caused by quinidine but cannot explain a sustained increase in serum digoxin concentration. A sustained increase in serum digoxin is possible only if the rate of digoxin elimination is slowed. Changes in volume of distribution will only lead to transient changes in plasma drug concentration (Lee, 1980; Fichtl and Doering, 1983). Quinidine produces a sustained increase in serum digoxin concentration (Leahey et al., 1978; Doering, 1979) so that a slowing of digoxin elimination itself would be sufficient to produce an increase in serum digoxin. The effect of changes in digoxin distribution, however, should not be discounted as a factor which might contribute to the rise in digoxin concentration immediately after quinidine administration. This notion is supported by studies in animal models which demonstrate that differences in serum digoxin concentration in the presence of quinidine become apparent within 2 hours (Gibson and Nelson, 1979; Kim et al., 1981a) when changes in elimination would have only a small impact on serum digoxin levels.

In summary, quinidine has complex effects on digoxin pharmacokinetics. In the presence of quinidine, serum digoxin concentration is elevated in patients receiving digoxin maintenance therapy. Quinidine appears to decrease the volume of distribution of digoxin within the body which may result in a transient increase in serum digoxin concentration. The sustained rise in digoxin levels must be due to a decrease in the rate of elimination of digoxin, and pharmacokinetic studies suggest that both renal and extrarenal clearance of digoxin is decreased.

C. Clinical Implications of the Quinidine/Digoxin Interaction

From a clinical perspective, the most important point to be resolved is whether the quinidine-induced elevation in serum digoxin concentration results in a comparable increase in the effect of digoxin on heart function. Studies of the quinidine/digoxin interaction in man and in experimental models have not led to concensus on this point.

In a series of restrospective and prospective case studies, Leahey and coworkers looked for evidence of increased glycoside effects in digitalized patients receiving quinidine. In their original paper (Leahey et al., 1978), 7 of 27 patients had ventricular arrhythmias develop or worsen after beginning quinidine administration. These adverse reactions and the incidence of nausea were lessened by decreasing the digoxin dose. Their subsequent studies confirmed the findings in the first paper. Patients on digoxin therapy, when administered quinidine simultaneously, had a lengthening of the PR interval of the electrocardiogram sometimes leading to A-V dissociation, a sign of increased glycoside action, as well as a high incidence of gastrointestinal distress. These changes were usually noted within 72 hr of initiating quinidine administration (Leahey et al., 1979; 1980b). Thus, increased serum digoxin concentration was associated with a greater incidence of untoward reactions and ECG evidence of a greater digoxin effect. These studies, however, did not directly address the question of whether the increased serum digoxin concentration was having a greater influence on ventricular performance. Although a greater incidence of ventricular arrhythmias and nausea in patients on quinidine/digoxin therapy might indicate a more pronounced effect of increased serum digoxin, quinidine itself has a high incidence of side

effects including arrhythmias and gastrointestinal disturbances (Cohen et al., 1977) so that the increased incidence of these symptoms might only reflect the presence of quindine. The increased prolongation of the P-R interval of the ECG, while indicative of a greater glycoside effect, is a measure of digoxin's anticholinergic action, not the drug's effect on ventricular contractility. Thus, these studies, while suggestive of an increased effect of digoxin in patients receiving both quinidine and digoxin, did not demonstrate an increased effect on cardiac performance.

Subsequent studies in man, examining the effects of digoxin on cardiac performance, are divided on whether increased serum glycoside concentration correlates with an increased effect on the heart. Some studies have demonstrated that the effect of digoxin on cardiac contractility was reduced after concomitant quinidine administration was begun. Cardiac contractility was measured indirectly by determining the effect of various drug regimens on the electromechanical systole of the heart. Electromechanical systole or the systolic time interval (STI) is shortened by inotropic concentrations of digoxin. Two studies demonstrated that the shortening of STI by digoxin was reduced after beginning quinidine administration (Hirsch et al., 1980; Steiness et al., 1980), which indicated that the inotropic effect of digoxin was decreased. These studies have been criticized, however, in that Steiness et al. (1980) did not report the effect of quinidine alone on STI and Hirsh et al. (1980) measured the effects of quinidine in less than half of the cases receiving digoxin and quinidine, though in those individuals studied, quinidine did not produce a significant effect on STI. Belz et al. (1982), conducting a double-blind, crossover study,

found that the effect of digoxin on STI shortening in volunteers receiving concomitant quinidine and digoxin was not greater than when they received digoxin alone; however, quinidine itself produced a significant lengthening of STI. Thus, the effect of digoxin on STI was greater with simultaneous quinidine administration when the effect of quinidine was taken into account. This finding was confirmed by elevating the serum digoxin concentration in the absence of quinidine to the same level as that seen with quinidine/digoxin administration. In both cases, STI were shortened to a similar extent by digoxin. Zaman et al. (1981), using echocardiographic techniques, also found evidence for an increased effect of digoxin on the rate of ventricular shortening when quinidine was administered simultaneously. These studies demonstrated that the increase in serum digoxin caused by quinidine was correlated with an enhanced glycoside effect on cardiac function, in opposition to the earlier studies (Hirsh et al., 1980; Steiness et al., 1980) which found no such correlation. The differences in these investigations may have resulted from the different protocols used.

Investigations using animal models of the quinidine/digoxin interaction have also given conflicting results concerning the effects of elevated serum digoxin levels. Kim et al. (1981b) found that occupancy of cardiac Na^+,K^+-ATPase, the putative pharmacologic receptor for cardiac glycosides (Akera, 1981), was increased in animals co-administered quinidine and digoxin at a time when serum digoxin concentration was significantly greater than in animals administered digoxin only. In dogs, elevated serum digoxin produced by concomitant quinidine administration was shown to have a greater pharmacologic effect as measured by inhibition of active monovalent cation transport (Leahey et

al., 1980a), which is proposed as the mechanism of glycoside action in the heart (Akera, 1981). In agreement with the above studies, acetylstrophanthidin tolerance (AcST) tests in dogs showed that digoxin had a greater pharmacologic effect when its serum concentration was elevated by quinidine (Wilkerson et al., 1984). The AcST test uses the inverse relationship between the pharmacologic effect of digoxin and the dose of acetylstrophanthin to produce premature ventricular beats to determine the degree of digoxin's pharmacologic effect on the heart. The test is complicated because quinidine alone increases AcST; however, when the effect of quinidine is considered, the reduction in AcST produced by digoxin in animals receiving both quinidine and digoxin is greater than the reduction in AcST produced by digoxin in dogs administered the same dose of digoxin only (Wilkerson et al., 1984). One study (Warner et al., 1984), though, shows a dissociation between the serum concentration of digoxin and its pharmacologic actions in dogs co-administered quinidine. In this study, quinidine increased serum digoxin levels 100 percent, but the inhibition of active monovalent cation transport in cardiac ventricular muscle from these dogs was the same as the inhibition of cation transport seen in cardiac muscle taken from dogs receiving digoxin only. Furthermore, when dogs on digoxin only were titrated to a serum glycoside concentration the same as that seen in animals receiving both quinidine and digoxin, the expected increase in inhibition of active cation transport was observed. Although the serum digoxin concentration was the same in both groups, the effect of digoxin on monovalent ion transport was significantly less in dogs administered quinidine. The reasons for the conflicting results in these studies is not clear. Thus, the possibility that quinidine interferes with the pharmacologic actions of digoxin remains unsettled.

D. Mechanism of the Positive Inotropic Effect of Cardiac Glycosides

Cardiac glycosides have been known to inhibit monovalent cation transport across cellular membranes for several decades (Schatzmann, 1957; Post et al., 1960). Thus, when a sodium, potassium-activated adenosine triphosphatase activity was identified in the microsomal fraction of crab neurons (Skou, 1957) and in other tissues (Hess and Pope, 1957; Post et al., 1960), the activity of which was blocked by cardiac glycosides, the hypothesis was soon advanced that the positive inotropic effect of the glycosides was due to inhibition of the cardiac sarcolemmal Na^+,K^+-ATPase (Repke, 1963; Glynn, 1964).

1. Determinants of glycoside inhibition of the Na^+,K^+-ATPase enzyme

Binding of cardiac glycosides to isolated Na^+,K^+-ATPase enzyme requires the presence of Mg^{2+} and is stimulated by ATP or inorganic phosphate (Matsui and Schwartz, 1968; Schwartz et al., 1968). The binding of digitalis is further enhanced by Na^+ but is antagonized by K^+ in the presence of Mg^{2+} and ATP, whereas, binding in the presence of Mg^{2+} and Pi is inhibited by both Na^+ and K^+ (Matsui and Schwartz, 1968; Schwartz et al., 1968; Akera and Brody, 1971). The ligand requirements for glycoside binding appear to be the same as those required for formation of the phosphorylated intermediate of the native Na^+,K^+-ATPase. Sodium, in combination with Mg^{2+} and ATP, promotes the formation of the phosphoenzyme and potassium enhances the rate of dephosphorylation of the enzyme (Sen et al., 1969; Skou and Hilberg, 1969). Inorganic phosphate also phosphorylates the enzyme in the presence of Mg^{2+} (Lindenmayer et al., 1968; Sen et al., 1969). Preferential binding of the glycoside, thus, appears to occur to a

phosphorylated conformation of the enzyme, E_2-P (Matsui and Schwartz, 1968; Sen et al., 1969; Tobin et al., 1972). Although ouabain and K^+ compete for binding to the same enzyme conformation, the glycoside binding site is distinct from that for K^+ (Matsui and Schwartz, 1966) and is located on the outer surface of the intact membrane (Perrone and Blostein, 1973).

Cardiac glycoside binding to the Na^+,K^+-ATPase leads to a stabilization of the phosphoenzyme (Lindenmayer et al., 1968; Sen et al., 1969) and further binding of ATP is blocked (Sen et al., 1969; Skou and Hilberg, 1969). Consequently, binding of cardiac glycosides correlates closely with inhibition of Na^+,K^+-ATPase activity, and, in intact cellular preparations, binding parallels drug-induced inhibition of active Na^+ and K^+ fluxes across the cell membrane of erythrocytes (Hoffman, 1969; Gardner and Kiino, 1973) and squid axon membranes (Baker and Willis, 1972). Recently, some cardiac muscle membranes have been shown to have two classes of glycoside binding sites, but only one class of sites, the low affinity binding sites, have been associated with inhibition of Na^+,K^+-ATPase activity and inhibition of active cation flux (Kazazoglou et al., 1983). Thus, cardiac glycosides specifically bind to the Na^+,K^+-ATPase and binding is modulated by the presence of Na^+ and K^+. The consequences of binding are inhibition of Na^+,K^+-ATPase activity and active monovalent cation transport in intact cells.

2. Positive inotropic effect of cardiac glycosides and binding to the Na^+,K^+-ATPase

The positive inotropic action of cardiac glycosides in the heart is believed to result from partial inhibition of the cardiac

sarcolemmal Na^+,K^+-ATPase. This hypothesis has been supported by several lines of evidence which show that cardiac glycosides bind to the Na^+,K^+-ATPase and inhibit monovalent cation transport at the time of their positive inotropic action in the heart. The Na^+,K^+-ATPase isolated from cardiac ventricular muscle of anesthetized dog administered ouabain has been shown to be inhibited to a degree related to the positive inotropic effect of the drug (Akera et al., 1970; Allen et al., 1975). Similar results were obtained in isolated in situ dog heart preparations (Besch et al., 1970). These studies have been supported by the finding that the glycoside is bound to the Na^+,K^+-ATPase at the time of the positive inotropic effect in isolated heart muscle preparations (Ku et al., 1974; Schwartz et al., 1974). Binding of the glycoside is also concentration-dependent (Allen et al., 1975) and the degree of binding correlates well with the onset and washout of drug effects (Ku et al., 1974; Schwartz et al., 1974). Furthermore, the function of other cellular components, the mitochondria and sarcoplasmic reticulum, which might alter cardiac muscle contractility, were not changed during cardiac glycoside inotropic action (Besch et al., 1970; Allen et al., 1975). Thus, digitalis binds to and is capable of inhibiting the Na^+,K^+-ATPase at the time of its positive inotropic effect.

The characteristics of the glycoside/enzyme complex formed in vivo and in heart muscle preparations show similar characteristics as the complex formed with isolated Na^+,K^+-ATPase. The glycoside/Na^+,K^+-ATPase complex formed in vitro in the presence of Na^+, Mg^{2+} and ATP is stabilized in the presence of KCl (Akera and Brody, 1971). Similarly, the dissociation of bound glycoside from tissue homogenates of canine

ventricular muscle exposed to ouabain for a period of time before homogenization is slower in the presence of KCl (Akera et al., 1976b). Potassium also antagonizes glycoside binding to the isolated Na^+,K^+-ATPase (Matsui and Schwartz, 1968; Akera and Brody, 1971). Likewise, when extracellular KCl is increased the rate of development of the positive inotropic effect and the increase in tissue content of digoxin in cat ventricular papillary muscles is delayed (Prindle et al., 1971). In anesthetized dog, hyperkalemia reduced the positive inotropic effect of digoxin and decreased the drug-induced inhibition of the Na^+,K^+-ATPase (Goldman et al., 1973).

The potency of cardiac glycosides to inhibit the Na^+,K^+-ATPase varies greatly depending on the source of the enzyme. The concentration of ouabain to produce a positive inotropic effect in isolated guinea pig and rat cardiac muscle correlates well with the concentration of drug required to inhibit the Na^+,K^+-ATPase isolated from guinea pig and rat heart. In both cases, the rat required an approximately 100 times greater drug concentration (Ku et al., 1976). Similar species-dependent effects of cardiac glycosides have been observed in dog, cat, and rabbit (Akera et al., 1973). In all cases, the concentration of digitalis which inhibits the activity of the cardiac Na^+,K^+-ATPase correlated well with the concentration to produce a positive inotropic effect. These studies, taken together, demonstrate that cardiac glycosides bound to the Na^+,K^+-ATPase during their positive inotropic action display characteristics similar to the drug bound to the Na^+,K^+-ATPase in vitro, which suggests that glycoside binding is closely related to the positive inotropic effect of these drugs.

3. Sodium pump inhibition and the positive inotropic effect of cardiac glycosides

The sodium pump, the functional correlate of the Na^+,K^+-ATPase, maintains the ionic gradients across the cellular membrane against passive leak of ions down their electrochemical gradients. Inhibition of the Na^+,K^+-ATPase by positive inotropic concentrations of digitalis should inhibit a fraction of the functional sodium units which would lead to an increase in intracellular sodium and a loss of intracellular potassium. Some studies, however, have shown that myocardial Na^+ and K^+ content does not change with inotropic concentrations of cardiac glycosides (Lee and Klaus, 1971; Bentfeld et al., 1977). Akera et al.(1976a) has postulated that monovalent cation content need not change markedly in the face of moderate sodium pump inhibition, because the "reserve capacity" of the pump, the difference between sodium influx rate and the capacity of the sodium pump to extrude sodium, will still allow the sodium pump to maintain the Na^+ and K^+ gradients across the membrane. Nevertheless, the magnitude of the transient increase in intracellular sodium which might be expected to occur following membrane excitation would be enhanced by moderate inhibition of the pump. More recent studies demonstrated that sodium content does increase in cultures of chick embryonic heart cells (Biedert et al., 1979; Kazazoglou et al., 1983) in a dose-dependent manner for positive inotropic concentrations of digitalis. Furthermore, diastolic free intracellular sodium concentration, measured with sodium-sensitive microelectrodes, has also been shown to increase after exposure of isolated heart muscle preparations to nontoxic concentrations of digitalis (Lee et al., 1980; Lee and Dagostino, 1982; Wasserstrom

et al., 1983). Alterations in intracellular sodium concentration do occur in the presence of positive inotropic concentrations of cardiac glycosides, a result consistent with sodium pump inhibition.

Direct confirmation that the sodium pump is inhibited by digitalis comes from measuring the rate of monovalent cation transport across the sarcolemmal membrane. The maximal rate of monovalent cation transport, the pump capacity, is decreased in a dose-dependent manner at subtoxic glycoside concentrations (Kazazoglou et al., 1983). The decrease in pump capacity also correlates well with the onset and washout of ouabain's positive inotropic effects in isolated heart muscle (Ku et al., 1974), and pump activity has also been shown to be decreased during the onset of drug effects (Hougen and Smith, 1978; Biedert et al., 1979). These studies demonstrate that pump inhibition is closely related to the positive inotropic actions of cardiac glycosides in the heart.

4. Determinants of glycoside binding to the sodium pump

As already discussed, digitalis binding to the Na^+,K^+-ATPase is modulated by the action of Na^+ and K^+ ions to induce the enzyme to take the K^+-sensitive phosphoenzyme conformation and to reduce this form of the enzyme, respectively. Similarly, in the intact cell, the binding of the glycosides to the sodium pump is regulated by the concentration of intracellular sodium and extracellular potassium, presumably reflecting the availability of the glycoside-sensitive conformation of the Na^+,K^+-ATPase. Raising extracellular potassium decreases the rate at which ouabain inhibits active cation transport in squid axon (Baker and Willis, 1972), decreases glycoside-induced inhibition of the Na^+,K^+-ATPase measured in canine cardiac muscle exposed to

ouabain (Goldman et al., 1973) and lessens the development of the positive inotropic actions of ouabain (Goldman et al., 1973; Prindle et al., 1973). Conversely, decreasing extracellular potassium enhances the rate of glycoside inhibition of active cation transport in squid axon (Baker and Willis, 1972). These studies show that binding of ouabain to the sodium pump is influenced by extracellular potassium which is likely to result from the K^+-induced reduction in the availability of glycoside-sensitive phosphoenzyme.

Maneuvers which increase intracellular sodium, on the other hand, promote glycoside binding to the sodium pump. Several studies (Moran, 1967; Park and Vincenzi, 1975; Bentfeld et al., 1977) have shown that the development of glycoside actions at different rates of electrical stimulation is dependent on the number of contractions not on the duration of glycoside exposure. This "beat dependency" of glycoside action was further shown to depend on the number of membrane depolarizations rather than the number of contractions (Akera et al., 1977). The rate of ouabain binding (Yamamoto et al., 1979; Temma and Akera, 1982) and inhibition of active monovalent cation transport (Yamamoto et al., 1979) are also increased by higher stimulation frequencies. These experiments demonstrate that the onset of cardiac glycoside action is highly dependent on the frequency of electrical stimulation. Increasing stimulation rates have been shown to increase diastolic intracellular sodium concentration (Cohen et al., 1982; January and Fozzard, 1984) and increase sodium pump activity (Yamamoto et al., 1979; Akera et al., 1981). Compounds which increase sodium influx rate such as monensin, a sodium ionophore (Meier et al., 1976)

and the sodium channel toxins, batrachatoxin (Albuquerque et al., 1973) and grayanotoxin (Narahashi and Seyama, 1974) which increase sodium influx via the sodium channel, increase the rate of development of the positive inotropic effect of ouabain (Akera et al., 1977) and enhance glycoside binding (Temma and Akera, 1982) in isolated cardiac muscle preparations. Thus, increased sodium transport by the sodium pump enhances the rate of digitalis action; again, presumedly by increasing the availability of the glycoside-sensitive conformation of the Na^+,K^+-ATPase.

The effects of lowering intracellular sodium on cardiac glycoside action are not as easily explained solely by postulating that glycoside binding is dependent on the availability of the K^+-sensitive phosphoenzyme conformation of the Na^+,K^+-ATPase. Isolated cardiac muscle preparations incubated in a low sodium (85 mM) salt solution showed a delayed onset of the positive inotropic action of ouabain when compared to muscles bathed in normal sodium (145 mM) solution (Akera et al., 1977). Lowering extracellular sodium concentration to 85 mM has been shown to decrease resting intracellular sodium activity by approximately 30% in sheep heart Purkinje fibers (Ellis, 1977). Incubating cardiac muscle preparations in sodium depleted (20-30 mM) or sodium-free buffers which decreases free intracellular sodium concentration more than 70% (Ellis, 1977), prevents the positive inotropic effect of cardiac glycosides (Linden and Brooker, 1980; Wiggins and Bentolila, 1980; Temma and Akera, 1983). Although Linden and Brooker (1980) believed that ouabain binding still occurred under these conditions, Temma and Akera (1983) demonstrated that ouabain binding to the Na^+,K^+-ATPase did not occur in guinea pig left atrial muscle exposed to the

glycoside in a 27 mM Na^+ buffer solution. These data are in agreement with the postulate that intracellular sodium ion concentration modulates glycoside binding to the sodium pump; however, other data appear to be at odds with this postulate. Inactivating the sodium channels by raising extracellular potassium concentration from 4 to 25 mM decreases free intracellular sodium concentration approximately 20% (Ellis, 1977), yet in cardiac muscle restored to excitability with isoproterenol, cardiac glycosides still have a positive inotropic effect (Besch and Watanabe, 1978). The magnitude and time course of the positive inotropic effect of ouabain observed in cat ventricular papillary muscle incubated in 22 mM K^+-containing buffer solutions is not significantly different than that seen in preparations incubated in 8 mM K^+ buffer solutions (Wiggins and Bentolila, 1980). The effect of isoproterenol on intracellular Na^+ concentration in the presence of high extracellular K^+ has not been examined. Moreover, epinephrine has been shown to stimulate glycoside binding to the Na^+,K^+-ATPase in rat soleus muscle immediately after addition of the catecholamine to the incubation medium (Clausen and Hansen, 1977). Therefore, the lack of an effect of high extracellular potassium on the inotropic actions of ouabain may not necessarily indicate that a reduction in intracellular sodium failed to affect the onset of the glycoside action. Isolated cardiac muscle preparations exposed to 3 μM tetrodotoxin, however, still show an increase in contractility in the presence of cardiac glycosides whose rate of development is not significantly different from that seen in the absence of tetrodotoxin (Wassermann and Holland, 1969), even though 3 μM tetrodotoxin is reported to decrease intracellular sodium by approximately ten percent (Deitmer and Ellis, 1980a).

The moderate decrease in free intracellular sodium ion concentration caused by tetrodotoxin may not be sufficient to produce a significant change in inotropic effect of the glycoside, unlike the more pronounced decrease in intracellular sodium ion concentration (30%) produced by lowering extracellular sodium to 85 mM. Alternatively, changing extracellular sodium ion concentration may affect digitalis binding to the Na^+,K^+-ATPase at an extracellular site in addition to its effect on intracellular sodium. In summary, increased intracellular sodium ion concentration promotes cardiac glycoside binding to the Na^+,K^+-ATPase; however, the effect of a decrease in intracellular sodium ion concentration on the binding and on the positive inotropic actions of digitalis are not as well established.

5. Mechanism of the positive inotropic effect of cardiac glycosides

Cardiac glycosides bind to the sarcolemmal Na^+,K^+-ATPase and, in the intact cell, inhibit the sodium pump producing a transient and/or sustained increase in intracellular sodium during the cardiac contraction cycle. The positive inotropic effect of the glycosides is mediated by an increased intracellular calcium transient during the cardiac action potential (Morgan and Blinks, 1982). The mechanism believed to link increased intracellular sodium to an increased intracellular calcium concentration is Na^+/Ca^{2+} exchange across the sarcolemma (Reiter and Seitz, 1968). Intracellular sodium is coupled to intracellular calcium by the electrochemical gradients for sodium and calcium across the sarcolemma (Mullins, 1979) so that an increase in intracellular sodium is translated into an increase in intracellular calcium by greater calcium influx across the sarcolemma (Glitsch et al., 1970; Ellis, 1977). Indeed, recent studies using cardiac cell

cultures suggest that calcium influx is increased by inotropic concentrations of cardiac glycosides (Biedert et al., 1973; Kazazoglou et al., 1983) and by veratridine, an agent which increases intracellular sodium independent of sodium pump inhibition (Fosset et al., 1977; Pang and Sperelakis, 1982). Thus, the hypothesis has been developed which states that digitalis inhibition of the Na^+,K^+-ATPase decreases the rate of sodium extrusion by the sarcolemmal sodium pump. The resulting increase in intracellular sodium augments net calcium influx during the action potential via sodium/calcium exchange. The increased calcium influx is manifested as the positive inotropic effect of the cardiac glycosides.

E. Possible Mechanisms of Quinidine Interaction with Digoxin

From the previous discussion, it is evident that quinidine could interfere with the inotropic actions of digoxin by several mechanisms. Quinidine could inhibit the binding of ouabain to the sodium pump either by competing for the glycoside receptor site of the Na^+,K^+-ATPase or by decreasing the availability of the glycoside sensitive conformation of the sodium pump. Alternatively, quinidine might interfere with the consequences of glycoside inhibition of the sodium pump.

Quinidine has been shown to affect many of the subcellular processes which control calcium metabolism in the heart. Quinidine binds to (Besch and Watanabe, 1977) and inhibits Ca^{2+} sequestration by isolated cardiac sarcoplasmic reticulum vesicles (Fuchs et al., 1968; Besch and Watanabe, 1977). Furthermore, quinidine is reported to inhibit membrane currents associated with Na/Ca exchange in frog heart

cells (Mentrard et al., 1984). Although inhibition of cardiac sarcoplasmic reticulum function or inhibition of sarcolemmal Na/Ca exchange might attenuate the effect of digoxin binding to the Na^+,K^+-ATPase in the heart, the quinidine concentrations ($>10^{-4}$M) which were effective at inhibiting these processes were 1-2 orders of magnitude greater than the therapeutic range (3-6 μM) of quinidine. Consequently, quinidine would not appear to interfere with the consequences of sodium pump inhibition by digoxin.

Quinidine might antagonize the inotropic actions of digoxin by decreasing binding of digoxin to the Na^+,K^+-ATPase in the heart. Displacing digoxin bound to its receptor site or preventing glycoside binding to the cardiac muscle Na^+,K^+-ATPase would antagonize inotropic actions of digoxin. Quinidine has been found to inhibit the activity of the Na^+,K^+-ATPase isolated from heart (Lowry et al., 1973; Besch and Watanabe, 1977) and brain (Lowry et al., 1973) as well as inhibit active monovalent cation transport in erythrocytes (Lowry et al., 1973; Ball et al., 1981). The concentration needed to produce fifty percent inhibition, however, was approximately 1 millimolar. Quinidine has been reported to decrease ouabain binding to the Na^+,K^+-ATPase. The drug (10^{-4} - 10^{-3}M) decreased the number of ouabain binding sites in beef heart sarcolemmal preparations without changing receptor site affinity (Straub et al., 1978). Another study (Ball et al., 1981), however, found that B_{max} for glycoside binding remained constant but that the apparent K_D increased due to a decrease in the association rate constant. Again, quinidine concentration was close to or equal to one millimolar. Quinidine inhibited Na^+,K^+-ATPase activity and decreased glycoside binding to the enzyme but only at very high

concentrations. Doering (1979), on the other hand, could not demonstrate any effect of lower concentrations (10^{-6} - 10^{-4}) of quinidine on ouabain binding to sheep heart Na^+,K^+-ATPase. It is interesting to note that even in the presence of high concentrations (millimolar) of quinidine, specifically-bound digoxin was not displaced from its receptor (Ball et al., 1981). Thus, quinidine is thought to act at a site distinct from the glycoside receptor -- a proposal supported by the data of Lowry et al. (1973) which showed the characteristics of quinidine inhibition of the Na^+,K^+-ATPase differed significantly from those of cardiac glycosides. The above reports, therefore, indicate that quinidine is capable of inhibiting digoxin binding to the Na^+,K^+-ATPase, but the concentrations required were much greater than those used therapeutically.

Quinidine could also inhibit digoxin binding by decreasing the availability of the glycoside-sensitive form of the sodium pump indirectly rather than competing at the glycoside receptor site. As discussed previously, the availability of the glycoside-sensitive form of the sodium pump is regulated by the levels of extracellular potassium and intracellular sodium. Quinidine probably has little effect on the effective extracellular potassium concentration (Note: Colatsky (1982) has demonstrated that quinidine inhibits the delayed rectifier current in rabbit Purkinje fibers, an effect which might influence extracellular potassium in the beating heart muscle). On the other hand, quinidine may well have significant effects on intracellular sodium concentration. Quinidine has local anesthetic actions and its antiarrhythmic effect has been attributed to its depression of the sodium current in heart muscle. Quinidine produces a significant decrease in the rate of

phase 0 depolarization of transmembrane action potentials recorded in cardiac atrial (Vaughan-Williams, 1958) and ventricular muscle fibers (Johnson, 1956; Johnson and McKinnon, 1957). The decrease in upstroke velocity reflects a decline in the rate of sodium entry through the sodium channel into the cardiac cell. The rate of sodium entry measured either by isotopic sodium flux (Choi et al., 1972) or indirectly, by the rate of potassium efflux (van Zwieten, 1969; Klein et al., 1960; Choi et al., 1972) is decreased by quinidine in heart muscle preparations. A similar decrease in sodium influx rate is believed to be responsible for the decrease in intracellular sodium ion activity produced by the local anesthetic agents, procaine and lidocaine, and by tetrodotoxin in cardiac Purkinje fibers (Deitmer and Ellis, 1980a; January and Fozzard, 1984). The effect of quinidine on intracellular free sodium ion concentration has not been investigated but it is expected to be similar to the other local anesthetic agents.

A quinidine-induced decrease in sodium influx and intracellular sodium ion activity in cardiac tissue might be expected to reduce the binding of cardiac glycosides to the myocardial sodium pump because of a decline in the digitalis-sensitive conformation of the pump. Studies in man have given conflicting results as to whether quinidine actually does decrease the inotropic actions of digoxin. Investigations using animal models of the quinidine/digoxin interaction also yield conflicting data on the effects of quinidine on digoxin binding to the cardiac Na^+,K^+-ATPase and inhibition of active monovalent cation transport. In vivo studies, however, are complicated by the fact that serum digoxin concentration increases markedly with co-administration of quinidine.

It is difficult then to discern whether quinidine does antagonize digoxin effects because the increased serum glycoside concentration may overcome the influence of quinidine. Although the apparent digoxin effect may be increased its effect might be less than in the absence of quinidine. Few studies have thoroughly evaluated this point (but see Belz et al., 1982; Warner et al., 1984). To avoid this complication, the effects of quinidine on the inotropic actions of digoxin have been investigated in isolated heart muscle preparations. In cat ventricular papillary muscle (Williams and Mathew, 1981), prior administration of quinidine decreased the positive inotropic effect of digoxin when compared to the same concentration of digoxin alone. Interestingly, if quinidine was added after digoxin, the net increase in contractility in the presence of both drugs was greater than with the glycoside alone, even though quinidine itself had no inotropic effect in this preparation. The reasons for the opposite effects of quinidine on the inotropic action of digoxin, depending on the order of drug addition, are unclear. Other studies in cultured heart cells (Horowitz et al., 1982), guinea pig and rat cardiac muscle (Kim et al., 1981a), and ferret papillary muscle (Lash et al., 1982), however, have found that quinidine did not influence the rate of onset or the magnitude of the positive inotropic actions of digoxin. In these studies, quinidine had no effect on glycoside inhibition of sodium pump activity (Horowitz et al., 1982; Kim et al., 1981a) or on glycoside binding to the Na^+,K^+-ATPase during incubation of the muscle preparation with digoxin (Kim et al., 1981a). The quinidine concentrations (3-15 μM) used in these studies were at or near therapeutic antiarrhythmic concentrations and were capable of elevating serum digoxin concentrations in vivo (Kim et

al., 1981c). These studies indicated that quinidine did not diminish the inotropic actions of digoxin in isolated heart muscle preparations.

In summary, the question of whether quinidine interacts with the direct effects of digoxin on cardiac muscle function remains unsettled. The hypothesis has been advanced that quinidine may be capable of diminishing the rate of digoxin binding to the Na^+,K^+-ATPase by decreasing sodium influx through the sarcolemmal sodium channel. The resulting decrease in intracellular sodium would reduce the glycoside-sensitive conformation of the sodium pump which would decrease the rate of digoxin binding and delay the onset of the positive inotropic effect of digoxin. Experimental studies in man and animals have found, most often, that quinidine does not diminish digoxin effects in the heart. Previous studies in this laboratory (Kim et al., 1981a) have also found that quinidine did not decrease the positive inotropic effect or specific binding of digoxin in isolated cardiac muscle preparations. The reasons for the lack of the expected quinidine/digoxin interaction in many experimental studies are unknown. Thus, the objective of this project was to investigate why quinidine apparently fails to interact with digoxin despite the above hypothesis and to determine if quinidine would reduce the positive inotropic effect of digoxin under certain experimental conditions.

The results of this study may help formulate a rationale basis to determine if the serum digoxin concentration in patients receiving combined quinidine/digoxin therapy should be "targeted" to a specified concentration or allowed to rise in the presence of quinidine.

MATERIALS AND METHODS

A. Inotropic Studies

Guinea pigs of either sex weighing 250-450 g were stunned by a sharp blow to the head and the hearts were rapidly removed. After retrograde perfusion of the aorta with a Krebs-Henseleit bicarbonate buffer solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 28 mM $NaHCO_3$, 1.0 mM KH_2PO_4, 1.2 mM $CaCl_2$ and 11 mM glucose) to wash out blood, the left atrial muscle was excised and hung vertically in a tissue bath at 32°C containing the above buffer solution saturated with 95% O_2-5% CO_2. Platinum electrodes were used for electrical field stimulation of the atrial muscle with square wave pulses of 4 ms duration and 20% above threshold. Resting tension was adjusted to 1 g and nearly isometric force of contraction was recorded continuously on a polygraph recorder (Grass Instrument Company, Quincy, MA; Polygraph 7B) equipped with a force-displacement transducer (Grass Instrument Company, FT-03C).

After an equilibration period of at least 60 minutes, a test drug or vehicle was added to the bathing medium. Quinidine was dissolved in water and benzocaine was dissolved in polethylene glycol. Polyethylene glycol content in the bathing medium did not exceed 0.1%. After the developed tension of the atrial muscle had reached a new steady state, digoxin, in the final concentration of 0.6 μM, was added to the bathing medium. The inotropic effects of digoxin were expressed as the change

in the force of contraction of the atrial muscle relative to force observed before glycoside administration. The rate of onset of the positive inotropic effect of digoxin was monitored and expressed as the time to half maximal drug effect. The inotropic effects of quinidine or benzocaine alone was also monitored.

B. Action Potential Studies

Left atrial muscle was isolated from guinea pig heart as described above. The atrial muscle was pinned to the bottom of a constant temperature bath maintained at 31°C and superfused at a rate of 3 ml/min with Krebs-Henseleit bicarbonate buffer solution saturated with 95% O_2/5% CO_2. Muscle preparations were stimulated by an extracellular electrode which delivered square-wave pulses of 4 msec duration, 20% above threshold.

Transmembrane potentials were recorded using 3 M KCl-filled microelectrodes connected to a high input impedance amplifier (WP Instruments, New Haven, CT; Model 707) via a Ag/AgCl wire (floating electrode). Electrode tip resistances were 10-30 MΩ. The output of the amplifier was electronically differentiated (Galveston Electronics Corp, Galveston, TX) to measure maximal upstroke velocity of the action potential. The outputs of the amplifier and differentiator were displayed on an oscilloscope (Tektronix Inc., Beaverton, OR; Model R5103N) and photographed with a kymograph camera (Grass Instruments Corp, Quincy, MA; Model C-3). In some experiments, the amplifier output was also stored and analyzed on a PDP-8 computer (Digital Equipment Corp, Maynard, MA). Drug effects on resting potential, maximal upstroke velocity ($\dot{V}_{max}$) of the action potential, action potential amplitude and

duration at 20 (T_{20}), 50 (T_{50}) and 90 percent (T_{90}) repolarization were monitored.

In the present experiments, continuous impalements were maintained in a single cell throughout the course of the experiment. After stable action potentials were recorded for at least 15 minutes from preparations perfused with a drug-free buffer solution, cumulative dose-response experiments were performed. Data are expressed relative to corresponding values observed during the control period. Where indicated, RbCl (5 mM) was substituted for KCl and KH_2PO_4 was replaced with NaH_2PO_4.

C. $^{86}Rb^+$ Uptake Studies

Sodium pump activity was measured in beating left atrial muscle preparations by a modification of the method of Yamamoto et al. (1979). Muscle preparations were isolated as described above and incubated in a Krebs-Henseleit bicarbonate buffer solution in which the following changes were made: KH_2PO_4 was replaced by NaH_2PO_4 and KCl was replaced with 5 mM RbCl. The RbCl-containing, K^+-free Krebs-Henseleit buffer solution (Rb^+ K-H) was continuously aerated with 95% O_2/5% CO_2 and maintained at 32°C. The atrial muscle were field-stimulated at the indicated frequency with platinum electrodes which delivered square-wave pulses of 4 msec duration, 50% above threshold from a Grass Instruments S-9 stimulator. After an equilibration period of at least 60 minutes, the test drug was added. After the actions of the drug had stabilized, tracer amounts of $^{86}RbCl$ were added to the buffer solution. Twenty minutes later, the atria were washed for 1 minute in a $^{86}Rb^+$-free, Rb^+ K-H (24°C), blotted, and weighed. Radioactivity in the

tissue was quantified using a gamma scintillation spectrometer (Tracor Analytic, Des Plaines, IL; Isocap 300). Ouabain-sensitive Rb^+ uptake was determined as the difference in the isotope uptake obtained in the absence and presence of 0.3 mM ouabain. Initial experiments showed that ouabain-sensitive ^{86}Rb uptake was almost linear up to 30 minutes (Figure 1, inset).

Ouabain-sensitive $^{86}Rb^+$ uptake in beating (not sodium-loaded) heart muscle preparations is thought to be a measure of on-going sodium pump activity because under steady-state conditions, sodium pump activity is in balance with the rate of sodium influx into the cardiac cell (Akera et al., 1981; Eisner et al., 1983b). For this reason, $^{86}Rb^+$ uptake was measured after drug effects had stabilized when steady-state conditions had been reached. Thus, ouabain-sensitive $^{86}Rb^+$ uptake can also be used as an indirect measure of sodium influx rate in beating atrial muscle preparations. To establish that the experimental conditions used could detect charges in sodium influx, the effect of alterations in the rate of sodium influx on $^{86}Rb^+$ uptake was examined. The rate of sodium influx was changed by stimulating the atrial muscle at different frequencies as Na influx rate into heart muscle fibers is reported to be increased by electrical stimulation (Langer, 1974). The results of preliminary experiments show that ouabain-sensitive $^{86}Rb^+$ uptake was linearly related to frequency of stimulation (Figure 1) which agrees with the data of Yamamoto et al. (1979) who also measured $^{86}Rb^+$ uptake in beating guinea pig left atrial muscle with a 2 mM RbCl Rb^+ K-H. Thus, sodium pump activity measured under the conditions outlined above may also serve as a measure of the rate of sodium influx in beating heart muscle.

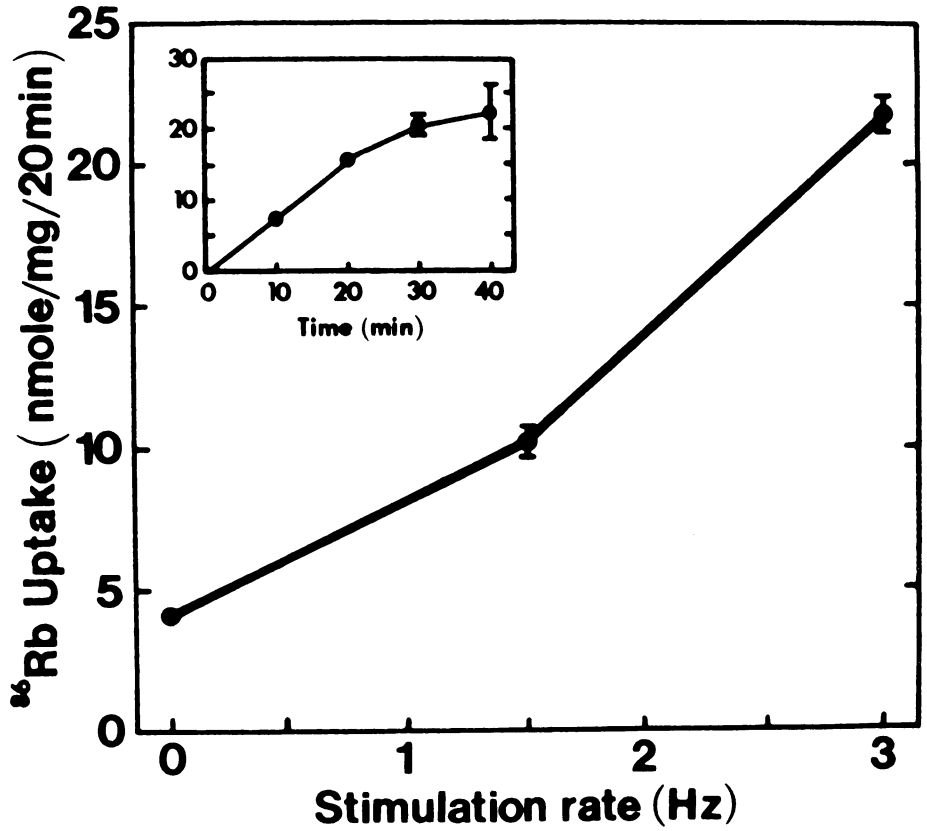

Figure 1. Effect of stimulation frequency on $^{86}Rb^{+}$ uptake. Guinea pig left atrial muscle was incubated in a 5 mM RbCl, K^{+}-free bicarbonate buffer solution at 32°C and electrically paced at the indicated frequency. Subsequently, tracer amounts of $^{86}Rb^{+}$ was added and uptake allowed to occur for 20 minutes. Inset: $^{86}Rb^{+}$ uptake as a function of time was measured in left atrial muscle stimulated at 1.5 Hz. The ordinate is ouabain-sensitive $^{86}Rb^{+}$ uptake expressed as nmoles Rb^{+}/mg wet weight. Ouabain-sensitive uptake was calculated as the difference in isotope uptake in the absence and presence of 0.3 mM ouabain. Data are expressed as the mean $\pm$ S.E.M., n=3.

D. [^{3}H]Ouabain Binding Studies

1. Estimation of digoxin binding to Na^+,K^+-ATPase in heart muscle preparations: Fractional occupancy

The binding of digoxin to the Na^+,K^+-ATPase in beating left atrial muscle preparations was estimated by homogenizing the atrial muscle and assaying the initial velocity of ATP-dependent binding of [^{3}H]ouabain to the homogenate. After a predetermined period of exposure to digoxin at 32°C, the muscle preparation was removed from the Krebs-Henseleit buffer solution and immediately homogenized in 3.5 ml of an ice-cold solution containing 1 mM EDTA and 10 mM Tris-HCl buffer (pH=7.5) using a Dounce ball-type homogenizer. The homogenate was transferred to a Potter-Elvehjem homogenizer, homogenized with a motor-driven Teflon pestle (3000 rpm, 12 sec), and then filtered through a stainless-steel wire mesh (150 μM pore size) to remove tissue debris (Temma and Akera, 1982). The final protein concentration of the homogenate was approximately 1 mg/ml.

An aliquot (0.1 ml) of homogenate was added to 0.9 ml of a prewarmed (37°C) incubation mixture containing 50 nM [^{3}H]ouabain, 100 mM NaCl, 5 mM $MgCl_2$ and 50 mM Tris-HCl buffer (pH 7.5) with or without 5 mM Tris-ATP. The binding reaction was terminated after 2 minutes by the addition of 6 ml of an ice-cold stopping solution containing 15 mM KCl, 0.1 mM unlabelled ouabain and 50 mM Tris-HCl buffer (pH 7.5). This mixture was passed through a nitrocellulose filter (Millipore Corporation, Bedford, MA; type AA, pore size 0.8 μm) to separate bound and unbound [^{3}H]ouabain. The filter was then washed with two additional 6 ml aliquots of stopping solution within 10 seconds. The radioactivity trapped on the filter was quantified by liquid scintillation spectrometry after dissolving the filter in 1 ml of ethylene

glycol monomethylether (Pierce Chemical Co., Rockford, IL; Piersolve).

The dissociation of bound cardiac glycoside from guinea pig heart Na^+,K^+-ATPase in ice-cold solution is relatively slow (Akera et al., 1973). Therefore, the release of bound digoxin during the preparation of the homogenate is anticipated to be slight; however, the time between the removal of the atrial muscle from the Krebs-Henseleit buffer solution and the addition of the homogenate to the binding medium was set at 6.5 min to avoid any variability in the amount of digoxin released from tissue binding sites during preparation of the homogenate.

ATP-dependent [^{3}H]ouabain binding was calculated by subtracting the amount of [^{3}H]ouabain bound in the absence of ATP (nonspecific) from the value observed in its presence. ATP-dependent [^{3}H]ouabain binding represents the specific binding of [^{3}H]ouabain to the glycoside receptor site on the Na^+,K^+-ATPase (Allen et al., 1971). A reduction in the initial velocity of ATP-dependent binding of [^{3}H]ouabain indicates previous occupancy of the glycoside receptor site by digoxin (Ku et al., 1974).

2. [^{3}H]Ouabain binding to guinea pig heart microsomes

In order to determine the effect of quinidine or benzocaine on the binding of cardiac glycosides to the Na^+,K^+-ATPase, [^{3}H]ouabain binding to guinea pig heart microsomal Na^+,K^+-ATPase was assayed in the presence of various concentrations of the drug. Guinea pig heart microsomes were isolated by a modification of the method of Akera et al. (1973). Guinea pig cardiac ventricular muscle was minced with scissors and homogenized with 4 volumes of an ice-cold solution

containing 0.25 M sucrose, 1 mM Na_2EDTA, and 5 mM dl-histidine (pH 7.0) using a Dounce ball-type homogenizer (both loose and tight pestle). The homogenate was transferred to a Potter-Elvehjem homogenizer and briefly (10 sec) homogenized with a motor-driven Teflon pestle (1000 rpm). The final homogenate was centrifuged at 10,000 x g for 15 min at 0°C to remove cell debris and the mitchondrial fraction. The supernatant was then centrifuged at 100,000 x g for 60 min at 0°C. This supernatant was discarded and the pellet was resuspended in an ice-cold solution containing 0.25 M sucrose, 1 mM EDTA, and 5 mM dl-histidine (pH 7.0). This crude microsomal preparation was frozen for later use.

[^{3}H]Ouabain binding to the microsomal Na^+,K^+-ATPase was assayed by adding 0.1 ml of the microsomal preparation to 0.8 ml of a prewarmed (37°C) incubation mixture containing 25 nM [^{3}H]ouabain, 20 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.5), and various concentrations of quinidine or benzocaine. In some assays, KCl, at indicated concentrations, was also present in the incubation mixture. After a further 5 min incubation at 37°C, the glycoside binding reaction was started by the addition of 0.1 ml of Tris-ATP (pH 6.8) so that the final ATP concentration was 5 mM. Nonspecific [^{3}H]ouabain binding was assayed by the addition of an equal volume of H_2O, instead of Tris-ATP. The binding reaction was terminated 3 minutes later by the addition of 6 ml of ice-cold stopping solution (see above). Bound [^{3}H]ouabain was separated by filtration on a nitrocellulose filter and the filters were washed with 2 aliquots (6 ml each) of stopping solution. ATP-dependent [^{3}H]ouabain binding was calculated as the difference in the amount of [^{3}H]ouabain bound in the presence and absence of ATP. Final protein concentration during the binding reaction was 100 μg/ml.

3. [^{3}H]Ouabain binding to guinea pig heart Na^+,K^+-ATPase

Cardiac Na^+,K^+-ATPase was obtained from ventricular muscle of guinea pigs by the method of Akera and Brody (1971) as later modified by Ku et al. (1976). Binding reactions were carried out essentially the same as those for guinea pig heart microsomes except for the following modifications: 1) final NaCl concentration was varied between 0-100 mM with choline chloride being used as an ionic and osmotic substitute so that NaCl plus choline chloride equalled 100 mM, 2) KCl was not present in the reaction mixture, 3) the nonspecific binding medium without ATP also contained 0.2 mM unlabelled ouabain, and 4) final protein concentration was 0.5 mg/ml.

E. Miscellaneous

All chemicals were reagent grade. Digoxin, quinidine hydrochloride and benzocaine (p-ethyl aminobenzoate) were purchased from Sigma Chemial Company (St. Louis, MO). [^{3}H]Ouabain and ^{86}RbCl were purchased from New England Nuclear (Boston, MA) or Amersham Corp. (Arlington Heights, IL). Nadolol was kindly supplied by E.R. Squibb and Sons (Princeton, NJ). Protein concentrations were estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Statistical analyses were performed where indicated by use of t-test or one-way or two-way analysis of variance with paired comparisons by least significant difference. Criterion for significance was a P value less than 0.05. Data are expressed as the mean $\pm$ S.E.M.

RESULTS

A. Effect of Quinidine on the Inotropic Action of Digoxin in Atrial Muscle Preparations of the Guinea Pig

Some studies in man (Hirsh et al., 1980; Steiness et al., 1980), experimental animal models (Warner et al., 1984), and isolated cardiac muscle preparations (Williams and Mathew, 1981) have indicated that quinidine lessened the effects of digoxin in cardiac muscle while others have found that quinidine did not influence the effect of digoxin on cardiac mechanical function in man (Zaman et al., 1981; Belz et al., 1982) and in isolated cardiac muscle preparations (Kim et al., 1981a; Horowitz et al., 1982; Lash et al., 1982). Investigations of the effect of quinidine on the positive inotropic action of digoxin in man and in live animal models have been complicated by changes in serum digoxin concentration. Therefore, the effect of quinidine on the inotropic actions of digoxin was studied in isolated left atrial muscle of the guinea pig so that cardiac glycoside concentration could be maintained at a constant level.

After an initial equilibration period of 60 minutes, the force of contraction of left atrial muscle preparations stimulated at 1.5 Hz remained stable for at least 2 hr. Developed tension after the equilibration period was 0.78±0.05 g (mean ± S.E.M. of 21 preparations), and addition of vehicle (H_2O) had no effect on developed tension. Quinidine

produced a positive inotropic effect at 10 and 30 μM (Figure 2). The peak effect on contractile force was reached within 15 minutes of drug addition. With 10 μM quinidine, the increase in force was sustained while in the presence of 30 μM quinidine developed tension decreased slightly towards controls after an initial increase and then stabilized by 40 minutes after drug addition. Higher concentrations of quinidine (100 μM; not shown in Figure 2) developed the initial increase in force which, however, was followed by a decline in force that did not stabilize. Often, these preparations could not be consistently excited at 1.5 Hz and/or had a substantial increase in the threshold voltage for stimulation. The instability of the atrial muscle preparations in the presence of high drug concentrations made their use in subsequent experiments impossible. The positive inotropic effect of quinidine and its biphasic effects on developed tension observed in the present study are similar to those reported earlier in guinea pig left atrial muscle (Nawrath, 1981).

Forty minutes after the addition of 3-30 μM quinidine to the incubation medium, developed tension had stabilized. At this time, digoxin in a final concentration of 0.6 μM was added and the magnitude as well as the rate of onset of positive inotropic effect of the glycoside was monitored.

In atrial muscle preparations which had not been treated with quinidine, 0.6 μM digoxin increased developed tension slowly, reaching a plateau within 30-40 minutes. The time to half maximal inotropic effect, $T^{on}_{1/2}$, which reflects the rate of onset of glycoside action, was 13±2 min (mean ± S.E.M. of four experiments) and the magnitude of the glycoside-induced increase in contractile force was 0.78±0.13 g. No

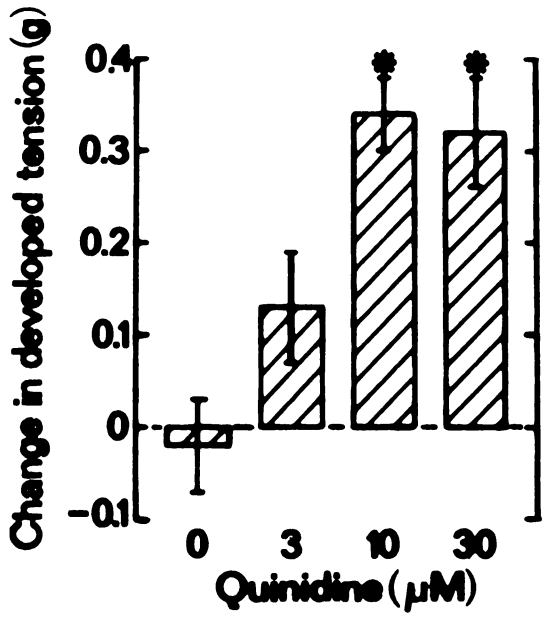

Figure 2. Effect of quinidine on developed tension of guinea pig left atrial muscle. Muscle preparations were stimulated at 1.5 Hz. After a 60-minute equilibration period, quinidine was added to the incubation medium at the final concentration of 0 (n=4), 3 (n=6), 10 (n=6), and 30 μM (n=5). The peak effect of quinidine on developed tension is expressed as the mean ± S.E.M. Asterisks denote a significant difference from 0 μM quinidine as determined by 1-way ANOVA.

change in resting tension was observed after digoxin administration. Figure 3 shows the positive inotropic effect of 0.6 μM digoxin in the presence of various concentrations of quinidine. The increase in developed tension produced by 0.6 μM digoxin in atrial muscle preparations treated with 3, 10, and 30 μM quinidine was 1.03±0.12, 1.08±0.15 and 0.95±0.12 g (mean ± S.E.M. of 5 or 6 experiments), respectively. The glycoside-induced increase in force was not significantly different in the absence or the presence of quinidine, at any concentration tested, as determined by a 1-way analysis of variance. The $T^{on}_{1/2}$ for the inotropic action of digoxin in the presence of 3, 10, and 30 μM quinidine was 14±2, 13±1 and 12± minutes, respectively. These values were also not significantly different from the $T^{on}_{1/2}$ of digoxin observed in the absence of quinidine. These data indicate that quinidine did not significantly influence the magnitude or the rate of onset of the positive inotropic effect of digoxin in left atrial muscle of the guinea pig.

The present results are in agreement with those of Kim and coworkers (1981a) who found that 6 μM quinidine did not affect the positive inotropic actions of digoxin in guinea pig heart Langendorff preparations. The question, then, is why the present experiments and those of others (Kim *et al.*, 1981a; Horowitz *et al.*, 1982; Lash *et al.*, 1982) have not demonstrated an interaction between quinidine and digoxin even though a quinidine-induced reduction in sodium influx is anticipated to decrease digitalis binding. One answer may be that quinidine did not decrease sodium influx into the cardiac muscle fibers under the conditions of the experiments, a point which has not been addressed in most published reports on the quinidine/digoxin

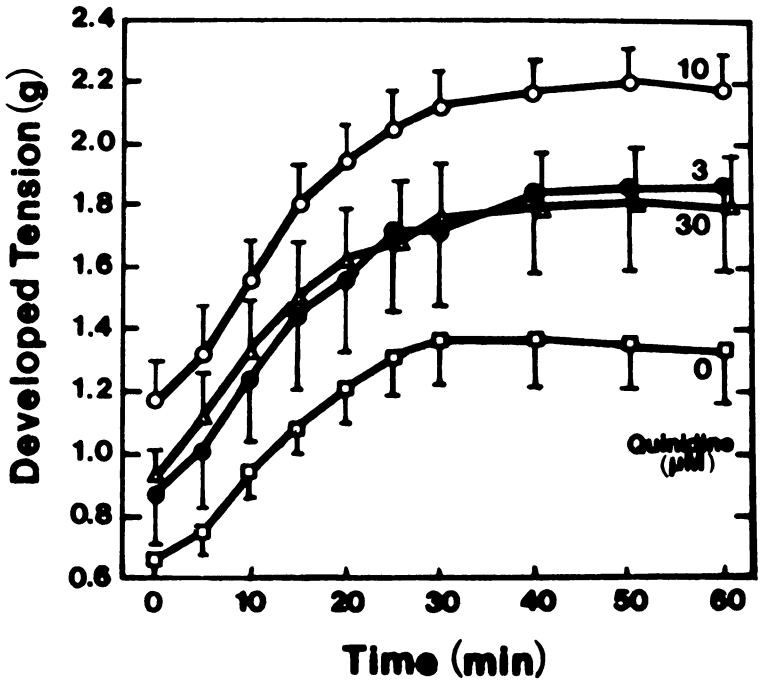

Figure 3. Effect of quinidine on the time course of the positive inotropic action of 0.6 μM digoxin. Guinea pig left atrial muscle was stimulated at 1.5 Hz. Forty minutes prior to addition of digoxin (0.6 μM), quinidine in the final concentration of 0 (n=4), 3 (n=6), 10 (n=6), and 30 μM (n=5) was added to the incubation medium. Data are expressed as mean ± S.E.M.

interaction. As a result, the objective of the following experiments was to evaluate the effect of quinidine on sodium influx and establish experimental conditions in which quinidine was likely to reduce the rate of sodium influx.

B. Effect of Quinidine and Benzocaine on the Transmembrane Action Potential

1. Quinidine

Quinidine has been shown to decrease the maximal upstroke velocity of the cardiac action potential (Johnson and McKinnon, 1957; Vaughan-Williams, 1958). The ionic current flowing during the cardiac action potential upstroke is believed to be carried by sodium ions moving down their electrochemical gradient into the cardiac cell (Weidmann, 1955). For this reason, measurements of the maximal upstroke velocity have been used by many authors (Chen et al., 1975; Hondeghem and Katzung, 1980; Weld et al., 1982) as an index of the fast sodium current; however, the validity of this relationship has recently been questioned (Strichartz and Cohen, 1978; Cohen et al., 1984). Thus, the measurements of maximal upstroke velocity in this study should be regarded as qualitative indicators of quinidine's effect on sodium current.

The action potentials recorded in guinea pig left atrial muscle superfused in drug-free buffer and stimulated at 0.5 Hz had the following parameters (mean $\pm$ S.E.M. of eight experiments): resting membrane potential, -78$\pm$1 mV; amplitude, 107$\pm$2 mV; maximal upstroke velocity, $\dot{V}_{max}$, 180$\pm$7 V/sec; duration at the time of 90% repolarization, T_{90}, 63$\pm$3 msec. The measured values of these action potential

parameters were similar to published values (Nawrath, 1981). Single impalements were maintained throughout the entire experiment. Action potential recordings in control experiments remained stable up to 3 hours with a single impalement, although T_{90} had a tendency to shorten slightly ($\leq$10%). Figure 4 shows the effects of cumulative doses of quinidine on an atrial muscle preparation stimulated at 0.5 Hz. Quinidine decreased action potential amplitude in a concentration-dependent manner beginning at 10 μM and increased action potential duration, measured at T_{90}, at a drug concentration as low as 3 μM (data not shown), while 100 μM quinidine increased T_{90} by 269±42% (mean ± S.E.M. of three experiments) compared to the value observed before drug addition. The effects of quinidine on action potential duration, measured at the time of 20 and 50% repolarization, was prolonged to a similar degree as that measured at T_{90} (data not shown). These results are consistent with previous reports in guinea pig atrial muscle (Nawrath, 1981), and rabbit (Colatsky, 1982) and canine Purkinje fibers (Mirro et al., 1981). Quinidine also produced a dose-dependent decrease in $\dot{V}_{max}$ (Figure 4, inset). In the experiment shown, $\dot{V}_{max}$ in the absence of quinidine was 171 V/sec. At a concentration of 3 μM, the alkaloid had no effect on $\dot{V}_{max}$, but 10, 30, and 100 μM quinidine reduced $\dot{V}_{max}$ to 154, 115, and 45 V/sec, respectively. These concentrations of quinidine were similar to those which have been reported to decrease $\dot{V}_{max}$ in guinea pig atrial (Nawrath, 1981) and ventricular muscle preparations (Johnson and McKinnon, 1957; Chen et al., 1975) as well as i_{Na} in rat ventricular myocytes (Lee et al., 1981). Quinidine also increased stimulus latency and threshold so that maintaining regular tissue excitation at high drug concentrations required increasing

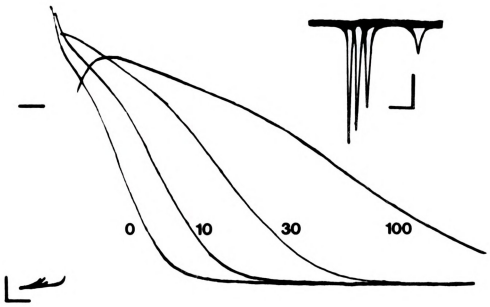

Figure 4. Effect of quinidine on transmembrane action potential. Guinea pig left atrial muscle was stimulated at 0.5 Hz with square-wave pulses of 4 msec duration, 20% above threshold by an extracellular electrode. At higher quinidine concentrations, it was necessary to increase stimulus voltage to maintain rhythmic excitation. In these cases, stimulus strength was readjusted to 20% above the new threshold. After stable action potentials had been recorded for 15 minutes quinidine concentration was increased to 10, 30, and 100 μM in the superfusing buffer solution every 40 minutes. The bar on the left of the figure marks 0 mV. The horizontal and vertical lines in the lower-left hand corner of the figure show 10 msec and 10 mV calibrations, respectively. Inset: Tracings of V_{max}. For clarity, the inverted records of V_{max} are offset from the action potential and displayed on an expanded time scale. The tracings of V_{max} from left to right are those recorded in the presence of 0, 10, 30 and 100 μM quindine. The calibration bar next to the V_{max} tracings are 50 Volts/sex (vertical) and 5 msec (horizontal).

stimulus strength more than two-fold. Resting membrane potential remained unchanged throughout the experiment except at the highest concentration tested, 300 μM, where a depolarization of a few millivolts occurred. The onset of the action of quinidine at each concentration was slow and the maximum effect was observed only after 30-40 minutes. Superfusion of the tissue preparation in drug-free buffer solution for 60 minutes did not completely reverse the drug effect on the action potential (data not shown). The mean data for the effects of cumulative doses of quinidine on $\dot{V}_{max}$ and T_{90} for atrial muscle stimulated at 0.5 Hz are shown in Figures 5A and 5B, respectively. These experiments indicate that in atrial muscle stimulated at 0.5 Hz, quinidine caused a decrease in $\dot{V}_{max}$ and a marked prolongation of the action potential.

Reuter and Scholz (1977) have postulated that sodium ions may pass through cardiac sarcolemmal calcium channels and, recently, Falk and Cohen (1983) have shown that sodium pump stimulation during repetitive electrical stimulation of canine Purkinje fiber is reduced by D-600, a calcium channel blocker, and that prolonged membrane repolarizations may also stimulate the sodium pump (Falk and Cohen, 1981). Thus, sodium entry during the plateau phase of the cardiac action potential may make a significant contribution to total sodium influx (Langer, 1974). The duration of the atrial muscle action potential is much shorter than that of the cardiac Purkinje fiber. Nevertheless, because quinidine prolongs action potential duration, the drug-induced decrease in sodium influx rate during the upstroke of the action potential might be offset by increased sodium influx during the action potential plateau. For this reason, the effects of quinidine on the action potential

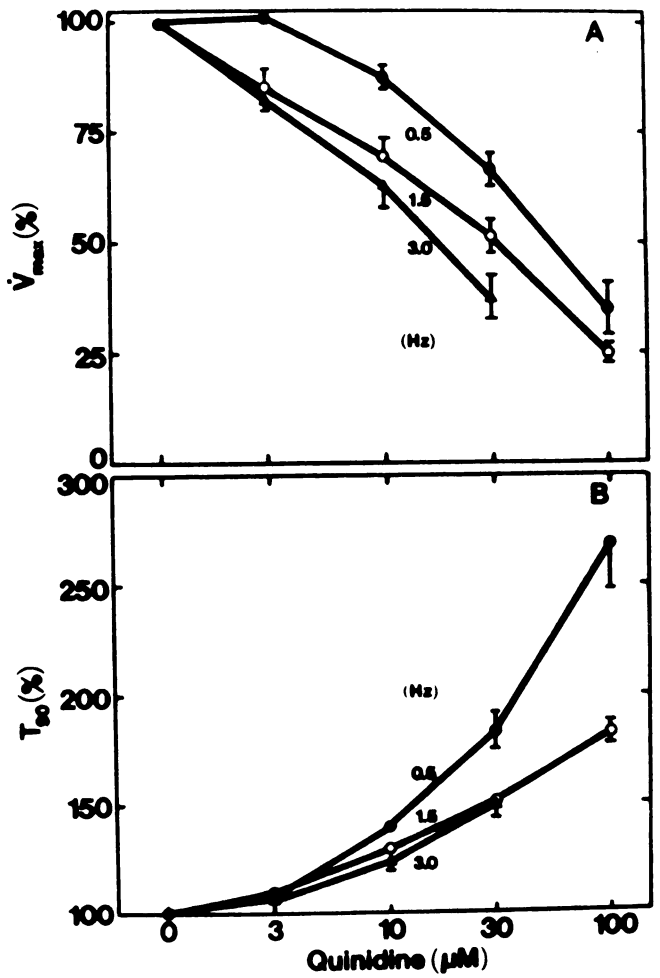

Figure 5. Effect of quinidine on atrial muscle action potential at various stimulation frequencies. Atrial muscle preparations were stimulated at 0.5 (n=3), 1.5 (n=5), and 3.0 (n=2) Hz. Cumulative concentrations of quinidine were added to the superfusing buffer solution as described in Figure 2. $\dot{V}_{max}$, maximal upstroke velocity (top panel), and T_{90}, time to 90% repolarization (bottom panel), is expressed as the mean percentage of the value before drug addition. The $\dot{V}_{max}$ and T_{90} of action potentials recorded prior to quinidine addition at all stimulation frequencies has been normalized to 100%.

were examined under conditions where the drug-induced depression of $\dot{V}_{max}$ would be more pronounced. Because quinidine has been shown to produce a frequency-dependent depression of $\dot{V}_{max}$ (Johnson and McKinnon, 1957; Hondeghem and Katzung, 1980), the effects of quinidine were investigated in preparations driven at higher stimulation frequencies. Figure 5A illustrates the effects of quinidine on $\dot{V}_{max}$ of preparations stimulated at 0.5, 1.5, and 3.0 Hz. Quinidine produced a progressively greater decrease of $\dot{V}_{max}$ at higher stimulation frequencies, in agreement with earlier studies (Hondeghem and Katzung, 1980). The dose-response curve for quinidine was shifted to the left at faster driving rates so that the drug concentration to cause a 50% decrease in $\dot{V}_{max}$ in preparations stimulated at 3.0 was approximately one-fourth that in atrial muscle stimulated at 0.5 Hz.

Figure 5B shows the effects of quinidine on T_{90} in atrial muscle preparations stimulated at different frequencies. Action potential duration, measured at T_{90}, was 63 ± 3 (n=8), 69 ± 1 (n=10), 65 ± 1 msec (n=6) for preparations stimulated at 0.5, 1.5, and 3.0 Hz, respectively. The apparent lack of a marked effect of stimulation frequency less than 3.0 Hz on the duration of the atrial muscle action potential was consistent with previous reports (Mendez *et al.*, 1956; Pasmooij *et al.*, 1976). In contrast to the effect of quinidine on upstroke velocity, action potential duration was increased less at higher frequencies. At higher stimulation rates, quinidine produced a greater decrease in $\dot{V}_{max}$ and a smaller increase in T_{90}. These characteristics of drug action tend to suggest that quinidine would cause a greater decline in net sodium influx in cardiac tissue paced at higher stimulation frequencies.

2. Benzocaine

Local anesthetics, such as benzocaine, have been shown to decrease I_{Na} (Schwarz et al., 1977), sodium influx rate (van Zwieten, 1969) and intracellular sodium ion concentration (Deitmer and Ellis, 1980a; Eisner et al., 1983a). Furthermore, benzocaine has been reported to slow the onset of the positive inotropic effect of strophanthidin in sheep cardiac Purkinje fibers (Bhattacharyya and Vassalle, 1981). These results suggested that the local anesthetic may also be capable of reducing glycoside binding by decreasing the rate of sodium influx, similar to the expected action of quinidine. To determine under what conditions benzocaine would reduce the rate of sodium influx into guinea pig atrial muscle, the effects of benzocaine on the action potential of guinea pig left atrial muscle were examined and compared to the effects of quinidine.

Benzocaine has been found not to display frequency-dependent depression of $\dot{V}_{max}$ in cardiac Purkinje (Gintant et al., 1983) and I_{Na} in ventricular fibers (Sanchez-Chapula et al., 1983). For this reason, the effect of benzocaine on the cardiac action potential was only examined in guinea pig left atrial muscle paced at 0.5 Hz. Superfusion of the preparations with vehicle, polyethylene glycol, at a final concentration of 0.1%, was found to have no significant effect on measured action potential parameters. Cumulative doses of benzocaine decreased action potential amplitude and $\dot{V}_{max}$ (Figure 6) in a concentration-related manner in atrial muscle stimulated at 0.5 Hz. The mean data for the effect of benzocaine on $\dot{V}_{max}$ are shown in Figure 7A. A fifty percent decrease in $\dot{V}_{max}$ was calculated to occur at a drug concentration of 900 μM, ten times that of quinidine to produce a decrease

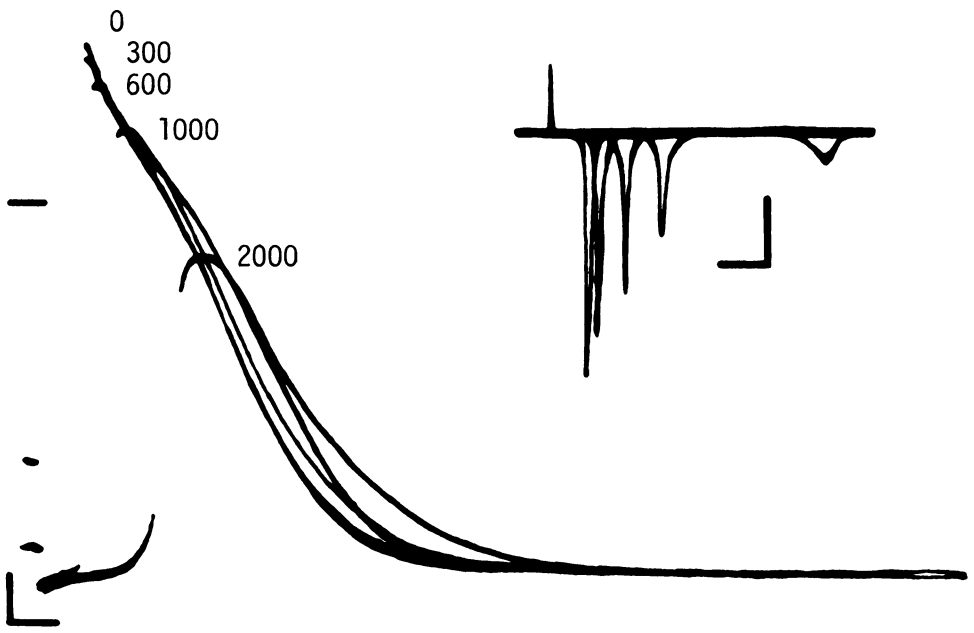

Figure 6. Effect of benzocaine on the transmembrane action potential. Guinea pig left atrial muscle was stimulated at 0.5 Hz with square-wave pulses of 4 msec duration, 20% above threshold with an extracellular electrode. Benzocaine, at concentrations greater or equal to 1000 μM, elevated stimulus threshold. Therefore, stimulus voltage was increased to 20% above the new threshold to maintain rhythmic excitation. After stable action potentials were recorded for 15 minutes, benzocaine concentration was increased in a step-wise manner to the indicated values (final concentration; μM) at 20 minute intervals. The bar on the left of the figure denotes 0 mV. Calibrations for 10 msec (horizontal line) and 10 mV (vertical line) are shown in the lower-left hand corner of the figure. Inset: Effect of benzocaine on the rate of depolarization during the action potential. V_{max} is displayed offset from the action potential recording and with an expanded time scale for clarity. From left to right the traces of V_{max} are for 0, 300, 600, 1000, and 2000 μM benzocaine. The spike at the beginning of the tracing is due to the end of the stimulus effect. The calibration bar next to the V_{max} tracings are 50 Volts/sec (vertical) and 5 msec (horizontal).

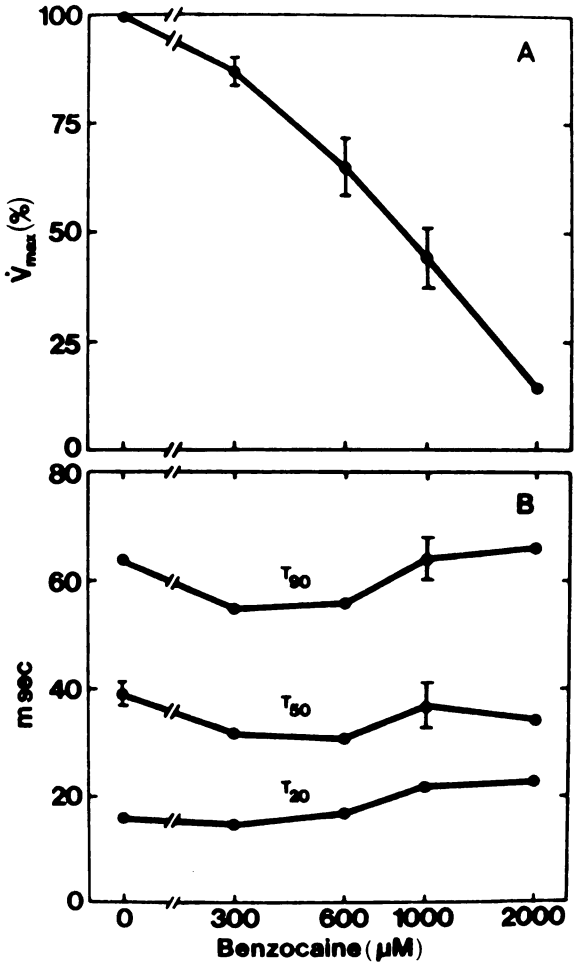

Figure 7. Effect of benzocaine on transmembrane action potential. Mean data for three experiments showing the effects of cumulative concentrations of benzocaine on the $\dot{V}_{max}$ (top panel) and action potential duration (bottom panel) of guinea pig left atrial muscle stimulated at 0.5 Hz. $\dot{V}_{max}$ is expressed as a percentage of the value recorded before drug addition. Action potential duration at 20 (T_{20}), 50 (T_{50}) and 90 (T_{90})% repolarization is expressed as milliseconds.

in $\dot{V}_{max}$ of similar magnitude. These concentrations were similar to those previously reported to decrease $\dot{V}_{max}$ (Gintant et al., 1983) and I_{Na} (Sanchez-Chapula et al., 1983) in canine cardiac Purkinje fibers and rat single ventricular myocytes, respectively. Like quinidine, the stimulus latency and threshold increased in the presence of benzocaine; however, resting membrane potential did not depolarize at any drug concentration tested (Figure 6). Benzocaine did not produce a significant increase in action potential duration (Figures 6 and 7B), instead, T_{90} was slightly decreased or not significantly different than control. Therefore, benzocaine decreased $\dot{V}_{max}$ without dramatic changes in action potential duration.

C. Effects of Quinidine and Benzocaine on Ouabain-Sensitive $^{86}Rb^{+}$ Uptake in Beating Atrial Muscle Preparations

In the beating heart, sodium influx must be balanced by sodium efflux in order to maintain cellular homeostasis under steady-state conditions. Increases or decreases in the rate of sodium influx then, should be matched by an increase or decrease in sodium pump activity, respectively, because the sodium pump is the major mechanism for sodium extrusion in the heart muscle cell. Akera et al. (1981) have shown that under certain conditions, sodium pump activity, measured by $^{86}Rb^{+}$ uptake, may be an indirect measure of sodium influx rate. Therefore, changes in sodium influx rate caused by quinidine and benzocaine were estimated by measuring $^{86}Rb^{+}$ uptake in beating left atrial muscle preparations.

1. Quinidine

Figure 8 shows the effect of quinidine on ouabain-sensitive $^{86}Rb^{+}$ uptake in electrically-driven quinea pig left atrial muscle. In these experiments, $^{86}Rb^{+}$ uptake was initiated 40 minutes after quinidine administration, at a time when the drug effect had stabilized so that the tissue had reached a new steady state. Under these conditions, $^{86}Rb^{+}$ uptake is not only a measure of sodium pump activity, but an index of sodium influx rate as well. In atrial muscle preparations stimulated at 1.5 Hz, ouabain-sensitive $^{86}Rb^{+}$ uptake was 11.1±0.5 nmol Rb^{+}/mg wet weight/20 min (mean ± S.E.M. of 6 experiments) in the absence of quinidine (Figure 8, left panel). Quinidine at 3 and 10 μM concentrations did not have a significant effect on $^{86}Rb^{+}$ uptake; however, 30 μM quinidine decreased uptake to 8.1±0.5 nmole Rb^{+}/mg wet weight/20 min (mean ± S.E.M. of five experiments). Thus, quinidine decreased $^{86}Rb^{+}$ uptake only at a concentration of 30 μM in atrial muscle stimulated at 1.5 Hz even though 3 and 10 μM drug concentrations significantly decreased $\dot{V}_{max}$ of action potentials recorded in preparations paced at the same rate.

The results of the action potential studies suggest that quinidine would decrease sodium influx more readily in atrial muscle stimulated at higher rates. This conclusion was re-examined by measuring ouabain-sensitive $^{86}Rb^{+}$ uptake in preparations stimulated at 3.0 Hz. In control preprations, $^{86}Rb^{+}$ uptake was 28.6±1.3 nmoles Rb^{+}/mg wet weight/20 min. This value is approximately two and one half times greater than the sodium pump activity observed in control preparations stimulated at 1.5 Hz. An increase in sodium pump activity at a higher

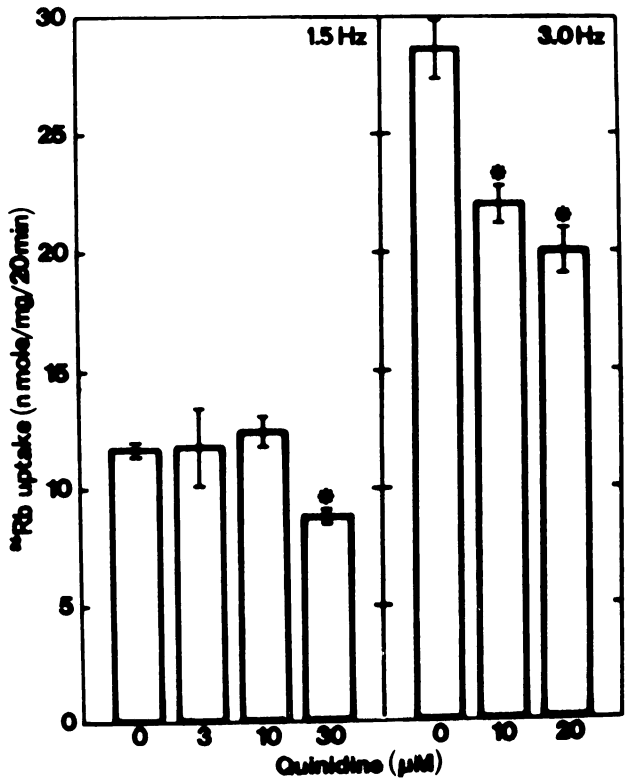

Figure 8. Effect of quinidine on ouabain-sensitive $^{86}Rb^{+}$ uptake. Guinea pig left atrial muscle were stimulated at 1.5 (left panel) or 3.0 Hz (right panel). Forty minutes after the addition of vehicle (H_2O) or the indicated concentration of quinidine, tracer amounts of $^{86}Rb^{+}$ were added to the 5 mM RbCl, K^{+}-free buffer solution. The $^{86}Rb^{+}$ uptake was discontinued after 20 minutes, at which time the tissue was briefly washed in $^{86}Rb^{+}$-free buffer solution, weighed and radioactivity estimated by gamma scintillation spectrometry. Ouabain-sensitive $^{86}Rb^{+}$ uptake was calculated as the difference between uptake values observed in the absence and presence of 0.3 mM ouabain. Data are expressed as the mean + S.E.M. of 5 or 6 experiments. Asterisks (*) denote a significant difference from 0 μM quinidine as determined by 1-way ANOVA.

stimulation frequency was expected because faster stimulation rates are believed to increase the rate of sodium influx (Langer, 1974; Yamamoto et al., 1979). Quinidine reduced $^{86}Rb^{+}$ uptake 23±3 and 30±3% at 10 and 20 μM concentrations, respectively, under these conditions. These data appeared to confirm the frequency-dependence of the effect of quinidine on sodium influx rate as quinidine produced a greater decrease in $^{86}Rb^{+}$ uptake at lower drug concentrations in atria stimulated at 3.0 Hz than 1.5 Hz.

The preceding experiment demonstrated that the effect of quinidine on sodium influx rate was dependent on both drug concentration and frequency of electrical stimulation. If the working hypothesis is correct, quinidine should reduce glycoside binding to the Na^{+},K^{+}-ATPase in the cardiac cell by decreasing the rate of sodium influx. Conversely, if quinidine does not decrease the rate of sodium influx, digitalis binding should be unaffected. For this reason, it was desirable to establish a set of experimental conditions such that under one condition quinidine would decrease sodium influx rate and under another it would not. The frequency-dependent effect of quinidine suggested that conditions may be selected such that a given concentration of drug would decrease $^{86}Rb^{+}$ uptake in atrial muscle stimulated at a fast rate but have no effect on $^{86}Rb^{+}$ uptake in atria stimulated at a slow rate. This point was investigated by observing the effects of 20 μM quinidine on ouabain-sensitive $^{86}Rb^{+}$ uptake in atrial muscle preparations stimulated at 0.5 and 3.0 Hz. Figure 9 shows the effects of 20 μM quinidine on $^{86}Rb^{+}$ uptake in atria stimulated at 0.5 Hz. Included for comparison are the data from the previous experiment showing that 20 μM quinidine significantly decreased sodium pump

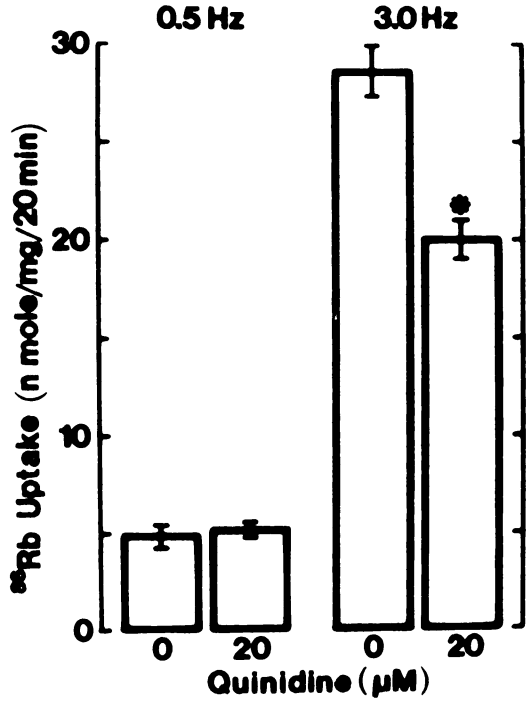

Figure 9. Effect of 20 μM quinidine on ouabain-sensitive $^{86}Rb^{+}$ uptake at different stimulation frequencies. Atrial muscle preparations were stimulated at 0.5 or 3.0 Hz either in the absence or presence of 20 μM quinidine. Experiments were performed as described in Figure 8. Data are expressed as the mean ± S.E.M. of 6 experiments. Asterisk denotes a significant difference from 0 μM quinidine as determined by 2-way ANOVA.

activity when preparations were stimulated at 3 Hz. In control preparations stimulated at 0.5 Hz, $^{86}Rb^{+}$ uptake was 4.9±0.6 nmole/mg wet weight/20 min, several times smaller than sodium pump activity in atrial muscle stimulated at 3 Hz, consistent with the beat-dependent influx of sodium. The $^{86}Rb^{+}$ uptake in preparations in the presence of 20 μM quinidine was 5.2±0.4 nmole/mg wet weight/20 min, a small but not statistically significant increase (Figure 9). These data confirm that a single concentration of quinidine could have different effects on sodium pump activity depending on the frequency of electrical stimulation.

Catecholamine release has been reported to stimulate $^{86}Rb^{+}$ uptake in canine cardiac ventricular tissue (Hougen et al., 1981). Sodium pump activity was measured in atrial muscle stimulated with field electrodes using large currents. Under these conditions, both muscle and nerve endings are stimulated in the atrial tissue. The local anesthetic actions of quinidine might antagonize catecholamine release from the nerve endings, similar to the effects of tetrodotoxin in field-stimulated atria (Katz and Kopin, 1969). The apparent decrease in $^{86}Rb^{+}$ uptake caused by quinidine might, in part, or completely be due to inhibition of catecholamine-induced stimulation of $^{86}Rb^{+}$ uptake rather than a reduction in sodium influx into muscle fibers. In order to examine this possibility, left atrial muscle preparations stimulated at 3 Hz were incubated in a Krebs-Henseleit buffer containing 1 μM nadolol, a β-adrenoceptor antagonist, beginning 20 minutes before quinidine (20 μM) addition. This concentration of nadolol was sufficient to shift the concentration of isoproterenol which produced a half-maximal inotropic effect in atrial muscle preparations from 2 to

150 nM (data not shown). In preparations treated with nadolol only, $^{86}Rb^+$ uptake was 25.9±1.1 nmole Rb^+/mg wet weight/20 min (data not shown) which was slightly but not significantly less than $^{86}Rb^+$ uptake in untreated atrial muscle (28.6±1.3 nmol Rb^+/mg wet weight/20 min). In nadolol-treated preparations, 20 μM quinidine reduced $^{86}Rb^+$ uptake by 25±3% (data not shown), similar to the degree of reduction of active monovalent cation transport seen in the absence of β-adrenoceptor blockade (30±3%). These data suggest that most, if not all, of the reduction in $^{86}Rb^+$ uptake induced by quinidine can be attributed to a drug effect directly in the muscle fibers.

2. Benzocaine

The effect of benzocaine on $^{86}Rb^+$ uptake was also examined in atrial muscle preparations to establish the experimental conditions in which the local anesthetic agent decreased sodium influx rate. Because the reduction of action potential $\dot{V}_{max}$ by benzocaine is not beat-dependent, the effects of benzocaine on $^{86}Rb^+$ uptake were measured in preparations stimulated at 3 Hz only. Action potential experiments and preliminary experiments which examined the inotropic actions of benzocaine showed that drug effects had reached a plateau within 15 minutes. In these experiments, $^{86}Rb^+$ uptake was begun 20 minutes after benzocaine administration, when the atrial muscle preparations had reached a new steady state. Figure 10 shows the effects of 20 and 300 μM benzocaine on ouabain-sensitive $^{86}Rb^+$ uptake. The low concentration of benzocaine (20 μM) had no effect on $^{86}Rb^+$ uptake when compared to control values. Benzocaine at a concentration of 300 μM, which decreased action potential $\dot{V}_{max}$ approximately 15%, caused a 32±11% reduction in $^{86}Rb^+$ uptake. The highest concentration of this drug tested, 1

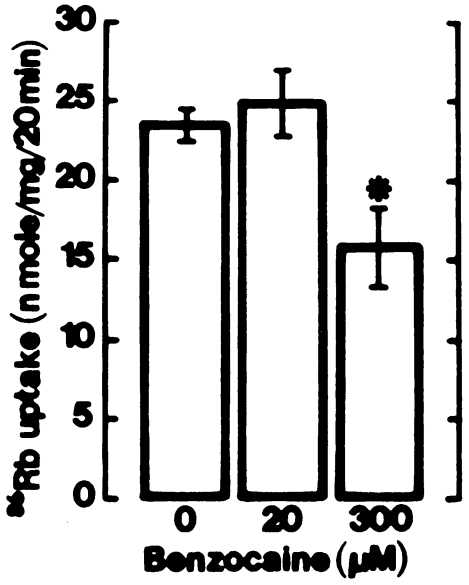

Figure 10. Effect of benzocaine on ouabain-sensitive $^{86}Rb^{+}$ uptake. Guinea pig left atrial muscle was stimulated at 3.0 Hz. Benzocaine, at the indicated concentration, or vehicle (polyethylene glycol) was added to the incubation medium 20 minutes before beginning $^{86}Rb^{+}$ uptake. Data is expressed as the mean $\pm$ S.E.M. of 3 experiments. Asterisks denote a significant difference in $^{86}Rb^{+}$ uptake from 0 μM benzocaine as determined by 1-way ANOVA.

mM (not shown in Figure 10), decreased $^{86}Rb^{+}$ uptake by 47±12%, but visual inspection of the preparations revealed that they were not rhythmically contracting at 3 Hz. Thus, high concentrations of benzocaine reduced sodium pump activity.

In summary, both quinidine and benzocaine reduced $\dot{V}_{max}$ of the action potential of guinea pig left atrial muscle fibers. The magnitude of the reduction of $\dot{V}_{max}$ by quinidine was dependent on the stimulation rate whereas that of benzocaine has been reported to be independent of frequency at stimulation rates as fast as 4 Hz (Gintant et al., 1983). Quinidine also caused a dose-dependent prolongation of the action potential which was more pronounced at lower stimulation frequencies in contrast to benzocaine which did not significantly increase action potential duration at the concentrations and stimulation frequency tested. Both drugs decreased sodium pump activity in beating muscle preparations. Quinidine produced a dose and frequency-dependent decrease in ouabain-sensitive $^{86}Rb^{+}$ uptake, similar to its frequency-dependent effect on $\dot{V}_{max}$. Benzocaine inhibited $^{86}Rb^{+}$ uptake at concentrations an order of magnitude higher than quinidine. Similar relative potencies of the two agents were noted during action potential studies. It seems reasonable, therefore, to examine 1) the effects of quinidine on the positive inotropic action of digoxin in atrial muscle preparations stimulated at higher frequencies and 2) the effects of high concentrations of benzocaine on the rate of onset and the magnitude of the positive inotropic action of digoxin.

D. Inotropic Action of Digoxin in the Presence of Quinidine or Benzocaine

1. Quinidine

Since the rate of onset of glycoside action varies with drug concentration (Park and Vincenzi, 1975), a concentration of digoxin was chosen so that, at the different stimulation rates examined, a substantial inotropic effect could be observed without the appearance of toxicity. In preliminary experiments, this concentration was found to be 0.6 μM.

Previous experiments demonstrated that the action of quinidine on both action potential $\dot{V}_{max}$ and sodium pump activity was frequency-dependent. Quinidine at a concentration of 20 μM, reduced sodium pump activity in atrial muscle stimulated at 3.0 Hz but not at 0.5 Hz. The goal of these experiments was to determine the effects of quinidine on the positive inotropic effect of cardiac glycosides under conditions in which the antiarrhythmic agent decreased the rate of sodium influx and in those which it did not decrease the rate of sodium influx. For this reason, the inotropic effects of digoxin were investigated in the presence and absence of 20 μM quinidine in left atrial muscle preparations of the guinea pig heart stimulated at 0.5 or 3.0 Hz. At the end of a 60 minute equilibration, atrial muscle preparations stimulated at 0.5 or 3.0 Hz produced a 0.26 ± 0.02 g (mean ± S.E.M. of 14 experiments) and 1.09 ± 0.07 g (mean ± S.E.M. of 22 experiments) developed tension, respectively. The atrial muscle preparations stimulated either at 0.5 or 3.0 Hz maintained stable force for at least 2 hours after the equilibration period. Quinidine produced a small positive inotropic effect in preparations which were stimulated at

either 0.5 or 3.0 Hz. In atrial muscle paced at 0.5 Hz, the inotropic effect developed slowly, reaching a plateau in approximately 30 minutes, at which time force was increased by 29%. The inotropic effect of quinidine in atrial muscle stimulated at 3.0 Hz developed rapidly, reaching a peak within 2 minutes. Thereafter, contractile force declined towards control levels and plateaued at a level not significantly different from control force by 30 minutes after quinidine addition. These results are similar to those observed under 1.5 Hz stimulation (Figure 2). Digoxin at a final concentration of 0.6 μM was added to the preparations only after force had stabilized.

In the absence of quinidine, the positive inotropic action of digoxin developed slowly and reached a plateau by approximately 100 minutes in preparations stimulated at 0.5 Hz (Figure 11). The $T^{on}_{1/2}$ for the inotropic action of digoxin in these experiments was 40±4 minutes (Figure 12) and the magnitude of the positive inotropic effect was 1.16±0.13 g (mean ± S.E.M. of 7 experiments). The positive inotropic effect of digoxin in atrial muscle stimulated at 3.0 Hz developed much more rapidly than that seen in preparations paced at 0.5 Hz. The $T^{on}_{1/2}$ was 8±1 min and the maximal change in developed tension was 0.89±0.07 g (mean ±S.E.M. of 11 experiments). A 2-way analysis of variance revealed that the magnitude of the positive inotropic effect of digoxin, expressed as change in grams developed tension, was not significantly different between preparations stimulated at 0.5 and 3.0 Hz; however, the rate of onset of the inotropic effect was significantly faster in atrial muscle stimulated at 3.0 Hz. The frequency-dependent onset of cardiac glycoside action is similar to that noted by

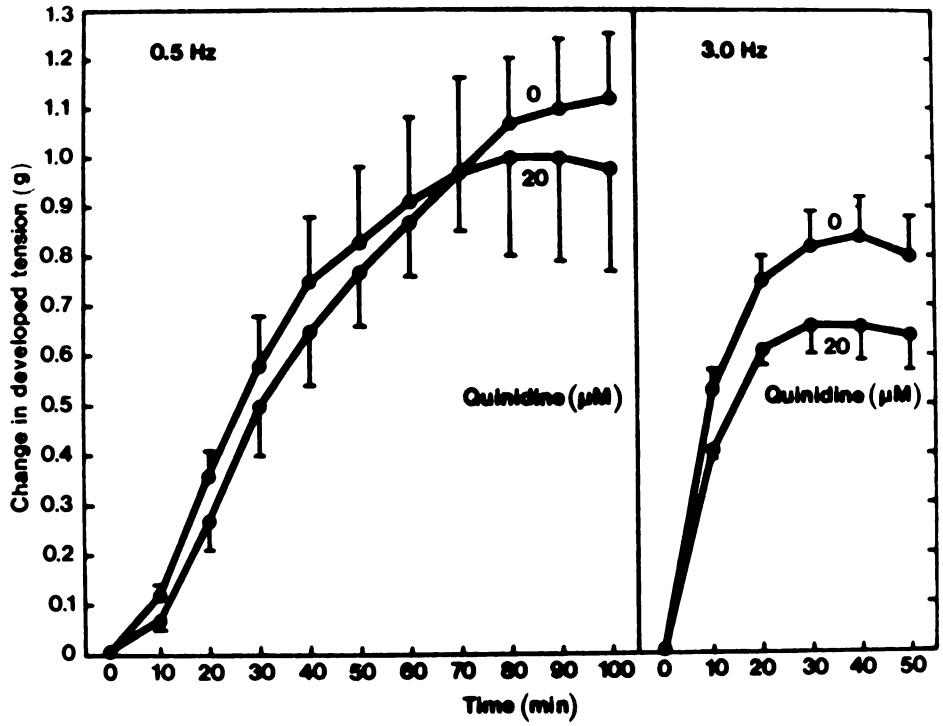

Figure 11. Effects of quinidine on the time course of the inotropic actions of digoxin in atrial muscle preparations stimulated at 0.5 and 3.0 Hz. Guinea pig left atrial muscle was stimulated at 0.5 (n=7) or 3.0 (n=11). After a 60-minute equilibration period, quinidine (20 μM) or vehicle (0 μM) was added to the incubation medium. After an additional 40 minutes, digoxin in a final concentration of 0.6 μM was administered (time zero). Data are expressed as mean ± S.E.M.

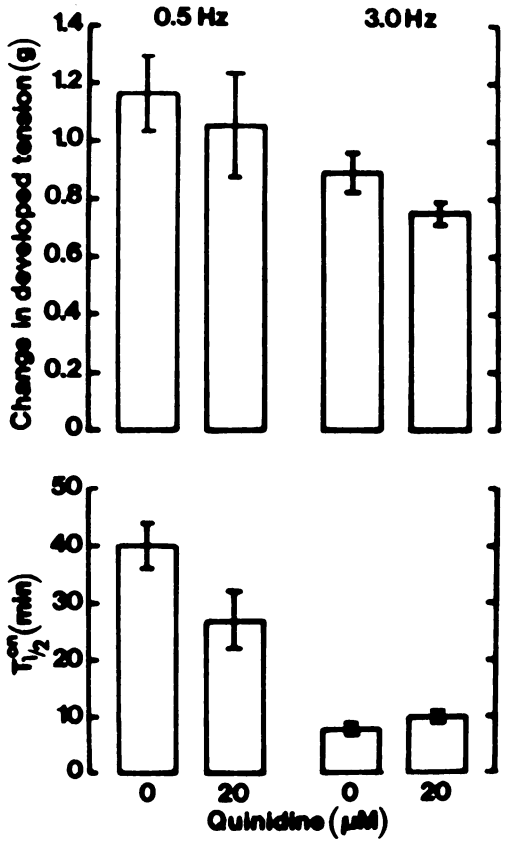

Figure 12. Effect of 20 μM quinidine on the positive inotropic effect of digoxin at different stimulation frequencies. Guinea pig left atrial muscle was stimulated at 0.5 or 3.0 Hz. Forty minutes after addition of 0 or 20 μM quinidine, digoxin (0.6 μM) was added to the incubation solution. The maximal change in developed tension (top panel) and the $T^{on}_{1/2}$, time to half-maximal effect (bottom panel), are shown as the mean $\pm$ S.E.M. for 6 experiments at 0.5 Hz and 11 experiments at 3.0 Hz.

others (Park and Vincenzi, 1975; Bentfeld et al., 1977; Temma and Akera, 1982).

The positive inotropic effect of 0.6 μM digoxin was examined in the presence of 20 μM quinidine. In atrial muscle stimulated at 0.5 Hz, in which the quinidine was found to have little effect on the rate of sodium influx, the magnitude of the positive inotropic effect of digoxin was not significantly different in the presence or absence of 20 μM quinidine (Figure 12). The rate of onset of glycoside action was more rapid in the presence of quinidine; however, the difference from the rate of onset in the absence of quinidine was not significant as determined by 2-way ANOVA. In preparations paced at 3.0 Hz, the magnitude of the glycoside-induced increase in developed tension in the presence of 20 μM quinidine was slightly, but not significantly, smaller than in the absence of quinidine. The rate of onset of digitalis action was also not significantly influenced by quinidine (Figure 12). The lack of an effect of quinidine on the positive inotropic action of digoxin in preparations stimulated at 0.5 and 3.0 Hz, confirmed the experimental results observed in atrial muscle stimulated at 1.5 Hz. These experiments, therefore, indicated that quinidine did not influence the positive inotropic effect of digoxin even in experimental conditions where quinidine apparently reduced the rate of sodium influx into cardiac muscle cells.

2. Benzocaine

Quinidine did not slow the development of the positive inotropic effect of digoxin under conditions where the alkaloid appeared to reduce sodium influx and, therefore, was expected to influence glycoside action. Benzocaine, like quinidine, was shown to be able to

decrease the rate of sodium influx in beating cardiac muscle. Thus, effects of benzocaine on the inotropic actions of digoxin were examined under experimental conditions where the local anesthetic agent decreased the rate of sodium influx. Atrial muscle preparations were stimulated at 3.0 Hz. Benzocaine, at a concentration of 20 μM, had no significant effect on the developed tension of the preparations, while a concentration of 300 μM produced a negative inotropic effect which plateaued within 15 minutes, at which time, developed tension had decreased by 28±5%. Digoxin was added to the bathing medium 20 minutes after benzocaine administration. In the presence of 20 and 300 μM benzocaine, the magnitude of the positive inotropic effect of 0.6 μM digoxin was slightly, though not significantly, greater than in the absence of the local anesthetic. The $T^{on}_{1/2}$ of the glycoside, though, was significantly slowed by 300 μM benzocaine (Figure 13), a concentration of drug shown to decrease the rate of sodium influx into beating atrial muscle fibers. These experiments demonstrated that the rate of onset of digoxin's positive inotropic effect could be delayed by benzocaine under conditions in which benzocaine decreased sodium influx rate.

E. Digoxin Binding in Beating Heart Muscle Preparations

The hypothesis that quinidine interacts with positive inotropic actions of digoxin is based upon the premise that quinidine would decrease the amount of sodium available to the sodium pump, and that rate of glycoside binding is determined by the amount of sodium. Therefore, the effect of quinidine on digitalis binding to the sodium pump was estimated in beating atrial muscle preparations.

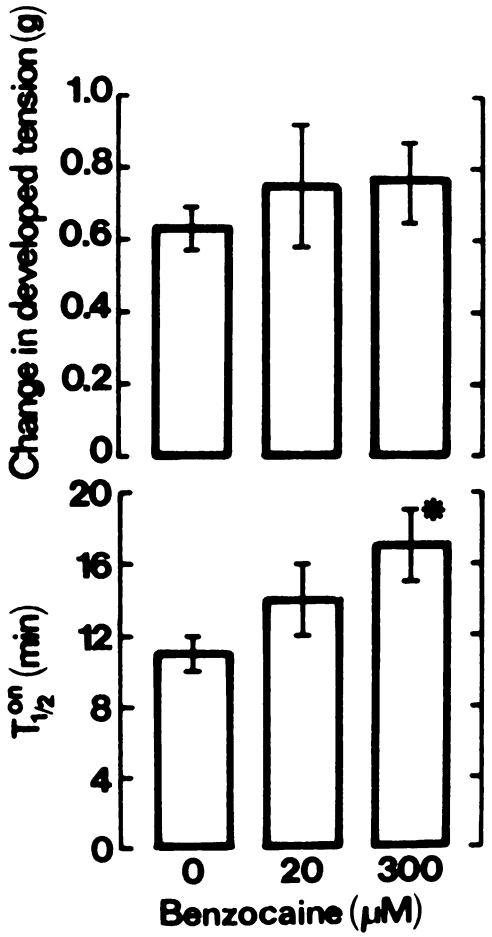

Figure 13. Effect of benzocaine on the positive inotropic action of digoxin. Guinea pig left atrial muscle was stimulated at 3.0 Hz. After 20 minutes in the presence of 0 (n=10), 20 (n=7) and 300 μM (n=10) benzocaine, digoxin (0.6 μM) was added to the bathing medium. Data for the maximal change in developed tension (top panel) and time to half-maximal effect (bottom panel) are expressed as mean ± S.E.M. The asterisk indicates a significant change from 0 μM benzocaine by 1-way ANOVA.

1. Quinidine

Digoxin binding to the Na^+,K^+-ATPase in the atrial muscle was examined by determining the fractional occupancy of glycoside receptor sites in preparations incubated with digoxin in the presence and absence of quinidine. Fractional occupancy was estimated from a reduction in the initial velocity of [^{3}H]ouabain binding reaction to homogenates of the atrial muscle. The initial velocity of ATP-dependent [^{3}H]ouabain is proportional to the number of unoccupied glycoside receptor sites in the homogenate which, in turn, is determined by the number of Na^+,K^+-ATPase enzyme molecules to which digoxin has bound during the prior incubation with the beating atrial muscle. Thus, digoxin binding to the heart muscle, the fractional occupancy, is inversely proportional to the initial velocity of [^{3}H]ouabain binding, i.e. a decrease in initial velocity corresponds to increased digoxin binding in the beating heart.

To determine if quinidine altered the rate of digoxin binding, fractional occupancy was examined at the time of the half maximal inotropic effect of digoxin. Previous experiments showed that the $T^{on}_{1/2}$ for digoxin in atrial muscle stimulated at 3 Hz was eight minutes. For this reason, fractional occupancy in atrial muscle driven at 3.0 Hz was estimated after an eight-minute incubation with digoxin. In these experiments, quinidine in a final concentration of 20 μM was added to the incubation medium 40 minutes prior to glycoside addition. Digoxin or vehicle was then added to the buffer solution and after an additional 8 minutes, the preparation was homogenized to measure fractional occupancy. The initial velocity of ATP-dependent binding to homogenates of preparations exposed to 20 μM quinidine only was not

significantly different from the initial velocity of binding in control preparations exposed to neither quinidine or digoxin. Table 1A shows that the initial velocity of [^{3}H]ouabain binding in homogenates obtained from atrial muscle after an 8 minute ($T^{on}_{1/2}$) incubation with 0.6 μM digoxin in the absence of quinidine was also not significantly different from control preparations. In contrast, the initial velocity of [^{3}H]ouabain binding in homogenates of preparations incubated with 0.6 μM digoxin and 20 μM quinidine was significantly less than that of atrial muscle incubated with 20 μM quinidine only or with 0.6 μM digoxin in the absence of quinidine. These observations did not support the hypothesis that quinidine would decrease digoxin binding to Na^+,K^+-ATPase in atrial muscle under conditions where quinidine decreased the rate of sodium influx. For this reason, digoxin binding was estimated again under the same experimental conditions except that the digoxin concentration was increased to 2.0 μM. Tissue homogenates of preparations incubated in the presence of 2 μM digoxin for 8 minutes showed a significant decrease in initial velocity of [^{3}H]ouabain binding; however, in contrast to the previous experiment, binding was not influenced by 20 μM quinidine. Digoxin binding was also estimated at the time of its maximal inotropic effect in atrial muscle stimulated at 3.0 Hz. In these experiments, tissue preparations were incubated with 0.6 μM digoxin for 45 minutes in the presence or absence of 20 μM quinidine. The initial velocity of [^{3}H]ouabain binding was significantly reduced in atria incubated with 0.6 μM digoxin. The presence or absence of 20 μM quinidine, however, did not influence digoxin binding (Table 1B). Taken together, these experiments indicate that 20 μM quinidine did not change digoxin binding to beating left atrial muscle.

TABLE 1

Effect of Quinidine on Fractional Occupancy by Digoxin in Beating Left Atrial Muscle

Digoxin (μM)	Quinidine (μM)	
	0	20
	ATP-dependent [^{3}H]ouabain binding (fmole/mg prot./2 min)	
A. Incubation Time: 8 minutes		
0	555±10	561±25
0.6	582±24	490±13*#
2.0	457±24*	449±18*
B. Incubation Time: 45 minutes		
0	593±14	604±47
0.6	510±13*	491±24*

Fractional occupancy of glycoside binding sites by digoxin was estimated from the reduction in the initial velocity of ATP-dependent [^{3}H]-ouabain binding to homogenates of guinea pig left atrial muscle. Guinea pig left atrial muscle, stimulated at 3 Hz, was incubated with or without 20 μM quinidine for 40 minutes prior to digoxin administration. After the indicated time in the presence of digoxin or vehicle (EtOH), the atrial muscle was homogenized and the initial velocity of ATP-dependent [^{3}H]ouabain binding was assayed as described in the Methods section. A significant difference between digoxin binding, as estimated by fractional occupancy, in atrial muscle incubated in the presence and absence of the indicated concentration of digoxin is designated by an asterisk (*) and a significant difference in digoxin fractional occupancy at a given digoxin concentration in the presence and absence of 20 μM quinidine is indicated by the cross-hatch (#). Statistical significance was determined by 2-way ANOVA. Data are expressed as mean ± S.E.M. of five experiments.

2. Benzocaine

Inotropic studies showed that benzocaine delayed the onset of the positive inotropic effect of digoxin. To determine if the decrease in the rate of onset was due to slower glycoside binding, the fractional occupancy of digoxin in atrial muscle stimulated at 3.0 Hz was estimated at the $T^{on}_{1/2}$ of digoxin (Table 2). No significant reduction in the initial velocity of ATP-dependent [^{3}H]ouabain binding was observed in the homogenates of atrial muscle exposed to 300 μM benzocaine only. The initial velocity of [^{3}H]ouabain binding to homogenates of tissue preparations incubated for 11 min ($T^{on}_{1/2}$) with 0.6 μM digoxin (data not shown in Table 2) was not significantly different from that in vehicle controls; however, initial velocity of [^{3}H]ouabain binding in homogenates of preparations incubated with 2.0 μM digoxin for 11 min was significantly decreased. The fractional occupancy of the Na^+,K^+-ATPase by digoxin, however, was not significantly changed by 300 μM benzocaine. These data indicate that the benzocaine-induced delay in the onset of the positive inotropic effect of digoxin might not be related to a decrease in the rate of glycoside binding.

In summary, quinidine caused a frequency and dose-dependent decline in both maximal upstroke velocity of the action potential and sodium pump activity which suggested that sodium influx into the cardiac cells was decreased by quinidine. A decrease in sodium influx rate has been postulated to diminish the rate of cardiac glycoside binding to the sodium pump. Quinidine would then be expected to delay the development of the positive inotropic effect of cardiac glycosides under conditions where the agent decreased sodium influx rate.

TABLE 2

Effect of Benzocaine on Digoxin Fractional Occupancy in Guinea Pig Left Atrial Muscle

Digoxin (μM)	Benzocaine (μM)	
	0	300
	ATP-dependent [^{3}H]ouabain binding (fmole/mg prot./2 min)	
0	531±30	573±24
2.0	443±31*	454±27*

Atrial muscle preparations stimulated at 3 Hz were incubated in the absence or presence of 300 μM benzocaine for 20 minutes before addition of digoxin. The atrial muscle was homogenized after an additional 11 minute incubation in the presence of 2.0 μM digoxin and fractional occupancy of the receptor site by digoxin was assayed as described in METHODS. A significant difference in the fractional occupancy between atrial muscle incubated in the 0 and 2.0 μM digoxin as determined by 2-way ANOVA is indicated by an asterisk. Data are expressed as the mean ± S.E.M. of six experiments.

Experimental results under these conditions showed that quinidine did not slow the development of the inotropic actions of digoxin nor decrease binding of digoxin to the Na^+,K^+-ATPase in beating atrial muscle preparations. Benzocaine also was shown to decrease action potential $\dot{V}_{max}$ and sodium pump activity but, unlike quinidine, the rate of onset of the positive inotropic action of digoxin was diminished by benzocaine under conditions where the local anesthetic agent decreased the rate of sodium influx. The rate of digoxin binding to the Na^+,K^+-ATPase in intact preparations, however, was not decreased by benzocaine. Thus, two drugs, both of which appeared to decrease the rate of sodium influx, did not influence the binding of digoxin to the sarcolemmal Na^+,K^+-ATPase in cardiac muscle fibers. These data do not support the working hypothesis that glycoside binding to the sodium pump is dependent on the level of intracellular sodium in the heart cell. The following experiments will examine possible reasons for the apparent discrepancy.

3. Effect of quinidine on the digoxin binding to beating heart muscle incubated in rubidium-containing buffer solutions

The working hypothesis is based on the concept that digoxin binding is dependent on the rate of sodium influx into cardiac muscle fibers, yet the data indicate that quinidine and benzocaine could decrease sodium influx rate without changing digoxin binding to the Na^+,K^+-ATPase in beating atrial muscle. This apparent discrepancy between the working hypothesis and the experimental data might be explained as an artifact of the different experimental procedures used to measure sodium influx rate and digoxin binding. The rate of sodium influx was estimated by the rate of $^{86}Rb^+$ uptake in beating atrial muscle. In these experiments, the cardiac tissue was incubated in a 5

mM RbCl, K^+-free bicarbonate buffer solution, whereas for the measurement of digoxin binding, muscle preparations were incubated in a 5.8 mM K^+, Rb^+-free bicarbonate buffer. Rubidium was used as an ionic substitute for potassium-activation of the Na^+,K^+-ATPase; however, Rb^+ has a slightly greater potency than K^+ for dephosphorylating the potassium-sensitive conformation of the Na^+,K^+-ATPase and the enzyme/cation complex formed after dephosphorylation is more stable in the presence of Rb^+ than in the presence of K^+ (Post et al., 1972). As a result, availability of the glycoside-sensitive conformation of the Na^+,K^+-ATPase would be less in the presence of Rb^+ than in the presence of potassium. Rubidium passes through sarcolemmal potassium channels; however, experiments in resting frog sartorius muscle (Sjodin, 1959) and squid axon (Hagiwara et al., 1972) suggest that membrane permeability for Rb^+ is less than that for potassium. The membrane permeability for Rb^+ might affect fluxes of other ions across the sarcolemma. These differences between Rb^+ and K^+ ions raised the possibility that the effects of quinidine on $^{86}Rb^+$ uptake in beating atrial muscle might not be entirely applicable to the effect of the alkaloid on sodium pump activity under the experimental conditions used to measure digoxin binding. For this reason, digoxin binding was measured in atrial muscle incubated in a Rb^+-containing buffer solution.

Quinidine (20 μM) significantly decreased $^{86}Rb^+$ uptake in atrial muscle stimulated at 3 Hz. Therefore, digoxin binding was measured in atrial muscle preparations incubated in a modified K-H bicarbonate buffer solution containing 5 mM RbCl and stimulated at 3 Hz. Preliminary experiments demonstrated that 20 μM quinidine did not significantly alter the fractional occupancy of glycoside binding sites

by digoxin in atrial muscle which had been incubated for 11 minutes with 0.6 or 2.0 μM digoxin. The initial velocity of [^{3}H]ouabain binding was significantly decreased in homogenates of atrial muscle exposed to 0.6 μM digoxin for 45 minutes (Table 3). Digoxin binding in those preparations incubated with digoxin and 20 μM quinidine was not significantly different from glycoside binding in preparations exposed to digoxin alone. These data demonstrate that under the same conditions where quinidine was shown to decrease $^{86}Rb^+$ uptake, digoxin binding in left atrial muscle was not influenced by the presence of quinidine. The discrepancy between the working hypothesis and the previous data, therefore, cannot be explained as an artifact of the different methods used to measure sodium pump activity and digoxin binding.

F. Effect of Quinidine on [^{3}H]Ouabain Binding to Na^+,K^+-ATPase

1. [^{3}H]Ouabain binding to guinea pig heart microsomes

The data to this point are inconsistent with the hypothesis that digoxin binding to the glycoside-sensitive form of the Na^+,K^+-ATPase in the intact cell is dependent on the rate of sodium extrusion by the sodium pump. The relationship between the rate of sodium extrusion by the sodium pump, the availability of the glycoside-sensitive conformation of the Na^+,K^+-ATPase and glycoside binding is complex and could be modified by changes in the effective affinity of the sodium pump for the glycoside. The data showing that quinidine caused a decrease in sodium pump activity with no change in digoxin binding could suggest that quinidine stimulated binding of the glycoside to the Na^+,K^+-ATPase. This could occur if quinidine stabilizes the glycoside-sensitive form of Na^+,K^+-ATPase thereby stimulating digoxin binding to

TABLE 3

Effect of Quinidine on Digoxin Fractional Occupancy in Atrial Muscle Incubated in Rb^+-Containing Buffer solution

Digoxin (μM)	Quinidine (μM)	
	0	20
	ATP-dependent [^{3}H]ouabain binding (fmole/mg prot./2 min)	
0	407±16	420±21
0.6	299±49*	269±19*

Left atrial muscle preparations of guinea-pig heart were bathed in a 5 mM RbCl, K^+-free buffer solution and electrically paced at 3 Hz. Fractional occupancy of glycoside receptor sites by digoxin was estimated in atrial muscle incubated with 0 or 0.6 μM digoxin for 45 minutes. Quinidine or vehicle (H_2O) was added to the bathing solution 40 minutes prior to digoxin. An asterisk indicates a significant difference in fractional occupancy between atrial muscle exposed 0 and 0.6 μM digoxin as determined by 2-way ANOVA. Data are expressed as the mean ± S.E.M. of four experiments.

Na^+,K^+-ATPase. In order to investigate this possibility, the binding of [^{3}H]ouabain to guinea pig heart microsomes was measured in the presence of various concentrations of quinidine.

Quinidine could stimulate binding to the Na^+,K^+-ATPase by increasing the apparent affinity (decreasing the apparent K_D) of digoxin for the enzyme. A decrease in apparent K_D could occur by decreasing the dissociation rate constant or increasing the apparent association rate constant. Quinidine, even in high concentrations, has been shown to have no effect on the rate of dissociation of digoxin from lamb kidney Na^+,K^+-ATPase (Ball et al., 1981). For this reason, the effect of quinidine on the rate of association of [^{3}H]ouabain with guinea pig heart Na^+,K^+-ATPase was investigated. The time course of ATP-dependent binding of 25 nM [^{3}H]ouabain in the presence of 20 nM NaCl, 5 mM $MgCl_2$, and 5 mM ATP was almost linear for up to 3 minutes (Figure 14, inset). Therefore, 3 minute binding times were used in subsequent experiments with cardiac microsomes as an approximation of the initial rate of ouabain binding to the Na^+,K^+-ATPase. Sodium concentration in these experiments was 20 mM, a concentration which submaximally stimulates ouabain binding. This low concentration was used to ensure that a quinidine-induced stimulation of binding could be observed and because the sodium pump is most likely to be activated by submaximal concentration of Na^+ in beating heart muscle preparations. In the absence of quinidine, the initial velocity of [^{3}H]ouabain binding to the Na^+,K^+-ATPase in these experiments was 784±19 fmole/mg prot./3 minutes (Mean ± S.E.M. of three experiments). Quinidine did not stimulate ouabain binding to the Na^+,K^+-ATPase at any concentration

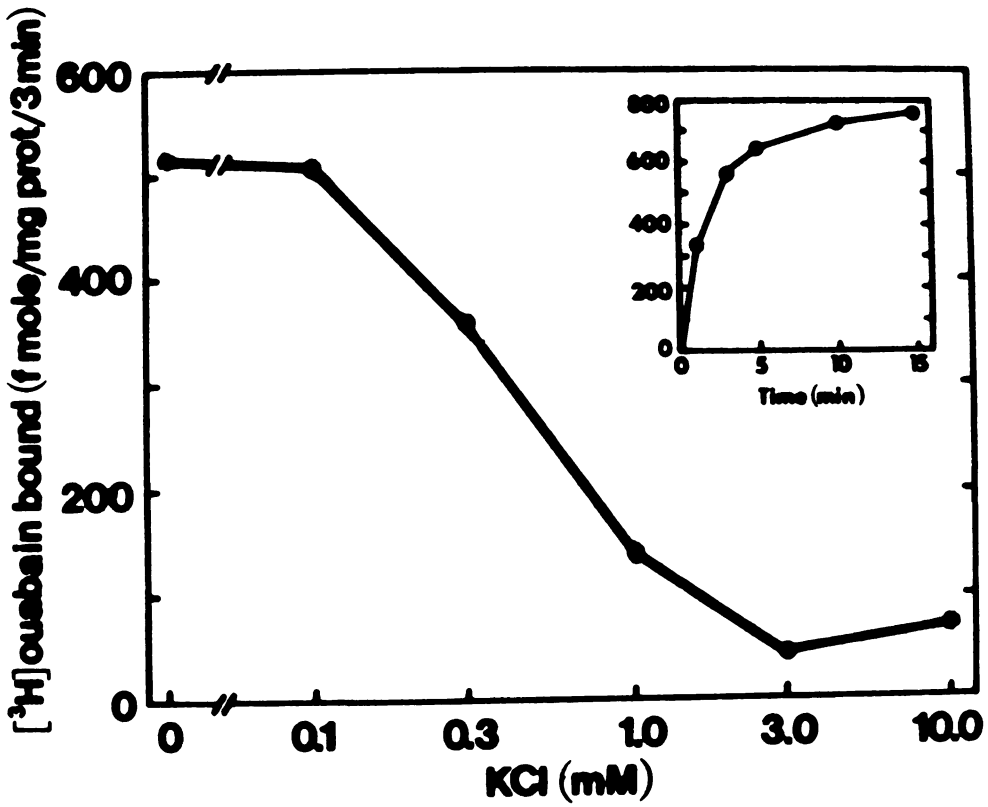

Figure 14. Effect of K^+ on ATP-dependent [^{3}H]ouabain binding to cardiac microsomes. Guinea pig heart microsomal preparations were preincubated for 5 minutes at 37°C in the presence of various concentrations of quinidine with 50 mM Tris-HCl (pH 7.5), 20 mM NaCl, 5 mM $MgCl_2$, and 25 nM [^{3}H]ouabain with or without 0.6 mM KCl. The binding reaction was started by the addition of 5 mM Tris-ATP (pH 6.8) or an equal volume of H_2O. Three minutes later the binding reaction was stopped by the addition of an excess volume of a solution containing 15 mM KCl, 0.1 mM ouabain and 50 mM Tris-HCl buffer (pH 7.5) and the mixture was immediately filtered to separate bound from unbound [^{3}H]ouabain. ATP-dependent binding was calculated as the difference between the amount of radioactivity bound in the presence and absence of ATP. Values are the mean of 2 experiments. Final protein concentration was 100 µg/ml. Inset: Time-course of [^{3}H]ouabain binding. Binding assays were performed as above in the absence of K^+ except that the binding reaction was stopped at the indicated times. Vertical axis: ATP-dependent [^{3}H]ouabain bound (fmole/mg prot./3 minutes).

tested. Instead quinidine caused a dose-dependent decrease in the initial velocity of [^{3}H]ouabain binding in the presence of 20 nM NaCl, 5 mM $MgCl_2$ and 5 mM ATP (Figure 15). The drug concentration to produce 50% inhibition of binding, however, was approximately 600 μM. Inhibition of binding was minimal at concentrations of quinidine shown to inhibit sodium pump activity in beating left atrial muscle preparations. The effect of high concentrations of quinidine on digoxin binding has been reported to be ligand-dependent (Ball et al., 1981). The above condition for binding reaction with Na^+, Mg^{2+} and ATP favored the formation of the phosphorylated intermediates of the Na^+,K^+-ATPase; however, in the beating heart muscle, K^+ ions are available and are most likely to act on the phosphorylated enzyme to promote dephosphorylation (Post et al., 1965; Sen et al., 1969) and to reduce the glycoside-sensitive conformation of the Na^+,K^+-ATPase (Matsui and Schwartz, 1968; Akera and Brody, 1971). Quinidine may antagonize the actions of K^+ on the Na^+,K^+-ATPase, so the effect of quinidine on [^{3}H]ouabain binding was examined in the presence of Na^+, Mg^{2+}, ATP, and K^+. Since the dephosphorylated forms of the Na^+,K^+-ATPase do not readily bind cardiac glycosides (Matsui and Schwartz, 1968), it was first necessary to find a concentration of K^+ which only partially inhibits [^{3}H]ouabain binding. Figure 14 shows that K^+ caused a concentration-dependent decrease in ATP-dependent ouabain binding. Half-maximal inhibition of binding occurred at 0.6 mM KCl, a concentration which was used in the following experiments. The rate of [^{3}H]ouabain binding measured in the presence of Na^+, Mg^{2+}, ATP, and K^+ was 387±18 fmoles/mg prot./3 minutes (Mean ± S.E.M. of three experiments). A

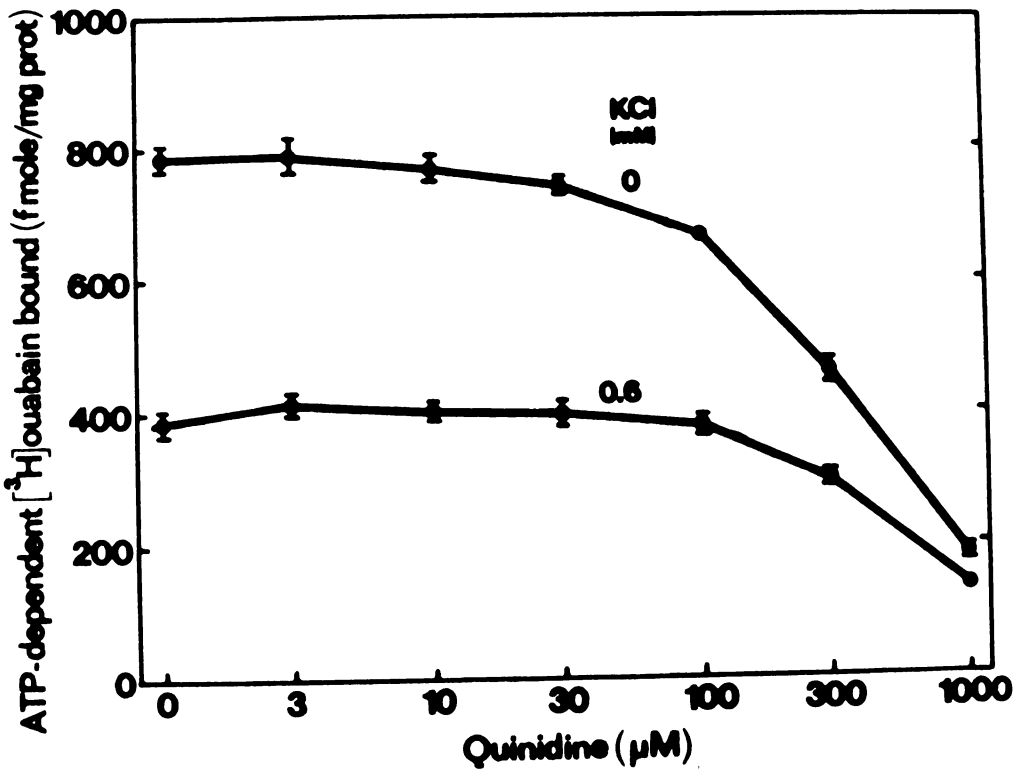

Figure 15. Effect of quinidine on ATP-dependent [^{3}H]ouabain binding to cardiac microsomes. Binding assays were performed as described in Figure 14, in the presence or absence of 0.6 mM KCl, except that various concenrations of quinidine were included. Data are expressed as mean $\pm$ S.E.M. of 3 experiments.

dose-dependent decrease in [^{3}H]ouabain binding was produced at high concentrations of quinidine. A slight though not statistically significant, stimulation of glycoside binding was noted at 3 and 10 μM drug concentration; however, the quinidine-induced stimulation of glycoside binding, if it occurs, seems to be too small to explain the discrepancy between the effects of quinidine on the rate of sodium influx and the lack of effect of the alkaloid on digoxin binding in beating cardiac muscle preparations.

2. [^{3}H]Ouabain Binding to Partially Purified Guinea Pig Heart Na^+,K^+-ATPase in the Presence of Low Sodium Concentrations

Sodium has been shown to stimulate the rate of cardiac glycoside binding to the Na^+,K^+-ATPase, but in these studies, the effects of sodium were examined at concentrations greater than the K_m for sodium binding to the enzyme (13.7 mM; Lindenmayer and Schwartz, 1973). Ouabain binding at nonsaturating concentrations ($\leq$10 mM), however, is greater than predicted by an enzyme model assuming a single sodium binding site. Instead, a model with a second, high affinity sodium binding site on the Na^+,K^+-ATPase with a K_m for sodium of less than 1 mM has been developed to explain the unexpectedly high glycoside binding at low sodium concentrations (Inagaki et al., 1974). These data point out that sodium stimulation of ouabain binding at low sodium concentrations, similar to those observed in cardiac muscle fibers with ion-sensitive electrodes (Cohen et al., 1982), cannot be extrapolated from the effect of high sodium concentrations of ouabain binding. Quinidine is expected to decrease intracellular sodium ion concentration, but the magnitude of this decrease is unknown as the effect of quinidine on intracellular sodium ion concentration has not been

measured. Quinidine (20 μM) decreased the rate of $^{86}Rb^+$ uptake by 30% in atrial muscle stimulated at 3.0 Hz. The decline in intracellular sodium ion concentration produced by this agent is, therefore, likely to be less than or equal to 30%. Because quinidine is expected to decrease intracellular sodium ion concentration, the effects of low sodium concentration on digitalis binding to the cardiac Na^+,K^+-ATPase were examined.

The rate of ouabain binding was measured in partially-purified Na^+,K^+-ATPase from guinea pig heart in the presence of Mg^{2+}, ATP and various concentrations (0-100 mM) of Na^+ to compare the stimulatory effect of low and high concentrations of sodium ion on the glycoside binding. Sodium contamination due to added reagents and protein was determined to be less than 0.1 mM in the absence of added NaCl by flame photometry. The final Na^+ concentrations in the reaction mixture were not adjusted for this slight error. In these experiments, choline chloride was used as an osmotic and ionic substitute for sodium. Figure 16 shows the effects of increasing Na^+ concentration on the initial velocity of ATP-dependent [^{3}H]ouabain binding to guinea pig heart Na^+,K^+-ATPase. From 0-5 mM NaCl, the velocity of binding decreased as Na^+ concentration was increased suggesting that low concentrations of Na^+ may inhibit rather than stimulate, glycoside binding. Similar effects of very low concentrations ($\leq$2 mM) of Na^+ ion on glycoside binding has been observed with rat brain Na^+,K^+-ATPase (Siegel and Josephson, 1972). Sodium concentrations in the range of intracellular Na^+ ion activities measured with ion-selective microelectrodes (Cohen et al., 1982), 5-15 mM, stimulated [^{3}H]ouabain binding; however, five-fold increase in Na^+ concentration from 5-25 mM produced only a

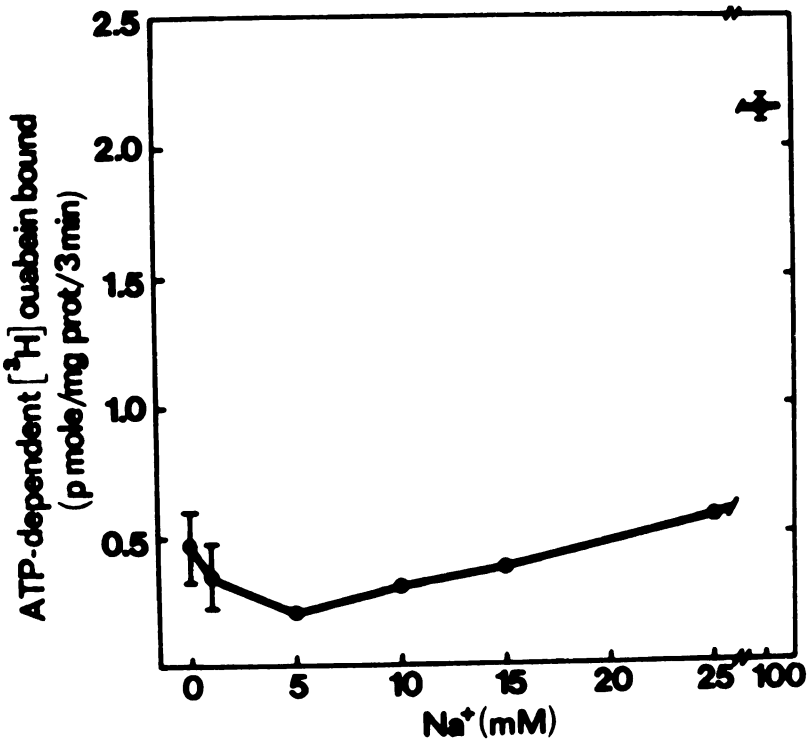

Figure 16. Effect of sodium ion on ATP-dependent [^{3}H]ouabain binding. Partially purified guinea pig heart Na^+,K^+-ATPase was incubated with 50 mM Tris HCl (pH 7.5), 5 mM $MgCl_2$, 25 mM [^{3}H]ouabain, 5 mM Tris-ATP (pH 6.8) and various concentrations of NaCl. Choline chloride was used as an ionic substitute for NaCl so that $[Na^+]+[choline^+]$ = 100 mM. ATP-dependent binding was calculated as the difference in [^{3}H]ouabain bound during a 3 minute reaction period in the presence and the absence of ATP. Data are expressed as mean $\pm$ S.E.M. Final protein concentration is 500 μg/ml.

three-fold increase in [^{3}H]ouabain binding. For comparison, rate of [^{3}H]ouabain binding is shown in the presence of 100 mM NaCl, a concentration which maximally stimulates binding. From these studies, a 30% decrease in Na^+ concentration, the magnitude of the maximal decrease of Na^+ concentration produced by quinidine, would be expected to reduce glycoside binding by 18%. These data indicate that in 5-15 mM sodium concentrations is capable of stimulating glycoside binding to the Na^+,K^+-ATPase; however, the change in Na^+ concentration produced by quinidine might be expected to produce only a small change in glycoside binding.

DISCUSSION

A. Influence of Quinidine and Benzocaine on the Positive Inotropic Action of Digoxin

1. The Effect of Quinidine on the Inotropic Action of Digoxin In Vivo

The effect of the quinidine-induced elevation in serum digoxin concentration on the cardiac actions of digoxin in humans remains unsettled. Several papers have reported that the effects of digoxin on the heart are increased after beginning simultaneous administration of quinidine (Leahey et al., 1978, 1979, 1980a; Belz et al., 1982), while others report that the quinidine-induced increase in serum digoxin concentration is not accompanied by the corresponding increase in the effect of the cardiac glycoside on the heart (Hirsh et al., 1980; Steiness et al., 1980). In these studies, the glycoside effect on cardiac contractility was determined by the degree of digoxin-induced shortening of the QRS complex of the electrocardiogram. Quinidine alone can prolong the QRS complex (Belz et al., 1982) so that the interpretation of the effect of digoxin on contractility is complicated. Judging the effect of digoxin solely on the degree of QRS shortening without accounting for the effect of quinidine may lead to an underestimation of the magnitude of glycoside action. This may account for the lack of an increased effect of digoxin on cardiac contractility with elevated serum digoxin concentration in the studies of Steiness et al. (1980)

who did not measure the effect of quinidine alone, and Hirsch et al. (1980) who measured the effect of quinidine in only 3 of 7 individuals.

In the above human studies, the effect of elevated serum digoxin concentration on the heart was estimated with indirect methods. To overcome the difficulty in measuring cardiac actions of digoxin in human subjects, studies using experimental animal models of the quinidine/digoxin interaction have been performed. A quinidine-induced increase in plasma digoxin concentration in anesthetized guinea pigs led to a greater degree of binding of digoxin to its putative inotropic receptor in the heart, the Na^+,K^+-ATPase (Kim et al., 1981b). In dogs, the digoxin-induced inhibition of active monovalent cation transport in cardiac ventricular tissue was increased when serum glycoside concentration was elevated by quinidine (Leahey et al., 1980b). A recent paper from the same investigators (Warner et al., 1984), however, found that the glycoside-induced inhibition of active monovalent cation transport in ventricular tissue of dogs receiving quinidine and digoxin simultaneously was not greater than the inhibition of active transport in cardiac tissue of dogs receiving the same dose of digoxin. The serum digoxin concentration in animals receiving quinidine and digoxin was, however, increased 100% compared to serum digoxin in dogs receiving digoxin only. The lack of a normal dose-response relationship in dogs receiving quinidine and digoxin contrasts with the results of their earlier report (Leahey et al., 1980b). The reason for the discrepancy between the two reports is not readily apparent. The experimental protocols used in the two studies were different resulting in a 50% lower serum digoxin concentration (1.2 ng/ml) in dogs receiving digoxin only in the report of Warner et al. (1984) than in the earlier

study (2.5 ng/ml digoxin) of Leahey et al. (1980b). Simultaneous administration of quinidine increased serum digoxin concentration to 2.4 and 3.1 ng/ml in the report of Warner et al. (1984) and Leahey et al. (1980b), respectively. Thus, quinidine administration in the more recent report (Warner et al., 1984) produced a greater increment in serum digoxin concentration so it is possible that quinidine would also have a greater effect on the pharmacologic actions of digoxin in the heart than in the earlier study (Leahey et al., 1980b). The conditions under which their experiments were performed appears to greatly affect their results. These studies leave open the possibility the quinidine does interfere with the pharmacologic actions of digoxin in the heart.

2. Effects of Quinidine on the Positive Inotropic Actions of Digoxin, In Vitro

Investigations of the effect of quinidine on the cardiac actions of digoxin, in vivo, are complicated by changes in serum glycoside concentration. For this reason, the present experiments were performed in isolated guinea pig left atrial muscle so that the effects of quinidine on the inotropic actions of digoxin could be observed in the absence of pharmacokinetic complications. In atrial muscle preparations stimulated at 1.5 Hz, quinidine in a final concentration of 3, 10 or 30 μM had no significant effects on the positive inotropic action of 0.6 μM digoxin. These results are in agreement with previously published reports in isolated rat and guinea pig cardiac muscle preparations (Kim et al., 1981a) and in chick heart cell cultures (Horowitz et al., 1982). Williams and Mathew (1981), however, have demonstrated that prior exposure of cat papillary muscles to quinidine decreases the positive inotropic action of digoxin and acetylstrophanthidin. If quinidine is added to the incubation medium at the time of

the maximal inotropic effect of digoxin, the positive inotropic effect of the glycoside is not decreased. Therefore, the order of drug addition determined the effect of quinidine on the actions of digoxin in these experiments in their study. The reason why the order of drug addition should influence the final drug effects is unclear. Experiments in guinea pig atrial muscle, however, showed that quinidine addition followed by digoxin, did not decrease the inotropic actions of the glycoside.

If the glycoside binding to Na^+,K^+-ATPase and ensuing positive inotropic effect are enhanced by intracellular sodium ion, then quinidine, which reduces the rate of sodium influx, is most likely to reduce the glycoside binding and the positive inotropic effect. Thus, an apparent lack of a quinidine-induced decrease in the positive inotropic effect of digoxin in guinea pig atrial muscle seems to warrant further investigation.

The binding of digitalis to the Na^+,K^+-ATPase, the proposed inotropic receptor for the cardiac glycoside, is stimulated by sodium (Matsui and Schwartz, 1968; Siegel and Josephson, 1972; Lindenmayer and Schwartz, 1973). The phosphorylated conformation of the Na^+,K^+-ATPase induced by sodium preferentially binds cardiac glycosides (Matsui and Schwartz, 1968; Sen et al., 1969). This explains why the rate of specific ouabain binding is greater in beating heart muscle preparations stimulated at higher frequencies (Yamamoto et al., 1979; Temma and Akera, 1982) and in cardiac muscle treated with batrachotoxin or monensin (Temma and Akera, 1982). Each of these experimental manipulations increase the rate of sodium influx into the cardiac muscle fiber. In the absence of toxicity, the cell will maintain a relatively low intracellular sodium concentration due to the fact that the sodium

pump is not functioning at maximal capacity under normal conditions, and an increase in the rate of sodium influx will be matched by an increased rate of active sodium extrusion (Akera et al., 1981; Eisner et al., 1983b). This point is supported by numerous studies on sodium pump current (Glitsch et al., 1978; Eisner et al., 1981a) and isotopic rubidium uptake (Yamamoto et al., 1979; Akera et al., 1981). An increase in pump activity promotes glycoside binding (Yamamoto et al., 1979; Temma and Akera, 1982), presumedly by increasing the availability of the sodium-induced, glycoside-sensitive form of Na^+,K^+-ATPase, similar to sodium-dependent stimulation of cardiac glycoside binding to the Na^+,K^+-ATPase enzyme observed in vitro (Matsui and Schwartz, 1968; Lindenmayer and Schwartz, 1973).

Because an elevation of sodium influx or intracellular sodium ion concentration enhances the glycoside binding to Na^+,K^+-ATPase, the rate of glycoside binding to its receptor site is expected to be decreased when the rate of sodium influx or intracellular sodium ion concentration is decreased. Indeed, the glycoside failed to bind to the Na^+,K^+-ATPase in cardiac muscle incubated in a low sodium (27 mM) buffer solution (Temma and Akera, 1983). Less dramatic decreases in extracellular sodium concentration (85 mM), delay the onset of the positive inotropic effects of cardiac glycosides (Akera et al., 1977). Glycoside binding under these conditions is slightly, though not significantly, slower than that seen in cardiac muscle incubated in normal sodium (145 mM) buffer solutions (Temma and Akera, 1983). Under most conditions the rate of digitalis binding to the Na^+,K^+-ATPase in intact cardiac muscle preparations appears to be dependent on the rate of sodium influx.

Quinidine is believed to exert its antiarrhythmic actions by decreasing the rate of sodium influx into cardiac cells. Quinidine has a direct depressant effect on the fast sodium current underlying the rapid upstroke of the cardiac action potential (Johnson and McKinnon, 1957; Chen et al., 1975; Hondeghem and Katzung, 1980; Lee et al., 1981) and on the rate of sodium uptake in beating heart muscle (Choi et al., 1972). It may be hypothesized, then, that quinidine would decrease cardiac glycoside binding to the Na^+,K^+-ATPase in the cardiac muscle and delay the onset of the positive inotropic effect of digitalis under conditions where the antiarrhythmic agent depressed the rate of sodium influx. Yet, in the initial experiments, quinidine had no effect on the inotropic actions of digoxin. One possible explanation for the lack of the anticipated effect of quinidine is that quinidine may not have decreased the rate of sodium influx into the cardiac cell during membrane excitation under the conditions of the present study. To test this possibility, the effects of quinidine on the $\dot{V}_{max}$ of the action potential and $^{86}Rb^+$ uptake in beating atrial muscle preparations were measured.

Quinidine produced a concentration- and frequency-dependent decrease in $\dot{V}_{max}$ and $^{86}Rb^+$ uptake in beating atrial muscle. In preparations, stimulated at 0.5 Hz, 20 μM quinidine did not have a significant effect on $^{86}Rb^+$ uptake and produced only a 20% decrease in $\dot{V}_{max}$, while the same drug concentration in atrial muscle stimulated at 3.0 Hz produced a 30% decrease in $^{86}Rb^+$ uptake and a 50% reduction in $\dot{V}_{max}$. The less pronounced effect of quinidine on $^{86}Rb^+$ than $\dot{V}_{max}$ may be due to the fact that quinidine produces a prolongation of the action potential, such that a decrease in sodium influx during the upstroke

may be offset by a greater sodium influx during latter portions of the action potential. These data suggest that 20 μM quinidine had little or no effect on sodium influx rate in atrial muscle paced at 0.5 Hz but produced a significant decrease in sodium influx in preparations stimulated at 3.0 Hz.

Quinidine is anticipated to decrease digoxin binding to the Na^+,K^+-ATPase in cardiac muscle when the antiarrhythmic agent decreases the rate of sodium influx. For this reason, the positive inotropic actions of digoxin and glycoside binding were measured in the presence and absence of quinidine. The positive inotropic effect of 0.6 μM digoxin was not significantly different in the presence or absence of 20 μM quinidine in atrial muscle stimulated at 0.5 Hz. The lack of an effect of quinidine under these experimental conditions was expected as the alkaloid did not decrease the rate of sodium influx. In atrial muscle preparations stimulated at 3.0 Hz, 20 μM quinidine also did not significantly influence the positive inotropic effect of 0.6 μM digoxin, in apparent conflict with the prediction that quinidine would decrease the inotropic actions of the glycoside under conditions where quinidine decreased sodium influx rate. Fractional occupancy of the Na^+,K^+-ATPase by digoxin at the time of the half-maximal inotropic effect of 0.6 μM digoxin in atrial muscle stimulated at 3.0 Hz; however, was significantly greater in the presence than in the absence of 20 μM quinidine. These data were unexpected because the quinidine-induced decrease in $^{86}Rb^+$ uptake under these experimental conditions suggested that digoxin binding would be decreased rather than increased. The decrease in the initial velocity of [^{3}H]ouabain binding (i.e., increase in fractional occupancy) which occurred in homogenates of

atrial muscle incubated with quinidine and digoxin in the absence of a significant fractional occupancy in atria exposed to digoxin only might be interpretted to suggest that quinidine stimulated the rate of digoxin to the Na^+,K^+-ATPase of the atrial muscle. This is unlikely, however, because quinidine did not significantly stimulate ouabain binding to guinea pig cardiac Na^+,K^+-ATPase (Figure 15) in agreement with the results of Doering (1979). Alternatively, quinidine may inhibit [^{3}H]ouabain binding to the Na^+,K^+-ATPase in homogenates. This interpretation, however, is not consistent with the present data (Figure 15) and published reports (Lowry et al., 1973; Ball et al., 1981) which demonstrate that much higher concentrations of quinidine are required to inhibit glycoside binding to the Na^+,K^+-ATPase. The increase in fractional occupancy noted in atrial muscle incubated with quinidine and digoxin compared to that in atrial muscle incubated with digoxin only, may also not be representative of true effect of 0.6 μM digoxin in the presence and absence of 20 μM quinidine. It was not possible to distinguish between these interpretations of the data or the interpretation that suggests the increase in fractional occupancy represents a true increase in digoxin binding. For this reason, further binding studies were performed. Results of studies in which the previous experiment was repeated but with 2.0 μM digoxin and those in which binding was measured at the maximal inotropic effect of 0.6 μM digoxin showed that 20 μM quinidine did not change glycoside binding to the Na^+,K^+-ATPase in the atrial muscle. Fractional occupancy of the glycoside-receptor sites by digoxin was also examined in atrial muscle stimulated at 3.0 Hz but incubated in a Rb^+-containing buffer solution, the same buffer solution used to measure sodium pump activity. Here,

too, 20 μM quinidine did not significantly change the binding of 0.6 μM digoxin. Taken together, these experiments demonstrate that quinidine did not affect the binding or the inotropic actions of digoxin, even under conditions where the alkaloid significantly decreased the rate of sodium influx into cardiac muscle.

3. Effect of Benzocaine on the Positive Inotropic Action of Digoxin, In Vitro

The failure of quinidine to reduce digoxin binding under the condition in which quinidine inhibits sodium influx may result from an additional action of quinidine which may mask the expected effects of quinidine on the glycoside binding. In order to examine if a decrease in the rate of sodium influx affects the binding or the positive inotropic actions of digoxin, the effect of another drug which reduces the rate of sodium influx was studied. Local anesthetics have been shown to decrease intracellular sodium ion concentration (Deitmer and Ellis, 1980a; Eisner et al., 1983a). Benzocaine, a local anesthetic agent, has been observed to delay the onset of the positive inotropic actions of strophanthidin in isolated canine cardiac Purkinje fibers (Bhattacharyya and Vassalle, 1981). For this reason, the effects of benzocaine on the inotropic actions of digoxin were examined in guinea pig left atrial muscle. The results of the action potential studies demonstrated that 300 μM benzocaine reduced $\dot{V}_{max}$ by 15% without significantly prolonging action potential duration in atrial muscle stimulated at 0.5 Hz. The actions of benzocaine on $\dot{V}_{max}$ (Gintant et al., 1983) as well as I_{Na} in cardiac (Sanchez-Chapula et al., 1983) and skeletal muscle cells (Schwarz et al., 1977) are reported to show little or no frequency dependence at stimulation frequencies below 7 Hz, so

that the action potential studies were not repeated at 3.0 Hz. The results of the $^{86}Rb^{+}$ uptake experiments suggested that benzocaine decreased sodium influx at concentrations greater than or equal to 300 μM in atrial muscle preparations stimulated at 3.0 Hz. In contrast to the above experiments with quinidine, the onset of the positive inotropic effect of 0.6 μM digoxin was significantly delayed by 300 μM benzocaine in the atrial muscle. The $T^{on}_{1/2}$ of 0.6 μM digoxin in the absence of benzocaine was 11 minutes whereas the $T^{on}_{1/2}$ was 17 minutes in the presence of 300 μM benzocaine. The delay in the onset of the inotropic actions of the glycoside is similar to that observed in dog cardiac Purkinje fibers (Bhattacharyya and Vassalle, 1981). Digoxin binding in atrial muscle stimulated at 3.0 Hz, however, was not significantly slowed in the presence of 300 μM. The magnitude of the decrease in digoxin binding which is responsible for the observed lengthening of the $T^{on}_{1/2}$ of 0.6 μM digoxin (from 11 minutes to 17 minutes by 300 μM benzocaine) is expected to correspond to approximately a 10% decrease in the fractional occupancy of digoxin measured after incubating the atrial muscle with digoxin for 11 minutes ($T^{on}_{1/2}$). The expected reduction of cardiac glycoside binding produced by benzocaine is smaller than the normal variability of the data about the mean. This suggests that the local anesthetic-induced decrease in binding would be difficult to observe against normal sample variation. Alternatively, these results might indicate that benzocaine delays the onset of the positive inotropic effect of digoxin by a mechanism subsequent to glycoside binding. The concentration of benzocaine used in these experiments is similar to concentrations at which other local anesthetic agents have been shown to decrease calcium accumulation and release

by isolated sarcoplasmic reticulum (Johnson and Inesi, 1969; Nash-Alder et al., 1980) and block calcium currents in K^+-depolarized cardiac muscle (Josephson and Sperelakis, 1976). It is quite possible, then, that benzocaine would delay the inotropic actions of digoxin by affecting the mobilization of calcium at a step subsequent to glycoside binding.

Both quinidine and benzocaine have been shown to reduce the rate of sodium influx in beating cardiac muscle preparations. Although benzocaine did significantly delay the onset of the positive inotropic action of digoxin, quinidine did not significantly influence glycoside actions in the isolated atrial muscle. Furthermore, neither agent produced a significant change in glycoside binding in beating heart muscle preparations. These data do not support the hypothesis that digoxin binding and positive inotropic actions will be reduced when quinidine (or benzocaine) decreases sodium influx into the cardiac muscle fiber. Possible reasons for this discrepancy will be discussed in the next section.

B. Possible Explanations for the Lack of Effect of Quinidine and Benzocaine on Digoxin Binding

Quinidine does not decrease digoxin binding to atrial muscle preparations under conditions where the antiarrhythmic agent decreases the rate of sodium influx in the atrial muscle preparations. Similar results are observed with a local anesthetic, benzocaine. It is anticipated, however, that the reduction in sodium influx would reduce the rate of glycoside binding to the Na^+,K^+-ATPase in cardiac muscle preparations. Several explanations may be possible to resolve this apparent conflict.

1. Effect of Quinidine and Benzocaine on Sodiun Influx into Atrial Muscle Preparations

a) Measurement of sodium influx

Quinidine is proposed to decrease glycoside binding to the Na^+,K^+-ATPase in atrial muscle under conditions where the alkaloid decreases the rate of sodium influx. For this reason, it is important to establish conditions in which quinidine decreases the rate of sodium influx into the beating atrial muscle preparations. The maximal upstroke velocity of the atrial muscle action potential and on-going sodium pump activity were used as estimates of the rate of sodium influx.

In this project, $\dot{V}_{max}$ was used as an indicator of the magnitude of the fast sodium current, though, recently, the use of $\dot{V}_{max}$ as a quantitative measure of sodium current has been questioned. For a uniform membrane action potential, $\dot{V}_{max}$ should be proportional to the total ionic current crossing the membrane at the time of $\dot{V}_{max}$ (Hodgkin and Huxley, 1952). The great majority of the ionic current flowing at the time of $\dot{V}_{max}$ in cardiac muscle is believed to be carried by sodium ions (I_{Na}) as nonsodium currents are expected to contribute little to $\dot{V}_{max}$ in the cardiac muscle action potential. As a result, $\dot{V}_{max}$ has been proposed to be a good measure of I_{Na} (Hondeghem, 1978). Experiments measuring both $\dot{V}_{max}$ and I_{Na} in the enzymatically-dispersed rat cardiac cells (Lee et al., 1979; Undrovinas et al., 1980) demonstrated that the observed $\dot{V}_{max}$ of the action potential was similar to the calculated $\dot{V}_{max}$ based upon membrane capacitance and the I_{Na} measured during voltage clamp experiments.

Strichartz and Cohen (1978), however, have argued that even when nonsodium currents are held at zero, $\dot{V}_{max}$ is still not linearly related to available sodium conductance (g_{Na}), where available g_{Na} is defined by the ratio of I_{Na} to maximal I_{Na}. Even with zero nonsodium currents, $\dot{V}_{max}$ would only be linearly related to g_{Na} if the kinetics of sodium channel inactivation were much slower than channel activation so that little inactivation would have occurred at the time of $\dot{V}_{max}$. The model system that Strichartz and Cohen used for the cardiac action potential assumed kinetics of the fast sodium current similar to those found during voltage clamp experiments in squid axon (Cohen and Strichartz, 1977). Hondeghem (1978) used a model system in which the kinetics for the sodium current were manipulated to reconstruct a ventricular muscle action potential (Beeler and Reuter, 1977), because, at the time, it was not possible to study the kinetics of the fast sodium current in the heart. The disagreements between these two model systems lies in the fact that in the squid axon sodium channel inactivation is significant at $\dot{V}_{max}$ (Hodgkin and Huxley, 1952), whereas in the model of Beeler and Reuter (1977), inactivation is much slower and is minimal at the time of $\dot{V}_{max}$. Recent voltage-clamp experiments using enzymatically-dispersed rat heart ventricular cells (Brown et al., 1981; Bodewei et al., 1982) and cultured cardiac cells (Cachelin et al., 1983) demonstrate that kinetics of sodium channel inactivation are faster than those calculated in the model of Beeler and Reuter (1977) so that significant inactivation may occur at $\dot{V}_{max}$. Experimentally, a nonlinear relationship between I_{Na} and $\dot{V}_{max}$ has been demonstrated in rabbit Purkinje fiber (Cohen et al., 1984). In this study, changes in $\dot{V}_{max}$ greatly underestimated the depression of the sodium

current produced by tetrodotoxin or steady membrane depolarization in Purkinje fibers. The above arguments point out that the use of $\dot{V}_{max}$ as an estimate of I_{Na} during the action potential upstroke remains controversial.

Another source of error in relating $\dot{V}_{max}$ to I_{Na} is due to latency changes with increasing local anesthetic drug concentration. Latency is defined as the time between the end of the electrical stimulus and the moment of $\dot{V}_{max}$. An increase in latency results from a stimulus which raises the membrane potential above the thresold voltage more slowly. This allows additional time for sodium channel inactivation to occur before $\dot{V}_{max}$, so that $\dot{V}_{max}$ may appear smaller than if latency is held constant (Walton and Fozzard, 1979). Latency did increase in the presence of both quinidine and benzocaine (see Figures 4 and 6), especially at high drug concentrations. Thus, the drug-induced decrease in $\dot{V}_{max}$, in part, may result from an increase in stimulus latency. Overall, the above discussion points out that the actions of quinidine or benzocaine on $\dot{V}_{max}$ should not be regarded as quantitative indicators of drug effect on sodium influx rate during the action potential upstroke; the reduction in $\dot{V}_{max}$ may underestimate the actual reduction in the rate of sodium influx. Nevertheless, these data should be viewed as relative measures of the effect of the drug at various concentrations and/or stimulation frequencies.

Is ouabain-sensitive rubidium-uptake in beating heart muscle a good measure of sodium influx? This depends upon two factors: 1) that the sodium pump is the only mechanism for sodium extrusion in the cardiac muscle cell and 2) that $^{86}Rb^{+}$ uptake is an accurate measure of sodium extrusion by the pump. The sodium pump is believed to be the

major mechanism for maintaining intracellular sodium and potassium ion concentrations (Skou, 1957; Post et al., 1960). Cardiac glycosides can block active monovalent cation transport across the cell membrane (Schatzmann, 1957; Post et al., 1960) and blockade of the pump by removing extracellular potassium or by toxic concentrations of cardiac glycosides causes a rapid increase in intracellular sodium ion concentration (Ellis, 1977; Eisner et al., 1981a,b). This indicates that the sodium pump is the major mechanism for maintaining low intracellular sodium ion concentration. The sodium pump, however, is not the only route for sodium efflux from the cardiac cell. The sarcolemmal sodium/calcium and sodium/hydrogen exchange mechanisms may also extrude sodium from the cells. A mechanism for regulating intracellular pH via trans-sarcolemmal exchange of sodium and hydrogen ion has been identified in cardiac Purkinje fibers bathed in bicarbonate-free buffer solution (Deitmer and Ellis, 1980b). The importance of this exchange mechanism on sodium flux under physiological conditions is not likely to have a substantial impact on total sodium influx as the hydrogen ion concentration at physiological pH range is submicromolar (0.1 μM). Even assuming substantial intracellular buffer capacity, the net sodium flux for sodium/hydrogen ion exchange is likely to be very small. Increasing extracellular calcium concentration has been shown to increase the rate of sodium efflux from isolated tissue preparations (Deitmer and Ellis, 1978). The magnitude of this mode of sodium/calcium exchange (calcium in and sodium out) is not well characterized in the heart. More appropriate, however, is whether the sodium flux via sodium/calcium exchange remained constant between the various manipulations; stimulation frequency and drug treatment. Increasing stimulation rate

is believed to augment calcium influx (sodium efflux) by the exchange mechanism. Faster driving rates have been shown to increase intracellular sodium ion concentration (Cohen et al., 1982) which could increase sodium/calcium exchange. A linear relationship between intracellular sodium ion activity and tension development in cardiac Purkinje fibers (Eisner and Lederer, 1980; Eisner et al., 1981a) presumedly reflects increased calcium influx in exchange in intracellular sodium. This phenomenon may underlie the positive force-frequency relationship observed in many mammalian cardiac muscle preparations (Langer, 1967). Increasing stimulation frequencies, then, might be expected to enhance sodium efflux from the cardiac cell by mechanisms other than the sodium pump. Nevertheless, sodium pump activity, as measured by $^{86}Rb^{+}$ uptake (Figure 1 and Akera et al., 1981), is roughly proportional to stimulation rate, so that whatever the contribution of the sodium/calcium exchange mechanism to net sodium efflux, it does not appear to dramatically alter the relationship between sodium influx and sodium pump activity. The effects of quinidine-like agents on the relative importance of sodium efflux unrelated to the sodium pump is not known. Thus, the sodium pump may be considered as the major mechanism for sodium extrusion in cardiac muscle cells.

The second criterion for $^{86}Rb^{+}$ uptake to be a measure of sodium influx is that isotopic rubidium uptake should accurately estimate sodium extrusion by the sodium pump. For ouabain-sensitive $^{86}Rb^{+}$ uptake to estimate sodium extrusion, the coupling ratio between sodium and rubidium must remain constant. Experiments in isolated canine (Gadsby, 1980) and sheep (Eisner et al., 1981a,b) Purkinje fibers have demonstrated that when the sodium pump is reactivated by potassium

(Gadsby, 1980) or rubidium (Eisner et al., 1981a,b) after a short period of sodium-loading in a potassium-free buffer, a glycoside-sensitive outward current can be measured by voltage-clamp techniques. This current decays in a monoexponential fashion with a rate constant that is dependent on the potassium (rubidium) concentration. Analysis of this "sodium pump" current has demonstrated that the coupling ratio for sodium and potassium transport by the sodium pump is fixed under a variety of cation concentrations. Other modes of sodium pump transport such as uncoupled sodium ion efflux or potassium:potassium (rubidium) exchange, however, could distort the relationship between measured $^{86}Rb^{+}$ uptake and sodium extrusion. Uncoupled sodium efflux which is ouabain-sensitive, has been demonstrated in erythrocytes in the absence of extracellular potassium and sodium (Robinson and Flashner, 1979); however, this mode of sodium pump activity will be negligible under the present experimental conditions. Potassium:potassium exchange is also ouabain-sensitive (Glynn and Luthi, 1968) and it is expected to be increased at low intracellular sodium concentrations. Nevertheless, potassium:potassium exchange in erythrocytes is inhibited almost completely at intracellular sodium concentrations greater than 4 mM (Simons, 1974). Because intracellular sodium concentration is higher than 4 mM, this exchange mechanism was probably insignificant in the present studies. The effect of quinidine and local anesthetics on the coupling ratio of the sodium pump has not been studied. Quinidine has significant effects on active transport of $^{86}Rb^{+}$ and $^{22}Na^{+}$ by erythrocyte membranes (Lowry et al., 1973; Ball et al., 1981) but only at concentrations much higher than those used in the present experiments. Thus, ouabain-sensitive $^{86}Rb^{+}$ uptake should be an accurate measure of the rate of sodium extrusion by the sodium pump and is a good estimate

of the rate of sodium influx into beating atrial muscle preparations under steady-state conditions.

b) Effect of quinidine and benzocaine on sodium influx rate

Quinidine produced a dose-dependent decrease in the $\dot{V}_{max}$ of the guinea pig left atrial muscle which was enhanced at more rapid rates of stimulation. This data is in agreement with the frequency-dependent depression of $\dot{V}_{max}$ observed in guinea pig ventricular muscle (Johnson and McKinnon, 1957; Chen et al., 1975; Hondeghem and Katzung, 1980). And although a rate-dependent block of I_{Na} by quinidine has not been demonstrated, lidocaine and its structural analogues which also produce frequency-dependent depression of $\dot{V}_{max}$ of the cardiac action potential, cause a frequency-dependent block of I_{Na} in isolated nerve preparations (Courtney, 1975) and use-dependent block in nerve (Hille, 1977) and cardiac Purkinje fiber (Bean et al., 1983) preparations. The increased effect of quinidine on $\dot{V}_{max}$ at faster driving rates, then probably reflects a greater depression of I_{Na} in the atrial muscle. These results are in agreement with the "modulated receptor" hypothesis of sodium current blockade by antiarrhythmic agents (Hondeghem and Katzung, 1984).

The present experiments demonstrated that quinidine reduced sodium pump activity in beating atrial muscle preparations in a dose- and frequency-dependent manner, similar to the drug's effect on action potential $\dot{V}_{max}$. That the quinidine produces a similar shift in two independent measures of sodium influx, strengthens the view that the reduction in $\dot{V}_{max}$ and ouabain-sensitive ^{86}Rb uptake reflected a decrease in the rate of sodium influx.

The drug-induced decrease in $^{86}Rb^+$ uptake is not paralleled by a reduction in $\dot{V}_{max}$ of similar magnitude. Quinidine decreases $^{86}Rb^+$ uptake only at concentrations which substantially decrease $\dot{V}_{max}$. For instance, in atrial muscle stimulated at 1.5 Hz, sodium pump activity was reduced approximately 25% by 30 μM quinidine, a drug concentration which decreased $\dot{V}_{max}$ 50%, yet 3 and 10 μM quinidine did not decrease $^{86}Rb^+$ uptake even though $\dot{V}_{max}$ was reduced 15 and 30%, respectively. Benzocaine also produced a dose-dependent decrease in $^{86}Rb^+$ uptake, but, unlike quinidine, the decrease in sodium pump activity was observed at a concentration (300 μM) which only minimally decreased $\dot{V}_{max}$.

The difference in the relative changes in $\dot{V}_{max}$ and $^{86}Rb^+$ uptake may arise for the following reason: $\dot{V}_{max}$ is an estimate of peak in sodium current (influx) during the action potential upstroke whereas ouabain-sensitive $^{86}Rb^+$ uptake is a time average of net sodium influx. Measurements of $\dot{V}_{max}$ are insensitive to changes in sodium influx occurring at times other than that during the upstroke. Conversely, $^{86}Rb^+$ uptake is apt to be less sensitive to changes in sodium influx during the upstroke, particularly if sodium influx at other times during cardiac muscle excitation is significant. Quinidine has been shown to decrease the calcium current in cat ventricular papillary muscle at the concentration of 10^{-4}M (Nawrath, 1981); however, in the concentrations used in the present experiments, quinidine may decrease $\dot{V}_{max}$ without blocking other mechanisms of sodium influx such as the calcium current. Quinidine may even increase sodium influx occurring after the upstroke because it prolongs action potential duration. Benzocaine, on the other hand, is used at much higher concentrations

(300 μM), similar to those at which other local anesthetics have been shown to block the calcium current (Josephson and Sperelakis, 1976). This suggests that quinidine may reduce sodium influx specifically by blockade of the sodium current whereas benzocaine may reduce sodium influx in a more nonspecific manner. If this is the case, quinidine might need to produce a substantial decrease in $\dot{V}_{max}$ before a significant reduction in total sodium influx, measured by $^{86}Rb^{+}$ uptake occurred, whereas the effect of benzocaine on total sodium influx might be greater than its reduction of sodium current alone. These arguments, however, are very speculative because, not only have mechanisms of sodium influx into cardiac atrial muscle fibers not been discretely identified, but the comparison of drug effects on action potential $\dot{V}_{max}$ are tentative as $\dot{V}_{max}$ is reliable only as a qualitative variable in these studies. In spite of this, quinidine depresses $\dot{V}_{max}$ and $^{86}Rb^{+}$ uptake at similar concentrations, both measures of sodium influx rate demonstrated a rate-dependent effect. These results are in agreement with those of Lee et al. (1981) who showed that quinidine decreases I_{Na} in single rat cardiac myocytes. Benzocaine also reduced $\dot{V}_{max}$ and $^{86}Rb^{+}$ uptake at similar concentrations, though these concentrations are approximately ten-fold greater than the effective concentrations of quinidine. Thus, the data indicated that these agents did decrease the rate of sodium influx in atrial muscle fibers.

That substantial sodium influx occurs at times during the cardiac action potential other than during the upstroke is unsettled. In Purkinje fibers, tetrodotoxin significantly decreases action potential duration at concentrations which have little effect on

$\dot{V}_{max}$ (Coraboeuf et al., 1979; Elharrar et al., 1984). The decrease in action potential duration has been attributed to the block of a steady-state inward "window" current carried by sodium ions (Attwell et al., 1979). The existence of the window current may vary between different types of cardiac muscle fibers as tetrodotoxin has little effect on action potential duration or background currents in guinea pig ventricular myocytes (J. Hume, personal communication).

Sodium may also enter the muscle fiber via calcium channels. Reuter and Scholz (1977) calculated the ionic permeabilities the calcium channel in cow ventricular trabeculae by using the constant field equations of Goldman, Hodgkin and Katz (Hodgkin and Katz, 1949) which predict that the reversal potential of a membrane current is the product of the permeability and concentration gradient of each ion species which contributes to the current. Using these equations, the permeability of the slow current for calcium was calculated to be 100 times that for sodium, yet because extracellular sodium concentration (150 mM) was much greater than extracellular calcium concentration (1.8 mM), sodium ions might account for 25% of the total current. When extracellular sodium was decreased the expected shift in the reversal potential predicted by the constant field equation was observed. The reversal potential for the calcium current, however, may have been less positive than predicted due to contamination of the calcium currents by outward potassium currents and the presence of a large series resistance in the voltage clamp circuit. In fact, recent experiments in guinea pig ventricular myocytes have demonstrated that reversal of current flow through the calcium channel occurs at +50 to +70 mV (Lee and Tsien, 1982, 1984). If this is the case, the calculated sodium

permeability of Reuter and Scholz (1977) may be too high. Furthermore, the current carried by sodium ions through the calcium channel in a calcium-free solution has been shown to be substantially reduced by low concentrations of calcium ion (300 μM) in guinea pig ventricular myocytes (Hess and Tsien, 1984). A recent paper by Matsuda and Noma (1984) also demonstrated that the removal of extracellular sodium did not have a significant effect on peak calcium current in guinea pig ventricular myocytes (in 1.8 mM calcium buffer solution). These observations indicate that at the calcium concentration (1.2 mM) in the present experiments, sodium influx via the calcium channel may not make a substantial contribution to the calcium current. In spite of this, sodium ions entering through the calcium channel have been reported to be significant as stimulation of the sodium pump current by repetitive trains of stimuli can be reduced by D-600 (NOTE: D-600 may inhibit Na current at concentrations greater than 10 μM; Dayer et al., 1975), a calcium channel antagonist (Falk and Cohen, 1983), which suggests that sodium influx via the calcium channels is significant. Recent experiments by January and Fozzard (1984) suggest that inactivation of the sodium channel, which was caused by maintaining the resting membrane potential at -50 mV in sheep Purkinje fibers, prevents the increase in intracellular sodium ion concentration which can be observed after rapid stimulus trains in Purkinje fibers with a more negative resting membrane potential. This suggests that sodium influx via non-sodium currents does not contribute to the increase in intracellular sodium ion concentration during the frequent stimulations. The reasons for the discrepancy between the results of Falk and Cohen (1983) and those of January and Fozzard (1984) are not

clear, although Purkinje fibers from different species were used and their protocols were also different. Thus, it is possible that sodium influx occurs via the slow calcium channels; however, the amount of sodium entering the cell via this pathway is unknown. If either inhibition of the slow calcium channels by D-600 or inhibition of the sodium channels by the partial depolarization is sufficient to markedly reduce the stimulus induced intracellular sodium ion accumulation, one may conclude that both mechanisms of the sodium influx are substantial and contribute to the accumulation of sodium ions observed during a rapid stimulus train.

The present experiments also suggest that sodium ions enter the cardiac cell at times other than the upstroke or plateau phase of the action potential. Sodium pump activity is beat-dependent, yet in quiescent atria, ouabain-sensitive $^{86}Rb^{+}$ uptake is 3.9 nmoles/mg wet weight/20 min (Figure 1), which is about 35% of the ouabain-sensitive $^{86}Rb^{+}$ uptake measured at 1.5 Hz. A substantial basal sodium leak is also suggested by measurements of the atrial muscle resting potential. The average resting potential, -78 mV, is less than the calculated potassium reversal potential, E_K, of -86 mV (assuming intracellular potassium concentration equals 150 mM) for the present experiments. This leviation of the resting potential from E_K is believed to be due to a low permeability of the resting membrane to sodium ions (Sperelakis, 1979). Thus, even in the absence of electrical stimulation, a baseline sodium leak still occurs. Electrical stimulation might, then, increase sodium influx via the sodium channel and other ionic channels. Studies in beating rabbit ventricular muscle have also shown that the rate of sodium efflux measured by labelled sodium ion exchange studies may exceed the amount of sodium

influx which occurs during the fast sodium current (Langer, 1974). Thus, net influx in the heart muscle may be the sum of sodium entering via the sodium channel and other pathways. This may account for discrepancy between the effects of quinidine and benzocaine on $\dot{V}_{max}$ and $^{86}Rb^{+}$, as the two drugs may have different effects on sodium influx which occurs at other times than the upstroke of the action potential.

In spite of the difference in the effects of quinidine and benzocaine on $\dot{V}_{max}$ and $^{86}Rb^{+}$ uptake, both drugs appear to decrease the rate of sodium influx in atrial muscle preparations under specific experimental conditions. Digoxin binding, however, is not significantly influenced by either drug under these specific experimental conditions. Thus, the lack of quinidine or benzocaine-induced effect on glycoside binding cannot be attributed to the absence of a decrease in sodium influx produced by quinidine or benzocaine.

2. Effect of the Positive Inotropic Action of Quinidine on Digoxin Binding

Another possible explanation for the lack of a quinidine-induced decrease in digoxin inotropic effect is that the positive inotropic effect of quinidine itself, may be responsible for changing glycoside binding so that a decrease in sodium influx would not produce a decline in total digoxin binding to the Na^{+},K^{+}-ATPase. In guinea pig left atrial muscle, quinidine produced a positive inotropic effect. At higher concentrations, a biphasic effect on developed tension was observed. The effects of quinidine on contractility of cardiac muscle appear to be species and tissue-dependent. Quinidine produces a negative inotropic effect in dog (Folle and Aviado, 1966; Pruett and Woods, 1967) and guinea pig ventricular muscle (Kim et al., 1981a); however, it produces a positive inotropic effect in rat atrial and ventricular

muscle (Kim et al., 1981a) and ferret papillary muscle (Lash et al., 1982). Nawrath (1981) also reported that quinidine produces a positive inotropic effect in guinea pig left atrial muscle which is not influenced by atropine. The positive inotropic effect was then followed by a decline in developed tension. The positive inotropic effect of quinidine observed in the present study, therefore, is in agreement with earlier published reports.

It is unlikely that the change in baseline contractility of the atrial muscle produced by quinidine altered the binding of digoxin. Changing extracellular calcium concentration from 1.25 to 2.5 mM, which increases force development more than two-fold, does not change the rate of onset of the positive inotropic action of ouabain in guinea pig left atrial muscle (Akera et al., 1977). Furthermore, although increasing stimulation frequency from 0.5 to 2.0 Hz increases the rate of glycoside binding to the Na^+,K^+-ATPase in beating atrial muscle, the extent of binding at peak inotropic effect is not significantly different between those preparations driven at 0.5 Hz and those at 2.0 Hz (Temma and Akera, 1982). These data suggest that merely changing the baseline inotropic state of the cardiac muscle will not alter glycoside action unless sodium influx is also changed. The positive inotropic action of quinidine does not appear to be due to an increase in sodium influx, as measured by $^{86}Rb^+$ uptake. Because quinidine produces a positive inotropic effect in some cardiac muscle preparations and a negative inotropic effect in others, it is possible to examine the influence of the inotropic actions of quinidine on the digoxin-induced increase in cardiac contractility. In cardiac muscle preparations in which quinidine has a positive inotropic effect (Kim et al., 1981a)

and a negative inotropic effect (Kim et al., 1981a; Horowitz et al., 1982), the positive inotropic effect of digoxin and the effect of glycoside-induced inhibition of the sodium pump has been found to be unchanged in the presence of quinidine. Furthermore, in guinea pig ventricular muscle, 6 μM quinidine does not influence the positive inotropic effect or the extent of binding of digoxin under experimental conditions very similar to those employed in the present study. Quinidine (6 μM) also produced a 35 percent decrease in force development in these preparations (Kim et al., 1981a). It is unlikely that the binding of digoxin or the positive inotropic effect of the glycoside would be altered due to the inotropic actions of quindine.

3. Effect of Quinidine on the Fractional Occupancy of Digoxin in Rubidium-Containing Buffer Solutions

Those experiments which examined the effect of quinidine on the inotropic actions and binding of digoxin were performed in a bicarbonate buffer containing 5.8 mM K^+, while sodium pump studies were carried out in a buffer solution containing 5 mM Rb^+. Rubidium acts as an ionic substitute for K^+ but it has greater affinity for the Na^+,K^+-ATPase than K^+ (Post et al., 1972) and membrane permeability for Rb^+ is less than that for K^+ (Sjodin, 1959; Hagiwara et al., 1972). For this reason, the effect of quinidine or benzocaine on $^{86}Rb^+$ uptake might not accurately reflect the actions of these two agents on sodium pump activity in K^+-containing solutions. Fractional occupancy of the Na^+,K^+-ATPase by digoxin in atrial muscle preparations stimulated at 3 Hz and incubated in a 5 mM Rb^+-containing buffer solution, however, was not significantly influenced by 20 μM quinidine, similar to the lack of effect of quinidine on digoxin binding in atrial muscle incubated in

K^+-containing buffer solutions. These results confirm the finding that quinidine does not influence digoxin binding under conditions where quinidine decreases the rate of sodium influx. Thus, the failure of the present finding to support the hypothesis which suggests glycoside binding will be decreased when quinidine reduces sodium influx cannot be resolved as difference in effects of quinidine on the rate of sodium influx under the different experimental protocols used to measure glycoside binding and the rate of sodium influx.

4. Quinidine-induced Stimulation of Digoxin Binding to Na^+,K^+-ATPase

Quinidine fails to significantly alter digoxin binding under conditions in which the quinidine appears to decrease sodium influx rate in atrial muscle preparations. The lack of an effect on digoxin binding raises the possibility that the decrease in digoxin binding which does occur due to a reduction in sodium influx rate is offset by a second effect of quinidine to stimulate glycoside binding. The net effect then will be that cardiac glycoside binding is not changed in the presence of quinidine. A stimulation of digoxin binding can occur by increasing the apparent affinity of digoxin for the Na^+,K^+-ATPase or by stabilizing the glycoside-sensitive form of the enzyme. An increase in the apparent affinity of the Na^+,K^+-ATPase for digoxin can result either from a decrease in the rate of dissociation or an increase in the rate of association of the glycoside. The dissociation of bound digoxin from lamb kidney Na^+,K^+-ATPase is reported to be insensitive to even millimolar concentrations of quinidine (Ball et al., 1981). At quinidine concentrations greater than or equal to 300 μM, the rate of ouabain binding was decreased in the presence or absence of 0.6 mM

K^+, consistent with published reports on the effect of the alkaloid on cardiac glycoside binding to the Na^+,K^+-ATPase (Lowry et al., 1973; Ball et al., 1981). A slight stimulation (5-10 percent) of the rate of [^{3}H]ouabain binding was observed with 3 and 10 μM quinidine when the binding reaction was performed in the presence of 0.6 mM K^+. This stimulation, though not statistically significant, may indicate that a similar 5-10 percent stimulation of digoxin binding could occur in beating atrial muscle preparations. The magnitude of this stimulation, however, does not appear to be large enough to offset the expected quinidine-induced inhibition of glycoside binding. These results indicate that a quinidine-induced stimulation of glycoside binding to the Na^+,K^+-ATPase would not account for a lack of the anticipated quinidine/digoxin interaction in guinea pig atrial muscle preparations.

5. Sodium Dependence of Glycoside Binding to the Na^+,K^+-ATPase

The absence of a quinidine-induced stimulation of ouabain binding to the Na^+,K^+-ATPase suggests that the relationship between the availability of glycoside-sensitive form of the sodium pump and glycoside binding will also remain unchanged in the presence of quinidine. The working hypothesis suggests that the availability of the glycoside-sensitive form of the sodium pump is proportional to the rate of sodium influx under steady state conditions. Quinidine is, therefore, expected to decrease digoxin binding to the sodium pump in atrial muscle fibers under conditions where the antiarrhythmic agent decreases sodium influx: yet, the results do not fulfill this expectation. For this reason, the working hypothesis was re-examined.

The concept that cardiac glycoside binding to the Na^+,K^+-ATPase in the cardiac cell is determined by the rate of sodium influx, is formulated from the data showing that digitalis binding and positive inotropic actions in cardiac muscle tissue are enhanced by maneuvers which increase the rate of sodium influx, such as rapid stimulation (Park and Vincenzi, 1975; Bentfeld et al., 1977; Yamamoto et al., 1979; Temma and Akera, 1983) and the treatment with agents which increase passive sodium leak across the sarcolemma (Akera et al., 1977; Temma and Akera, 1982). Conversely, incubation of cardiac muscle preparations in buffer solutions with moderate reductions in sodium concentration slows the onset of the positive inotropic effect of ouabain (Akera et al., 1977) and a further reduction to less than 30 mM sodium, abolishes the positive inotropic effect (Linden and Brooker, 1980; Wiggins and Bentolila, 1980; Temma and Akera, 1983) and specific binding (Temma and Akera, 1983) of cardiac glycosides. The above concept is also consistent with the fact that sodium enhances glycoside binding to the isolated Na^+,K^+-ATPase in the presence of Mg^{2+} and ATP (Matsui and Schwartz, 1968; Lindenmayer and Schwartz, 1973), ligand conditions similar to those believed to promote binding in the beating heart (Akera et al., 1976b). These binding studies, though, used sodium concentrations considerably higher than those measured in quiescent and beating heart muscle preparations (4-15 mM) with sodium-sensitive microelectrodes (Ellis, 1977; Cohen et al., 1982; January and Fozzard, 1984). The results of the present experiments which assayed ATP-dependent ouabain binding to Na^+,K^+-ATPase isolated from guinea pig heart showed that at sodium concentrations greater than 5 mM, ouabain

binding was stimulated with increasing sodium concentration. The concentration of sodium to half-maximally stimulate ouabain binding in the absence of K^+ has been reported to be 13-20 mM (Siegel and Josephson, 1972; Lindenmayer and Schwartz, 1973). Sodium stimulation of ouabain binding to Na^+,K^+-ATPase obtained from guinea pig heart does occur at concentrations similar to those measured in cardiac muscle cells. Furthermore, quinidine does not appear to alter the relation between sodium concentration and glycoside binding, as [^{3}H]ouabain binding to guinea pig microsomal Na^+,K^+-ATPase in the presence of 20 mM sodium, a concentration which will half-maximally stimulate glycoside binding, is not influenced by quinidine except at concentrations greater than 100 μM (Figure 15). A decrease in intracellular sodium ion concentration produced by quinidine would then be expected to decrease the rate of glycoside binding to the Na^+,K^+-ATPase as predicted by the working hypothesis.

The fact that quinidine and benzocaine did not change glycoside binding suggests several possible explanations to reconcile the experimental data and the working hypothesis. First, the hypothesis that a quinidine-induced decrease in the rate of sodium influx will decrease the rate of cardiac glycoside binding to the Na^+,K^+-ATPase may be unsubstantiated. As pointed out previously, however, a large body of experimental evidence supports the hypothesis that the rate of cardiac glycoside binding to the Na^+,K^+-ATPase in intact cardiac muscle is dependent on the rate of sodium influx into the heart cell. Alternatively, the lack of an effect of quinidine on digoxin binding even under conditions where the alkaloid decreased the rate of sodium influx may suggest that the stimulation of glycoside binding by sodium cannot

be explained only by postulating that the rate of sodium influx determines the rate of digitalis binding. A reduction of extracellular sodium concentration to 85 mM decreases the rate of onset of the positive inotropic action of digitalis (Aker et al., 1977) and slightly reduces glycoside binding (Temma and Akera, 1983), while lowering extracellular sodium concentration to less than 30 mM abolishes the positive inotropic effects of cardiac glycosides (Linden and Brooker, 1980; Wiggins and Bertolila, 1980; Temma and Akera, 1983). Yet, quinidine and benzocaine do not influence digoxin binding under conditions where they decrease the rate of sodium influx by 25-30%. Reduction of extracellular sodium concentration to 50 mM from 145 mM also reduces $^{86}Rb^{+}$ uptake by 30% in guinea pig left atrial muscle stimulated at 1.5 Hz (Yamamoto et al., 1979). These data point out that although the reduction in the sodium pump activity is similar, the effect of decreased extracellular sodium on glycoside binding is greater than the effect of either quinidine or benzocaine. This implies that the concentration of extracellular, as well as intracellular, sodium may be important in determining the rate cardiac glycoside binding to the sodium pump. Similar schemes have been proposed to explain high and low affinity binding sites for sodium ion stimulation of digitalis binding in squid axon (Baker and Willis, 1972) and isolated Na^{+},K^{+}-ATPase (Inagaki et al., 1974). In brief, extracellular sodium ion may stabilize the conformation of the Na^{+},K^{+}-ATPase, which binds the cardiac glycoside, or stabilize the glycoside-enzyme complex. Thus, when extracellular sodium ion concentration is reduced, the reduction in the stabilizing effects due to reduced extracellular sodium ion concentration and the reduced stimulatory effect of intracellular sodium ions may

act additively. If this were the case, the effect of quinidine or benzocaine, which reduces intracellular sodium only, is anticipated to be smaller. Finally, the difference in the fractional occupancy of the Na^+,K^+-ATPase by digoxin in the presence and absence of quinidine (or benzocaine) may be too small to be distinguished from sample variation. Quinidine and benzocaine decrease the rate of sodium influx by 25-30%, so that the maximal decrease in intracellular sodium ion concentration would be 30%, assuming the rate of sodium extrusion by the sodium pump remains unchanged. However, sodium pump activity decreases as intracellular sodium ion concentration falls (Eisner et al., 1981a,b), so that the actual decrease in intracellular sodium ion concentration is expected to be less than 30%. A concentration of procaine (0.1 mM), which decreases the rate of sodium influx by 30%, reduces intracellular sodium ion concentration less than 10% in quiescent Purkinje fibers (Deitmer and Ellis, 1980a). The sodium-induced stimulation of [^{3}H]-ouabain binding to guinea pig cardiac Na^+,K^+-ATPase suggests that a 10-30% reduction in sodium would reduce glycoside binding by 6-18%. Digoxin binding to the Na^+,K^+-ATPase has a similar sodium dependence as ouabain binding (Fricke and Klaus, 1978), which suggests that a similar reduction in digoxin binding would occur with a 30% reduction in sodium influx rate in atrial muscle preparations. Sample variation was typically 10-15% around the mean in fractional occupancy experiments. Thus, a change in fractional occupancy much larger than that which is expected would be required to discern a significant effect on binding. Therefore, it is possible the decrease in binding produced by quinidine and benzocaine is too small to be seen against sample variation.

Furthermore, this small decrease in binding may not be pharmacologically significant. This may explain why quinidine does not influence the positive inotropic actions of digoxin in the guinea pig left atrial muscle preparations.

In conclusion, the influence of quinidine on the positive inotropic effects of digoxin was examined in isolated left atrial muscle of the guinea pig heart under a variety of experimental conditions. Under none of the experimental conditions examined was the expected interaction between quinidine and digoxin observed. The rate of digoxin binding to the Na^+,K^+-ATPase in the atrial muscle and the resulting positive inotropic effect were unchanged in the presence of quinidine, even in experimental conditions where the quinidine decreased sodium influx rate. These results suggest that in the clinical setting, the rise in serum digoxin concentration in the presence of quinidine will be accompanied by a greater pharmacological effect of the glycoside. These findings indicate that the "target" concentration for digoxin in plasma should not be altered when the combination of digoxin and quinidine is to be administered.

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