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Membrane Adenosinetriphosphatase: A Digitalis Receptor?

The enzyme is a good model for receptor binding studies because of its known functional correlates.

Tai Akera

Summary. The enzyme Na^+,K^+-ATPase is a good model for receptor studies because of its known functional correlates. The binding of digitalis to the enzyme observed in vitro satisfies the criteria for receptor binding. Studies of the relationship between the digitalis binding and the drug action reveal an impressive correlation between these events but fail to provide proof of a causal relationship. Studies with other Na^+,K^+-ATPase inhibitors and agents that affect transmembrane Na^+ movements (steps that would follow Na^+,K^+-ATPase inhibition) provide further supportive evidence that sodium pump inhibition and the resulting enhancement of intracellular Na^+ transients cause the inotropic action of digitalis.

Pharmacological effects of a drug may be regarded as ultimate consequences of physicochemical interactions between that drug and functionally important molecules in the living organism. The molecules with which drugs combine may be called receptors. The pharmacological receptors, then, may be defined as specific, ligand-recognition sites on a macromolecule that also contains, or is biologically coupled to, a response-generating molecule or molecules. Several studies have shown the presence of such receptors for various endogenous and exogenous ligands (*1*).

Since radiolabeled compounds with high specific activities have become available, there have been numerous studies of the saturable binding of drugs to tissue preparations. This saturable binding is generally termed receptor binding, implying that it represents the binding of a drug to a specific macromolecule that has a specific high affinity for the drug and also initiates a response when combined with agonists. The latter event, however, is difficult to demonstrate because the consequences of receptor binding cannot be observed in vitro in most instances. The interaction between digitalis (*2*) and the form of adenosinetriphosphatase which is activated by $Na^+ + K^+$ (Na^+,K^+-ATPase) is an excellent model for drug receptor studies, since the function of the binding macromolecule (the enzyme) is known and the biochemical and physiological consequences of the interaction can be studied in vivo and in vitro.

Saturable Binding in vitro

For saturable binding observed in vitro to be considered as receptor binding several criteria have to be met: (i) the saturable binding sites must be distributed in areas of tissue that contain the site of drug action; (ii) the kinetic parameters of drug-receptor complexes observed in vitro (for instance, reversibility) must parallel those of drug action observed in vivo or in isolated tissues (the reversibility of drug action); and (iii) the specificity and structure-activity relationships must be comparable—that is, the pharmacological potency (an index of affinity) must parallel the affinity of the drug for in vitro binding sites.

The enzyme system Na^+,K^+-ATPase (Fig. 1) was first considered responsible for the coupled, active transport of Na^+ and K^+ across the cytoplasmic membrane (*3*). For this reason, it is often overlooked that this enzyme satisfies all the criteria for receptor binding (*4*). It is distributed in cardiac sarcolemma (*5*), which appears to be the site of the positive inotropic action of digitalis and its derivatives (*6*). It binds ^3H-labeled cardiac glycosides under specific ligand conditions (*7, 8*). The binding is characterized by high affinities, and the glycosides may be displaced by pharmacologically active, but not by inactive, congeners (*7*). A quantitative relationship has been observed between the dissociation rate constants of the drug-enzyme complexes observed in vitro and the rate constants for loss of the drug action in isolated heart preparations (*9*). Thus, Na^+,K^+-ATPase may be regarded as a high-affinity, saturable binding site for digitalis. Whether it represents the receptor for the positive inotropic action of digitalis must be judged from studies of the consequences of the drug binding.

Binding of digitalis to Na^+,K^+-ATPase results in enzyme inhibition (*8, 10*), and the subsequent release of digitalis from the enzyme results in reactivation (*11*). Inhibition of myocardial Na^+,K^+-ATPase has been demonstrated in anesthetized dogs treated with cardiac glycosides (*12*). Such inhibition would, in turn, cause inhibition of active sodium extrusion (sodium pump activity) in myocardial cells (*3, 13*). Thus, many investigators have extensively studied the binding of digitalis to Na^+,K^+-ATPase, inhibition of the sodium pump activity, myocardial sodium concentrations, and the inotropic response in attempts to establish relationships among these events.

The author is a professor of pharmacology at Michigan State University, East Lansing 48824.

Binding in vitro and Drug Action

If the binding of digitalis to Na^+,K^+-ATPase is causally related to the inotropic action of the drug, these two events may share common features. Na^+,K^+-ATPase is an allosteric enzyme, which undergoes a cycle of conformational changes as it hydrolyzes adenosine triphosphate (ATP) and translocates Na^+ and K^+. Cardiac glycosides preferentially bind to the enzyme when it is in a particular conformation (*14*). When the enzyme completes the translocation of Na^+ in the presence of Na^+, Mg^{2+}, and ATP, it assumes the digitalis binding conformation, whereas K^+ induces further conformational change to a form in which the binding sites for cardiac glycosides appear to be less accessible (*11, 15*). Since Na^+,K^+-ATPase is normally affected by intracellular Na^+ and extracellular K^+ as shown in Fig. 1, intracellular Na^+ should stimulate and extracellular K^+ inhibit the binding of digitalis to the enzyme in intact cells.

The development of the positive inotropic action of cardiac glycosides is a slow process, corresponding to a slow binding of digitalis to Na^+,K^+ATPase in vitro (*14*). It is enhanced under conditions that would increase the intracellular Na^+ concentration, such as twin-pulse stimulation (where each electric pulse is followed 100 to 150 milliseconds later by another one, causing two membrane depolarizations for each beat) or the presence of grayanotoxins, which increase the Na^+ influx (*16*), and is delayed under conditions that would decrease intracellular Na^+, such as a low Na^+ concentration in the medium (*16*) or the presence of tetrodotoxin (*17*). High K^+ concentrations in the medium also delay the development of the inotropic action of cardiac glycosides in isolated heart preparations (*18*). A unique feature of the inotropic action of glycosides is that its development appears to be dependent on the number of cardiac contractions, rather than the time of exposure to the drug (*19*). This may be accounted for by the intracellular Na^+ requirement for digitalis binding to the inotropic receptor, since twin-pulse studies have revealed that the development of the inotropic action of glycosides is dependent on the number of membrane depolarizations, rather than the number of contractions (*16*). In cardiac cells, the rate of Na^+ influx is greatly increased by membrane depolarizations (*20*).

In addition to inhibiting the binding of glycosides to Na^+,K^+ATPase, potassium also delays their release from the enzyme in vitro (*11, 15*). Similarly, potassium delays the loss of the inotropic effect of cardiac glycosides in isolated perfused heart during washout with a drug-free solution (*21*). The equilibrium concentration of bound glycosides in vitro is slightly reduced (*22*) and that of bound aglycones is markedly reduced by potassium (*21*), because it affects drug binding more than it does drug release. Similarly, the inotropic effects of glycosides are slightly reduced and those of aglycones are markedly reduced at higher K^+ concentrations in the medium (*21*).

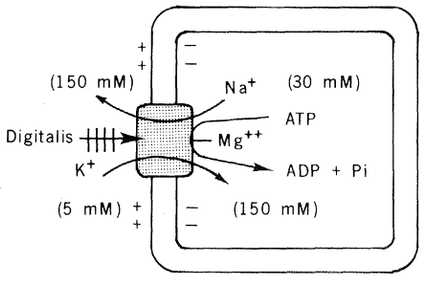


Fig. 1. Schematic model of membrane Na^+,K^+-ATPase. The enzyme is localized in the cell membrane. Adenosine triphosphate binds to the enzyme from the interior of the cell. When Na^+ and Mg^{2+} bind to the ATP-enzyme complex, the terminal phosphate of ATP transfers to the enzyme. This is immediately followed by a conformational change of the phosphoenzyme molecule, translocating the enzyme-bound Na^+ to outside the cell membrane, where it exchanges with K^+. The binding of K^+ to the phosphoenzyme causes a second conformational change, translocating K^+ from outside to inside the cell membrane. After this event, the phosphate bond is hydrolyzed and K^+ is released into the cell, and the enzyme enters another cycle. Digitalis binds to the enzyme from outside the cell membrane, preferentially when the enzyme is assuming the Na^+-induced conformation.

A remarkable feature of the inotropic action of digitalis is the difference in sensitivity to the drug observed between different species. Such species-dependent differences may be explained in part by variations in the rates of metabolism and elimination of the drug or in basic properties of the excitation-contraction coupling mechanism (*23*). However, they are mostly due to differences in the sensitivity of cardiac Na^+,K^+-ATPase to the inhibitory action of digitalis (*12, 24–26*). These differences in digitalis sensitivity have been shown to result from differences in the stability of the digitalis-enzyme complex (*9, 25*). In moderately digitalis-sensitive species, such as guinea pig or rabbit, the half-time for dissociation of the digitalis-enzyme complex in vitro was 10 to 16 minutes at 27°C, whereas in highly digitalis-sensitive species, such as dog or cat, it was 67 to 85 minutes. The half-time for loss of the inotropic response in isolated perfused heart preparations was 7 to 10 minutes in the former and 85 to 100 minutes in the latter species (*9*). Considering the difference in experimental conditions, these results indicate a good correlation between the release of digitalis from Na^+,K^+-ATPase and the loss of the inotropic effect.

In contrast, the association rate constants for the digitalis-enzyme complex in vitro are remarkably similar regardless of the source of the enzyme (*25*). Consistent with this finding, the binding of glycosides to isolated Na^+,K^+-ATPase and the development of positive inotropic effects in isolated perfused heart preparations are similar in different animal species for a particular concentration of digitalis (*9, 26*).

With various digitalis derivatives, a correlation was observed between the potency to inhibit Na^+,K^+-ATPase and to produce an inotropic response (*27*). Different affinities of Na^+,K^+-ATPase for the digitalis derivatives appear to result again from differences in stability of the digitalis-enzyme complexes (*28, 29*), which are consistent with differences in rate constants for the loss of the inotropic effect in isolated heart preparations during washout with a drug-free solution (*29, 30*).

These observations are summarized in Table 1. The digitalis binding to Na^+,K^+-ATPase observed in vitro and the digitalis-induced positive inotropic effect behave in a similar way in every known instance.

Binding in Beating Heart

The evidence outlined above indicates that there is an intimate relation between the saturable binding of digitalis to Na^+,K^+-ATPase observed in vitro and the inotropic response of cardiac tissue to these agents observed in isolated heart preparations. The next step is to demonstrate binding of digitalis to cardiac Na^+,K^+-ATPase in beating hearts and to compare the binding quantitatively with the drug action. Attempts to approach this problem by measuring the uptake of ^3H-labeled drug by cardiac tissue have met with limited success because there is also extensive drug uptake that is unrelated to Na^+,K^+-ATPase or the inotropic action (*31*).

Cardiac Na^+,K^+-ATPase was observed to be inhibited in digitalis-treated dogs (*12*). When plasma K^+ concentrations were increased, a larger dose of

digoxin was required to produce comparable inotropic effects. Despite differences in dose or time of exposure to digoxin, the degree of inhibition of cardiac Na^+,K^+-ATPase was similar when inotropic effects were comparable (*32*). Inhibition of cardiac Na^+,K^+-ATPase by digitalis was also observed in isolated heart studies (*33, 34*). Since it is reasonable to assume that the observed Na^+,K^+-ATPase inhibition is the result of digitalis binding, it appears that digitalis binds to this enzyme at the time of the drug action. Subsequently, Ku *et al.* (*30*) observed that the binding of digitalis to Na^+,K^+-ATPase was parallel to the development of inotropic effects and the release of digitalis was accompanied by loss of these effects in isolated perfused hearts. Ku *et al.* (*30*) further demonstrated that the binding of glycosides to Na^+,K^+-ATPase in beating hearts results in a substantial inhibition of the sodium pump activity. Thus, the binding of digitalis to Na^+,K^+-ATPase and the ensuing sodium pump inhibition appear to be related to the positive inotropic action of the drug.

Functional correlates of binding. At this point, it should be determined whether sodium pump inhibition unequivocally produces the positive inotropic effect. Inhibitors of Na^+,K^+-ATPase such as *N*-ethylmaleimide, *p*-chloromercuribenzoate, ethacrynic acid, fluoride, sanguinarine, and adriamycin (*35, 36*) have been shown to produce positive inotropic effects in isolated hearts (*36, 37*). We have recently demonstrated that the positive inotropic effects of *N*-ethylmaleimide, *p*-chloromercuribenzoate, and *p*-chloromercuribenzenesulfonic acid are related to sodium pump inhibition (*38*).

The *Erythrophleum* alkaloid cassaine and the prednisolone derivative prednisolone-3,20-bis(guanylhydrazone) share many of the pharmacologic actions of digitalis, including positive inotropic action, yet lack the structural characteristics typical of digitalis such as a cyclopentanophenanthrene nucleus with A-B *cis*, B-C *trans*, and C-D *cis* fusion of the four-ring structure, a C-14 hydroxyl group, and an unsaturated lactone ring in the β configuration on C-17 (see Fig. 2). These agents inhibit Na^+,K^+-ATPase in a manner related to its positive inotropic effect (*39*).

Several monovalent cations are also capable of inhibiting Na^+,K^+-ATPase activity in the presence of Na^+ and K^+ (*40*). These cations have the advantage that several of them with differential effects on Na^+,K^+-ATPase are available and thus appropriate controls may be obtained. Among them, Rb^+ and Tl^+ inhibited Na^+,K^+-ATPase activity in the presence of Na^+ and K^+, and produced sustained positive inotropic effects in isolated heart preparations (*26, 41*). Furthermore, these studies indicate that concentrations of Tl^+ and digitalis which are equally effective in inhibiting sodium pump activity appear to cause the same magnitude of increase in cardiac contractility. The inotropic response to Rb^+ developed rapidly, as did Na^+,K^+-ATPase inhibition by this agent. With Rb^+ and Tl^+, the development of the inotropic effect was independent of the number of membrane depolarizations, which is consistent with the lack of sodium dependence of these agents for binding to Na^+,K^+-ATPase. No species difference in Na^+,K^+-ATPase inhibition was observed with Rb^+, or Tl^+, and similarly no species difference in inotropic response to these ions was observed. Other monovalent cations such as K^+, Cs^+, NH_4^+, Na^+, and Li^+ in similar concentrations produced essentially no effect on the Na^+,K^+-ATPase activity or slightly stimulated it in the presence of Na^+ and K^+, and failed to produce sustained positive inotropic effects.

At high concentrations, Li^+ produced

Table 1. Relationship between binding of digitalis to Na^+,K^+-ATPase in vitro and positive inotropic action.

Parameter	Binding in vitro	Inotropic action
Onset rate	Slow Inhibited by K^+ Stimulated by Na^+ Dependent on intracellular Na^+ No species difference	Slow Delayed by K^+ Facilitated by Na^+ Dependent on depolarizations No species difference
Equilibrium	Glycosides: slightly decreased by K^+ Aglycones: markedly decreased by K^+ Marked species difference	Glycosides: slightly decreased by K^+ Aglycones: markedly decreased by K^+ Marked species difference
Offset rate	Dependent on chemical structure Delayed by K^+ Marked species difference	Dependent on chemical structure Delayed by K^+ Marked species difference

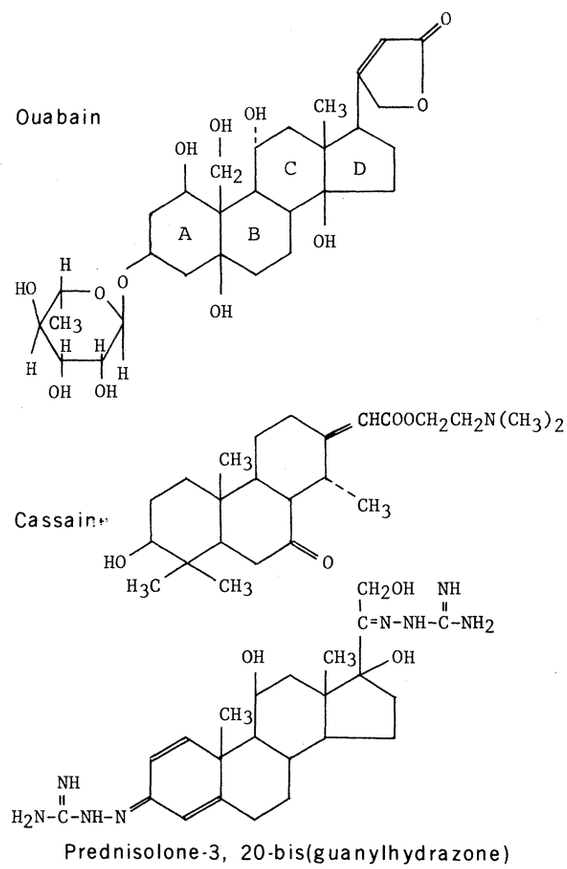


Fig. 2. Chemical structures of ouabain, cassaine, and prednisolone-3,20-bis(guanylhydrazone).

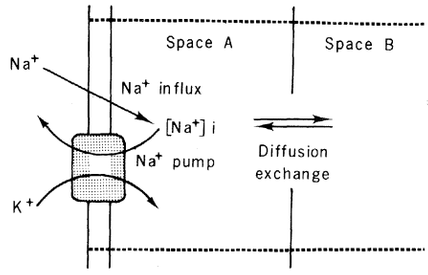


Fig. 3. Model for computer simulation of intracellular Na^+ concentration $[Na^+]_i$, at the inner surface of sarcolemma during a cycle of myocardial function. Changes in $[Na^+]_i$ in space A are calculated as the rate of Na^+ influx minus the rate of Na^+ efflux plus (or minus) the diffusion exchange of Na^+ with space B. The rate of Na^+ influx was adapted from Langer (*20*) (see Fig. 5). The relationship between $[Na^+]_i$ and the sodium pump activity was estimated from the activity of a partially purified dog heart Na^+,K^+-ATPase preparation assayed with various concentrations of sodium and ouabain (*53*). For each molecule of ATP hydrolyzed, three sodium ions are transported (*56*). The rate of diffusion exchange between space A and space B was arbitrarily selected so that half-time for equilibrium is 100 μsec when the volume of space A is 0.1 percent that of space B. Factors that convert transmembrane Na^+ movements to Na^+ concentration in space A were selected so that the average Na^+ concentrations in space A and space B are 30 m*M*. The change in $[Na^+]_i$ with time was calculated at each half-millisecond in the cardiac cycle for a total of 500 msec (corresponding to a heart rate of 120 beats per minute). Results are shown in Fig. 4.

a positive inotropic effect in isolated atrial preparations (*41*). Since Li^+ causes an uncoupling of ATPase and sodium pump activities at these higher concentrations (*42*)—that is, Li^+ stimulates Na^+,K^+-ATPase with a concomitant inhibition of the sodium pump activity—it appears that inhibition of cardiac Na^+,K^+-ATPase per se is not an absolute requirement for a positive inotropic response; instead, it is the activity of the sodium pump that determines the magnitude of increase in cardiac contractility.

Opposing Evidence

Despite the facts that it fulfills the currently used criteria for in vitro receptor binding and that the binding of digitalis to the enzyme observed in vitro has been correlated with the pharmacologic action of the drug, not all scientists are convinced that Na^+,K^+-ATPase is the pharmacologic receptor for digitalis. Several reports have claimed that the binding of digitalis to Na^+,K^+-ATPase or the resulting enzyme inhibition is unrelated to the inotropic action (*33, 43*). In some studies (*43*) the dissociation of these events was apparently caused by an experimental design which neglected the fact that the binding is a slow process that requires ATP in the presence of Na^+ (*44*), or the fact that the reversibility of the digitalis-enzyme complex is highly dependent on the source of the enzyme (*9*). Other investigators (*33*) reported that the enzyme inhibition by digitalis is essentially irreversible, whereas the inotropic action is easily reversible. These results, however, were not confirmed by later studies (*30, 34*): a more detailed discussion of this subject may be found in (*30*).

Long-term treatment of guinea pigs with digitoxin has been reported to cause an adaptive induction of cardiac Na^+,K^+-ATPase, presumably responding to the initial enzyme inhibition (*45*). This finding appears to be inconsistent with the hypothesis that Na^+,K^+-ATPase is the inotropic receptor for digitalis, because the development of neither tolerance nor reverse tolerance is observed during digitalis therapy in humans. Our experiments, however, indicate that cardiac Na^+,K^+-ATPase is not altered during long-term treatment of dogs with either nontoxic or toxic doses of digoxin, which clearly cause enzyme inhibition throughout the treatment (*46*). The reason for this discrepancy is not known.

Cardiac glycosides appear to stimulate Na^+,K^+-ATPase under certain conditions (*6, 47*). The stimulation of the sodium pump activity at low concentrations of digitalis and the inhibition at higher concentrations superficially resemble the positive inotropic action of digitalis at low therapeutic concentrations and the negative inotropic action at higher toxic concentrations. Thus, several investigators speculated that the stimulation of Na^+,K^+-ATPase is related to the positive inotropic action of these agents [for example, see (*6*)]. It may be easier to envision that the stimulation of the enzyme leads to hyperfunction (increased contractility) of the cardiac muscle. Positive inotropic concentrations of digitalis, however, inhibit myocardial Na^+,K^+-ATPase in beating hearts (*12, 29, 30, 32–34*). Thus, we should seek the relation between the enzyme inhibition and the inotropic response.

Is Na^+,K^+-ATPase inhibition related to the inotropic action of digitalis? Most studies of the intracellular distribution of Na^+ and K^+ have indicated that cardiac concentrations of these electrolytes are not altered by digitalis at the inotropic stage (*6*). Even when increases in intracellular Na^+ were observed with inotropic doses of digitalis, the changes were disproportionately small compared to the 30 to 40 percent inhibition of the enzyme activity observed with comparable doses (*12, 30, 32, 34*). If sodium pump inhibition does not have a biochemical consequence, its physiological significance is open to question.

To explain how the binding of digitalis to Na^+,K^+-ATPase causes its inotropic action despite its apparent lack of biochemical consequence (namely, marked myocardial Na^+ accumulation), a mechanism has been proposed in which membrane Na^+,K^+-ATPase transports cardiac glycosides to the intracellular sites of action (if these sites are inside the cell) (*48*). This hypothesis is based on the finding that digitalis stimulates Na^+,K^+-ATPase under certain conditions (when digitalis is transported, it may stimulate the turnover of enzyme) and that the highest concentration of digitalis after perfusion of isolated hearts is found in sarcoplasmic reticulum. This concept is weakened by recent data indicating that the "sarcoplasmic reticulum" preparation used in these studies contained a large amount of sarcolemma (*49*), but is supported by the observation that heart rate and temperature affected the onset of the inotropic action of glycoside, but not aglycone, in isolated rabbit atria (*50*). Since membrane depolarization stimulates Na^+,K^+-ATPase activity through Na^+ influx and temperature affects transport rates, it was suggested that the more polar glycosides are transported across the membrane by an active process, presumably involving Na^+,K^+-ATPase, whereas the lipid-soluble aglycones gain access to intracellular receptors by a passive diffusion mechanism which does not involve Na^+,K^+-ATPase. Whether the binding of digitalis glycosides to Na^+,K^+-ATPase is required for the first step of the transport or for the inhibition of the activity is not known at this time.

Another theory is based on the premise that the digitalis-enzyme binding causes a specific conformational change in the enzyme protein (*51*). According to this hypothesis, the change in protein conformation alters the affinity of calcium for lipids associated with Na^+,K^+-ATPase, and increases the amount of calcium released into the cell, independent of sodium pump inhibition. If this theory is correct, the amount of the digitalis-enzyme complex formed, rather than the sodium pump activity, determines the myocardial contractility. Thus, partial sodium pump inhibition, which may be insignificant for Na^+ movements, may cause a significant change in Ca^{2+} movements and hence an inotropic response.

Intracellular Sodium Transients

Is sodium pump inhibition unrelated to the inotropic action of digitalis? One way to investigate this question is to create ionic conditions similar to those associated with sodium pump inhibition and to observe their consequences. Such conditions may be produced by counterbalancing the sodium pump activity without affecting it directly. Agents such as grayanotoxins, veratrum alkaloids, angiotensin II, batrachotoxin, sodium ionophores, and germitrine and sodium loading of cardiac cells have been shown to produce positive inotropic effects, which are associated with the increase in transmembrane Na^+ influx (*52*). Thus, it appears that alterations in transmembrane Na^+ movements—either enhancement of Na^+ influx or inhibition of Na^+ efflux—cause positive inotropic effects.

The next step, therefore, is to determine the consequence of sodium pump inhibition. Specific questions are why the up to 40 percent inhibition of sodium pump activity observed with positive inotropic doses of digitalis (*12*) fails to cause myocardial sodium accumulation (*6*); and what happens to the intracellular Na^+ concentration, $[Na^+]_i$, when the sodium pump activity is only partially inhibited. It is not possible to measure $[Na^+]_i$ at the inner surface of the sarcolemma with a time resolution sufficient to follow changes in it during each cycle of myocardial function; however, it is possible to simulate it with a computer (Fig. 3) (*53*).

The results of such simulation studies are shown in Fig. 4. The rate of Na^+ influx during membrane excitation is markedly greater than that in the resting state. Thus, the rate of Na^+ influx is higher during the earlier part of each cycle of myocardial function and lower during the later part. This would result in fluctuations of $[Na^+]_i$ at the inner surface of the sarcolemma. Since $[Na^+]_i$ primarily determines sodium pump activity (*54*), the sodium pump may be markedly activated early in each cycle of myocardial function. Simulation studies indicate that the initial rapid Na^+ influx associated with each membrane depolarization results in maximal sodium pump activation. Since the rate of Na^+ influx exceeds sodium pump capacity during the early phase of the action potential plateau, $[Na^+]_i$ increases further and reaches a peak at 50 milliseconds, when the decreasing rate of influx equals the rate of sodium pump capacity. After this time the rate of Na^+ efflux exceeds the rate of influx, and therefore $[Na^+]_i$ decreases and returns to preexcitation levels approximately 350 msec after membrane excitation—the time when the decreasing sodium pump activity equals the low, resting Na^+ influx rate. The sodium pump functions markedly below capacity during the later part of each cycle of myocardial function because of the low $[Na^+]_i$ and thus may be considered to have a reserve capacity.

Inhibition of Na^+,K^+-ATPase by ouabain increases the peak $[Na^+]_i$ and also increases the time during each cycle when the sodium pump operates at a higher rate—that is, a rate close to the inhibited maximal velocity (Fig. 4). This is because the sodium pump does not have any reserve capacity during the early phase in each cycle. With 40 percent inhibition of Na^+,K^+-ATPase, which may be observed with therapeutic doses, the peak $[Na^+]_i$ is approximately

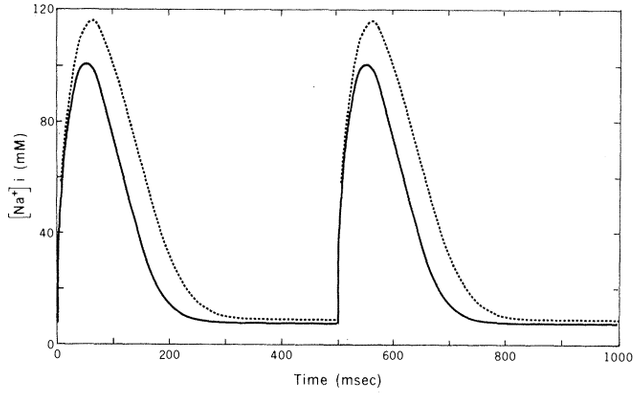
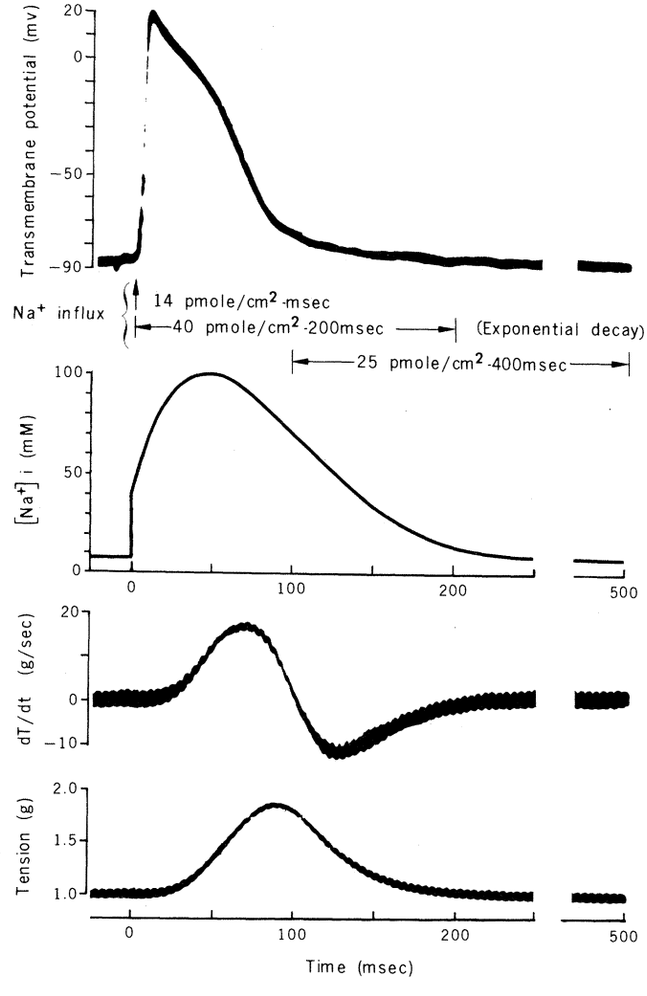


Fig. 4 (left). Computer simulation of $[Na^+]_i$ at the inner suface of sarcolemma during two cycles of cardiac function in the absence and presence of digitalis (see Fig. 3). (Solid line) Changes in $[Na^+]_i$ under the normal condition; (dotted line) changes in $[Na^+]_i$ when sodium pump activity is inhibited 40 percent by the inotropic concentration of ouabain. Fig. 5 (right). Temporal relationship between transmembrane action potential, Na^+ influx, $[Na^+]_i$, and tension development. The rate of Na^+ influx is adapted from Langer (*20*). During the upstroke of the action potential (~1 msec), 14 pmole of Na^+ enter per square centimeter of the sarcolemma. During the subsequent plateau phase, a total of 40 pmole of Na^+ enter per square centimeter, with an average influx rate of 0.2 pmole cm^{-2} $msec^{-1}$. An exponential decay of the Na^+ influx rate over a 200-msec period (half-time for the exponential process = 30 msec) was assumed for the computer simulation study represented in Fig. 4. The resting Na^+ influx is 0.0625 pmole cm^{-2} $msec^{-1}$, or the total of 25 pmole cm^{-2} in the 400-msec period. Values of $[Na^+]_i$ were obtained from the computer simulation study. The quantity *dT/dt* is the first derivative of the developed tension, which is presumably related to the intracellular free Ca^{2+} concentration. Tracings of transmembrane potential, *dT/dt*, and developed tension were supplied by K. Temma. These recordings were obtained with a guinea pig left atrial preparation.



15 percent higher than the control value, but $[Na^+]_i$ approaches preexcitation levels 400 msec after the membrane excitation (Fig. 4). Thus, inotropic doses of digitalis will enlarge the sodium transient (a transient increase in $[Na^+]_i$ at the inner surface of the sarcolemma associated with membrane excitation), but will not cause progressive myocardial sodium accumulation at relatively slow heart rate. Some of the results of the computer simulation were experimentally confirmed (*53*).

If an intracellular sodium transient occurs during each cycle of myocardial function, and the effect of a moderate sodium pump inhibition is to enhance such a transient without causing progressive sodium accumulation, then one can understand why sodium pump inhibition by therapeutic doses of digitalis increases myocardial contractility without sodium accumulation. The concept also explains why the sodium-calcium exchange reaction (Ca^{2+} influx coupled with Na^+ efflux) proposed by several investigators (*55*) operates only during the early phase of each cycle of myocardial function, causing an intermittent Ca^{2+} influx and cycles of myocardial contraction and relaxation. Temporal relationships among these events are shown in Fig. 5. If this is the mechanism by which the membrane excitation causes an increase in intracellular Ca^{2+} concentrations (the so-called excitation-contraction coupling mechanism), then enhanced sodium transients should increase calcium transients and hence myocardial contractility. Such a sequence of events has yet to be experimentally demonstrated.

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