Question of Norepinephrine Release in the Action of Cardiac Glycosides

I read with great interest the article by Jan Koch-Weser entitled "Beta Receptor Blockade and Myocardial Effects of Cardiac Glycosides," which appeared in Circulation Research (p. 109, February 1971). I wish to take issue with several facets of his study.

First, the author describes in his introduction a controversy as to whether norepinephrine release contributes to the action of cardiac glycosides on the electrical performance of the heart and indicates that support for the concept is based on (1) findings of lowered myocardial norepinephrine concentration after exposure to cardiac glycosides and (2) decreased ability of cardiac glycosides to induce arrhythmias after either pretreatment with reserpine or after beta-receptor blockade. This summary ignores a number of other important supporting findings, namely: (1) a causal relationship between digitalis excitation of the sympathetic nervous system and the development of serious ventricular rhythmic disorders (Gillis, Science 166:508, 1969; McLain, Int J Neuropharmacol 8:379, 1969); (2) propranolol conversion of digitalis-induced arrhythmias correlates with depression of digitalis-induced neural hyperactivity (Gillis, Science 166:508, 1969); (3) drugs effective for conversion of digitalis-induced arrhythmias to sinus rhythm are neuro-depressants (Standaert et al., Eur J Pharmacol 6:209, 1969); (4) surgical interruption of autonomic cardiac pathways protects the heart from otherwise cardiotoxic doses of digitalis (Erlij and Mendez, J Pharmacol Exp Ther 144:97, 1964; Boyajy and Nash, Toxicol Appl Pharmacol 9:199, 1966; Raines et al., Arch Int Pharmacodyn Ther 170:485, 1967; Wallace et al., Bull NY Acad Med 43:1119, 1967); (5) bretylium, a drug known to impair sympathetic nerve terminal function but possessing no significant effects on the electrophysiological properties of the myocardium is effective in treating digitalis-induced cardiac arrhythmias (Bigger and Jaffe, Am J Cardiol 27:82, 1971; Papp and Williams, Br J Pharmacol 37:380, 1969); and (6) drugs which normally counter digitalis arrhythmias fail to do so when the heart is deprived of sympathetic influence (Levitt and Roberts, Circ Res 19:622, 1966; Raines et al., Eur J Pharmacol 11:293, 1970).

Second, the author attempts to evaluate the role of norepinephrine in the action of cardiac glycosides by studying isolated heart muscles. In such preparations, most of the sites with which digitalis would interact to release norepinephrine are not present (e.g., central nervous system structures, sympathetic ganglia, and adrenal medulla). The evidence the author cites which supports the contention that norepinephrine release is involved in the ability of digitalis to disturb the electrical performance of the heart was obtained from in vivo studies (McLain, Int J Neuropharmacol 8:16, 1969). It is difficult for me to see how the author's in vitro studies can be used to refute the results obtained from the whole animals.

Third, the author reports that propranolol in a concentration of 10^-6 M markedly reduced the ability of cardiac glycosides to induce ectopic impulse formation in isolated myocardium and concludes that quinidinelike properties are largely responsible for this action. However, the author demonstrated that 10^-8 M propranolol is an effective beta-receptor-blocking dose and failed to demonstrate that it produces a negative inotropic effect; the latter occurs pari passu with the antiarrhythmic effect of quinidine. Therefore, the experimental data actually suggest that the beta-receptor-blocking action was responsible for reducing the ectopic impulse formation induced by digitalis. These experiments thus indicate involvement of norepinephrine release in digitalis toxicity even in preparations free from extrinsic sympathetic control.

Fourth, the author reports that cardiac glycosides do not influence the myocardial catecholamine content. However, the doses of digitalis tested were not in the arrhythmogenic range, and hence the negative results obtained have no bearing on the role of
norepinephrine release in the disturbance of the electrical performance of the heart by digitalis materials. Indeed, the author points out that investigators who have found that digitalis administration lowers the myocardial norepinephrine concentration, employed concentrations which were toxic to lethal.

In summary, I cannot agree with the author's conclusion that "release of myocardial norepinephrine plays no role in the actions of cardiac glycosides on the heart." In fact the author's data strongly suggest that norepinephrine release is involved in digitalis-induced ectopic formation even in isolated myocardial preparations.

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REPLY TO THE ABOVE LETTER

I should like to comment on each of the interesting points raised by Richard Gillis. The studies cited in the first part of his letter clearly have no bearing on the mechanism of the therapeutic or toxic mechanical effects of digitalis which were the primary subject of my study. Do they really contain "important supporting findings" for the concept that norepinephrine release from cardiac sympathetic nerves is responsible for the electrical actions of cardiac glycosides?

1. No "causal relationship" between excitation of the sympathetic nervous system by digitalis and the development of ventricular arrhythmias has been shown. Gillis (Science 166:508, 1969) described inhibition or enhancement of activity in sympathetic nerves of decerebrate cats after intravenous injection of toxic doses of ouabain (50–100 μg/kg). He stated: "I noted a correlation between the ouabain-induced increase in sympathetic activity and the occurrence of ventricular tachycardia" but presented no data to support this statement, and some of his animals developed ventricular arrhythmias without receiving ouabain. Other investigators have found that digitalis administration decreases efferent activity in sympathetic fibers (Abiko, Jap J Pharmacol 13:305, 1963; Daggett and Weisfeldt, Am J Cardiol 16:394, 1965). Altered activity of the autonomic nervous system is one of the direct effects of high doses of digitalis on the central nervous system. This does not prove that sympathetic hyperactivity mediates the actions of nontoxic doses of cardiac glycosides on the electrical performance of the heart, nor is there convincing evidence that it is the cause of digitoxic arrhythmias.

2. My paper emphasized that under some conditions administration of beta-receptor blockers, pretreatment with reserpine, surgical denervation, and other interventions which interfere with the normal action of the sympathetic nervous system on the myocardium may increase the maximum dose of digitalis tolerated without the development of cardiotoxicity. It simply does not follow that digitalis causes arrhythmias by releasing norepinephrine. Even under normal circumstances the sympathetic nerves in the heart continuously release norepinephrine and under many experimental conditions sympathetic nervous system tone is abnormally high. While such tonic release has nothing to do with cardiac glycosides, the electrophysiologic actions of the released norepinephrine summate with many of the arrhythmogenic effects of digitalis (Raper and Wale, Eur J Pharmacol 6:223, 1969).

3. While some drugs which counteract digitalis-induced arrhythmias are "neurodepressants," it is probably more to the point that all of them have characteristic direct effects on the electrical behavior of myocardial fibers. The relative effectiveness of these drugs against digitalis arrhythmias correlates well with their specific actions on heart muscle.

4. Bretylium prolongs the action potential and lengthens the effective refractory period of ventricular muscle and of Purkinje fibers (Wit et al., J Pharmacol Exp Ther 173:344, 1970; Bigger and Jaffe, Am J Cardiol 27:82, 1971); these are significant electrophysiologic effects. Furthermore, the drug is less effective
against digitalis arrhythmias than agents such as diphenylhydantoin or lidocaine which do not impair sympathetic function. Finally, dextropropranolol, which has "quinidinelike" properties but very weak beta-receptor-blocking potency, is effective against digitalis arrhythmias, while sotalol, a beta-receptor-blocking drug with weak direct electrophysiologic effects, is relatively ineffective (Lucchesi et al., in Cardiovascular Beta Adrenergic Responses, Univ of Calif Press, pp 21-43, 1970; Somani et al., J Pharmacol Exp Ther 151:32, 1966).

In regard to Dr. Gillis's second point, it is obvious that my experiments have no bearing on possible extracardiac actions of cardiac glycosides. However, to my knowledge, the effects of all concentrations of cardiac glycosides on the mechanical and electrical behavior of the myocardium in vivo can also be observed in isolated heart preparations.

Third, Dr. Gillis errs in suggesting that antiarrhythmic agents which lack beta-receptor-blocking action influence the electrical behavior of myocardium only in concentrations high enough to depress myocardial contractility. Effective antiarrhythmic activity without negative inotropic action has been demonstrated for such compounds under conditions ranging from the therapeutic situation to experimentation on isolated heart muscles (Austen and Moran, Am J Cardiol 16:701, 1965; Lieberman et al., Am J Cardiol 22:375, 1968; O'Rourke et al., Am J Cardiol 23:238, 1969; Nahas et al., Can J Physiol Pharmacol 47:1038, 1969; Strauss et al., Circ Res 23:463, 1968). We have found that quinidine, diphenylhydantoin, procainamide, lidocaine, and dextropropranolol in concentrations which have no negative inotropic effect reduce the ability of cardiac glycosides to induce ectopic impulse formation in isolated atrial strips and papillary muscles.

Fourth, the concentrations of ouabain and digoxin which during our in vitro and in vivo experiments failed to influence myocardial catecholamine concentrations produced nearly the maximum possible positive inotropic effect and induced arrhythmic activity in over 40% of the experiments. The effect of lethal concentrations of cardiac glycosides on myocardial catecholamine content does not seem pertinent to their usual mechanism of action. One suspects that myocardial catecholamines may become partially depleted during a variety of agonal states.

In summary, I remain unconvinced by Dr. Gillis's arguments that release of myocardial norepinephrine is responsible for the various cardiac actions of digitalis.

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Determination of Electrical Sources in the Mammalian Heart from Intracellular Action Potentials

The object of this letter is the development of a quantitative relationship between the intracellular ventricular action potential, the electromotive activation wave source in the heart, and resultant bipolar waveforms utilizing the idealized cardiac activation model shown in Figure 1 (1). The individual cells have the dimensions of about 15μ x 15μ x 100μ long. Electrophysiological

FIGURE 1
Wave of activation and spatial action potential superimposed on a section of cardiac cells. (Wave width on same scale as cells should be eight times wider; it is narrowed for clarity.)

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investigation of the activation of the outer portion of the heart wall shows a uniform propagating wave (2, 3). Spatial and temporal events can be interchanged since potentials must be of the form \( f(l - vt) \), where \( l \) is the direction of propagation and \( v \) is the corresponding velocity. To provide greater generality, the direction of propagation shown in Figure 1 is not along the fiber axis, but is arbitrarily oriented. Superimposed on the cell geometry is the "leading edge" of the ventricular action potential plotted spatially. It is tacitly assumed that at all membrane points on a plane normal to the direction of propagation, the transmembrane potential is the same and corresponds to the abscissa in the spatial action potential curve.

Each cell can be considered as an electrical source, consisting of a double layer, \( K \), on the membrane with an orientation normal to the surface. \( K \) equals \((V_i - \sigma_i - V_o - \sigma_o) / \sigma_o\), where \( V_i \) and \( V_o \) are the membrane potentials just inside and just outside the membrane and \( \sigma_i \) and \( \sigma_o \) are the intracellular and interstitial conductivities (4). The electrical potential due to such a source is

\[
\Phi_v = \frac{1}{4\pi} \int K \nabla \left( \frac{1}{r} \right) \cdot dS,
\]

where \( S \) is the surface of the cell. Although \( K \) is defined only on \( S \), we can arbitrarily choose its value on a planar surface normal to the direction of propagation (within \( S \)) to be that on \( S \). This permits a transformation of Eq. 1 to

\[
\Phi_p = \frac{1}{4\pi} \int dA \int \frac{\partial K}{\partial l} \nabla \left( \frac{1}{r} \right) \cdot d\vec{a} dZ
\]

(see Eq. 5.79 in ref. 3) where \( A \) is the oblique sectional area of each cell, and \( Z \) is in the axial direction. A physical interpretation of Eq. 2 is that \( \partial K / \partial l \) is the double layer density per unit length in the \( l \) direction (i.e., a dipole moment per unit volume) and \((\partial K / \partial l) dl \) is an elementary double layer lamina of thickness \( dl \).

For the special case of \( \sigma_i = \sigma_o \), then \( K = V_m \), the transmembrane potential. The double layer density, \( \partial V_m / \partial l \) is plotted in Figure 2b corresponding to \( V_m(l) \) in Figure 2a; its functional form is, roughly, an error function. This corresponds to the experimental results of Solomon et al. (5). The activation wave extends a finite thickness corresponding to \( \partial V_m / \partial l \neq 0 \) (i.e., it lies between resting tissue and that in the idealized plateau state). It consists of stratified double layers with a density that varies as an error function. Using a rise time of \( 2 \times 10^{-8} \) sec.

**FIGURE 2**

(a) The leading edge of the ventricular action potential. Because of assumed propagation, the abscissa represents both time (t) and a spatial coordinate (l) along the direction of propagation, where \( l = -ct \). b: The double layer source density in the direction of propagation is given by \( \partial V_m / \partial l \), the derivative of the curve in a.

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for the transmembrane action potential, $V_m(t)$, and a propagation velocity of 400 mm/sec, then the spatial rise time corresponds to 0.8 mm. For many purposes this thickness is of little importance, so that one can collapse the distributed source into a single total double layer. The strength of the net double layer $K_i$, is simply the sum

$$K_i = -\int \frac{\partial V_m}{\partial t} \, dt \frac{dA}{dS} = \frac{A}{S} (V_{pK} - V_r), (3)$$

where $dA/dS$ is the ratio of the area occupied by each fiber to the total area (fiber plus adjoining interstitial area). This ratio is included so that $K_i$ is an effective and continuous double layer. Assuming $dA/dS$ to be uniform and equal to $A/S$, then the integral can be evaluated with the result as shown. In Eq. 3, $V_{pK}$ is the maximum potential (plateau) and $V_r$ is the resting potential. Since $A/S = .85$, the value of $K_i$ is around 100 mv.

The potential expected from bipolar intramural electrode measurements can be deduced from Eq. 1 and 3. For an assumed uniform excitation, each electrode potential is proportional to the sum of solid angles subtended by each double layer lamina comprising the composite source. When both electrodes are on the same side of a lamina, since in general its lateral extent is large compared to electrode separation, both electrodes “see” roughly the same solid angle and the potential difference (p.d.) is small (zero). No p.d. should be recorded until the activation wave passes wholly or in part between the electrodes. Then the p.d. is the full $4\pi$ solid angle difference times the net (included) lamina strength. Thus the potential variation is simply the integral of that portion of the “error function” lying between the recording electrodes. For electrodes wider than the wave, the potential will maintain its maximum value (a flat peak) until the activation wave begins crossing the second electrode. If the spacing is less than the wave thickness, since one cannot realize both electrodes on opposite sides of the total composite double layer source, the peak potential is reduced. Actual measurements by Durrer (6) follow this expectation in waveform. The theoretical predictions of Selvester et al. (7) differ from these, but since their assumptions and method of computation were not given it is not possible to comment on the discrepancy.

When each electrode is on opposite sides of the composite double layer then, from Eq. 1 and 3,

$$\text{p.d.} = \frac{A}{S} (V_{pK} - V_r) \approx 100 \text{ mv.} \quad (4)$$

This represents a peak value and corresponds to surface measurements reported by Vander Ark and Reynolds (8), but Scher (2) and Selvester et al. (7) using intramural electrodes recorded only 10 to 60 mv.

A point of view that does not depend on assuming $\sigma_i = \sigma_a$ is to consider the myocardi- um as composed of long parallel fibers, as suggested by unimpeded transmission between cells. A reasonable electrical representation is the core conductor model. Consequently, for uniform propagation, the potential waveform interstitially and intracellularly are similar and are related by the external ($R_e$) and internal ($R_i$) longitudinal resistances per unit length. Specifically, the external potential $V_e = R_e V_m/(R_i + R_e)$, and the internal potential is $V_i = R_i V_m/(R_i + R_e)$. The longitudinal resistances can be found from the conductivities and the cross-sectional area of the fiber, $a$, and the interstitial space $(s-a)$. Thus $R_e = 1/\sigma_e(s-a)$, $R_i = 1/\sigma_a$, and the double layer strength of $(\sigma_i V_i - \sigma_a V_o)/\sigma_a$ thus evaluates to $\sigma_i V_i/[\sigma_a + \sigma_e(s-a)]$. The effect of unequal conductivities is seen to introduce the coefficient of $V_m$. For $\sigma_o = 2\sigma_i$ and for $a/s = .85$, the coefficient evaluates to .87.

Another approach is to substitute the expressions for $R_e$ and $R_i$ in the above equation for $V_o$ which gives

$$V_o = V_m \left[ \frac{\sigma_o}{\sigma_o + \sigma_e(s-a)} \right] \quad (5)$$

This is seen to correspond to the double layer result except for a multiplicative factor $(a/s)$. Eq. 5 gives a corrected source density,
replacing \((V_{pK} - V_r)\) of Eq. 4; it describes
the axial interstitial potential variation as
simply \(V_m\) reduced by perhaps 15%. Analysis of
cardiac muscle using cable theory implicitly
assumes infinite fiber length, thus the ap-
proach is valid only as long as the sources lie
well within the heart wall (however, the
double layer correction factor should remain
valid). Unipolar action potentials by Scher
(2) show leading edges similar to \(V_m\), but the
duration is limited by breakthrough and the
amplitudes are only 40 mv.

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