DIGITALIS

Effects of Digitalis on Myocardial Ionic Exchange

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SUMMARY

Prior to consideration of the effects of digitalis compounds on ionic exchange the basic mechanisms of active Na⁺ and K⁺ transport are discussed. It is generally accepted that digitalis is a specific inhibitor of the Na⁺–K⁺ activated ATPase located primarily in the sarcolemmal membrane. Present evidence supports the concept that positive inotropic effects of digitalis are not seen unless evidence of inhibition of the enzyme and, therefore, of the "Na pump" leads to a small increase of intracellular Na⁺. This leads to augmented activity of a Na⁺ transport system which is coupled, not to K⁺, but to Ca⁺⁺. Evidence for the existence of Na⁺–Ca⁺⁺ coupling at excitable membranes is accumulating, and this is reviewed. The possibility that administration of digitalis, through its inhibition of the Na⁺–K⁺ coupled system produces an increase in Na⁺–Ca⁺⁺ coupled transport and thereby an increase of influx of Ca⁺⁺ to the myofilaments is discussed and is presented as a possible basis for the mechanism of digitalis action. The toxic electrophysiologic effects of digitalis are discussed in terms of the effects on K⁺ exchange induced by the drug.

Additional Indexing Words:
Active transport Sodium Potassium Calcium exchange
Sarcolemmal ATPase Cardiac contractility Glycoside toxicity

FORTY YEARS ago Calhoun and Harrison1 studied the effect of administration of a digitalis preparation on the potassium content of dog heart. They remarked, "At present time we are not in a position to understand the significance of the fact that even in therapeutic doses there seems to be a slight tendency for digitalis to diminish the potassium content of the heart muscle. The close relation of calcium to digitalis action is well known . . . and it is conceivable that digitalis may act in such a way as to readjust the ionic balance, even at the expense of loss of base from the muscle . . ." The suggestion that digitalis might alter ionic balance was made a quarter of a century before the first definitions of active sodium transport2 and the first outlines of the excitation-contraction coupling sequence in muscle were developed. As more has been learned of the complexities of ionic transport and exchange and their relationship to myocardial tension development over the past decade, Calhoun's and Harrison's concept has gained support. The information which forms the foundation of present ideas of digitalis action comes from the application of diverse technics using a variety of experimental preparations. It is necessary that this information be reviewed in order to place present concepts of digitalis action in proper perspective.
The "Sodium Pump"

The first support of the presence of an energy-liberating system located in the membrane of cells stems from the studies of Skou\(^2\) in crab nerve membrane. He discovered the presence of an enzyme associated with this membrane which was capable of hydrolyzing adenosine triphosphate (ATP). The characteristics of adenosine triphosphatase (ATPase) were defined over the next few years.\(^4\) It requires the presence of \(Mg^{++}\) and both \(Na^+\) and \(K^+\) in the medium for full activation. Steinbach\(^5\) had previously demonstrated that \(Na^+\) was actively extruded from frog skeletal muscle, and Hoffman\(^6\) had demonstrated that ATP was the source of energy for \(Na^+\) pumping in the red blood cell. Therefore the concept was formed that \(Na^+\) was actively transported from inside to outside the cell and that the energy for this transport was supplied by the hydrolysis of ATP regulated by membrane ATPase. A membrane ATPase which demonstrated the above characteristics was then identified in cardiac muscle.\(^7\),\(^8\)

Work, again in the red blood cell,\(^9\),\(^10\) disclosed some further characteristics of the ATPase with respect to the importance of the distribution of the physiologic cations. The enzyme-containing membrane was found to be asymmetric with respect to its response to \(Na^+\) and \(K^+\). The \(K^+\) ion stimulated the ATPase only at the outer surface of the membrane whereas \(Na^+\) was effective only at the inner surface. This asymmetry appears to obtain in other tissues as well, including the myocardium.\(^11\) It is this characteristic which constitutes the basis for the feedback control of the \(Na^+\) pump and is requisite to the understanding of the mechanism of action of the digitalis glycosides.

In cardiac muscle, electrical excitation of the cell precedes and triggers contraction. The development of a regenerative excitation depends upon an abrupt and marked increase in \(Na^+\) permeability. This accounts for cellular depolarization and is accompanied by a movement of \(Na^+\) into the cell. This movement is a passive one down both an electrical and chemical gradient. (The potential across the resting membrane, during diastole, is 80–90 mv with the inside of the cell negative. The concentration of \(Na^+\) outside is approximately 150 mmole and inside the cell approximately 30mmole.) With each excitation approximately 50 \(\mu\)moles of Na/kg of tissue is calculated to move into the cell.\(^12\) This results, during the course of a single excitation, in an increment of about 0.3% in intracellular \(Na^+\) content. If the \(Na^+\) accumulated during excitation were not removed from the cell, intracellular \(Na^+\) would double over the course of slightly more than 300 beats.

\(Na^+\) is not the only cation of importance in excitation of the cell. Following the period of increased \(Na^+\) permeability which leads to depolarization and influx of \(Na^+\), the \(Na^+\) permeability declines and, after a period of 150–250 msec, the cell begins to repolarize rapidly. This repolarization is based upon an increase in \(K^+\) permeability which returns the cell to its resting (diastolic) potential. Associated with this period, there is a movement of \(K^+\) out of the cell. Therefore at the end of the action potential, the cell has accumulated \(Na^+\) and lost \(K^+\). If the cellular content is to remain in a steady state, the "Na pump" must remove \(Na^+\) and restore \(K^+\). The pump is, then, actually a \(Na^+ - K^+\) pump and is best visualized as a molecular "carrier" which actively transports \(Na^+\) outward and \(K^+\) inward; i.e., \(Na^+\) and \(K^+\) transport are coupled.

Increased requirements of the pump are produced by a gain of intracellular \(Na^+\) and a loss of intracellular \(K^+\). The latter may result in a transient increase in \(K^+\) concentration at the outside of the membrane.\(^12\) These are exactly the conditions which were indicated to stimulate the membrane ATPase and, thereby, stimulate the activity of the pump. Therefore a condition which produces an elevation of intracellular \(Na^+\) and/or elevation of external \(K^+\) will result in a stimulation of pump activity. The asymmetry of the membrane ATPase is, in part, the basis of the ability of the pump to respond to fluctuating demands. For example, an increase in heart rate produces an increase in the rate of \(Na^+\) influx because there are an increased number of
membrane depolarizations. In order that intracellular Na and K achieve a new steady state, the activity of the pump must be augmented. As influx is increased there is evidence that internal Na\(^{+}\) increases for a short period of time and then reaches a steady state at a slightly higher level if the increased heart rate is sustained.\(^{13}\) Intracellular K\(^{+}\) declines and stabilizes. Given the properties of the membrane ATPase it is reasonable to conclude that the increment in internal Na\(^{+}\) plays a significant role in the feedback stimulus which results in the augmentation of Na\(^{+}\)-pump activity and thereby the reestablishment of the new steady-state levels of cellular Na\(^{+}\) and K\(^{+}\) in the face of a sustained augmentation of Na\(^{+}\) influx.

**Effect of Digitalis on the Pump**

Schätzmann\(^{14}\) produced the first clear demonstration that digitalis acts directly on the ion-transport system discussed above. Since that time a great deal of evidence\(^{15}\) supports the view that digitalis compounds are specific in their inhibition of the Na–K activated membrane ATPase of various tissues including heart. Radioactively labeled digitalis compounds are found to bind specifically to the enzyme,\(^{16}\) resulting in a stable complex unable to function in the transport of Na\(^{+}\) and K\(^{+}\). It would seem that the glycosides do not compete for the K\(^{+}\) or Na\(^{+}\) sites on the enzyme but bind to an allosteric site which then affects the conformation of the enzyme leading to inhibition of its transport function.\(^{17}\)

There is little doubt that glycosides are specific for the membrane ATPase and in binding to it inhibit its activity to a certain degree. It would be expected that administration of digitalis would result in an accumulation of Na\(^{+}\) and a loss of K\(^{+}\) from the heart muscle. Such has not been consistently demonstrated with doses of glycoside which clearly produce a positive inotropic response. If this is the case, i.e. contractile force increases without evidence of inhibition of Na–K transport, then the specific effect of the drug on the membrane ATPase cannot be related to its therapeutic action. It would then be necessary to define some other, as yet unknown, action of the drug and relate this action to the functional response of the myocardium.

**Effect of Digitalis on Myocardial Na and K Exchange**

A number of studies during the early 1960’s\(^{18–20}\) indicated that a positive inotropic effect could be obtained upon administration of the glycosides without evidence for an effect upon Na\(^{+}\)–K\(^{+}\) exchange. These studies were primarily directed at evaluation of cellular K\(^{+}\) exchange. The early studies found evidence for glycoside-induced net loss of K\(^{+}\) (indicative of Na\(^{+}\)-pump inhibition) only with toxic doses of the drug well beyond the point of maximum inotropic effect. The problem, however, with most of these studies was their inability to discern relatively small (total net losses of K\(^{+}\) less than 1 mmole/kg wet tissue) changes in K\(^{+}\) flux. The possibility remained that alterations in pump activity were occurring prior to the onset of toxic effects of the glycosides but were not discernible with the technics used. In 1965 Müller\(^{21}\) worked with sheep and calf myocardial bundles which were labeled for 4–5 hours with \(^{42}\)K in order to achieve complete labeling of the total K\(^{+}\) content of the tissue. He then administered both therapeutic and toxic doses of ouabain. Again, the net K\(^{+}\) losses were quite high but, unlike the previous studies, he could not demonstrate an increase in contractile tension unless some K\(^{+}\) loss was demonstrable. We recently studied the effect of glycosides on Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) exchange in our laboratory using the isolated, arterially perfused rabbit interventricular septum.\(^{22}\) With this technic it is possible to detect net changes in tissue K\(^{+}\) amounting to as little as 0.2 mmoles/kg. The results of this study clearly indicated that no inotropism could be elicited by digitalis unless evidence of net K\(^{+}\) loss was detected. Significant (20–30%) increases in dP/dt occurred coincident with net K\(^{+}\) losses in the range of only 0.5 mmoles/kg. Maximum inotropic effects (prior to the onset of irritability and progressive increase in diastolic tension) were achieved when total K\(^{+}\) loss was approximately 1.5 mmoles/kg.
These values indicate that the net K\(^+\) losses coincident with nontoxic, positively inotropic responses to the glycosides are small, amounting to less than 2% of the total cellular K\(^+\) content. It should be emphasized, however, that though the effects on K\(^+\) flux are small, some effect is detectable if a positive inotropic response is elicited. We would, therefore, agree with Glynn: 23 "Because the heart is high in potassium, slight inhibition of the pump will cause only minimal changes in intracellular potassium concentration, and this limitation may account for the failure of certain other investigators to detect any change in potassium content in hearts showing a positive inotropic response."

Most investigators have concentrated on the examination of the effects of glycoside on K\(^+\) flux rather than Na\(^+\) flux. This is because, though detection of small changes in K\(^+\) flux are difficult, detection of similar changes in Na\(^+\) flux are essentially impossible in whole myocardium unless special technics are applied. This is due to the high extracellular Na\(^+\) content which obscures small changes attributable to the cell. Nevertheless, it has been possible to demonstrate directly, in the perfused rabbit septum, that glycoside inhibits cellular Na\(^+\) efflux and, thereby, produces an increase in internal sodium. 22 The fact that net loss of K\(^+\) occurs is an almost certain indication that a similar gain in Na\(^+\) is produced, and now this has been directly corroborated.

The evidence supports the statement that coincident with onset of the inotropic action of digitalis compounds there is an inhibition of the activity of Na-K activated membrane ATPase leading to a small accumulation of cellular Na\(^+\) and a small loss of cellular K\(^+\). At this point in our discussion it is pertinent to ask how these effects might be related to the augmentation of contractile tension in the heart. We can immediately discard one possibility—namely that the decrease in cellular K\(^+\) or increase in cellular Na\(^+\) has a direct effect on the contractile proteins. The studies of Hajdu in 1953 24 on the ionic shifts associated with abrupt increases in frequency of contraction (Bowditch staircase) raised the possibility that a loss of cellular K\(^+\) had a direct effect on the interaction of contractile proteins and that this, in some way, produced an increment in tension. Recent studies on the ionic sensitivity of the contractile proteins 25 indicate that a direct effect of alterations in intracellular potassium or sodium on the contractile proteins is very unlikely. It should also be stated, in passing, that there is no convincing evidence of a direct effect of the glycosides themselves on contractile protein interaction. 25 It therefore becomes necessary to examine other possibilities.

**Importance of Calcium**

Although there has been some difference of opinion on the role played by Na\(^+\) and K\(^+\) in digitalis action, it is almost unanimous among investigators that Ca\(^{2+}\) is critically involved in the mechanism of the myocardial response to glycosides. It is generally agreed that doses of digitalis which produce increases in diastolic tension (contracture) are associated with a net uptake of Ca\(^{2+}\) by the cell. 26 At glycoside levels which produce positive inotropism without contracture it has been possible to demonstrate clearly that the exchangeability or "turnover" of Ca\(^{2+}\) is increased, but it is more difficult to validate a net gain of Ca\(^{2+}\) by the cell. 27, 28 With sensitive technics based on the ability to follow \(^{40}\)Ca uptake sequentially before and after glycoside administration, small net gains (approximately 3% increments in total tissue Ca\(^{2+}\)) are demonstrable in association with inotropic responses prior to onset of contracture. 22 Whether or not a net gain is demonstrable is not crucial to support the statement that therapeutic doses of glycoside augment Ca\(^{2+}\) influx. The fact that increased "turnover" occurs means that influx per unit time increases. If the increased influx results in no net gain it simply means that efflux increases proportionately; i.e., steady-state exchangeability or "turnover" is increased.

The Ca\(^{2+}\) ion does, of course, play the central role in the excitation-contraction coupling process in muscle. 12, 25, 29 Onset of con-
traction is dependent upon the release of Ca\(^{++}\) from storage sites to the region of the myofilaments. The free Ca\(^{++}\) concentration must exceed approximately 10\(^{-7}\)M in order that active tension start to develop. Above this concentration, Ca\(^{++}\) is believed to attach in sufficient quantity to inhibit the action of a protein called troponin. At Ca\(^{++}\) concentrations below 10\(^{-7}\)M, troponin (complexed with tropomyosin) inhibits the interaction of actin and myosin. As the Ca\(^{++}\) concentration increases, the "blocking" action of troponin is inhibited, tension-generating bridges form between actin and myosin, and active tension development ensues. Complete inhibition of troponin-tropomyosin is obtained with free intracellular Ca\(^{++}\) concentrations of about 5 \times 10\(^{-4}\)M, and it is at this concentration that full activation of actin-myosin interaction is achieved. The total number of bridges formed determines how rapidly the tension develops, i.e. dP/dt.\(^{30}\) In order to achieve full contractile unit activation (~5 \times 10^{-6}M Ca\(^{++}\)) from the fully relaxed state (<10^{-7}M Ca\(^{++}\)), approximately 50 \(\mu\)moles Ca\(^{++}\)/kg myocardium has to be released upon excitation. This amounts to about 1.5% of the total Ca\(^{++}\) content of the tissue which must be mobilized in a "free state" in order to achieve full contractile activation with each beat. It should be noted that the release of a relatively small amount of additional Ca\(^{++}\) will result in a significant increase in tension and dP/dt. The addition of 10 \(\mu\)moles Ca\(^{++}\)/kg/beat delivered to the troponin sites in a heart previously functioning with 20 \(\mu\)moles/kg would induce about a 50% increase in force development. Evidence now indicates that the "final pathway" of digitalis action is through the augmentation of that fraction of tissue Ca\(^{++}\) involved in the coupling of excitation to contraction.

The source of the "coupling Ca\(^{++}\)" in mammalian heart remains incompletely defined. As indicated, the actual amount involved in a single heart beat represents only a small fraction (1.5% or less) of the Ca\(^{++}\) present in the tissue at any one time. The immediate origin of the "coupling Ca" in skeletal muscle has been localized\(^{31}\) to the lateral elements of an intracellular tubular system called the sarcoplasmic reticulum. At present, evidence is accumulating that the "coupling Ca" in mammalian heart is much more superficially located, i.e. on or near the sarcolemmal (including "T" tubule) surface.\(^{32-34}\) This suggests that the action of digitalis results in the augmentation of a Ca\(^{++}\) store near the cellular surface of cardiac cells thereby resulting in increased influx upon excitation and the positive inotropism (increased tension and maximum dP/dt) typical of the glycosides. This suggestion is supported by the evidence\(^{35}\) which indicates a lack of effect of ouabain on the calcium accumulation of deeper membranous structures of the cell—the sarcotubules and mitochondria.

**Relationship of Ionic Movements to Digitalis Action**

It remains now to relate, if possible, the inhibition of the Na pump to the augmentation of Ca\(^{++}\) influx. The increment in Ca\(^{++}\) influx is associated with a gain in cellular Na\(^{+}\). Repke\(^{36}\) was among the first to suggest the possibility that an increment in cellular Na\(^{+}\) might result in an increase in Ca\(^{++}\) influx. Our laboratory has also proposed that accumulation of Na\(^{+}\) on the inside of the membrane results in augmented Ca\(^{++}\) influx, and that this is the fundamental ionic mechanism responsible for both the Bowditch staircase phenomenon\(^{37-39}\) and the action of the digitalis glycosides.\(^{22}\) Evidence for such a relationship between Na\(^{+}\) and Ca\(^{++}\) was indirect until the recent studies by Baker et al.\(^{40}\) The experiments were performed using the perfused squid axon. This study used a "tube of membrane" prepared by squeezing the axoplasm from the nerve. This permits perfusion of not only the outside of the membrane but also the inside with solution of any desired content. It was, therefore, possible specifically to increase the Na\(^{+}\) concentration on the inside and observe the effect on Ca\(^{++}\) influx. The influx was found to be strikingly increased. The squid axon, of course, far removed from the mammalian myocardial cell but it is not unreasonable to expect that its

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basic responses may be representative of most excitable membranes.

The information presently available indicates that digitalis may affect ionic exchange and contractile tension as illustrated in figure 1. The critical concept of the model is the relation between Na⁺ and Ca²⁺ exchange. It is proposed that an element of Na⁺ efflux is linked not only to K⁺ influx through the Na–K activated membrane ATPase but, as proposed for the squid axon,⁴⁰ linked through another system to Ca²⁺ influx. Glycoside exerts its primary effect by the inhibition of Na-K exchange. This results in a small increment in Na⁺ at the inside of the membrane. This accumulation is proposed to stimulate the Na⁺–Ca²⁺ exchange, so that Na⁺ efflux and Ca²⁺ influx are augmented via the Na⁺–Ca²⁺ coupled route. Therefore, a portion of Na⁺ efflux from the augmented cellular Na⁺ pool is now coupled to Ca²⁺ influx, resulting in an increase in the quantity of Ca²⁺ delivered to the myofilaments and enhancement of contractility.

Toxic Effects of Digitalis

A consideration of digitalis toxicity from the point of view of ionic movements focuses on

![Diagram](https://example.com/diagram.png)

**Figure 1**

Proposed effect of digitalis upon ionic movements. (A) Prior to digitalis. Na⁺ at position 1 is transported from the cell via two routes. The cycle to the left involves a carrier coupled to K⁺ and the cycle to the right involves a carrier coupled to Ca²⁺. The flux of the Na⁺–K⁺ cycle is proportional to the activity of Na⁺–K⁺ activated ATPase and plays a major role in the Na⁺ transport (indicated by thickness of the arrows). A portion of the Ca²⁺ involved in the Na⁺–Ca²⁺ cycle influxes to the myofilaments and therefore affects tension. (B) After digitalis. Digitalis partially blocks the Na⁺–K⁺ cycle through its inhibition of Na⁺–K⁺ activated ATPase. This results in a small increase in Na⁺ at position 1 and a shift of a portion of outward Na⁺ transport to the Na⁺–Ca²⁺ cycle. Note increased thickness of the Na⁺–Ca²⁺ cycle arrows as compared to (A). This results in an increase of the influx of Ca⁺⁺ to the myofilaments and increased contractile tension.
the K+ ion. The discussion to this point has emphasized that K+ probably does not play a primary role in the movements of Ca++ leading to the therapeutic inotropic action of the glycosides. It is well recognized, however, that K+ shifts have relatively great effects on the electrophysiologic aspects of cellular function.41 If the therapeutic dose of digitalis is exceeded, K+ continues to be lost from the cells,22 and this loss will become manifest in the form of electrical effects. As K+ loss becomes excessive the level of the membrane resting potential becomes less, i.e., less negative, and the cells approach threshold for excitation. During the initial stages of this process the cell will become more irritable and/or automatic as the potential change necessary to achieve threshold becomes less. This situation gives rise to the appearance of ectopic arrhythmias—one of the manifestations of digitalis toxicity. As more potassium is lost from the cell, the membrane potential falls even closer to threshold, and this produces a somewhat paradoxical electrophysiologic effect. Though closer to threshold, it becomes much more difficult to produce a regenerative excitation of the cell. This is attributable to the fact that the Na+ current responsible for the spike is proportional to the magnitude of the resting potential. Therefore, as K+ loss continues, the membrane resting potential comes closer to threshold, sufficient Na+ current cannot be developed for a regenerative depolarization, and block occurs. Thus, as K+ loss progresses, the cells pass through a period of irritability and/or increased automaticity into a period of inexcitability or from a period of ectopic arrhythmia to the appearance of block.

In addition, the effect of glycoside is to shorten the action potential, largely during the segment which accounts for the effective refractory period. This allows the cell to respond to a second stimulus after a shorter interval of time. The combination of block and shortened refractory period sets the stage for reentry and the appearance of fibrillatory episodes.42

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