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COMMENTARY

MECHANISM OF ACTION OF THE CARDIAC GLYCOSIDES ON THE HEART*

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We have now arrived at the 200th anniversary of the "modern age" of the use of digitalis as a therapeutic agent. William Withering's descriptions in the 1780s of his applications of the foxglove for the treatment of "dropsy" are classic. One might think that the scientific basis for the positive inotropic effect of the cardiac glycosides would have long since been established. Not so. It is only now, in the 1980s, that some consensus is forming with respect to the mechanism of action of the cardiac glycosides. The reason for this long latency between empirical application and scientific understanding is obvious. It is now clear that the action of the drug is mediated through events at the sarcolemmal membrane of the myocardial cell. The membrane is extremely complex and it is only within the recent past that insights have been gained into its structure and function. Before the drug's site of action, i.e. the sarcolemma, was better defined, it was impossible to gain a clear concept of the sequence of events precipitated by the drug's application.

Therefore, before I proceed to comment directly upon glycoside action, I will very briefly discuss some important pertinent developments in our knowledge of the myocardial sarcolemma and its superficial coating, the glycocalyx.

The sarcolemmal–glycocalyx complex

A component of the sarcolemma is the lipid "unit membrane" or bilayer. This bilayer contains phospholipid with hydrophobic fatty acid chains directed inward from the outer and inner surfaces. Hydrophilic polar heads of the phospholipids, sugars and amino acids extend into the aqueous phase at both inner and outer surfaces. Embedded in the lipid bilayer are the so-called integral proteins and these are an important feature of the currently well-accepted "fluid mosaic model" of the membrane [1]. These proteins are amphipathic (having both hydrophilic and hydrophobic segments). Some of these proteins extend through the entire bilayer from outer to inner surface, others extend only part way from the outer surface. It is believed that as these proteins "float" in the lipid bilayer many of them abut on one another forming triads or tetrads of higher order combinations. As the proteins come into apposition, it is proposed that they form a central, open core or hydrophilic channel of a few angstroms in diameter. It is these channels, formed of proteins which extend through the bilayer, which are believed to be selectively conductive for the various ions [e.g. sodium (Na^+), potassium (K^+) and calcium (Ca^{2+})] which cross the membrane. Passage of ions through the channels is controlled by a "gating" process which opens or closes the channels. The "gate" can be controlled by the transmembrane potential—opening or closing dependent upon the level of membrane polarization. It also seems to be controlled by the level of phosphorylation of the integral proteins making up the channels [2]. It is through these channels that ions are conducted as charged species.

In addition to the channels described above, the membrane contains other systems involved in moving ions and these are termed pumps or carriers. In contrast to the channels in which charged ions flow dependent upon potential and concentration gradients, the pumps and carriers move ions "uphill" against concentration gradients with the expenditure of energy. The classic pump is the Na^+–K^+ pump which moves $3Na^+$ out of the cell in exchange for $2K^+$ inward and is controlled by the Na^+–K^+ ATPase. This system is, of course, critically important in the action of cardiac glycosides.

Another carrier system of importance in discussion of the glycosides is a more recently defined system—the Na^+–Ca^{2+} carrier or exchanger. In 1964, Repke [3], in a consideration of the mechanism of action of digitalis, and Langer [4], in a consideration of the rate-staircase response, raised the possibility that intracellular accumulation of Na^+ might be linked to augmented Ca^{2+} influx. Then in 1968, Reuter and Seitz [5] found evidence for a membrane carrier system in the heart which involved Na^+ and Ca^{2+}, and they demonstrated that the Ca^{2+} efflux rate was sensitive to the concentration gradient for Na^+ across the sarcolemma. A year later, Baker *et al.* [6] demonstrated (in perfused squid axon) that, in addition to inward Na^+ movement coupled to outward Ca^{2+} movement, exchange also occurred in the opposite direction, i.e. elevation of internal Na^+ increased Ca^{2+} influx. There is now clear evidence for the existence of a Na^+–Ca^{2+} carrier in intact cardiac tissue [7–10] and in fractionated sarcolemmal vesicles from the heart [11–13]. There is a great deal of evidence which indicates that the Na^+–Ca^{2+} carrier plays a significant role in the response of the heart to cardiac glycosides (see below).

The myocardial cellular surface includes more than the sarcolemmal component of the lipid bilayer. External to the bilayer is the *glycocalyx* which has

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two components: an inner *surface coat* and an outer *external lamina*. The surface coat represents an integral part of the cell membrane since glycoprotein chains extend outward from the unit membrane to contribute to the coat [14]. The external lamina, located superficial to the surface coat, can be separated from the surface coat by perfusion of the tissue with solution which contains no Ca^{2+} [15]. The glycocalyx consists of glycoproteins, glycolipids and mucopolysaccharides. Many of the constituents are acidic in nature and are, therefore, negatively charged at physiological pH levels. The glycocalyx invaginates with the bilayer to fill the transverse tubules of the cell. Its specific role is not yet defined, but initial studies indicate that it may be of importance in the control of Ca^{2+} permeability and may contribute to cationic binding at the cellular surface [16]. Its role in the control of cationic flux, especially Ca^{2+}, should not be ignored. Since Ca^{2+} flux is central to the action of the cardiac glycosides, the glycocalyx may play a role.

Primary action of the cardiac glycosides

The relationship of the glycosides to sarcolemmal Na^+–K^+ ATPase has been comprehensively reviewed by Schwartz *et al.* [17]. There is no doubt that low concentrations of the drug inhibit the enzyme. The interaction with the enzyme is at the external surface of the sarcolemma and the Na^+–K^+ ATPase is closely associated with the drug's receptor since the most highly purified enzyme (only two major polypeptides) retains sensitivity to the drug. A majority of studies [18–22] support the concept that inhibition of the enzyme is basic to the inotropic action of the glycosides in contrast to a few studies which claim dissociation of enzyme inhibition and positive inotropy [23]. The possible reasons for this dissociation are discussed by Schwartz *et al.* [17] and one comes to the conclusion that when Na^+–K^+ ATPase inhibition can be demonstrated a positive inotropic response is the result.

There remains, however, another series of studies which raises doubts concerning the concept that inhibition of the Na^+–K^+ ATPase is a necessary condition for the production of increased myocardial force development. These studies have been extensively reviewed recently by Noble [24]. Godfraind's laboratory [25, 26] has shown evidence that the Na^+–K^+ pump is *stimulated* by low doses (10^{-9}–10^{-8} M) of glycoside and that there is, at these low doses, positive inotropy. Noble [24] reports that increased force of contraction occurs in Purkinje fibers with low-dose glycoside when there is no reduced K^+ gradient and even an increased K^+ gradient (as derived from reversal potential measurements). This is consistent with no change or stimulation of Na^+–K^+ pump activity coincident with augmentation of force. The stimulatory action of the glycosides is most evident at higher levels of $[K^+]_0$ and is more difficult to demonstrate if $[K^+]_0$ is in the physiological range (3–4 mM).

On initial appraisal it would seem difficult to reconcile the demonstration of increased Na^+–K^+ pump activity with the proposal that pump inhibition is basic to the production of positive inotropy. An analysis of the relationship between the glycosides and myocardial catecholamines seems, however, to provide a reasonable explanation. A number of studies in the mid-1960s [27–29] demonstrated that reserpinization of heart muscle to reduce the level of endogenous catecholamine depressed the inotropic action of the glycosides. In addition, it has been shown that the positive inotropic effect of ouabain is markedly reduced by β-adrenoceptor blockade of guinea pig atria [30]. It is also clear that ouabain diminishes noradrenaline content of heart muscle [31] probably by inhibition of uptake of the catechol in sympathetic nerve terminals by interacting with Na^+–K^+ ATPase of the neuronal membrane [32]. Inhibition of uptake would be expected to augment free catechol concentration at the cellular surface.

With these results in mind, the recent study of Smith and Hougen [33] reconciles the stimulatory effect of low-dose glycoside on Na^+–K^+ ATPase and the inhibitory effect of higher dose—both associated with positive inotropy. They found, in guinea pig left atria and in agreement with other studies, that 10^{-8} M isoproterenol stimulated the Na^+–K^+ pump as monitored by $^{86}Rb^+$ uptake. Ouabain (3×10^{-9} M) also caused a significant *increase* in pump activity, but at concentrations greater than 10^{-8} M the pump was consistently inhibited. β-Blockade (10^{-6} M propranolol) blocked both the stimulatory effects of low-dose ouabain and isoproterenol. If the atria were depleted of endogenous catecholamine by administration of reserpine or 6-hydroxydopamine, then 3×10^{-9} M ouabain produced only a decrease in pump activity. These results strongly indicate that low-dose glycoside causes a release of catecholamine and/or a reduction in its reuptake. This increased level of catechol augments Na^+–K^+ pump activity which obscures a smaller, direct inhibitory effect of glycoside. The increased level of catechol would be expected to produce a positive inotropic response. Since KCl administration also causes a release of endogenous catecholamines [34, 35], it is not surprising that elevated levels of $[K^+]_0$ augment the effect of low-dose glycoside in the production of increased catechol levels.

In summary, then, there is no doubt that the primary action of the glycosides is to inhibit the Na^+–K^+ pump. In addition, however, low-dose glycoside is capable of producing an increase in free catecholamine levels in the myocardium. The latter stimulates the Na^+–K^+ pump while producing the well-known catecholamine-induced inotropy and is the probable explanation for the inotropy associated with pump stimulation at low-dose levels of glycoside.

Model for response to therapeutic doses of glycoside

Administration of a therapeutic dose (absence of arrhythmia and contracture) of glycoside results in an increased concentration of Ca^{2+} at the force-producing myofilaments during the course of active tension development. On the basis of the studies reviewed above, it is likely that glycoside affects Ca^{2+} movements across the sarcolemma by affecting both the "channel" and "carrier" systems.

Low-dose glycoside increases the level of catecholamine at the sarcolemmal surface. This increases cyclic AMP through activation of adenylate

cyclase. The increased cyclic AMP activates a protein kinase leading to increased phosphorylation of the channel-forming proteins and increased conductance of Ca^{2+} [36]. The increased Ca^{2+} conductance would lead to the augmented force development associated with initial action or low-dose of glycoside. This formulation is consistent with the recent study of Weingart *et al.* [37] which shows an increased slow inward (Ca^{2+}) current which developed during the early inotropic effect of 10^{-6} M strophanthidin administered to calf heart Purkinje fibers.

At higher doses of glycoside the other sarcolemmal system comes into play. This is the Na^+–Ca^{2+} carrier. As we indicated in 1970 [38], administration of higher doses of glycoside inhibits the Na^+–K^+ pump, leading to an increase of Na^+ within the cell. As reviewed previously, this increase further activates Na^+–Ca^{2+} exchange. The 1970 study showed that even high doses of glycoside did not affect the rate of Ca^{2+} efflux. Since there was a net increase of Ca^{2+} produced, it is evident that Ca^{2+} influx was augmented. This indicates stimulation of movement of Na^+ outward and Ca^{2+} inward via the carrier. Therefore, the primary action of inhibition of the Na^+–K^+ pump leads to stimulation of the Na^+–Ca^{2+} carrier, increase of Ca^{2+} influx, and further positive inotropy. The details of this formulation have been presented previously [39].

In addition to glycoside-induced increase in cellular Na^+, there is a loss of cellular K^+ attributable to depressed active influx and enhanced passive efflux [38]. It has been proposed [40] that the increased Ca^{2+} influx is coupled to the K^+ efflux rather than to Na^+ via the Na^+–Ca^{2+} exchanger. The K^+–Ca^{2+} coupling has been shown to be very unlikely, however. Using respiratory acidosis (30% CO_2, pH = 6.65) to virtually eliminate the passive K^+ leak induced by glycoside, Poole-Wilson and Langer [41] found that significant inotropy still occurred. The glycoside was still active in depressing K^+ influx which indicated that inhibition of the Na^+–K^+ pump was, indeed, produced and likely to be correlated with the increased force of contraction through augmentation of intracellular Na^+ leading to enhanced Na^+–Ca^{2+} exchange (see above).

Locus of glycoside-induced Ca^{2+} *pool*

Nayler [42] administered ouabain in doses from 10^{-8} to 10^{-7} M to dog trabecular muscle. The dose range used produced positive inotropy without contracture. There was an increment in Ca^{2+} content of the tissue which was largely displaceable by the lanthanum ion (La^{3+}). It has been well documented that La^{3+} is a potent competitor with Ca^{2+} for binding sites at the cell surface and does not enter the intact myocardial cell. The demonstration that glycoside seemed to increase Ca^{2+} at superficial sites within the sarcolemmal–glycocalyx complex was surprising but more recent studies support the finding.

Biedert *et al.* [43] used 2.5×10^{-7} to 10^{-6} M ouabain to produce positive inotropy in cultured chick embryo ventricular cells. They found the degree of Na^+–K^+ pump inhibition to be closely related to the magnitude of the positive inotropic effect and to an increase in exchangeable Ca^{2+} content of the cells. The increased Ca^{2+} was kinetically localized in these cells entirely within the most rapidly exchangeable component (equilibrated within 5 min). This is consistent with, but by no means proof of, a superficial locus for the glycoside-induced Ca^{2+} increment.

Studies in progress in our laboratories at UCLA also support the concept that the glycosides augment Ca^{2+} in a pool at the cellular surface. Using a technique whereby $^{45}Ca^{2+}$ uptake can be followed continuously on-line in monolayer myocardial cultures [44], administration of ouabain produces a net uptake of Ca^{2+} of some 15–20 per cent. Most of this increment is La^{3+}-displaceable which indicates that it is localized within the sarcolemmal–glycocalyx complex.

Glycoside toxicity

The foregoing discussion has focused on the therapeutic inotropic actions of digitalis. Toxic levels of the drug lead to arrhythmias which can be life-threatening. Although the alterations produced in K^+ exchange seem to have little to do with the therapeutic actions of the drug, they have a great deal to do with the electrical toxicity produced. As glycoside dosage is increased, large net losses of K^+ occur which produce decreases in $[K^+]_i$ and increases in $[K^+]_0$ [38]. This leads to a progressive decline in the membrane resting potential (E_m) as determined by the Nernst relationship: $E_m = RT/F \ln [K^+]_i/[K^+]_o$ (R = gas constant, T = abs. temp., F = Faraday). Such reduction brings the cells closer to excitation threshold and with a concomitant increase in the rate of diastolic depolarization or phase 4 slope (mechanism not well-defined) causes extrasystoles to appear. This is a frequent early manifestation of digitalis toxicity. As E_m declines (becomes progressively less negative), the spike of the action potential (phase 0) is slowed. The rate of rise of the spike (dV/dt) is directly proportional to the magnitude of E_m. This relationship is based on the influence of E_m on the membrane gates which control Na^+ conductance during spike generation. Therefore, as E_m declines, the action potential spike slows. Speed of conduction from cell to cell is, in turn, dependent upon the speed of the spike. Thus, as toxicity develops, conduction rate from cell to cell falls. This, along with the vagotonic effect of digitalis, slows atrio-ventricular as well as ventricular conduction. Finally, the duration of the action potential plateau decreases. This is based upon an increase in K^+ conductance and is probably based upon an increase of Ca^{2+} within the cell and a rise in $[K^+]_o$—both of which augment K^+ conductance. A shortened plateau leads to a shortened refractory period. The combination of slowed conduction and shortened refractory period sets the condition for development of reentrant arrhythmias. One of these is, of course, ventriclular fibrillation—a not infrequent manifestation of digitalis toxicity.

Conclusion

Knowledge about the mechanism of action of the cardiac glycosides has been evolving over the past 200 years. At present the weight of evidence supports two components in the mechanism. Low dosage (10^{-9}–10^{-8} M) glycoside is associated with increases in myocardial catecholamine level, stimulation of the

Na^+–K^+ pump, and positive inotropy based on the catecholamine elevation. Higher dosage ($> 10^{-8}$ M) inhibits sarcolemmal Na^+–K^+ ATPase and the Na^+–K^+ pump. This produces elevation of intracellular Na^+ which, in turn, activates the trans-sarcolemmal Na^+–Ca^{2+} exchange system thereby augmenting Ca^{2+} influx and producing further positive inotropy. Toxic responses to the drug are based on further Na^+–K^+ pump inhibition leading to large net losses of cellular K^+. These losses are responsible for the various arrhythmogenic effects.

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