This is to certify that the

dissertation entitled

Mechanism of enhancement of the arrhythmogenic effects of digitalis by ischemia

presented by

Donghee Kim

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Pharmacology
Pharmacology and Toxicology

Tai Akera
Major professor

Date Sept. 7, 1982

MECHANISM OF ENHANCEMENT OF THE ARRHYTHMOGENIC EFFECTS OF DIGITALIS BY ISCHEMIA

By

Donghee Kim

AN ABSTRACT OF A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology and Toxicology

1982

ABSTRACT

MECHANISM OF ENHANCEMENT OF THE ARRHYTHMOGENIC EFFECTS OF DIGITALIS BY ISCHEMIA

By

Donghee Kim

Myocardial ischemia sensitizes the heart to the arrhythmogenic effects of digitalis thereby reducing the therapeutic usefulness of digitalis for the treatment of heart failure in the presence of myocardial ischemia. Therefore, mechanisms by which this phenomenon occurs were studied. Ligation of the left anterior descending (LAD) coronary artery in isolated guinea-pig hearts reduced the time to onset of arrhythmias during perfusion with a toxic concentration of digoxin. The period of occlusion was less than 80 min in all preparations. Kinetic parameters of (^{3}H)ouabain binding to Na,K-ATPase, a putative receptor for the glycoside, and the enzyme activity itself, were unchanged by 2-hr of partial or complete ischemia. Thus, the enhanced sensitivity of the LAD artery ligated guinea-pig heart to digitalis appears to be due to mechanisms other than an altered glycoside binding to the enzyme. LAD artery ligation in anesthetized cats also reduced the dose of digoxin required to produce arrhythmias. Removal of the autonomic nervous system input to the heart by bilateral vagotomy and spinal section or by propranolol pretreatment

failed to influence the enhanced sensitivity of LAD artery ligated animals to digitalis.

The direct effects of ischemia on the heart that might enhance the arrhythmogenic actions of digitalis were further examined using Langendorff preparations of guinea-pig heart. Reserpine or cimetidine pretreatment failed to abolish the increased digitalis sensitivity caused by LAD artery ligation. Infusion of a high (10.6 mM) K^+ solution, but not a 5.8 mM K^+ solution into the LAD coronary artery and perfusion of the rest of the heart with digoxin significantly enhanced the arrhythmogenic action of the glycoside. These results suggest that the ischemia-induced increase in digitalis sensitivity may be due to an interaction between K^+ ion and digitalis in certain areas of the heart.

to my lovely wife, Sachiko

ACKNOWLEDGEMENTS

I sincerely thank Dr. Tai Akera for his invaluable guidance during the graduate training. I thank him for his kindness, support, constructive criticism, advice, patience and concern.

I sincerely appreciate the advice and criticisms offered by Drs. Theodore M. Brody, Kyosuke Temma, and Richard H, Kennedy. I also would like to thank the other members of my graduate committee, Drs. Gerald L. Gebber, Gregory D. Fink and Lynne C. Weaver for their advice and friendship. I deeply value the friendships of Josh Berlin, Eddie Ng, Richard Kennedy, Sue Glashower, Dr. Yumi Katano, Paul Stemmer, Annie Han, and Cathy Berney.

I thank Katie Hilliker and Bonnie Baranyi for their help during the years of my graduate training, and Diane Hummel for her excellent secretarial help to complete my thesis dissertation.

I sincerely thank my parents, brother and sister for their love and constant emotional support.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

INTRODUCTION

A. General Background

A group of cardiac glycosides and aglycones that are found in the digitalis leaves, strophanthus seeds and squill bulbs are collectively referred to as digitalis. Although digitalis has been in use for many hundreds of years, the first written account of its beneficial effects in cardiac insufficiency was that by William Withering in 1785. Later in 1912, Herrick advocated digitalis therapy in acute myocardial infarction as a means of improving cardiac function. Early clinical observations, however, indicated that treatment of myocardial infarction with digitalis was largely associated with the development of ventricular arrhythmias. These clinical observations prompted several investigators to examine the possibility that myocardial infarction may sensitize the heart to digitalis, resulting in toxicity.

The earliest of such studies in experimental animals demonstrated that the arrhythmogenic doses of digitalis preparations were significantly reduced in coronary artery occluded animals compared to that in non-occluded animals (Bellet et al., 1973; Travell et al., 1938). In another series of studies, experimental animals with acute myocardial ischemia (Kumar et al., 1970; Ku and Lucchesi, 1979) as well as chronic myocardial infarction (Hood et al., 1967; Morris et al., 1969; Moss et al., 1981) showed greater sensitivity to the arrhythmogenic effects of digitalis.

In addition, pathologic conditions producing hypoxemia, an important component of ischemia, were also observed to enhance digitalis-induced rhythm disturbances (Baum et al., 1959; Hargreave, 1965; Harrison, 1968; Williams et al., 1968; Beller et al., 1971; Mason et al., 1971; Friedman et al., 1972; Beller and Smith, 1975). Thus, in patients with acute or chronic pulmonary insufficiency with or without cor pulmonale, or in experimental animals with artificially-induced acute hypoxemia, the sensitivity to the arrhythmogenic actions of cardiac glycosides were significantly increased.

It appears then that ischemic conditions enhance the arrhythmogenic potential of digitalis. The mechanism of these phenomenon is not completely understood at present. It is of clinical significance to understand how ischemia sensitizes the heart to the arrhythmogenic activity of digitalis since these drugs are of immense value in the treatment of certain types of heart failure and arrhythmias, such as congestive heart failure, in acute pulmonary disease, and in supraventricular tachycardia with or without myocardial infarction (Selzer, 1965; Karliner and Braunwald, 1972). In the present study, attempts were made to elucidate the mechanism by which ischemia enhances the toxic (arrhythmogenic) actions of digitalis.

B. Effects of Ischemia on the Direct Effects of Digitalis on the Heart

1. Mechanism of inotropic and toxic actions of digitalis

In 1938, Cattell and Gold demonstrated that digitalis increased the force of contraction of isolated, electrically-driven cat papillary muscle. Since then, the direct positive inotropic action of

digitalis on the heart has been firmly established. It was further observed that cardiac glycosides specifically inhibit the active transport of sodium and potassium ions across the cell membrane (Schatzmann, 1953; Glynn, 1957). The ATP-dependent sodium-potassium ATPase, also known as the sodium pump was highly sensitive to the inhibitory effects of the cardiac glycosides (Skou, 1957; Glynn, 1964; Skou, 1965). The amount of cardiac glycosides bound to Na,K-ATPase was demonstrated to strongly and inversely correlate with Na,K-ATPase activity (Matsui and Schwartz, 1968; Hansen et al., 1971). These two different effects by digitalis, sodium pump inhibition and positive inotropy, have therefore resulted in speculation that they may be causally related.

Further studies on the relationship between Na,K-ATPase inhibition and positive inotropy produced by digitalis have shown that the two effects are positively and temporally correlated (Repke, 1964, 1965; Tobin and Brody, 1972; Akera et al., 1969; Allen and Schwartz, 1969; Hougen and Smith, 1978). Therefore, it is generally accepted that inhibition of Na^+,K^+-ATPase is the mechanism by which cardiac glycosides produce their positive inotropic effects, and that Na,K-ATPase is the putative pharmacological receptor for the inotropic action of digitalis (Repke, 1964; Schwartz et al., 1975; Akera and Brody, 1977). Other mechanisms for the positive inotropic actions of digitalis have been proposed (Dutta et al., 1968; Schwartz, 1976). Schwartz (1976) has proposed that digitalis binds to Na,K-ATPase and alters the affinity of enzyme-associated lipids for Ca^{++}. Dutta et al. (1968) proposed that Na,K-ATPase transports digitalis to

intracellular space. These mechanisms for the positive inotropic effect of digitalis, however, have received little support.

Further investigations of the relationship between digitalis-induced Na^+,K^+-ATPase inhibition and subsequent positive inotropic effect have revealed that a transient increase in intracellular sodium concentration may be responsible for the observed positive inotropic effect following enzyme inhibition. For example, increasing the intracellular sodium concentration by sodium ionophores, veratrum alkaloids, or grayanotoxins, similar to the outcome of sodium pump inhibition, was followed by a positive inotropic effect (Horackova and Vassort, 1974; Akera et al., 1976; Howard et al., 1976; Ku et al., 1977).

The relationship between digitalis cardiotoxicity and Na,K-ATPase inhibition has not been as well defined as that of the positive inotropic effect and enzyme inhibition. For example, the arrhythmogenic actions of cardioactive glycosides could be dissociated from their inotropic actions. The percent increases in contractility by various glycosides did not correlate with the percent development of arrhythmias (Haustein and Hauptmann, 1974). It is generally accepted, however, that an excessive sodium pump inhibition is responsible for the toxic effects of cardiac glycosides. Toxicity occurs when the reserve capacity of the sodium pump is exhausted and further inhibition of the sodium pump results in accumulation of Na^+ and loss of K^+ (Akera and Brody, 1977; Akera and Brody, 1982). Akera et al. (1976) have observed that an approximately 60% inhibition of sodium pump occurred with a toxic dose of digitalis whereas a lesser

inhibition (40%) occurred with a positive inotropic dose. The involvement of Na,K-ATPase in digitalis toxicity is further supported by the findings that K^+ was lost to the extracellular space with toxic doses of digitalis (Langer and Serena, 1970; Lee and Klaus, 1971). Such large increases in extracellular K^+ and the resulting electrophysiological changes were therefore proposed to be responsible for digitalis toxicity (Langer, 1972). Thus, a K^+-induced reduction in the membrane potential, which may reach the threshold level for spontaneous firing has been proposed to be responsible for the arrhythmogenic actions of digitalis. More recent studies have demonstrated that the digitalis-induced arrhythmias can be developed from delayed afterdepolarizations which are observed with a toxic concentration of digitalis (Ferrier et al., 1973; Rosen et al., 1975; Ferrier, 1977). Although the exact ionic mechanism by which delayed afterdepolarizations occur is not known, it is well established that digitalis-induced inhibition of the sodium pump is the first step in the process. Thus, the toxic or arrhythmogenic effects of digitalis result from sodium pump inhibition; however, the steps following the sodium pump inhibition appear to be very complex.

2. Factors Involving Na,K-ATPase that May Enhance Digitalis Toxicity

Ischemia may affect glycoside binding to the sarcolemmal Na,K-ATPase in several ways. It may alter the affinity of the binding sites on the enzyme for the glycoside, the number of functioning enzyme units, or both. It has been shown that ischemia or hypoxia increases the membrane permeability to certain ions such as K^+ and

Ca^{++} (Nayler et al., 1979; Nayler, 1981; Shine, 1981). Such effects will alter the ionic environment immediately surrounding the Na,K-ATPase. Since various ions determine the activity of the enzyme and therefore the turnover rate, the affinity for the glycoside will concomitantly change (Clausen and Hansen, 1977). It has been well demonstrated that stimulation of the sodium pump by increasing the intracellular sodium concentration either by high frequency stimulation of cardiac muscle (Yamamoto et al., 1979) or using pharmacological agents that specifically increase Na-K-pump activity (Clausen and Hansen, 1977) enhances glycoside binding to Na,K-ATPase. By this mechanism ischemia may enhance digitalis toxicity.

Alternatively, ischemia itself may cause inhibition of the cardiac Na,K-ATPase activity and contribute to digitalis toxicity. Beller et al. (1976) reported that following 2-hr of coronary artery ligation in dogs, Na,K-ATPase activity in the partially purified enzyme obtained from ischemic tissues was significantly reduced. Ku and Lucchesi (1979) observed, however, that the sodium pump activity in ventricular slices obtained from partially ischemic and completely ischemic tissues in coronary artery ligated dog heart was increased and was also more sensitive to the inhibitory effects of digitalis. In other studies, no changes in Na,K-ATPase activity following a 24-hr occlusion in dogs were observed (Schwartz et al., 1973).

Studies with hypoxia, an important component of ischemia, also showed contrasting results on Na,K-ATPase activity. Hypoxic perfusion for 15 min caused significant reduction in Na,K-ATPase activity in the rat heart (Balasubramanian et al., 1973) whereas a

much longer period (60 min) of anoxia failed to affect Na pump activity in rabbit heart (Rau et al., 1977). McDonald and MacLeod (1971, 1973) reported that in electrically stimulated guinea-pig papillary muscle maintained at 35°C, 8 hr of anoxic perfusion had a stimulatory effect on sodium pump activity that was responsible for the maintenance of the resting membrane potential in spite of a large K^+ leakage to the extracellular space. These investigators further reported that sensitivity of the sodium pump to the inhibitory effect of ouabain was markedly greater in 8 hr anoxic when compared with 10 min anoxic tissues. This effect was attributed to the increased sodium pump activity in 8 hr anoxic tissues. This latter finding is supported by the results that increased sodium pump activity enhanced the inhibitory effects of digitalis by causing greater glycoside binding to Na,K-ATPase (Yamamoto et al., 1979).

Thus, the above contrasting results suggest that there may be species-specificity with respect to the effects of ischemia on Na,K-ATPase or sodium pump activity. If so, ischemia may or may not alter the glycoside effect on Na,K-ATPase, depending on the animal species. Ischemia may also reduce the reserve capacity of the sodium pump and enhance the toxic effects of digitalis. It has been proposed that digitalis toxicity occurs when glycoside-induced inhibition of the sodium pump exceeds its reserve capacity, and the remaining sodium pump activity becomes inadequate for maintaining a low intracellular sodium ion concentration (Akera and Brody, 1977). Membrane depolarization may subsequently occur and give rise to an action potential

when the membrane potential reaches the threshold potential for spontaneous activity.

Therefore, an increase in the affinity of the Na,K-ATPase for glycoside, a decrease in the number of functioning enzyme molecules or a reduction in the reserve capacity of the sodium pump may enhance digitalis-induced toxicity in the heart.

C. Effects of Ischemia on the Indirect Action of Digitalis on the Heart

1. Role of Autonomic Nervous System in Toxic Actions of Digitalis

In addition to the direct action of digitalis on cardiac tissues, the extracardiac effects have been shown to significantly contribute to the toxic effects of digitalis, i.e. to the development of ventricular arrhythmias. Although neuroexcitatory effects of digitalis have been known for a long time, it was not until 1937 that Korth et al. emphasized a specific effect of digitalis to increase central sympathetic outflow to the heart. Since then a vast amount of research has accumulated implicating the sympathetic nervous system as one of the causes for digitalis-induced arrhythmias.

A strong correlation between digitalis-induced increase in sympathetic activity and the occurrence of ventricular arrhythmias was observed by several investigators (Gillis, 1969; Gillis et al., 1972; McLain, 1969; Pace and Gillis, 1976). Procedures that interfered with the central sympathetic outflow to the heart, such as intraventricular administration of propranolol, abolished neurally associated cardiac arrhythmias (Lewis and Haeusler, 1975). Using hypothalamic stimulation to augment sympathetic outflow, Evans and Gillis (1975) were

able to produce arrhythmias with a dose of ouabain that would not normally be arrhythmogenic.

Studies utilizing spinal cord transection techniques also demonstrated the involvement of the sympathetic nervous system in digitalis-induced arrhythmias. Raines et al. (1967) observed that C-1 section increased the dose of ouabain to produce ventricular tachycardia and fibrillation. Similar results were obtained by many other investigators (Gillis et al., 1972; Cagin et al., 1974; Levitt et al., 1973; Somberg et al., 1978). Acute or chronic cardiac denervation in dogs decreased the sensitivity of the heart to the arrhythmogenic actions of digitalis (Mendez et al., 1961; Cooper et al., 1961; Solti et al., 1965).

In addition to surgical methods, drugs that modify sympathetic nervous system function have been extensively studied. Drugs that depress central sympathetic outflow such as clonidine (Gillis et al., 1972); drugs that block cholinergic transmission, both nicotinic and muscarinic, such as hexamethonium and atropine (Gillis et al., 1975); beta-adrenergic receptor blocking agents, such as dl-proprano-lol (Evans et al., 1976), or sotalol (Kelliher and Roberts, 1974); or drugs that interfere with storage and release of norepinephrine at the postganglionic sympathetic nerve endings, such as reserpine (Boyajy and Nash, 1965; Ciofalo et al., 1967; Nadeau and Champlaine, 1973), 6-hydroxydopamine (Saito et al., 1974), guanethidine (Raines et al., 1968), or bretylium (Papp and Vaughn Williams, 1969; Gillis et al., 1973), all increased the dose of digitalis required to produce ventricular arrhythmias either in dogs, cats, or guinea-pigs. In

conjunction with these results was the finding that exogenously administered catecholamines enhanced the arrhythmogenic effect of digitalis (Morrow, 1967; Raper and Wale, 1969; Lum et al., 1977). These observations strongly suggest the importance of the sympathetic nervous system in digitalis-induced ventricular arrhythmias.

The role of the parasympathetic nervous system in digitalis-induced arrhythmias, however, remains controversial. The vagus is reported to have a protective effect (LoSasso and Paradise, 1969; Levitt et al., 1970), an enhancing action (Robinson and Wilson, 1918; Kreuger and Unna, 1942), or no effect (McLain et al., 1958; Boyazy and Nash, 1966; Lechat and Schmitt, 1982) on digitalis-induced arrhythmias.

2. Factors that May Enhance Digitalis Toxicity

From above studies, it may be concluded that if ischemia affects autonomic nerve activity, the toxic actions of digitalis on the heart may be altered. In 1967, Brown noted that coronary artery occlusion activated sympathetic cardiac afferent nerves in cats. Since then, a series of studies by other investigators have confirmed this observation and further demonstrated coronary artery occlusion-induced increases in sympathetic cardiac efferent nerve activity (Malliani et al., 1969; Uchida and Murao, 1974; Felder and Thames, 1979; Bosnjak et al., 1979). Such a sympathetic reflex was also present in C-1 spinal-sectioned animals (Malliani et al., 1969), an observation that led to the proposal that a cardiocardiac sympathetic reflex may be present during myocardial ischemia. In addition to changes in cardiac efferent nerve activity, coronary artery occlusion

caused changes in renal nerve activity (Weaver et al., 1981). Therefore, coronary artery occlusion, by activating afferent cardiac sympathetic nerves, influenced efferent sympathetic nerve activity not only to the heart, but also to other organs. On the other hand, many investigators observed a coronary artery occlusion-induced inhibitory influence on cardiac sympathetic nerve activity in similar animal models (Constantin, 1963; Thoren, 1973; Kedzi et al., 1974; Felder and Thames, 1979). Kedzi et al. (1974) recorded postganglionic sympathetic nerve activity and blood pressure following occlusion of circumflex coronary artery of anesthetized dogs. Although blood pressure decreased following occlusion, postganglionic sympathetic nerve activity was also reduced. Cutting the vagus nerves resulted in an immediate increase in sympathetic nerve activity and blood pressure. Thus, in these experiments, coronary artery occlusion, by activating vagal afferents, reflexly inhibited sympathetic outflow.

The reason for these disparate observations on sympathetic cardiac nerve activity induced by coronary artery occlusion is not clear. The area of myocardial ischemia may be an important determinant of the direction of change in sympathetic nerve activity, since sympathetic or vagal afferents in the heart may be unequally distributed (Oberg and Thoren, 1973; Thoren, 1973; Uchida et al., 1974; Corr et al., 1976). Of particular interest are the findings of Lathers et al. (1978) that coronary artery occlusion produced increases, decreases or no change in discharge within a bundle of postganglionic cardiac sympathetic nerves. They therefore postulated that such non-uniform changes in nerve activity may cause arrhythmias to develop.

In both human patients and experimental animals with ischemic heart and coronary artery ligation respectively, the catecholamine concentration in the blood and urine were significantly elevated (Forssman et al., 1952; Valorie et al., 1967; Klein et al., 1968; Staszewska-Barczak and Ceremuzynski, 1968; Hayashi et al., 1969; Siggers et al., 1971; Staszewska-Barczak, 1971). In many cases, the amount of catecholamine released into the blood was closely correlated with morphological changes in the ischemic heart. Conversely, administration of exogenous catecholamines produced features similar to myocardial ischemia (Raab et al., 1962; Waldenstrom et al., 1978). These findings suggest that during the ischemic process there is increased sympathetic activity and release of catecholamines. Such effects may predispose the heart to digitalis-induced toxicity.

D. Release of Endogenous Substances that may Enhance Digitalis Toxicity

It has been demonstrated that ischemia can release endogenous substances such as catecholamine and histamine into the circulating blood. These substances have been shown to produce changes in biochemical and physiological functions of the heart. Ischemia also impairs ionic movements across the cell membrane and within the cell (Nayler et al., 1979). Therefore, electrical events during excitation-contraction coupling may be depressed. These effects may alter the toxic actions of digitalis on the heart.

1. Catecholamines

It is well known that catecholamines potentiate the arrhythmogenic effects of digitalis. As described in section C of the

Introduction, beta-adrenergic blocking agents, catecholamine depleting drugs, and sympathectomy all reduced digitalis toxicity. Consistent with these findings are the results that exogenously administered catecholamines potentiated digitalis toxicity. Thus, if ischemia increases the level of catecholamines in the heart, the arrhythmogenic actions of digitalis will be enhanced.

In addition to increases in catecholamine release by sympathetic stimulation, ischemia (Lammerant et al., 1966; Ceremuzynski, 1969; Shahab et al., 1969; Abrahamsson et al., 1981) or hypoxia (Wollenberger and Shahab, 1965; Penna et al., 1965) has also been shown to cause local release of catecholamines from the nerve terminals in isolated heart muscle.

Digitalis has also been shown to release catecholamines from nerve terminals in isolated cardiac tissues. In isolated perfused rat or guinea pig hearts, ouabain increased spontaneously occurring norepinephrine release (Linmar and Loffelholz, 1974; Harvey, 1975). A large number of experiments have also been performed to study the effects of digitalis on the uptake of norepinephrine. These studies have indicated that digitalis inhibited the uptake of norepinephrine by neuronal membranes of heart tissues (Dengler et al., 1962; Berti and Shore, 1967; Stickney, 1976; Sharma and Banerjee, 1977). Ischemia may augment these effects and elevate digitalis toxicity. Thus, ischemia-induced release of catecholamines in the heart or its enhancing effects on the release and inhibition of uptake of norepinephrine by digitalis may potentiate the arrhythmogenic actions of digitalis in the ischemic heart.

2. Histamine

Histamine is found in cardiac tissues of many mammalian species including humans. Using H_1 and H_2 antagonists, both H_1 and H_2 receptors and responses of cardiac tissues to histamine were identified (Powell and Brody, 1976; Levi et al., 1976). Although the physiological role of endogenous cardiac histamine is uncertain, when released from the cardiac tissues during immediate hypersensitivity reactions (Levi, 1972), histamine produced marked changes in cardiac function by altering contractility, conduction, automaticity and coronary flow (Rocha and Silva, 1966; Levi, 1972). The arrhythmogenic actions of histamine at high doses were demonstrated to be by an action on H_2-receptors of cardiac tissues.

The interaction between digitalis and histamine to potentiate their effects on atrioventricular conduction block and ventricular automaticity was observed by Levi and Capurro (1974). In their study, ouabain enhanced histamine-induced changes in the above parameters in isolated guinea-pig hearts. In a related study, Somberg et al. (1980) found that both H_1 and H_2 antagonists were able to protect the cat heart from ouabain-induced cardiotoxicity. Histamine administration abolished the increase in lethal doses of ouabain caused by H_2 antagonists, but not that caused by H_1 antagonists. Thus, although the mechanism of the antiarrhythmogenic action of histamine antagonists is still not understood, the above studies indicate that histamine, when released, may augment the toxic actions of digitalis. Thus, ischemia may sensitize the heart to digitalis-induced toxicity by causing histamine release.

3. Potassium Ion

It has been clearly demonstrated that ischemia causes increases in extracellular K^+ concentration as a result of leakage from the intracellular space (Harris et al., 1954; Hill and Gettes, 1980; Hirche et al., 1980). Harris (1954) has proposed that such an effect is a major cause for the ventricular arrhythmias observed early in acute myocardial ischemia. The exact cause for the genesis of ventricular ectopic activity was attributed to a local increase in K^+ concentration within a normal region such that a K^+ gradient was present and a current of injury was flowing at the boundary between ischemic and normal tissue (Harris, 1954, 1966; Hoffman, 1966; Hill and Gettes, 1978; Hirche et al., 1980; Weiss and Shine, 1981). However, the precise mechanism by which coronary artery occlusion produced such ectopic activities has not been identified.

Several mechanisms have been proposed for the genesis of ventricular arrhythmias in early myocardial ischemia. In parallel with the loss of intracellular K^+, the resting membrane potential of ischemic cells was decreased (Kleber et al., 1978). It is well established that a reduction of the resting membrane potential to values lower than approximately -65 mV inactivates the fast inward sodium current without affecting the slow inward current carried mainly by calcium. It also was discovered that the slow inward current can under certain conditions generate propagated and automatic action potentials, called "slow responses" (Carmeliet and Vereecke, 1969; Pappano, 1970; Cranefield et al., 1971; Aronson and Cranefield, 1973). In the presence of high concentrations of beta-adrenergic

agonists which enhance the inward calcium movement and therefore the slow inward current, slow responses were observed (Wit et al., 1972; Sherlag et al., 1974). Thus, K^+-induced depolarizations may generate such propagated "slow" action potentials and contribute to ectopic activities observed in acute myocardial ischemia, and catecholamines may augment these effects of K^+ (Wit and Bigger, 1975; Zipes et al., 1975).

In ischemic tissues, an elevation of extracellular K^+ concentrations was also closely related to the shortening of the action potential duration and lengthening of conduction time (Weiss and Shine, 1981). Such localized changes in these parameters during regional ischemia have been hypothesized to be an important mechanism that sets a condition for re-entrant arrhythmias (Wit and Bigger, 1975; El-Sherif et al., 1977). In this mechanism, slow responses need not be present for arrhythmias to occur, but the presence of non-uniform changes in action potential duration and conduction time is sufficient for development of arrhythmias. Thus, in ischemic hearts with no arrhythmic activities, the addition of digitalis may help to bring the above processes into play and cause ventricular ectopic activities to appear.

In summary, digitalis alters ion movements across the cell membrane and also alters ion concentrations inside the cell to cause inotropic and toxic effects. Several possibilities exist that may enhance digitalis toxicity in the ischemic heart. Any one or a combination of these may be responsible for this phenomenon.

E. Objectives

The objective of the present study was to elucidate the mechanisms responsible for the enhanced arrhythmogenic effects of digitalis in ischemic hearts. The possible mechanisms were divided into three major categories and each was examined.

1. Ischemia may enhance the direct effects of digitalis on the heart. Since digitalis has been proposed to bind to cardiac sarcolemmal Na,K-ATPase to produce its pharmacological and toxic effects, the possibility that ischemia alters Na,K-ATPase activity, glycoside binding to the enzyme, or reserve capacity of the sodium pump was studied.
2. The toxic (arrhythmic) effect of digitalis is enhanced by the sympathetic nervous system. Therefore, ischemia may alter sympathetic activity and potentiate digitalis toxicity. Thus, the role of the sympathetic nervous system in ischemia-induced sensitization to arrhythmogenic actions of digitalis was examined.
3. Endogenous substances such as cardiac histamine and catecholamines are known to potentiate digitalis toxicity. Enhanced digitalis toxicity observed in ischemic hearts may be due to release of such substances. Ischemia also causes an increase in membrane permeability to K^+ and possibly to other ions. The possibility that such changes elevate digitalis-induced toxicity were studied.

MATERIALS AND METHODS

A. Materials

Tritium-labeled ouabain (generally labeled, specific radioactivity, 14 Ci/mmol) and ^{3}H-labeled digoxin (12α-labeled, specific radioactivity, 14 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Potassium hydroxide (^{42}K-labeled, specific radioactivity, 0.6 mCi/mmol) was prepared at the Nuclear Reactor Laboratory of Michigan State University. ^{42}KOH was neutralized with HCl before use.

Ouabain octahydrate (strophanthin-G), digoxin, Tris-ATP, rubidium chloride, reserpine, α-chloralose, dl-propranolol-HCl, isoproterenol-HCl, Patent Blue Violet dye, gallamine triethiodide, bovine serum albumin, tyramine-HCl were all purchased from Sigma Chemical Company, St. Louis, MO. Dihydrodigoxin was purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN. Rubidium chloride (ultrapure grade) was obtained from Alfa Division, Danvers, MA. Other chemicals were of analytical reagent grade.

Radioimmunoassay kits for digoxin were obtained from Clinical Assays, Cambridge, MA. Biofluor (liquid scintillation counting solution) was purchased from New England Nuclear, Boston, MA. Nitrocellulose filters were obtained from Millipore Filter Corporation, Bedford, MA type AA, pore size 0.8 μm). Gas mixtures were obtained from AIRCO, Inc., Montvale, NJ.

B. Isolated Heart Studies

An isolated perfused heart is a good model to study the direct effects of pathophysiological or pharmacological interventions on cardiac function. Various physiological parameters such as heart rate, temperature or perfusion rate may be precisely controlled, making the interpretation of data easier and accurate. In the present study, isolated heart preparations of guinea pigs were used primarily because preliminary results have indicated that this animal model is suitable for this study, and for other practical reasons such as size of the heart and availability.

Guinea pigs of either sex weighing 300-400 g were stunned by a sharp blow to the head, and the hearts were quickly removed and immersed in a bath containing Krebs-Henseleit bicarbonate buffer (pH 7.5) solution containing 118 mM NaCl, 27.2 mM $NaHCO_3$, 4.8 mM KCl, 1.0 mM KH_2PO_4, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$ and 11.1 mM glucose, and saturated with a 95% O_2-5% CO_2 gas mixture at room temperature. The aortic root was then cannulated and the heart was allowed to hang from a Langendorff perfusion apparatus. The hearts were perfused at a constant flow rate of approximately 2.5 ml/g tissue/min or at a constant pressure of 20 mmHg with the oxygenated Krebs-Henseleit buffer (pH 7.5) solution maintained at either 32°C or 36°C.

When the visible blood in the heart was washed away, both left and right atrial muscles were excised. The hearts were electrically stimulated at 1.5 Hz with square wave pulses of 5 msec duration at a voltage 30% above threshold by a pair of platinum electrodes placed near the atrioventricular node. One end of a thin silk thread was tied to the apex of the heart and the other end was connected to a

force-displacement transducer via a pulley. Resting tension was adjusted to 1.0 g and the force of contraction and twitch tension curves were recorded on a polygraph recorder using a force-displacement transducer (Grass Instrument Co., model 7D and FT-03C, respectively).

For studies of ischemia-induced changes in digitalis sensitivity, the hearts were equilibrated for 30 min and when no arrhythmic beats were observed, the left anterior descending coronary artery, approximately 1 cm above the apex of the heart, was completely occluded by passing a silk thread through the muscle surrounding the artery with a needle, and tying the ends of the threads tightly around the artery. The control preparations were subjected to the same procedure, but the thread was not tied. Following coronary artery occlusion, the hearts were perfused via the aorta for an additional 40 min. When no arrhythmic contractions were present during this period, digoxin was added to the perfusing solution (final digoxin concentration, 1.8 or 2.5 μM) and twitch tension curves were monitored to determine glycoside-induced arrhythmias. Control preparations were similarly perfused with digoxin solution. Approximately 5% of all heart preparations produced arrhythmic beats before or after coronary occlusion, but not after digoxin perfusion and therefore were discarded.

In another set of experiments, guinea pigs were intraperitoneally injected with 5 mg/kg reserpine solution 24 hr prior to sacrifice. To test catecholamine depleting effects of reserpine, a dose-response curve for the positive inotropic action of tyramine-HCl (10^{-6} - 10^{-4}M) was obtained in reserpine-treated and control heart preparations. In

histamine studies, cimetidine (10^{-5}M), an H_2-receptor blocker, was added to the perfusing solution 60 min prior to addition of digoxin to block effects of endogenously released histamine. The H_2-receptor blocking effects of cimetidine were tested by obtaining a dose-response curve for the positive inotropic effect of histamine in the presence and absence of cimetidine-pretreatment. The time to the onset of digoxin-induced arrhythmias was similarly monitored from twitch-tension recordings.

To study the non-uniform effects of digitalis on the heart, the left anterior descending coronary artery was carefully cannulated with a 27 gauge needle and normal Krebs-Henseleit bicarbonate buffer (pH 7.5) solution (5.8 mM K^+) was infused through the needle at a constant rate of 1.0 ml/min. After a 40-min equilibration period, digoxin was added to the solution that perfused the rest of the coronary arteries via the aorta. In another group of hearts, a modified Krebs-Henseleit bicarbonate buffer containing 10.6 mM K^+ instead of 5.8 mM K^+, was infused through the cannulated LAD artery. Digoxin was similarly added to the solution perfusing the rest of the heart via the aorta and the time to onset of arrhythmias was monitored. Heart preparations with the cannulated artery infused with a solution containing either 5.8 mM K^+ or 10.6 mM K^+ without digoxin perfusion were also monitored for comparable time periods for development of arrhythmias. In order to confirm the non-uniform distribution of digoxin in the heart by color staining, Patent Blue Violet dye was added to either solution perfusing the heart via the aorta or the solution perfusing the cannulated artery. At the end of the experiment, the heart was

cut in cross-section to visually identify areas of stained and non-stained tissues.

For global ischemia studies, Langendorff preparations of guinea-pig heart were maintained at 36°C in a humidified chamber and perfused at control flow rate of 2.5 ml/g tissue/min. Following a 20-min period, the flow rate was either maintained at control rate, or reduced to 5% of the control flow rate or the flow was completely stopped for 2 or 6 hr. For reperfusion studies, perfusion of the hearts were completely stopped for 2 or 5 hr and reperfused at control flow rate for 20 min or 1 hr. For the transition from no-flow to reperfusion, the rate of increase of flow rate was 0.5 ml/g tissue/min. At the end of global ischemia or reperfusion, ventricular muscles were dissected and homogenized. The homogenates were immediately assayed for Na,K-ATPase activity and (^{3}H)ouabain binding (see below).

C. Whole Animal Studies

Cats of either sex weighing 1.9-3.2 kg were anesthetized by intravenous injection of 60 mg/kg α-chloralose. A tracheostomy was performed and the animals were artificially respired with room air supplemented with approximately 30% oxygen. Femoral artery and vein were cannulated with polyethylene tubing for blood pressure and drug administration, respectively. Blood pressure and electrocardiogram from lead II were recorded using a pressure transducer and EKG-amplifier on a polygraph recorder (Statham-Gould, Oxnard, CA, model P23-ID and Grass Instruments Co., model 7D). Animal body temperature was

maintained at 36-38°C using a heating pad. A left thoracotomy was then performed and the heart was exposed by cutting open the pericardium. The left anterior descending coronary artery was identified and a silk ligature was passed around the artery approximately 1 cm below the lower tip of the left atria and left untied. The animals were then either left neurally intact, bilaterally vagotomized, or bilaterally vagotomized and spinal-cord sectioned at C1 or pretreated with dl-propranolol. After 60 min, when the blood pressure became stable, the artery was either untied (control) or ligated (LAD ligation) by tying the thread around the artery.

Digoxin was first dissolved in 50% alcohol and further diluted with 0.9% saline solution. The final alcohol content was approximately 0.5%. This digoxin solution was continuously infused at a rate of 60 μg/kg/hr after 40 min of LAD artery ligation. In some cats with LAD artery ligation, 0.9% saline solution containing similar amounts of alcohol with no digoxin was infused. For digoxin infusion in the presence of dl-propranolol, isoproterenol (0.1, 0.3 or 1.0 μg/kg) was infused intravenously and the changes in heart rate were noted for each dose of isoproterenol. A loading dose of dl-propranolol (1 mg/kg, i.v.) followed by a continuous infusion of 1 mg/kg/hr was started and the effect of isoproterenol on the heart rate were examined again after 30 min. This dose of dl-propranolol completely inhibited isoproterenol-induced changes in heart rate.

At the onset of digoxin-induced arrhythmias in both coronary artery occluded and non-occluded animals, 5 ml blood samples were collected and then 3 ml of Patent Blue Violet dye (100 mg dissolved in

3 ml of 0.9% saline solution) was infused via a femoral vein. When the heart turned blue, it was cut in cross-section to visualize dye-stained and non-stained areas. Darkly stained, lightly stained and non-stained tissues were visually delineated and small pieces of each type were cut out and immediately homogenized for biochemical assays and myocardial digoxin uptake determinations (see below). Darkly-stained, lightly-stained or non-stained tissues were designated as non-ischemic (NI), partially ischemic (PI) or completely ischemic (CI), respectively.

D. Plasma and Myocardial Digoxin Concentration Studies

Digoxin concentrations in plasma were determined by either radio-immunoassay for digoxin or by assaying for radioactivity of the plasma following an intravenous infusion or ^{3}H-digoxin. At appropriate times before and after digoxin infusion to anesthetized cats, blood samples were obtained from the femoral artery and placed in heparinized tubes. Blood samples were then centrifuged for 10 min at 2000 rpm to obtain plasma samples which were quickly frozen at -20°C. The plasma samples were subsequently analyzed for digoxin using the radioimmunoassay method (Clinical assays, Cambridge, MA; kit for digoxin). In one series of experiments, digoxin solution containing tracer amounts of 12α-^{3}H-digoxin was infused into the animals. Plasma digoxin concentrations of these cats were estimated by dissolving the plasma samples in 20 ml of Biofluor (New England Nuclear, Boston, MA) and assaying for radioactivity using a liquid scintillation spectrometer. At the onset of digoxin-induced ventricular arrhythmias, Patent Blue Violet

dye was infused into the animals via a femoral vein. Hearts were removed several minutes later and ischemic, partially ischemic and non-ischemic areas of the heart were dissected after they were visually identified from the intensity of dye staining. The respective tissues were homogenized in distilled water (1 g/ml) and a 200 µl aliquot of each homogenate was dissolved in 1.0 ml of NCS tissue solubilizer (Amersham/Searle). To each sample, 15 ml of Dimilume-30 solution (Packard Instrument Company, Inc., Downers Grove, IL) was added and radioactivity was assayed. Counting efficiency (approximately 30%) was monitored by the external standard channel ratio method. Background radioactivity was assayed using homogenates or plasma samples obtained from saline-infused animals.

E. (^{3}H)Ouabain Binding Studies

Affinity of glycoside binding sites on Na,K-ATPase for (^{3}H)-ouabain and the number of glycoside binding sites were estimated by the method developed by Akera and Cheng (1977). Ventricular muscles obtained in ischemic studies were homogenized at 0°C in 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA using a Potter-Elvehjem glass homogenizer with a motor driven Teflon pestle to the final concentration of approximately 40 mg of tissue per 1 ml of buffer solution. The ventricular muscle homogenates (final concentration in incubation medium, 0.3-0.4 mg protein/ml) were added to a pre-warmed (37°C for 5 min) mixture containing final concentrations of 1 mM $MgCl_2$, 1 mM Tris-phosphate, 10 mM Tris-HCl buffer (pH 7.5), 10 nM (^{3}H)ouabain and various concentrations of non-labeled ouabain (0, 20, 50, 100, 200, 500 or 1000 nM). The mixture was incubated at 37°C for 90 min to

attain equilibrium of the binding reaction. After incubation, the (^{3}H)ouabain binding reaction was terminated by the addition of non-labeled ouabain (final concentration, 0.1 mM). Bound and free (^{3}H)-ouabain were separated by filtering the aliquot through nitrocellulose Millipore filters. The filters were washed twice with 5 ml each of an ice-cold solution containing 0.1 mM nonradioactive ouabain, 15 mM KCl and 50 mM Tris-HCl buffer (pH 7.5) and dissolved in 1 ml of ethyleneglycol monomethyl ether. The radioactivity on the filter (bound ouabain) was assayed using a liquid scintillation spectrometer. Counting efficiency (approximately 30%) was monitored by the external standard channel ratio method. Saturable ouabain binding was calculated by subtracting the non-saturable binding observed in the presence of 0.1 mM non-labelled ouabain from the total binding. From these values, the number of specific (^{3}H)ouabain binding sites and the affinity of these sites for (^{3}H)ouabain were calculated by the method of Akera and Cheng (1977).

Fractional occupancy of the glycoside binding sites on Na,K-ATPase in non-ischemic, partially ichemic and ischemic tissues by digoxin during digoxin or saline infusion was estimated from the reduction in ATP-dependent (^{3}H)ouabain binding to the respective tissue homogenates. Tissues were homogenized in a 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA. The homogenates were added to a prewarmed (37°C) incubation mixture yielding final concentrations of 0.5-0.8 mg of protein per ml, 200 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH 7.5) and 20 nM (^{3}H)ouabain with or without 5 mM Tris-ATP. After a 2-min incubation at 37°C, the binding reaction was terminated

by adding a cold solution containing excess non-labeled ouabain which inhibits further binding of (^{3}H)ouabain. The mixture was immediately filtered through a nitrocellulose filter (Millipore Filter Corporation, Bedford, MA; type AA, pore size, 0.8 μm) to separate bound and free (^{3}H)ouabain. The filter was washed with two 5-ml aliquots of an ice-cold solution containing 0.1 mM nonradioactive ouabain, 15 mM KCl and 50 mM Tris-HCl buffer (pH 7.5) within 10 sec after the termination of the binding reaction. The radioactivity trapped on the filter (bound ouabain) was assayed using a liquid scintillation spectrometer after dissolving the filter in ethylene glycol monomethyl ether. The ATP-dependent (^{3}H)ouabain binding is the difference in values observed in the presence and absence of ATP. This value represents the binding of (^{3}H)ouabain to the glycoside binding sites on Na,K-ATPase in tissue homogenates (Allen et al., 1971) and its reduction indicates the previous occupancy of these sites by a glycoside (Ku et al., 1974). Since the digoxin-Na,K-ATPase complex has a relatively long half-life (Akera et al., 1974), the observed values are reasonable estimates of fractional occupancy of the glycoside binding sites by digoxin which takes place during digoxin infusion.

To estimate ischemic-induced changes in the effects of K^+ or Na^+ on the glycoside binding to Na,K-ATPase, ventricular muscle homogenates were incubated in the presence of 200 mM NaCl, 5 mM $MgCl_2$, 5 mM Tris-ATP, 50 mM Tris-HCl buffer (pH 7.5) and 0-150 mM KCl (medium A), or in the presence of 5 mM $MgCl_2$, 5 mM Tris-ATP, 50 mM Tris-HCl buffer (pH 7.5) and 0-300 mM NaCl (medium B). (^{3}H)ouabain binding was assayed as above and the specific binding was calculated as the difference in values observed in the presence and absence of ATP. The

affinity ($K_{0.5}$ value) of Na,K-ATPase for K^+ or Na^+ was estimated from the concentration of K^+ to cause a half-maximal inhibition of the specific (^3H)ouabain binding observed in Medium A, or from the concentration of Na^+ to cause a half-maximal stimulation of the specific (^3H)ouabain binding in Medium B.

F. Na,K-ATPase Studies

ATPase activity of the ventricular muscle homogenates was assayed by measuring the amount of inorganic phosphate (P_i) released from ATP (Bonting et al., 1961). Ventricular muscle homogenates were prepared as described above. Homogenates were added to a prewarmed (37°C) incubation mixture (final concentration of protein, 0.3-0.4 mg/ml) containing 5 mM $MgCl_2$ 5 mM sodium azide, 50 mM Tris-HCl buffer (pH 7.5), 5 mM Tris-ATP, 100 mM NaCl, 15 mM KCl and an indicated concentration of dihydrodigoxin in the presence or absence of 0.1 mM vanadate. After a 10-min incubation, the reaction was terminated by adding 1 ml of an ice-cold 0.8 M perchloric acid solution. The amount of inorganic phosphate (P_i) released from ATP was assayed by adding a color reagent and reading the light absorption of the mixture at 700 nM using a spectrophotometer (Gilford, Oberlin, OH). Mg-ATPase activity, assayed in the presence of 0.1 mM vanadate was subtracted from the value observed in its absence to calculate the Na,K-ATPase activity. Sodium azide was used to inhibit Mg-ATPase activity and the possible regeneration of ATP by oxidative phosphorylation (Ismail-Beigi and Edelman, 1971). Since Ca-ATPase activity was negligible under the present assay conditions, the vanadate-sensitive ATPase

activity could be taken as the representative of Na,K-ATPase activity. Essentially, the same results were obtained by using 1 mM ouabain.

G. $^{42}K^+$ or $^{86}Rb^+$ Uptake Binding

Sodium pump activity was estimated from the ouabain-sensitive $^{42}K^+$ or $^{86}Rb^+$ uptake by strips of right ventricular muscle or slices of left ventricular muscle, respectively. Langendorff preparations of guinea-pig heart were perfused with Krebs-Henseleit bicarbonate buffer solution (pH 7.5) at a control flow rate of 2.5 ml/g tissue/min, or at 5% of the control flow rate, or not perfused at all for 2 hr. Several preparations of the last group were reperfused for an additional 20-min period at the control flow rate. From the first two groups of preparations, thin ventricular strips were obtained and suspended in a tissue bath containing the above Krebs-Henseleit bicarbonate buffer solution maintained at 36°C and saturated with a 5% O_2-90% N_2-5% CO_2 gas mixture. The strips were electrically stimulated at either 1.5 or 7 Hz. Tracer amounts of $^{42}K^+$ were then added to the incubation medium and the $^{42}K^+$ uptake during a 10-min incubation period was assayed. The tissues were rinsed in a $^{42}K^+$-free solution, blotted on filter paper, and the radioactivity in the tissue was estimated using a gamma scintillation spectrometer (Searle Analytic Inc., Des Plaines, IL; model 1185). Ouabain-sensitive $^{42}K^+$ uptake is the difference in values observed in the presence and absence of 0.3 mM ouabain.

Ventricular strips obtained from the reperfused hearts did not respond to electrical stimulation, making it impossible to evaluate the viability of preparations on the effect of electrical stimulation.

Furthermore, in noncontracting tissues, the exchange of substances between interstitial fluid and the medium may be limited (Lullmann et al., 1979). Therefore, sodium pump activity was estimated from the more conventional method involving $^{86}Rb^{+}$ uptake using thin slices of the ventricular muscle. Approximately 0.5 mm thick slices were prepared by using a Stadie-Riggs tissue slicer (A.H. Thomas, Philadelphia, PA). The slices were incubated in a prewarmed (37°C) Krebs-Henseleit bicarbonate buffer solution containing 2 mM RbCl with tracer amounts of $^{86}Rb^{+}$ (specific activity, 0.16 Ci/mmol) and no K^{+}. After a 10-min incubation at 37°C, the slices were rinsed twice by immersing in separate K^{+}-free solutions for 20 sec each and blotting each time with filter paper. The radioactivity remaining in the slices was assayed using a gamma scintillation spectrometer. Ouabain-sensitive $^{86}Rb^{+}$ uptake is the difference in values observed in the absence and presence of 0.3 mM ouabain.

H. Nerve Activity Studies

Cats weighing 1.8-3.6 kg were anesthetized and prepared as described above. During surgical preparation, cats were immobilized with gallamine triethiodide (4 mg/kg) which caused muscle relaxation. In all animals, the first and second ribs on the left were removed extrapleurally and a thin postganglionic segment, the inferior cardiac efferent nerve, arising from the left stellate ganglion was carefully dissected out and mounted on a paired platinum electrode for monitoring electrical activity. A left thoracotomy was then performed and the pericardial sac opened to expose the left anterior descending

coronary artery. A silk thread was then passed through the myocardium surrounding the artery just below the left atrium. The ends of the silk sutures were fitted through a short piece of polyethylene tubing and the artery was occluded, when needed, by pulling on the suture through the tubing.

Neural discharges from the cardiac nerve were monitored with an oscilloscope, and the mean discharge rates were determined using a window discriminator and rate analyzer (Frederick Haer, Brunswick, ME) and displayed on a polygraph recorder. The femoral arterial blood pressure and lead II electrocardiogram were also recorded on the polygraph. After an equilibration period of 30 min, when the nerve activity was stable, the LAD was occluded for 60 sec and released. Following recovery of the occlusion-induced changes in nerve activity, digoxin infusion (60 μg/kg/hr) was started. When approximately 80-85% of the arrhythmogenic dose of digoxin had been administered, the LAD was again occluded for another 60 sec period and the electrical activity of the nerve was monitored. When ventricular arrhythmias finally developed, the 60 sec occlusion was again performed. When the blood pressure was reduced by LAD artery occlusion, animals were hemorrhaged, following release of occlusion and recovery of nerve activity, by collecting blood from the femoral artery until the blood pressure decreased to the same level as observed during occlusion. The blood was infused back into the animal after the hemorrhage experiment.

I. Miscellaneous Methods

Protein concentrations were determined by the method of Lowry et al., (1951), using bovine serum albumin as the standard.

J. Statistical Analysis

Statistical evaluations of the data were performed by student's t-test, paired t test, linear regression and two-way analysis of variance with or without block design. Criterion for significance was a probability value of less than 0.05.

RESULTS

A. Ischemia-induced Enhancement of the Arrhythmogenic Actions of Digitalis: Effects of Ischemia, and Reperfusion on Cardiac Sarcolemmal Na,K-ATPase Activity, on the Kinetic Parameters of Na,K-ATPase for (^{3}H)Ouabain Binding and on Sodium Pump Activity

The arrhythmogenic effects of digitalis glycosides have been shown to result from both a direct action on the heart and an indirect action involving the autonomic nervous system. A direct action of the glycoside on the heart has been shown to result from its binding to sarcolemmal Na,K-ATPase, a putative receptor for the glycoside (Akera and Brody, 1977). Since ischemia is well known to produce membrane damage, electrolyte imbalance and abnormal metabolism (Jennings et al., 1981), such effects may augment the direct action of the glycoside on the heart, by altering Na,K-ATPase activity, the sensitivity of Na,K-ATPase to the inhibitory effects of digitalis, or by reducing the reserve capacity of the sodium pump. Digitalis toxicity is proposed to occur when glycoside-induced inhibition of the sodium pump exceeds its reserve capacity, and the remaining pump becomes inadequate for maintaining a low intracellular sodium ion concentration. Therefore, these possibilities were studied using Langendorff preparations of guinea-pig hearts.

1. Isolated-Heart Perfusion Studies

In order to determine whether ischemia augments the arrhythmogenic effects of digitalis in the isolated guinea-pig heart, the

left anterior descending (LAD) coronary artery was completely occluded for 40 min before perfusion with an arrhythmogenic concentration of digoxin.

The perfusion of the heart at a constant flow rate of 2.5 ml/g tissue/min did not produce arrhythmias and the force of contraction remained stable for at least three hours. Coronary artery occlusion by itself also did not produce arrhythmias during the perfusion under the experimental condition (Figure 1a). The perfusion at a constant flow rate with either 1.8 or 2.5 μM digoxin produced a positive inotropic effect and then arrhythmias. Although the rate of onset of the positive inotropic effect of digoxin was similar in both control and LAD artery ligated animals, the onset of arrhythmias was significantly earlier in the LAD artery ligated animals for both concentrations of digoxin (Figure 2). The types of digoxin-induced arrhythmic contractions observed in control and the LAD artery ligated hearts are shown in Figure 1 (b-f). Extra beats following the normal stimulation-induced beat were present in all digoxin-induced arrhythmias. During the extra beat, the muscle failed to respond to electrical stimulation, probably due to refractoriness during repolarization. When the extra beat was complete, stimulation was again followed by contractions. All types of arrhythmic contractions shown were observed in both control and LAD-ligated hearts.

In order to examine the possbility that changes in the time to onset of digoxin-induced arrhythmias are due to alterations in the flow distribution caused by LAD artery ligation, several heart preparations were perfused under a constant pressure of 20 mmHg. In this

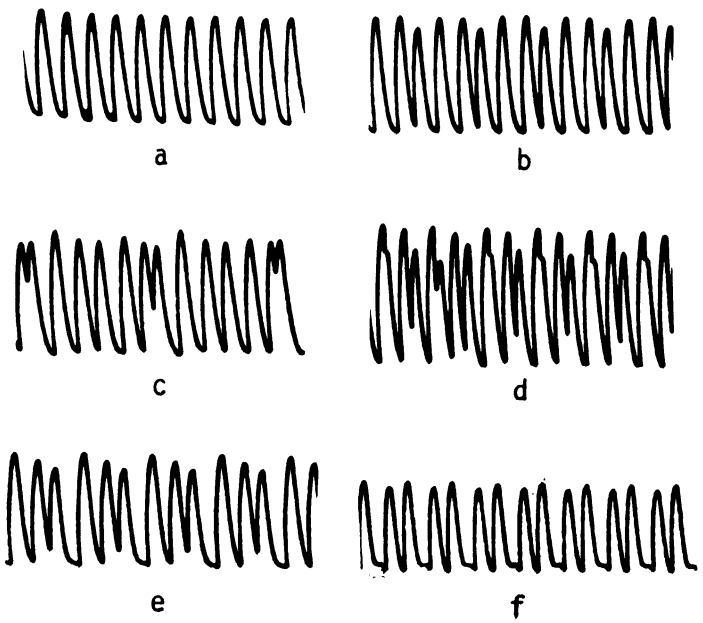

Figure 1

Figure 1. Effects of digoxin on the isometric twitch tension curves observed in Langendorf preparations of guinea-pig heart. The heart preparations were electrically stimulated at 1.5 Hz and perfused with Krebs-Henseleit bicarbonate buffer (pH 7.5) solution at 32°C. Following an equilibration period, the left anterior descending coronary artery was occluded, and digoxin (final concentration, 1.8 or 2.5 μM) was added to the perfusing solution 40 min later. Development of digoxin-induced arrhythmias was monitored by the twitch tension curves. a: Non-arrythmic beats observed before the onset of arrythmias in all preparations perfused with or without digoxin. b-f: Typical arrhythmic beats observed at the onset of digoxin-induced arrhythmias in both control (b,d,e) and occluded (c,f) preparations. Typical tracings are shown from several experiments.

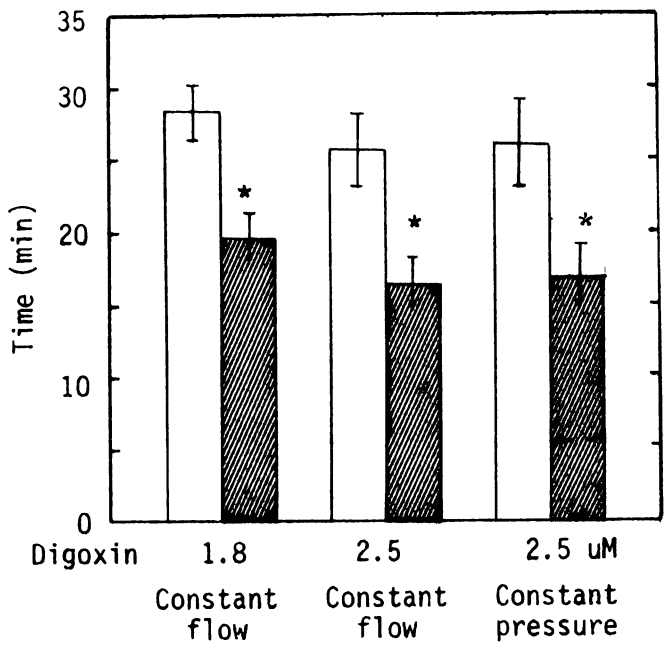

Figure 2

Figure 2. Effects of LAD artery ligation on the time of digoxin perfusion required to produce arrhythmias. See legend to Figure 1. Open bars: Control hearts without occlusion. Shaded bars: LAD artery occluded hearts. The rate of perfusion of the hearts were either at 2.5 ml/g tissue/min or at constant pressure of 20 mmHg. Each bar represents the mean of 6 experiments. Vertical lines indicate S.E. *Significantly different from the corresponding control value ($p<0.05$).

model too, 2.5 μM digoxin produced arrhythmias earlier in the LAD artery ligated than in control preparations (Figure 2). In these hearts, there were no clear differences between the two groups with respect to the types of contractions. These results demonstrate that LAD artery ligation sensitizes the isolated heart preparations to the arrhythmogenic actions of digoxin and that Langendorff preparations of guinea-pig hearts may be used to study the mechanisms of increased sensitivity of ischemic heart to digitalis.

2. Na,K-ATPase Studies

An enhanced digitalis sensitivity of the ischemic heart may be due to a change in Na,K-ATPase activity or its sensitivity to the inhibitory actions of the glycoside. Thus, enzyme activity of homogenates obtained from ischemic hearts and the sensitivity of the enzyme to the inhibitory actions of dihydrodigoxin were examined using globally ischemic guinea-pig heart preparations. Perfusion of Langendorff preparations was completely stopped or they were perfused at 5% of the control flow rate for 2 or 6 hr. Since digitalis action on the ischemic border zone may be important, heart preparations were perfused with severely-reduced flow rates (5% of the control flow rate). Subsequently, ventricular muscle homogenates were prepared and assayed for Na,K-ATPase in the absence and presence of dihydrodigoxin. This glycoside was selected because of its rapid rate of binding to the glycoside binding sites on Na,K-ATPase.

Perfusion of Langendorff preparations for 2 hr with a reduced flow rate (5% of the control rate) or no perfusion (0% of the control rate) failed to significantly alter Na,K-ATPase activity in

homogenates obtained from these preparations (Figure 3). Perfusion of Langendorff preparations for 6 hr with a reduced flow rate failed to alter enzyme activity whereas 6 hr of zero-perfusion caused a significant decrease in Na,K-ATPase activity (Figure 4). Dihydrodigoxin inhibited the enzyme in concentrations ranging from 30 to 300 μM. Sensitivity of Na,K-ATPase to the inhibitory action of dihydrodigoxin was not altered by ischemia, as indicated by the similar dihydrodigoxin concentrations necessary to cause a 50% inhibition (approximately 100 μM) of Na,K-ATPase in homogenates obtained from control or ischemic tissues (Figures 3 and 4).

The effect of reperfusion of ischemic tissues on Na,K-ATPase activity was examined since ischemia-induced myocardial damage may recover or may be further enhanced during reperfusion. In these studies, perfusion was completely stopped for 2 or 5 hr, and subsequently, the preparations were reperfused at a control flow rate (2.5 ml/g tissue/min) for an additional 1 hr period. After 2 or 5 hr of complete ischemia and 1-hr reperfusion, Na,K-ATPase activity of ventricular muscle homogenates, assayed as above, was significantly reduced (Figure 5). It should be noted that 2-hr ischemia failed to cause significant decreases in Na,K-ATPase activity. Therefore, reperfusion of ischemic tissues apparently enhanced the reduction in Na,K-ATPase activity caused by ischemia. Sensitivity of the remaining active enzymes to the inhibitory actions of dihydrodigoxin was unaffected by ischemia and reperfusion.

It is possible that ischemic tissues obtained from globally ischemic hearts may not be similar to the ischemic tissues formed in

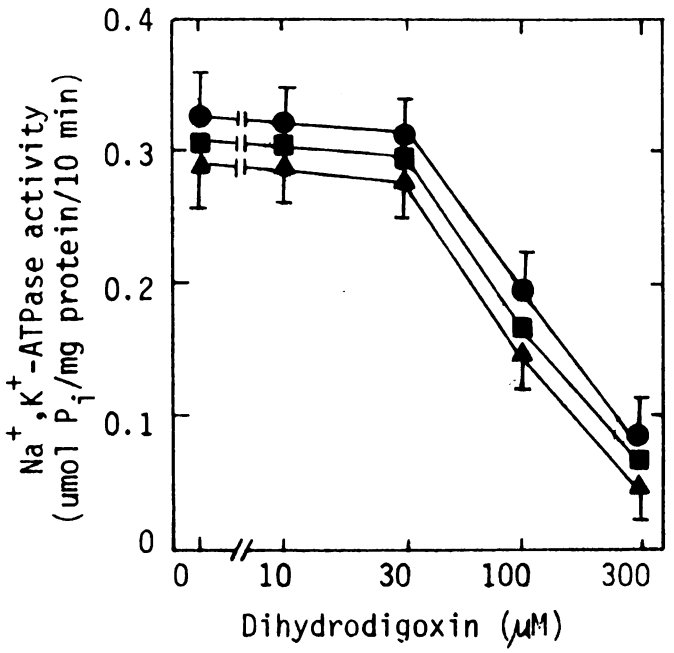

Figure 3

Figure 3. Effects of global ischemia on Na,K-ATPase activity and its inhibition by dihydrodigoxin. Langendorff preparations of guinea-pig hearts were perfused at control flow rate of 2.5 ml/g tissue/min (●), or 5% (■) or 0% (▲) of the control flow rate for 2 hr. Subsequently, ventricular muscle was homogenized and assayed for Na,K-ATPase activity. Na,K-ATPase activity was calculated as the difference in values observed in the absence and presence of 0.1 mM vanadate. Each point represents mean of 6 experiments and vertical lines indicate S.E. None of the values were significantly different from the corresponding control values ($p<0.05$).

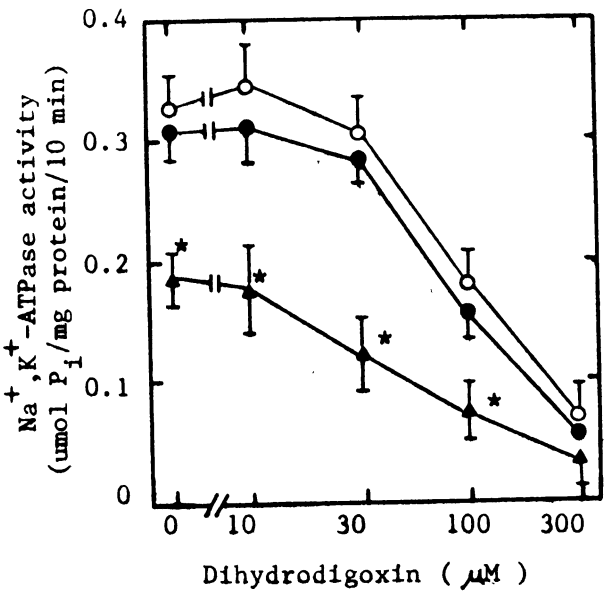

Figure 4

Figure 4. Effects of global ischemia on Na,K-ATPase activity and its inhibition by dihydrodigoxin. Langendorff preparations of guinea-pig hearts were perfused at control flow rate of 2.5 ml/g tissue/min (○), or 5% (●) or 0% (▲) of the control flow rate for 6 hr. Subsequently, ventricular muscle was homogenized and assayed for Na,K-ATPase activity. Na,K-ATPase activity was calculated as the difference in values observed in the absence and presence of 0.1 mM vanadate. Each point represents mean of six experiments and vertical lines indicate S.E. *Significantly different from the corresponding control value ($p<0.05$).

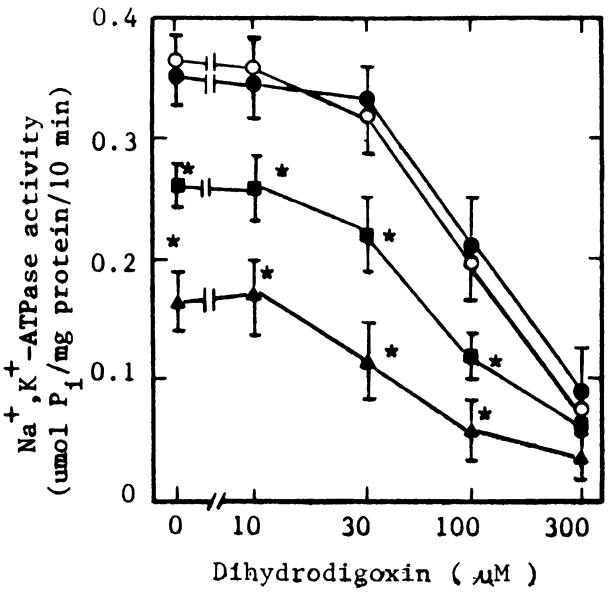

Figure 5

Figure 5. Effects of reperfusion on Na,K-ATPase activity and its inhibition by dihydrodigoxin. Langendorff preparations of guinea-pig heart were perfused at 0% of the control flow rate for 2 hr (■) or 5 hr (▲) and subsequently reperfused at control flow rates for 1 hr. Control preparations were perfused for 3 hr (○) or 6 hr (●). Na,K-ATPase activity of the ventricular homogenates were assayed, Na,K-ATPase activity was calculated as the difference in values observed in the absence and presence of 0.1 mM vanadate. Each point represents the mean of six experiments and vertical lines indicate S.E. *Significantly different from the corresponding control values ($p<0.05$).

coronary artery occluded hearts with respect to uniformity of ischemia and metabolic events. In particular, the ischemic border zone, proposed to exist in ischemic hearts, may not be mimicked simply by reducing the perfusion flow rate. Therefore, the effect of coronary artery occlusion-induced ischemia on Na,K-ATPase activity was examined using ischemic tissues obtained from LAD artery occluded hearts of anesthetized cats. This animal species was used because preliminary studies (see Part B) have indicate that LAD artery ligation enhanced the arrhythmogenic effects of digitalis, and the size of hearts is relatively large, allowing sufficient quantities of ischemic tissues to be obtained for analysis.

Following LAD artery ligation, either digoxin was infused intravenously at a rate of 60 μg/kg/hr until the onset of arrhythmias or 0.9% saline solution was infused for a similar period of time. A dye solution was infused and non-ischemic (NI), partially ischemic (PI) and completely ischemic (CI) ventricular tissues were obtained (see Figure 6), and the homogenates of these tissues were assayed for Na,K-ATPase activity. Animal preparations without LAD artery occlusion were also infused with digoxin and heart tissues obtained at the onset of arrhythmias.

Na,K-ATPase activities of the homogenates obtained from perfused, partially perfused and non-perfused tissues in saline infused animals were all similar (Figure 7). Na,K-ATPase activities of the homogenates obtained from partially ischemic and non-ischemic tissues in digoxin-infused animals were significantly reduced compared to the corresponding control values in saline-infused animals. Na,K-ATPase activity of the homogenates obtained from completely ischemic

Figure 6

Figure 6. A cross-section of the heart showing stained and non-stained ventricular muscle following infusion with a dye at the onset of digoxin-induced arrhythmias in LAD artery ligated cats.

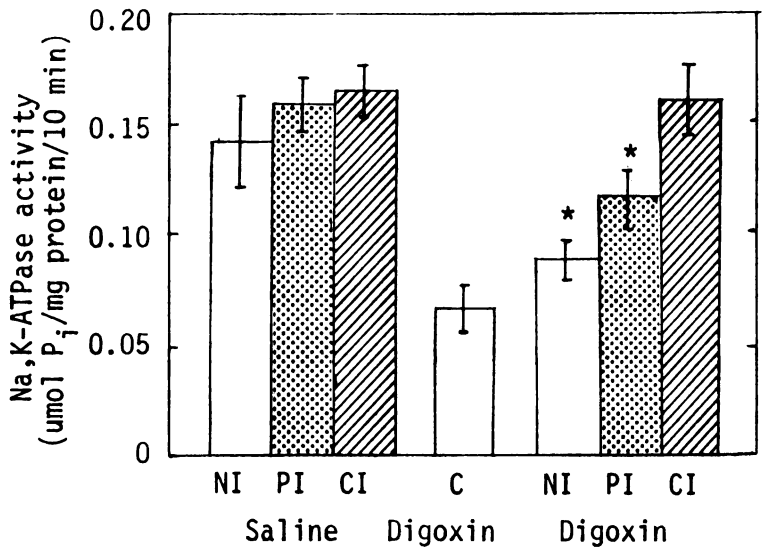

Figure 7

Figure 7. Effects of ischemia on Na,K-ATPase activity in ventricular muscle homogenates obtained from anesthetized cat hearts. LAD artery was completely ligated and either digoxin or similar volume of 0.9% saline was infused 40 min later. At the onset of arrhythmias in digoxin-infused animals or 3 hr later in saline-infused animals, dye was infused and completely stained (CI), partially stained (PI) or nonstained (NI) ventricular tissues were prepared. Homogenates obtained from these tissues were assayed for Na,K-ATPase activity. Na,K-ATPase activity is the difference in values observed in the presence and absence of 0.3 mM ouabain. Open bars: Na,K-ATPase activity in non-ischemic (NI); dotted bars: in partially ischemic (PI); and shaded bars: in completely ischemic (CI) tissue homogenates. The smallest open bar (C) represents Na,K-ATPase activity in tissue homogenates obtained from control (no coronary artery occlusion), digoxin infused animals. Each bar represents the mean of five experiments. Vertical lines indicate the S.E. *Significantly different from the corresponding control value ($p<0.05$).

tissues was, however, similar to the control value suggesting that ischemia itself did not inhibit the enzyme activity. In digoxin-infused animals with no LAD-ligation, Na,K-ATPase activity of the ventricular homogenates was lower than that from LAD artery ligated. hearts. This is probably due to a longer infusion period in control animals, and therefore a larger dose of digoxin infused for the development of arrhythmias.

Thus, ischemia produced globally, or by coronary artery occlusion, failed to alter Na,K-ATPase when the period of ischemia was 2 or 3 hr, respectively. A longer period of ischemia (6 hr) or reperfusion following 2 hr of ischemia reduced Na,K-ATPase activity by decreasing the number of active Na,K-ATPase molecules, but the sensitivity of the residual enzyme to the inhibitory effects of digitalis remained unaltered. In animals in which LAD coronary artery was ligated and infused with digoxin, the completely ischemic tissue were not reduced in enzyme activity. This is probably due to absence of digoxin in this tissue.

3. (^3H)Ouabain Binding Studies

The elevated sensitivity of the ischemic heart to the toxic actions of the cardiac glycosides may result from a change in glycoside binding to sarcolemmal Na,K-ATPase. Therefore, possible changes in the number of glycoside binding sites and their affinity for (^3H)-ouabain binding were determined. Initial velocity of (^3H)ouabain binding reaction was also estimated to determine whether ischemia alters the rate of glycoside binding to Na,K-ATPase.

Ventricular muscle homogenates obtained from globally ischemic Langendorff preparations were incubated with (^{3}H)ouabain for 90 min, allowing the binding reaction to reach equilibrium. The number of (^{3}H)ouabain binding sites (Bmax) and the affinity of the sites for ouabain (Kd value) were calculated as described previously (Akera and Cheng, 1977). A reduced perfusion rate (5% of the control flow rate) for 2 or 6 hr, or a zero-perfusion for 2 hr, failed to significantly alter either Bmax or Kd value in ventricular muscle homogenates (Table 1); however, zero-perfusion for 6 hr, or 2 or 5 hr of zero-perfusion followed by 1 hr of reperfusion, significantly reduced the number of binding sites, without affecting the affinity of the remaining binding sites for ouabain.

Ventricular muscle homogenates obtained from hearts of anesthetized cats in which the LAD artery was ligated and then infused with either saline or digoxin were prepared as described above, and assayed for initial velocity of ATP-dependent (^{3}H)ouabain binding. (^{3}H)Ouabain binding was similar in ventricular muscle homogenates obtained from non-ischemic (NI), partially ichemic (PI) and completely ischemic (CI) tissues (Figure 8). In digoxin-infused animals, ATP-dependent (^{3}H)ouabain binding to the non-ischemic and partially ischemic ventricular muscle homogenates was significantly less than the values observed in similar tissues obtained from saline-infused animals (control). (^{3}H)Ouabain binding to completely ischemic muscle homogenates was slightly, but not significantly, reduced from the control value. This is probably because of the absence of digoxin in the ischemic tissues as a result of lack of perfusion. Therefore, the

TABLE 1

Effect of Global Ischemia and Reperfusion on Kinetic Parameters of Na,K-ATPase for (^{3}H)Ouabain Binding Reaction

Langendorff preparations of guinea-pig heart were perfused at control flow rate of 2.5 ml/g/min or 5% or 0% of the control flow rate for either 2 or 6 hr. In some preparations, hearts were perfused at 0% for 2 or 5 hr and reperfused at control rate for 1 hr. Ventricular muscle was then homogenized and incubated with (^{3})ouabain in the presence of 1 mM $MgCl_2$, 1 mM Tris-PO_4, 10 mM Tris-HCl buffer (pH 7.5) and various concentrations of non-labeled ouabain (0-1000 nM). After a 90-min incubation, specific (^{3}H)ouabain binding was calculated by subtracting non-specific binding observed in the presence of 0.1 mM ouabain from the total binding observed in its absence. The affinity of the binding sites for ouabain (Kd value) and the method of Akera and Cheng (1977). Each value represents mean ± S.E. of six experiments.

Perfusion	(time)	Bmax[1]	Kd
		Pmol/mg protein	nM
Control[2]	(2 hr)	6.30±0.38	110.9±5.5
5%	(2 hr)	5.85±0.45	95.1±7.9
0%	(2 hr)	5.30±0.34	91.1±7.0
Control[2]	(6 hr)	5.54±0.43	90.2±8.3
5%	(6 hr)	4.41±0.27[3]	80.8±3.4
0%	(6 hr)	3.80±0.21[3]	103.0±6.6
Control[2]	(3 hr)	6.05±0.51	105.8±4.2
0% (2 hr) plus control[2]	(1 hr)	4.20±0.36[3]	107.5±7.5
Control[2]	(6 hr)	6.18±0.40	102.0±3.7
0% (5 hr) plus control[2]	(1 hr)	3.50±0.41[3]	101.2±7.4

[1]Bmax (binding site concentration) and Kd (apparent dissociation constant).

[2]2.5 ml/g tissue/min.

[3]Significantly different from control values ($p<0.50$).

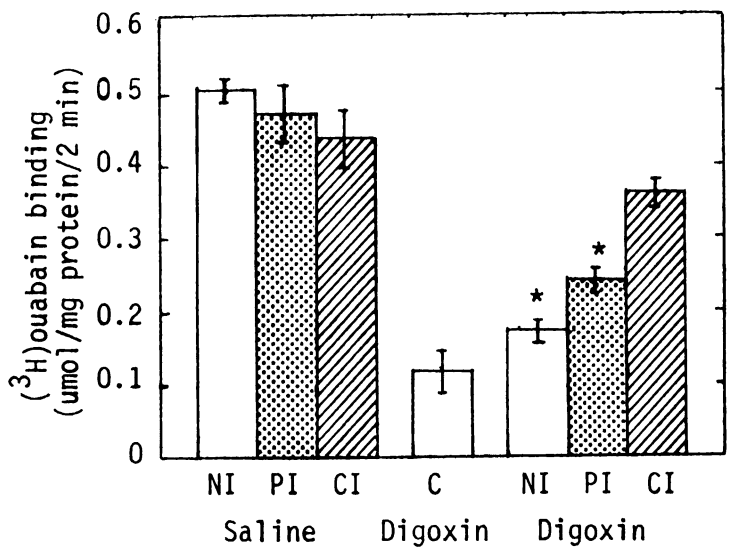

Figure 8

Figure 8. Effects of ischemia on the ATP-dependent (^{3}H)ouabain binding to ventricular muscle homogenates obtained from anesthetized cat hearts. See legend to Figure 6. Homogenates obtained from completely ischemic (CI, shaded bars), partially ischemic (PI, dotted bars) and non-ischemic (NI, open bars) ventricular muscle were assayed for (^{3}H)ouabain binding. Specific (^{3}H)ouabain binding is the difference in value observed in the presence and absence of ATP. The smallest open bar (C) represents (^{3}H)ouabain binding to homogenates obtained from control, digoxin-infused animals. Each bar represents the mean of five experiments. Vertical lines indicate the S.E. *Significantly different from the corresponding control value ($p<0.05$).

glycoside binding sites were not occupied by digoxin. In digoxin-infused animals in which the LAD artery was not occluded, the initial velocity of (^{3}H)ouabain binding to the ventricular muscle homogenates prepared at the onset of glycoside-induced arrhythmias was slightly lower than the value observed in homogenates obtained from occluded animals. Thus, fractional occupancy of the glycoside binding sites on Na,K-ATPase by digoxin was non-uniform in LAD artery ligated hearts and was less in ischemic tissues than in non-ischemic tissues.

The binding of the cardiac glycoside to Na,K-ATPase is elevated by increased intracellular Na^+ or decreased extracellular K^+ (Akera and Brody, 1977). Therefore, possible changes in the effects of these cations to stimulate or inhibit the specific (^{3}H)ouabain binding were examined in ventricular muscle homogenates obtained from globally ischemic heart preparations. After a reduced perfusion (5% of control flow rate) or zero-perfusion for 6 hr, or after 2 or 5 hr of zero-perfusion followed by 1 hr of reperfusion, the concentration of Na^+ to cause a half-maximal stimulation of the glycoside binding or the concentration of K^+ to cause a half-maximal inhibition of the binding was unchanged (Table 2). Thus, a 2-hr ischemia, whether produced globally by reducing or stopping the perfusion of the whole heart, or produced locally by coronary artery occlusion, failed to affect the number of glycoside binding sites or their affinity for ouabain. A much longer ischemia (6 hr) or reperfusion following 2- or 5-hr ischemia markedly reduced the number of binding sites without affecting their affinity for ouabain, Na^+ and K^+.

TABLE 2

Concentrations of K^+ and Na^+ Affecting Specific Binding of (^{3}H)Ouabain to Na,K-ATPase During Ischemia

Langendorff preparations of guinea-pig heart were perfused as described in the legend to Table 1. The ventricular muscle homogenates were incubated in a medium containing 200 mM NaCl, 5 mM $MgCl_2$, 5 mM Tris-ATP, 50 mM Tris-HCl buffer (pH 7.5), 10 nM (^{3}H)ouabain and KCl (0-150 mM), or in a similar medium without KCl containing 0-300 mM NaCl. Each value represents the mean ± S.E. of six experiments.

Perfusion	(time)	K^+ [1] (mM)	Na^+ [2] (mM)
Control	(3 hr)	2.75±0.10	32.8±1.0
Control	(6 hr)	2.90±0.20	30.5±1.5
5%	(6 hr)	2.80±0.10	30.6±1.7
0%	(6 hr)	3.00±0.10	31.6±1.3
0% (2 hr) + control	(1 hr)	3.10±0.26	30.2±2.9
0% (5 hr) + control	(1 hr)	2.60±0.15	32.0±4.0

None of the values were significantly different from the corresponding control values ($p<0.05$).

[1] The concentration of K^+ to cause a 50% inhibition of the specific ouabain binding.

[2] The concentration of Na^+ to cause a half-maximal stimulation of the specific ouabain binding.

4. $^{42}K^+$ or $^{86}Rb^+$ Uptake Studies

Myocardial ischemia may elevate the digitalis sensitivity of the heart by decreasing either the sodium pump activity or the reserve capacity of the sodium pump. In order to examine these possibilities, ventricular slices were prepared from isolated guinea-pig hearts which were perfused at 5% of the control flow rate or not perfused for 2 hr, or subjected to zero-perfusion for 2 hr and subsequently reperfused at the control flow rate for 20 min. Activity of the sodium pump was estimated from the specific (ouabain-sensitive) $^{86}Rb^+$ uptake. In these preparations, specific $^{86}Rb^+$ uptake accounted for approximately 50% of the total uptake, the remaining 50% representing nonspecific uptake (Figure 9). The specific $^{86}Rb^+$ uptake was unaffected by either the reduced perfusion or zero-perfusion for 2 hr; however, 2 hr of zero-perfusion followed by 20 min reperfusion significantly reduced specific $^{86}Rb^+$ uptake by ventricular slices obtained from these preparations. It should be noted that nonspecific $^{86}Rb^+$ uptake was also reduced in these preparations.

The reserve capacity of the sodium pump may be estimated from the difference in the specific $^{42}K^+$ uptake by the tissues when stimulated at 7 and 1.5 Hz. Since thin slices of ventricular muscle do not respond to electrical stimulation by visible contraction, right ventricular strips were used in these experiments. In ventricular strips obtained from non-ischemic preparations, the specific $^{42}K^+$ uptake observed at 7 Hz stimulation was significantly greater than that observed at 1.5 Hz stimulation (Figure 10). The specific $^{42}K^+$ uptake by ventricular muscle strips obtained from Langendorff

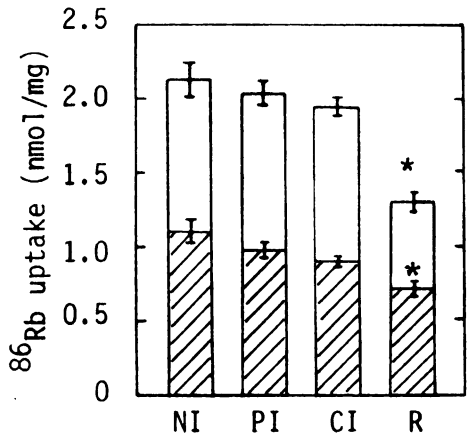

Figure 9

Figure 9. Effects of ischemia, and reperfusion on $^{86}Rb^{+}$ uptake in ventricular slices of guinea-pig heart. Langendorff preparations of guinea-pig heart were perfused at control flow rate of 2.5 ml/g tissue/min, 5% (PI) or 0% (CI) of the control rate for 2 hr, or 0% of the control rate for 2 hr and reperfused at control rate for 20 min (R). Ventricular slices were prepared and incubated at 37°C in modified Krebs-Henseleit bicarbonate buffer (pH 7.5) solution containing 2 mM RbCl and no K^{+}, and $^{86}Rb^{+}$ uptake by the tissues was estimated 10 min later. Specific $^{86}Rb^{+}$ uptake (open bars) is the difference in values observed in the absence (whole bars) and presence (shaded bars) of 0.3 mM ouabain. Each bar represents the mean of six experiments. Vertical lines indicate S.E. (Significantly different from the control value ($p<0.05$).

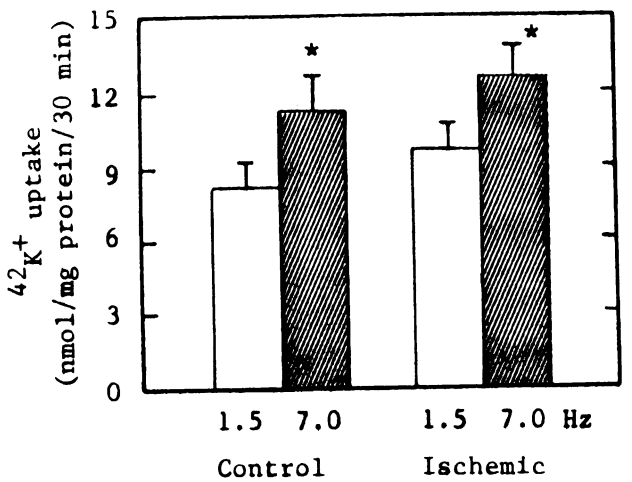

Figure 10

Figure 10. Effects of ischemia on the specific $^{42}K^+$ uptake by ventricular strips of guinea-pig heart. Langendorff preparations of guinea-pig heart were perfused at the control flow rate of 2.5 ml/g tissue/min or 5% of the control rate for 2 hr. Subsequently, right ventricular strips were prepared and incubated in Krebs-Henseleit bicarbonate buffer solution (pH 7.5) containing $^{42}K^+$. Preparations were electrically stimulated at either 1.5 (open bars) or 7 Hz (shaded bars) and $^{42}K^+$ uptake was estimated 10 min later. Specific $^{42}K^+$ uptake is the difference in uptake observed in the absence and presence of 0.3 mM ouabain. Each bar represents the mean of seven experiments. Vertical lines indicate S.E. *Significantly different from the control value ($p<0.05$).

preparations perfused for 2 hr at a reduced flow rate (5% of the control flow rate) was slightly higher than corresponding values observed with control (non-ischemic) preparations. The difference in the specific $^{42}K^{+}$ uptake observed at 1.5 and 7 Hz stimulation, however, was not altered by partial ischemia. These results indicate that sodium pump activity was not significantly affected by the partial reduction or complete stopping of the perfusion for 2 hr. The reserve capacity of the sodium pump was also unchanged by 2 hr of reduced perufsion.

In summary, coronary artery occlusion significantly enhanced the arrhythmogenic actions of digitalis in isolated perfused guinea-pig heart. This effect occurred within 80 min following coronary occlusion. However, Na,K-ATPase activity, its sensitivity to the inhibitory effects of digitalis, number of glycoside binding sites and their affinity for ouabain, K^{+} and Na^{+}, sodium pump activity, and the reserve capacity of the sodium pump were not affected by 120 min of ischemia. Therefore, although the primary mechanism of digitalis sensitization appears to be by a direct action on the heart, it is not by an enhanced glycoside effect on the cardiac sarcolemmal Na,K-ATPase. It is possible, however, that the lack of effect of digoxin in completely ischemic tissues may contribute to the reduced tolerance of the heart to digitalis by causing a non-uniform digitalis effect on the heart. This problem was further examined and is described in Part C.

Although the direct action of digitalis is sufficient to account for the enhanced toxicity in the ischemic heart, one cannot rule out the additional influence of indirect effects of digitalis via

the autonomic nervous system. Therefore, the indirect effects of digitalis on the sensitivity of the ischemic hearts to the glycosides were examined next.

B. Ischemia-induced Enhancement of Arrhythmogenic Actions of Digitalis: Involvement of the Sympathetic Nervous System

Results of studies in Part A have demonstrated that ischemia enhances the arrhythmogenic effects of digitalis by a direct action on the heart. It has been shown that in patients with myocardial ischemia, as well as in experimental animals with coronary artery occlusion, the catecholamine concentrations in the blood are significantly elevated (Valorie et al., 1967; Staszewska-Barczak, 1971). In animals, coronary artery occlusion has been shown to produce alterations in sympathetic discharge (Brown and Malliani, 1971) and vagal afferent nerve activities (Kedzi et al., 1974). Since the effect of digitalis on the heart has been demonstrated to be affected by the autonomic nervous system (Gillis and Quest, 1979), the possibility that the nervous system might influence the arrhythmogenic effects of digitalis on the ischemic heart strongly exists. Therefore, intact animals were employed to examine this possibility.

1. Whole Animal Studies

In order to study the role of the sympathetic nervous system in the reduced tolerance of the ischemic heart to the toxic effects of digitalis, sympathetic discharge to the heart was interrupted surgically or pharmacologically. Anesthetized cats were either left neurally intact, bilaterally vagotomized, or bilaterally vagotomized and spinal cord sectioned at C-1 or pretreated with dl-propranolol. After a 40-min occlusion of the left anterior descending coronary

artery, digoxin (60 μg/kg/hr) was infused until the onset of ventricular arrhythmias. In four animals in which the coronary artery was occluded, the intravenous infusion of 0.9% saline solution did not produce arrhythmias for the entire experimental period of 4 hr. Figure 11 shows typical electrocardiograms and blood pressure in neurally-intact (control) and LAD artery ligated animals infused with digoxin. In all animal preparations, digoxin infusion caused a gradual rise in blood pressure, and a gradual fall in heart rate. Blood pressure was not significantly altered by occlusion itself although in some animals, a slight and transient fall was observed. The mean blood pressures observed before and at various time points after digoxin infusion were similar in both coronary artery occluded and non-occluded hearts. Coronary artery occlusion caused S-T segment elevation which became more pronounced with time. The onset of digoxin-induced arrhythmias was accompanied by QRS complexes with markedly greater amplitudes and disappearance of P waves in both LAD artery occluded and control animals. However, in either neurally intact or bilaterally vagotomized animal preparations, the onset of arrhythmias was earlier in LAD artery occluded than in control, non-occluded preparations (Figure 12). The differences in the dose of digoxin required to produce arrhythmias in control and LAD artery ligated animals were similar in intact and vagotomized animals.

Bilateral vagotomy caused an initial rise in blood pressure that returned to control levels within 30 min. In these animals, spinal cord section at C-1 produced a dramatic and immediate increase in blood pressure and heart rate followed by a gradual decline to a

Figure 11. Electrocardiogram (EKG) and femoral arterial blood pressure (BP) during digoxin infusion in coronary artery occluded and non-occluded anesthetized cats. Panel A (from left to right): Electrocardiogram and blood pressure observed before digoxin infusion, after 100 min of digoxin infusion, and at the onset of arrhythmias. Panel B: Electrocardiogram and blood pressure observed 20 min after LAD occlusion, after 100 min of digoxin infusion with occlusion, and at arrhythmia. Typical tracings are shown from several experiments.

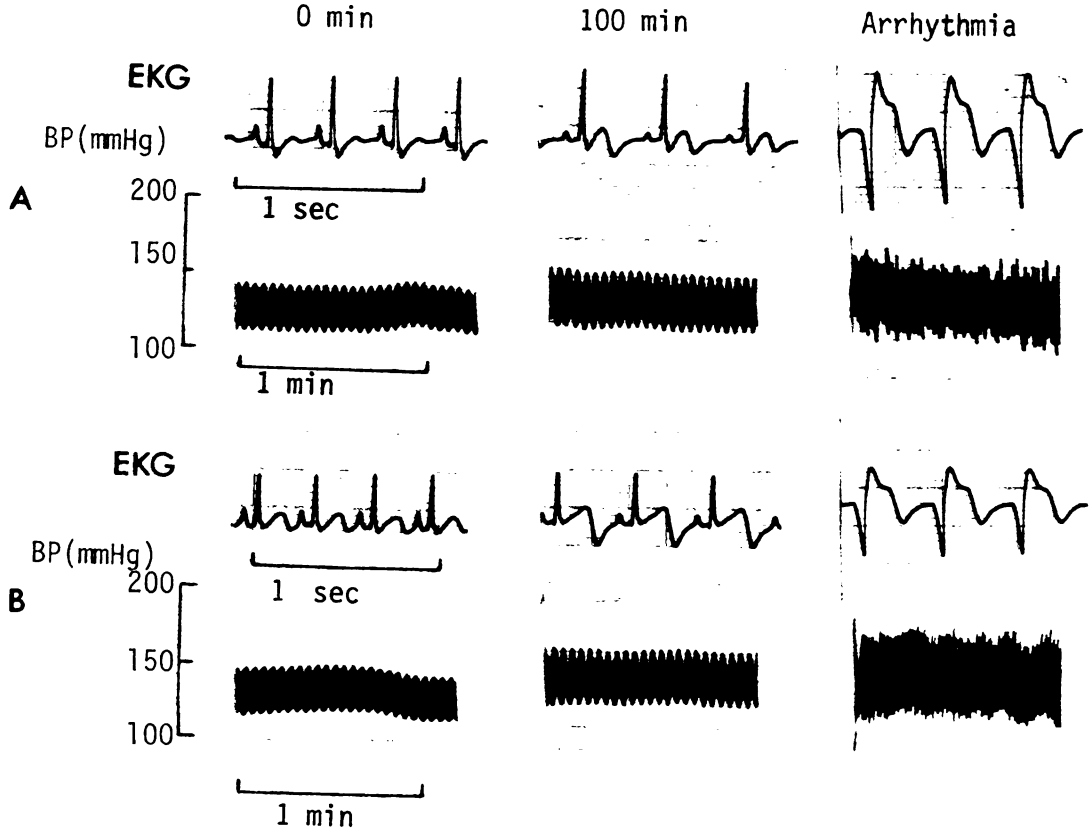
0 min
100 min
Arrhythmia
EKG
BP(mmHg)
A
200
150
100
1 sec
1 min
EKG
BP(mmHg)
B
200
150
100
1 sec
1 min

Figure 11

level approximately 60 mmHg below the pre-surgical blood pressure. The mean blood pressure remained at approximately 80-100 mmHg. Such effects indicated success of C-1 spinal section. In these C-1 sectioned animal preparations, the dose of digoxin required to produce arrhythmias was significantly greater than that in neurally-intact animals in both coronary artery occluded and non-occluded animals (Figure 14). The differences in the dose of digoxin required to produce arrhythmias in control and LAD artery ligated animals were similar in neurally-intact and C-1 sectioned animals. These results indicated that C-1 spinal section did not influence the ischemia-induced sensitivity to the arrhythmogenic actions of digitalis.

In animal preparations pretreated with dl-propranolol, the effect of isoproterenol-HCl on the heart rate was examined to test the completeness of beta-adrenergic receptor blockade (Figure 13). Concentrations of isoproterenol-HCl (0.1-1.0 μg/kg) produced marked dose-dependent increases in heart rate which were completely blocked by the dl-propranolol dose-regimen used in this study. In these animals without LAD artery ligation the dose of digoxin required to produce arrhythmias was greater than that in control, untreated animals. The effect of dl-propranolol pretreatment on the dose of digoxin required to produce arrhythmias was slightly greater than the effect of C-1 section, but the difference in dose was not statistically significant. These results demonstrate that bilateral vagotomy and/or sympathectomy produced by either spinal transection or beta-adrenergic receptor blockade failed to influence the increase in digitalis sensitivity of the ischemic heart.

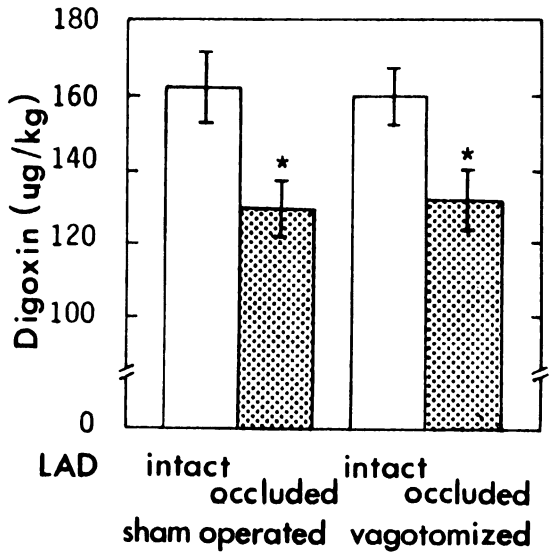

Figure 12

Figure 12. Effects of LAD artery ligation on the dose of digoxin required to produce arrhythmias in neurally intact or bilaterally vagotomized anesthetized cats. Sixty min following bilateral vagotomy or sham operation, the left anterior descending coronary artery was occluded. Forty min later, digoxin infusion (60 µg/kg/ hr) was started in LAD artery occluded (shaded bars) and non-occluded animals (open bars) and the time to the onset of glycoside-induced arrhythmias was monitored. Each bar represents the mean of six experiments. Vertical lines indicate the S.E. *Significantly different from control values ($p<0.05$).

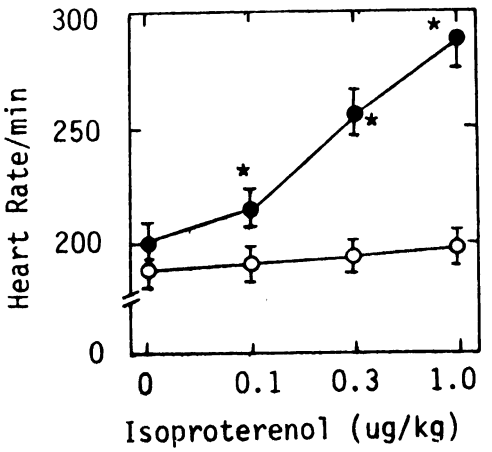

Figure 13

Figure 13. Effects of isoproterenol-HCl on the heart rate in the absence and presence of dl-propranolol-HCl in anesthetized cats. An intravenous loading dose of dl-propranolol (1 mg/kg) was followed by a slow continuous infusion (1 μg/kg/min). Thirty min later, an intravenous bolus injection of 0.1, 0.3 and 1.0 μg/kg isoproterenol-HCl were given at 20 min intervals and the changes in the heart rate was monitored. Control animals were similarly tested with isoproterenol-HCl. Peak chronotropic effects were noted and plotted against the isoproterenol concentrations. Each point represents the mean of six experiments. Vertical lines indicate S.E. *Significantly different from the corresponding control values ($p<0.05$).

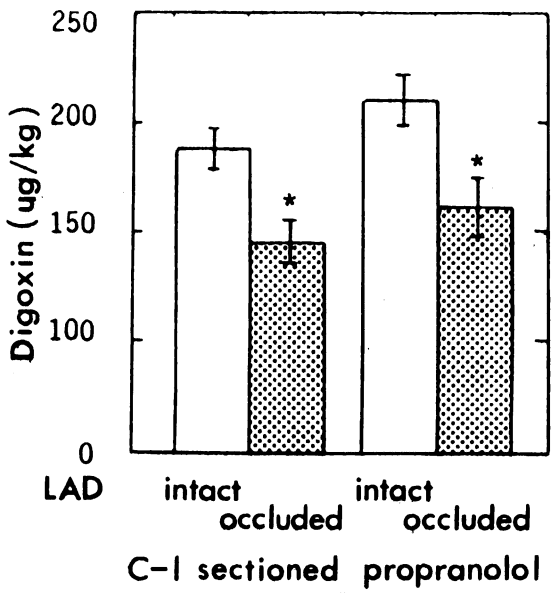

Figure 14

Figure 14. Effects of LAD artery ligation on the dose of digoxin required to produce arrhythmias in C-1 sectioned or dl-propranolol-treated anesthetized cats. Sixty min following bilateral vagotomy and C-1 section or propranolol treatment, LAD was occluded. Digoxin infusion (60 μg/kg/hr) was started in both LAD-occluded (shaded bars) and control (open bars) preparations 40 min later and the time to the onset of glycoside-induced arrythmias was monitored. Each bar represents the mean of five experiments. Vertical lines indicate S.E. *Significantly different from the control values ($p<0.05$).

2. Plasma and Myocardial Digoxin Concentration Studies

In order to examine whether the animals in which the coronary artery was occluded have altered digoxin pharmacokinetics, the plasma digoxin concentration was assayed at various time points following digoxin infusion. Either specifically labeled 12α-^{3}H-digoxin or unlabeled digoxin was infused and the plasma digoxin was estimated by assaying for radioactivity or by radioimmunoassay, respectively. An intravenous infusion of digoxin (60 μg/kg/hr) to control animals caused a linear increase in plasma glycoside concentration (Figure 15). Similar digoxin infusion to coronary-occluded animals changed neither the rate of increase nor the concentration of digoxin in plasma at any time period from the corresponding control values. The plasma digoxin concentration at the onset of arrhythmia was, however, significantly less in LAD artery occluded than in control animals. Since the method of assay for digoxin was the same for any paired group of animals (Table 3), these results indicate that a lower plasma glycoside concentration was present at the onset of arrhythmias in LAD artery occluded animals than control animals and that this was not due to altered digoxin pharmacokinetics. It is possible that other factors such as heart rate may affect digoxin binding to cardiac tissues since the rate of glycoside binding has been shown to be frequency-dependent (Yamamoto et al., 1979). This possibility was examined by determining myocardial digoxin uptake in LAD artery occluded and control animals infused with radioactive digoxin. Cardiac tissues were prepared from both groups of animals and assayed for radioactivity to estimate tissue-bound digoxin.

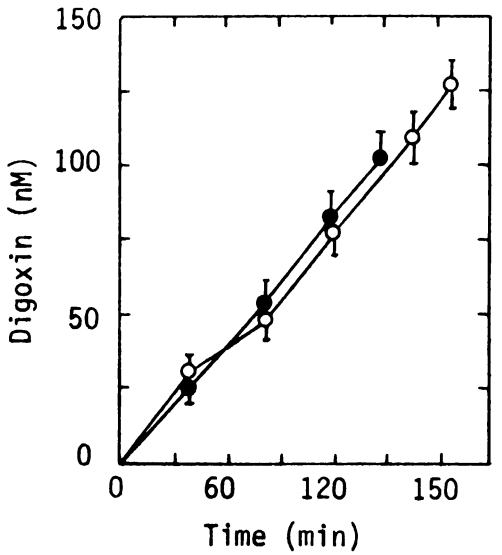

Figure 15

Figure 15. Effects of LAD artery ligation on plasma digoxin concentration on anesthetized cats. Digoxin solution was infused at a rate of 60 μg/kg/hr, and blood samples were obtained at various time points and digoxin concentration in the plasma was determined by radioimmunoassay. Open circles: control, unoccluded animals. Filled circles: the left anterior descending coronary artery was occluded 40 min prior to digoxin infusion. Each point represents the mean of six experiments. Vertical lines indicate S.E. None of the values were significantly different from the corresponding control values ($p<0.05$).

TABLE 3

Plasma Digoxin Concentrations at the Onset of Digoxin-induced Ventricular Arrhythmias in Coronary-occluded and Non-occluded Anesthetized Cats

See legends for Figures 11 and 13. Blood samples obtained from the femoral artery at the onset of ventricular arrhythmias were centrifuged at 1000 rpm for 10 min. The plasma samples thus obtained were assayed for digoxin by radioimmunoassay or by assaying for the radioactivity in the plasma samples obtained from animals infused with (^{3}H)digoxin (the last pair of groups in the table). Each value represents the mean ± S.E. of five experiments.

Animal Preparations	Digoxin (nM)
Neurally intact	127.9± 4.6
Intact with occlusion	108.8± 2.9*
Bilateral vagotomy	116.4± 5.6
Vagotomy with occlusion	94.4± 4.8*
C-1 section + vagotomy	151.3± 9.9
C-1 section with occlusion + vagotomy	132.8± 3.8*
dl-propranolol + vagotomy	128.8± 9.7
dl-propranolol with occlusion + vagotomy	93.9±11.2*

*Significantly different from the corresponding control values ($p<0.05$).

Figure 16 shows myocardial digoxin bound at the onset of arrhythmia in both LAD artery occluded and control animals. Since the time to onset of arrhythmias was longer in control than LAD artery occluded animals, the total amount of digoxin infused to the LAD artery occluded animals was less than that of the control animals. Digoxin concentrations in hearts of control animals were significantly greater than that in LAD artery occluded animals. Furthermore, ischemic areas of the heart contained much less digoxin than non-ischemic areas, probably due to reduced blood flow to these tissues following coronary occlusion. These data indicate that the amount of digoxin bound to the LAD artery occluded heart at the time of arrhythmias is less than that bound to non-occluded hearts and therefore, the increased sensitivity of the ischemic heart to the arrhythmogenic effects of digitalis is not by an enhanced uptake of the glycoside by the ischemic myocardium.

3. Nerve Activity Studies

In order to further examine the role of sympathetic outflow on the increased digitalis sensitivity of ischemic heart, the effects of digitalis and coronary artery occlusion on the cardiac efferent sympathetic nerve activity were studied. The inferior cardiac efferent sympathetic nerve arising from the left stellate ganglion was isolated and its electrical activity measured during a 60-sec occlusion of the left anterior descending coronary artery, in the absence and presence of subtoxic and toxic doses of digoxin. LAD artery occlusion before digoxin infusion to anesthetized cats caused variable effects on nerve activity (Figure 17), i.e., increases, decreases or no change were observed. The mean changes in nerve activity from the

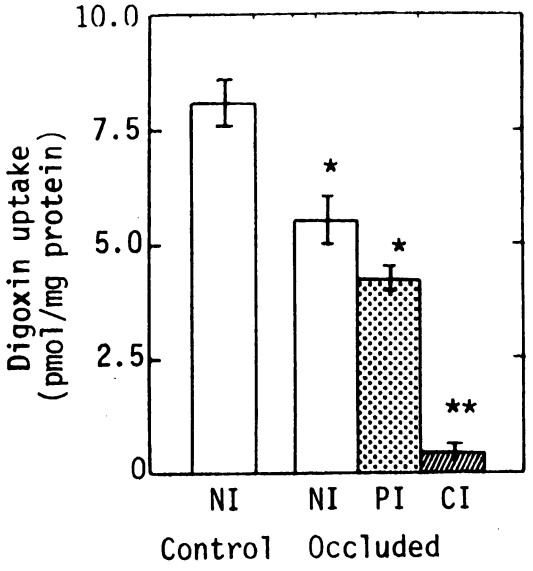

Figure 16

Figure 16. Effects of LAD artery ligation on myocardial digoxin uptake. Anesthetized cats were infused with digoxin solution (60 μg/kg/hr) containing (^{3}H)digoxin, and at the onset of arrhythmia, a dye was further infused. Non-ischemic (NI) tissues from LAD artery occluded and control animals, and partially (PI) and completely (CI) ischemic tissues from occluded animals were obtained from stained, medium-stained and non-stained tissues, respectively. Tissue digoxin content was determined by assaying for the radioactivity of tissue homogenates. Each bar represents the mean of five experiments. Vertical lines indicate S.E. *Significantly different from the control value observed in unoccluded animals ($p<0.05$). **Significantly different from the control values observed in both occluded and non-occluded animals ($p<0.05$).

Figure 17. Effects of LAD artery ligation on inferior cardiac sympathetic nerve activity in anesthetized cats. Electrical nerve activity of cardiac nerve was continuously monitored by the procedure previously described (Weaver, 1977). Control nerve activity was recorded and LAD was occluded for 60 sec and quickly released. Mean cardiac nerve discharge rate and the percent change from control values observed before the occlusion were calculated. Each line represents nerve activity changes observed in one animal. Data points illustrate average activity during a 60-sec control period (C), during three continuous 20-sec intervals of occlusion ( ) and during a 60-sec recovery (R) period. Digoxin infusion was started (60 μg/kg/hr) and when the total digoxin reached 83.0±4.3% of the mean arrhythmic dose, another 60 sec occlusion was performed (middle panel). At the onset of arrhythmia, a third 60-sec occlusion was performed. Arrows indicate that the nerve activities were significantly different from the control activity in that period ($p<0.05$).

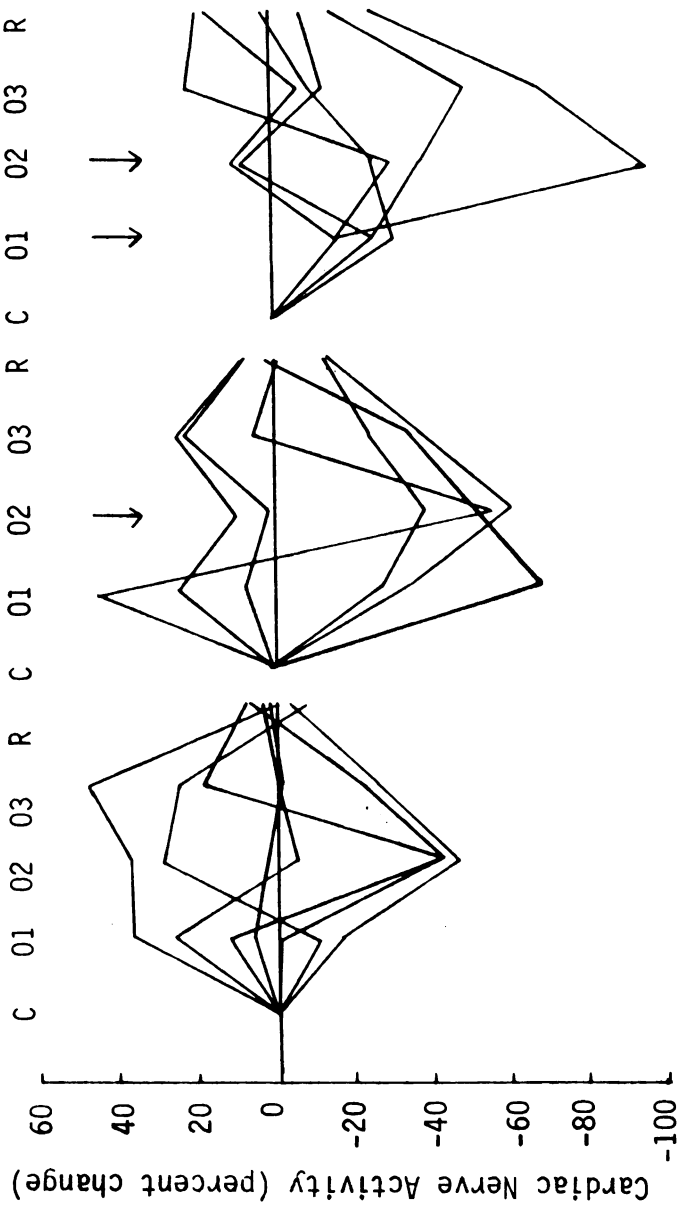
Cardiac Nerve Activity (percent change)
60
40
20
0
-20
-40
-60
-80
-100
C 01 02 03 R C 01 02 03 R C 01 02 03 R

Figure 17

control during the three successive 20 sec periods were 109 + 8%, 91 + 11%, and 109 + 12%, respectively. Release of the occlusion returned the nerve activity to control levels observed before the occlusion. When digoxin infusion was started and the amount of infused digoxin reached 83.0+4.3% of the arrhythmogenic dose, a similar 60-sec LAD artery ligation caused either reduction or elevation of nerve activity; however, nerve activity tended to be lower than the response observed prior to digoxin infusion. During the second 20-sec occlusion period, reduction of nerve activity caused by LAD artery ligation was statistically significant (66+12% vs. 91+11%). LAD artery ligation during digoxin-induced ventricular arrhythmias caused a further decrease in the activity of the nerve. Thus, temporary LAD artery ligation reduced the mean cardiac sympathetic nerve activity when near-toxic or toxic concentrations of digoxin were present in the animal.

LAD artery ligation was generally followed by either a moderate decrease or no change in blood pressure. The blood pressure decrease, when present, was transient. To determine whether such changes in blood pressure might alter the response of the electrical activity of the nerve caused by coronary artery occlusion, the blood pressure was reduced to a similar extent by hemorrhage. As expected in a baroreceptor-intact animal, reduction in blood pressure by hemorrahge increased the firing of the nerve (data not shown). These results demonstrate that reduction in the nerve activity observed during LAD artery ligation was not due to changes in blood pressure.

It appears from above studies that ischemia-induced changes in sympathetic input to the heart or vagal activity does not play a significant role in the enhancement of arrhythmogenic effects of digitalis on the ischemic heart. These results further support the observation that a direct action of digitalis on the ischemic heart is of primary importance in the increased sensitivity to digitalis.

C. Ischemia-induced Enhancement of the Arrhythmogenic Actions of Digitalis: Involvement of Endogenously-Released Substances and non-Uniform Digitalis Effect

It has been shown that coronary artery occlusion causes release of endogenous catecholamines from the nerve terminals in the early stages of myocardial ischemia (Lammerant et al., 1966; Shahab et al., 1969; Staszewska-Barczak, 1971). Reperfusion of the ischemic hearts has been also shown to cause a marked increase in norepinephrine concentration in the perfusate (Abrahamsson et al., 1981) suggesting that the catecholamine concentration in the extracellular space was elevated during ischemia. Coronary artery occlusion may also release endogenous cardiac histamine. Histamine has been shown to be released from the heart during immediate hypersensitivity reactions (Levi and Burke, 1980). Since catecholamine and histamine at high concentrations are arrhythmogenic in themselves and are known to potentiate toxic digitalis actions on the heart, ischemia-induced release of these substances may enhance the arrhythmogenic actions of digitalis.

Results from studies in sections A and B demonstrate that digitalis is unevenly distributed within the ischemic heart. Additionally, extracellular K^+ concentrations have been shown to be elevated

in ischemic areas of the heart, as a result of increased K^+ permeability. Such effects have been proposed to be responsible for ischemia-induced arrhythmias (Harris, 1966), since automatic activity may be elicited by high K^+. Therefore, the possibility that such events cause elevation of toxic effects of digitalis on the ischemic heart was examined.

1. Isolated Heart Studies: Involvement of Catecholamine and Histamine

To examine the possibility that ischemia-induced release of endogenous catecholamines or histamine may enhance the arrhythmogenic effects of digitalis, guinea-pigs were pretreated with reserpine 24 hr prior to sacrifice, or Langendorff preparations were pretreated with 10^{-5}M cimetidine, a H_2 receptor blocker, respectively. In guinea-pig hearts, the H_2-receptors have been demonstrated to mediate histamine-induced idioventricular tachyarrhythmias (Levi et al., 1976).

Addition of cimetidine to the perfusing solution caused an approximately 15% reduction in developed tension of the heart. The catecholamine depleting effects of reserpine or the H_2-receptor blocking actions of cimetidine was tested by challenging with tyramine-HCl or histamine, respectively, and obtaining a dose-response curve for the positive inotropic effect of tyramine or histamine. The dose of reserpine or cimetidine employed in the present study completely inhibited the positive inotropic effect of 10^{-4}M tyramine or 10^{-6}M histamine in the guinea-pig heart preparation (data not shown).

Following an equilibration period, the left anterior descending coronary artery of the Langendorff preparation of guinea-pig

heart was completely occluded and 40 min later, the heart was perfused at a constant pressure with 2.5 μM digoxin. Digoxin produced a positive inotropic effect followed by arrhythmias in all preparations. The onset of arrhythmias was earlier in LAD artery occluded than in control preparations (Figure 18). Reserpine or cimetidine pretreatment did not significantly alter the time to onset of arrhythmias in either control or LAD artery ligated hearts. The types of digoxin-induced arrhythmic contractions observed in both LAD artery occluded and control preparations were similar to those shown in Figure 1. There were no clear differences between the two groups with respect to the types of arrhythmic contractions.

Although a separate effect of catecholamine depletion or histamine antagonism did not affect the sensitivity of the LAD artery occluded heart to the arrhythmogenic effect of digitalis, it is possible than an additive effect may abolish the increased digitalis sensitivity. Therefore, isolated heart preparations from guinea pigs pretreated with reserpine were perfused with a solution containing cimetidine. In these preparations also, the onset of digoxin-induced arrhythmias was earlier in LAD artery occluded preparations (Figure 18). These results indicate that the increased arrhythmogenic effects of digitalis in ischemic hearts can be observed in the absence of endogenously released cardiac catecholamine, histamine or both. These results partly support the earlier finding in intact cats that dl-propranolol treatment also failed to influence the ischemia-induced enhancement of digitalis toxicity.

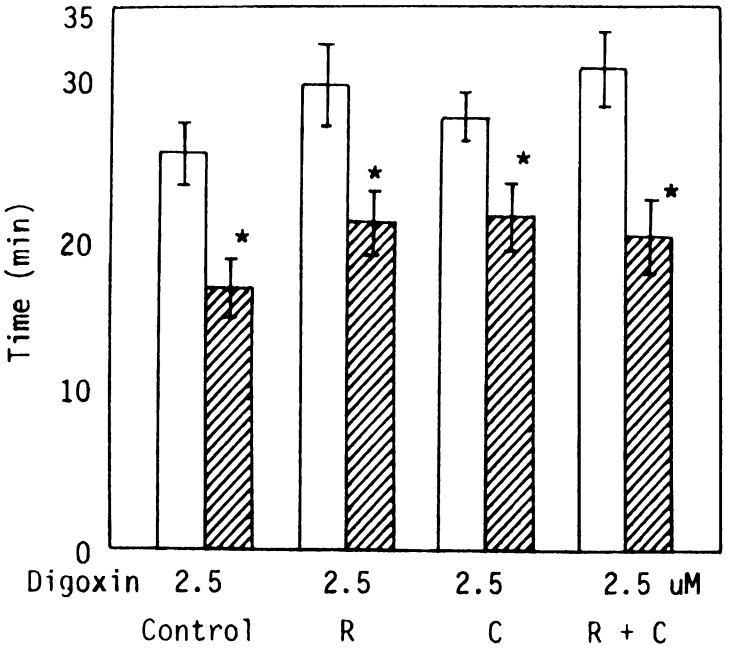

Figure 18

Figure 18. Effects of LAD artery ligation on the time to the onset of digoxin-induced arrhythmias in the isolated perfused guinea-pig heart. Following an equilibration period, LAD artery was ligated and digoxin added to the perfusing solution 40 min later (final concentration, 2.5 μM) (shaded bars). Control preparations were also perfused with a similar concentration of digoxin (open bars). Reserpine, when used, was injected 24 hr prior to sacrifice of the animal (R). Cimetidine (final concentration, 10 μM) was added 40 min prior to digoxin perfusion to either control (C) or reserpine-treated (R + C) hearts. Each bar represents the mean of six experiments. Vertical lines indicate S.E. *Significantly different from the corresponding control value ($p<0.05$).

2. Isolated Heart Studies: Non-uniform Digitalis Effect and K^+

In hearts in which the LAD coronary artery is ligated and then perfused with digoxin, an uneven glycoside distribution occurs due to areas that are only partially perfused or not perfused at all (Thompson et al., 1974). Therefore, in coronary artery-occluded hearts, non-homogeneous glycoside effects will occur and non-uniform electrophysiological characteristics such as an altered action potential duration or conduction velocity may result. It is believed by some investigators (Weiss and Shine, 1981; Wit and Bigger, 1975) that non-uniform alterations in these electrical events may cause the development of arrhythmias. Therefore, possible contributions of such non-uniform digitalis effects on the heart to the earlier generation of arrhythmias were examined using Langendorff preparations of guinea-pig heart.

To mimic a non-uniform digitalis distribution, the left anterior descending coronary artery of the isolated guinea-pig heart was cannulated. Thus, the heart was perfused via the coronary arteries by way of the aorta and also separately through the cannulated coronary artery. Following an equilibration period, an arrhythmogenic concentration of digoxin was added only to the solution perfusing the heart via the aorta such that the tissue areas perfused by the cannulated artery were digoxin-free. The non-homogeneity of glycoside distribution was visually identified by adding a dye only to the solution perfusing the heart via the aorta. Control heart preparations were similarly cannulated but the entire heart was perfused with

digoxin by adding the glycoside to the solution perfusing the heart via both the aorta and the cannulated artery. The time to the onset of digoxin-induced arrhythmias was similar in the two groups of preparations (Figure 19B). Thus, a simple, non-uniform digitalis distribution within the heart did not increase the sensitivity of the heart to digitalis-induced arrhythmias.

The extracellular K^+ concentration has been shown to be elevated in ischemic tissues (Hill and Gettes, 1980; Hirche et al., 1980). Although the estimated values of extracellular K^+ concentrations were varied, they were generally between 8 mM and 14 mM. The extracellular K^+ concentration remained elevated when it was measured 30 min following ischemia. Therefore, the effects of such an elevated extracellular K^+ concentration on the arrhythmogenic actions of digitalis were further examined using isolated perfused guinea-pig heart in which the left anterior descending coronary artery was cannulated and separately perfused. Normal Krebs-Henseleit bicarbonate buffer solution (pH 7.5) containing 5.8 mM K^+ was perfused through the aorta whereas a modified solution containing 10.6 mM K^+ was infused through the cannulated artery. Such perfusion of the heart with two different concentrations of K^+ solution to different coronary arteries did not cause arrhythmias to develop during the 90-min period under the experimental conditions employed. An arrhythmogenic concentration of digoxin (2.5 μM) was then added to the solution perfusing the heart via the aorta but not to the solution perfusing the cannulated coronary artery. Digoxin-induced arrhythmias appeared significantly

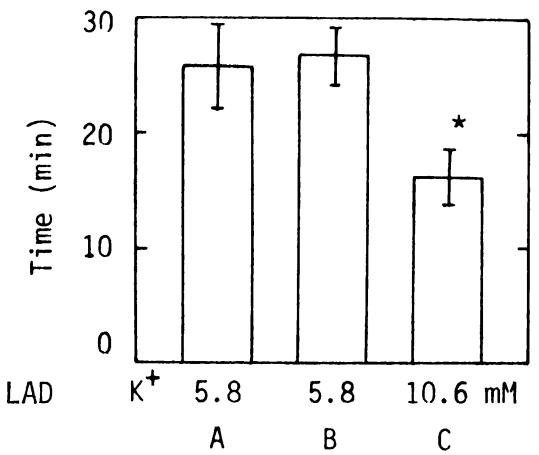

Figure 19

Figure 19. Effects of non-uniform digitalis perfusion with or without locally elevated K^+ concentration on the time to onset of arrhythmias in the isolated, perfused guinea-pig heart. Left anterior descending coronary artery was cannulated and Krebs-Henseleit bicarbonate buffer (pH 7.5) solution containing either 5.8 mM K^+ and 2.5 μM digoxin (control, A), 5.8 mM K^+ with no digoxin (B) or 10.6 mM K^+ with no digoxin (C) was infused while the rest of the heart was perfused with 2.5 μM digoxin solution through the coronary arteries via the aorta. Development of arrhythmias was monitored from twitch-tension curves. Each bar represents the mean of six experiments. Vertical lines indicate S.E. *Significantly different from the values of other bars ($p<0.05$).

earlier in these hearts than in the hearts entirely perfused with solution containing 5.8 mM K^+ and perfused with digoxin solution via the aorta (Figure 19C). When the whole heart was perfused with a solution containing 10.6 mM K^+, a similar concentration of digoxin added to the solution perfusing the aorta produced a positive inotropic effect with no arrhythmias occurring up to 90 min.

3. (^{3}H)Ouabain Binding

Non-uniform glycoside distribution and effects in hearts perfused with digoxin through the aorta but not through the cannulated LAD were further studied. Dye was perfused through the aorta at the onset of arrhythmias. The fractional occupancy of glycoside binding sites on Na,K-ATPase from the reduction in the initial velocity of ATP-dependent (^{3}H)ouabain binding to ventricular muscle homogenates obtained from dye-stained (S) and non-stained (NS) myocardial tissues were estimated. Control heart preparations were perfused for a similar period of time. Figure 20 shows the (^{3}H)ouabain binding to dye-stained and nonstained tissues. Since dye-stained tissues were perfused with digoxin, the glycoside binding sites were bound with "cold" digoxin and therefore the (^{3}H)ouabain binding was low. In the non-stained tissue, which was not perfused with digoxin, the (^{3}H)ouabain binding was high, indicating that glycoside binding sites were not bound, as expected. To test whether infusion of a solution via the cannulated artery in fact perfused the tissues, digoxin was infused to the cannulated artery. The tissue was then assayed for (^{3}H)ouabain binding. The (^{3}H)ouabain binding in this tissue was low, indicating

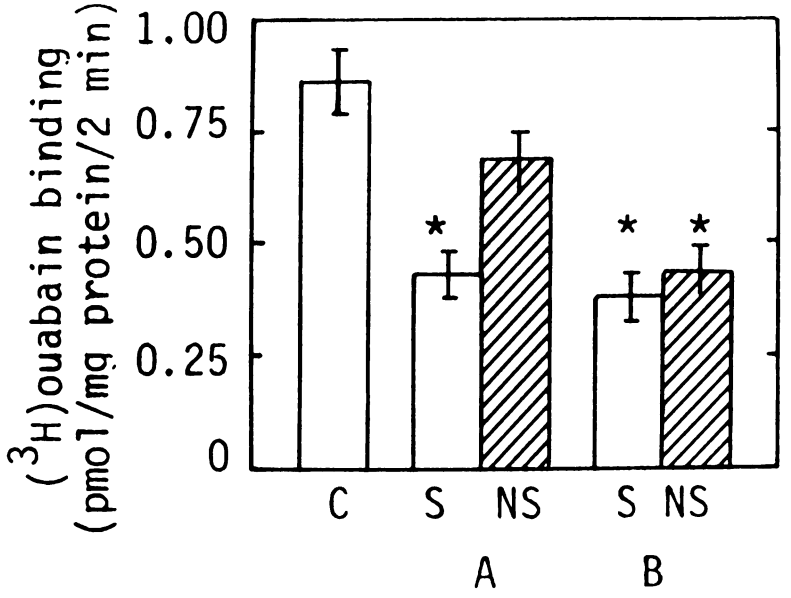

Figure 20

Figure 20. Effects of non-uniform digoxin perfusion on specific (^{3}H)ouabain binding to ventricular muscle homogenates. LAD artery of Langendorff preparations of guinea-pig heart was cannulated and infused with solution containing digoxin and 5.8 mM K^+ (B) or 5.8 mM K^+ (A) while the heart was perfused with a similar concentration of digoxin and 5.8 mM K^+. At the onset of arrhythmias, a dye was added to the solution perfusing via aorta and stained (S), and non-stained (NS) portions of ventricular homogenates, including that obtained from control hearts perfused with no digoxin (C) were assayed for (^{3}H)-ouabain binding. (^{3}H)ouabain binding shown is the difference in values observed in the presence and absence of ATP. *Significantly different from the control values ($p<0.05$).

that digoxin bound to the tissue normally perfused by the cannulated artery (Figure 20). The dye itself did not affect the (^{3}H)ouabain binding reaction. These results show that separate perfusion to the cannulated artery indeed produces a non-homogeneous glycoside effect on the heart.

In summary, the presence of an area of the heart with an elevated K^{+} concentration, adjacent to areas of the heart exposed to digoxin appears to enhance the arrhythmogenic effects of digitalis. It is tempting to speculate that similar conditions may be present in acute myocardial ischemia and predispose the heart to digitalis-induced arrhythmias.

DISCUSSION

A. Mechanisms of Toxic Actions of Digitalis in the Heart

Cardiac glycosides produce arrhythmias by a direct action on the heart. This is readily shown by perfusion of isolated heart preparations or superfusion of atrial, ventricular or papillary muscle, or Purkinje fibers with toxic concentrations of a glycoside which produce an initial positive inotropic effect followed by arrhythmias. Digitalis also acts on the nervous system to enhance its arrhythmogenic actions. In particular, the potentiating action of the sympathetic nervous system on digitalis-induced arrhythmias has been well established.

It is generally accepted that the initial event in the toxic action of cardiac glycosides is the binding to and inhibition of sarcolemmal Na,K-ATPase. As described in the Introduction section, the positive inotropic action of digitalis is closely related to enzyme or sodium pump inhibition; however, the mechanism by which toxic (arrhythmogenic) effects of digitalis are produced has not been elucidated although sodium pump inhibition is thought to be the primary event. Additional changes in ion movement are proposed to explain the toxicity.

The sodium pump actively counter-transports Na^+ and K^+ across the cell membrane. Therefore, inhibition of the sodium pump causes

accumulation of extracellular K^+ which in turn reduces the transmembrane K^+ gradient and partially depolarizes the cells. Digitalis-induced arrhythmias have been proposed to develop from the increase in the inhibition of the sodium pump and progressive reduction in the resting membrane potential that reach the threshold level for spontaneous depolarization and automatic activities. However, K^+ outside the cell may not accumulate enough to reduce the resting membrane potential to the thresold potential since excess K^+ may be washed out by coronary blood flow.

Recently, several groups of investigators have observed that digitalis causes the appearance of transient depolarizations (also known as oscillatory afterpotentials, or delayed afterdepolarizations) in Purkinje fibers treated with toxic concentrations of digitalis. When the transient depolarizations reached the threshold potential, action potentials were elicited (Ferrier et al., 1973; Rosen et al., 1975; Ferrier, 1977). Ferrier et al. (1977) noted while recording transmembrane potential simultaneously from canine Purkinje fibers and attached papillary muscle that the triggered action potentials arose from the myocardial conducting system (Purkinje fibers), conducted to the adjacent papillary muscle and produced contractions in that muscle. Papillary muscle, however, did not generate oscillatory afterpotentials. These observations lead to the concept that digitalis produces arrhythmias by causing development of oscillatory afterpotentials which are capable of producing extrasystoles or arrhythmias.

Even when transient depolarizations do not attain the threshold potential, they were shown to modify conduction (Rosen et al., 1975). Since transient depolarizations can vary in magnitude from one cycle to the next, they can modify the amplitude and upstroke velocity of successive action potentials and conduction velocity. Thus, bigeminy and "concealed" conduction were observed in myocardial tissues exhibiting oscillatory afterpotentials.

Several studies using voltage clamp techniques have suggested that the ionic mechanism responsible for the oscillatory afterpotentials is a transient inward current (Lederer and Tsien, 1976; Aronson and Gelles, 1977). The specific ion involved in the transient depolarization was further proposed to be Ca^{++} since calcium blockers such as verapamil depressed the depolarization. Other investigators have suggested that Na^{+} may also be involved since lowering of the extracellular sodium concentration or TTX treatment reduced the magnitude of the transient depolarizations (Tsien and Carpenter, 1978; Vassalle and Scida, 1979). These results suggest that although the mechanism is uncertain, the inward currents carried by Ca^{++} and/or Na^{+} are important for the development of digitalis-induced oscillatory afterpotentials.

It is known that toxic concentration of cardiac glycosides cause accumulation of intracellular calcium. It is possible that increases in calcium in the cell may contribute to the appearance of oscillatory afterpotentials. This view is supported by the observations that raising extracellular calcium or reducing extracellular sodium, both of which cause elevation of intracellular calcium, the latter probably

by the sodium/calcium exchange mechanism, were capable of producing oscillatory afterpotentials (Kass et al., 1978). Digitalis-induced increases in intracellular calcium may alter calcium fluxes within the cell, i.e., cause calcium redistribution between intracellular stores, and cause transient changes in membrane conductance to cations such as sodium.

Thus, the putative scheme for the development of digitalis-induced arrhythmias begins by the binding of a glycoside to sarcolemmal Na,K-ATPase and the inhibition of sodium pump which results in an elevation of intracellular sodium. Via the sodium/calcium exchange mechanism, sodium efflux is coupled to calcium influx and the intracellular calcium level rises. Calcium redistribution in the cell and possibly oscillatory movements of calcium between intracellular stores may cause changes in membrane conductance in a phasic manner. Transient inward movements of sodium or calcium then produce transient depolarizations. Toxic concentrations of digitalis inhibit the sodium pump to an extent such that the above events are enhanced and transient depolarizations succeed in reaching the threshold potential necessary to produce triggered action potentials or arrhythmias.

Another possible mechanism by which transient depolarization may occur is by accumulation of K^+ just outside the cell membrane, i.e., at the glycocalyx surface coat. The transient accumulation of the potassium ions released during an action potential may partially depolarize the cells. Digitalis, by inhibiting the sodium pump, may cause a greater transient elevation of K^+ outside the cell and cause transient depolarizations to occur.

Ischemia may alter the effect of digitalis on the development of transient depolarizations by interfering at any of the steps described above. The effect of reduced or no coronary blood flow to the cardiac tissue, or ischemia, has been intensively studied using the isolated perfused heart model. These studies show that ischemia impairs all aspects of cellular function, i.e., metabolic, electrophysiologic, mechanical and structural. In cardiac tissues that are partially perfused, however, cellular function may be depressed but not destroyed. In these cells, the effects of digitalis may be altered at a specific site if ischemia-induced damages are confined to a particular function. For example, an enzyme that has a low Km rather than a high Km for ATP may survive the partial ischemia when ATP synthesis is reduced. Therefore, depending upon the severity of ischemia, the sensitivity of the myocardial cells to ischemic insult may change. Thus, the sensitivity of the cell to the effects of digitalis may be changed by the severity of ischemia. In the present studies, the effect of ischemia on the effect of digitalis was examined in hearts perfused at a reduced rate of flow or zero flow. Some of the steps in the mechanism of action of digitalis were studied to determine whether ischemia caused any changes at glycoside binding sites.

B. Effects of Ischemia on Na,K-ATPase Activity, Sensitivity of the Enzyme to the Inhibitory Effects of Digitalis, and Glycoside Binding to Na,K-ATPase

Effects of ischemia on the sensitivity to the arrhythmogenic actions of digitalis was first examined using Langendorff preparations of guinea-pig heart to determine whether a direct action of the glycoside on the ischemic heart is an important mechanism for the

development of the early onset of arrhythmias. When the left anterior descending coronary artery of the isolated perfused heart was completely ligated and then the heart was challenged with a toxic concentration of digoxin, the onset of glycoside-induced arrhythmias was significantly earlier in the ligated preparation than in the control, non-ligated hearts. These results confirm the observations made by other investigators that coronary artery occlusion reduced the dose of digitalis required to develop ventricular arrhythmias in cats, dogs, and pigs (Hood et al., 1967; Balcon et al., 1968; Morris et al., 1969; Kumar et al., 1970; Ku and Lucchesi, 1979) and further demonstrate that altered sensitivity to digitalis can be observed in the isolated ischemic heart.

In myocardial ischemia, only a small portion of the heart becomes ischemic due to lack of blood flow to this area. Therefore, both ischemic and non-ischemic zones exist in the coronary artery-occluded heart. The existence of a "border zone" which lies between the ischemic and non-ischemic tissues has also been considered and studied using various techniques. Surface fluorescence studies, histological and ultrastructural studies, and histochemical and electrophysiologic studies have generally failed to prove or disprove the concept of a "border zone". Evidence for and against the existence of a border zone was obtained in the studies of many investigators (Sayen et al., 1952; Reimer et al., 1977; Hearse et al., 1977; Harken et al., 1978; Vokonas et al., 1978). However, flow studies (Lubbe et al., 1974; Malsky et al., 1977) and electrocardiographic studies (Braunwald et al., 1974; Janse et al., 1979) have indicated that zones with intermediate flow or S-T segment elevations clearly exist. These studies

all suggest that although the size of the border zone may be small, there probably exists a narrow zone of partially ischemic tissues during acute myocardial ischemia. This assumption is reasonable and experimentally sound since the infarct size in ischemic heart can be reduced by cardiac drugs such as propranolol, indicating that there exist border ischemic tissues that can be salvaged (Puri, 1974; Braunwald et al., 1974; Hearse et al., 1977).

In a coronary artery occluded heart, the completely ischemic tissues would not be perfused whereas the partially ischemic tissues present in the border zone would be perfused at a reduced rate. When digitalis is perfused to the heart, the partially ischemic tissues, however, may be exposed to a similar concentration of glycoside as the non-ischemic tissues. Therefore, if a border zone has an increased sensitivity to digitalis, toxic effects of the glycoside may appear earlier at this site.

The direct action of digitalis on the heart may be on cardiac sarcolemmal Na,K-ATPase, a putative receptor for the pharmacological and toxic actions of digitalis (Repke, 1964; Schwartz et al., 1975; Akera and Brody, 1977). Therefore, alterations in Na,K-ATPase activity, or sensitivity of the enzyme to digitalis may augment the cardiac response to the toxic effects of digitalis. One suggestion is that the cardiac electrogenic sodium pump is stimulated under anoxic conditions (McDonald and MacLeod, 1971). Such an increase in sodium pump activity has been shown to enhance the inhibitory effects of digitalis (Clausen and Hansen, 1977; Yamamoto et al., 1979). On the other hand, various results have been observed on the effects of ischemia on myocardial Na,K-ATPase activity. In failing rabbit hearts

produced by constriction of the aorta, Na,K-ATPase activity of ventricular muscle homogenates were significantly reduced (Yazaki and Fjuii, 1972). Coronary artery occlusion of the canine heart failed to alter Na,K-ATPase activity when assayed 24 hr later but did depress the enzyme activity after 7 days (Schwartz et al., 1973). In similar studies, Beller et al. (1976) noted that Na,K-ATPase activity of partially purified enzyme preparation from ventricular tissue obtained from ischemic areas of 2-hr coronary artery occluded canine heart was markedly lower than that in non-ischemic tissue. Hypoxia, an important component of ischemia, also decreased Na,K-ATPase activity in both isolated rat heart tissue (Balasubramanian et al., 1973) and dog heart muscle (Friedman et al., 1972). Rau et al. (1977), however, did not observe any alteration in enzyme activity following 60 min of anoxic perfusion of rabbit heart muscle. The reasons for these different results are not clear. It may be due to differences in the procedure of enzyme preparation since slightly different methods were employed by these investigators, the duration of the ischemia or other factors.

In the present study, the effects of ischemia on Na,K-ATPase activity and glycoside binding to the enzyme were examined. Globally ischemic guinea-pig hearts were utilized as the experimental model, since large quantities of uniformly ischemic tissues could be obtained and pathways of energy production and depletion were shown to be similar to those produced in vivo by ischemia (Jennings et al., 1981). As discussed above, the presence of a marginally ischemic zone may be important in ischemia-induced augmentation of glycoside toxicity. Therefore, cardiac tissues were made either partially or completely

ischemic by reducing the perfusion flow to the heart to either 5% of the control flow rate by completely stopping the flow, to represent marginally and totally ischemic zone, respectively. Results of the present study show that following a 2-hr of partial or complete ischemia, or 6-hr of partial ischemia, the Na,K-ATPase activity of the ventricular muscle homogenates remains unchanged. In hearts that were made completely ischemic for 6 hr, Na,K-ATPase activity and the number of (^{3}H)ouabain binding sites were significantly reduced. The affinity of the binding sites for (^{3}H)ouabain, however, was not affected. The sensitivity of the remaining and active Na,K-ATPase to the inhibitory effects of dihydrodigoxin was also unchanged. The affinity of Na,K-ATPase for Na^+ or K^+, i.e., the sensitivity of the enzyme for K^+-induced inhibition or Na^+-induced stimulation of glycoside binding, was also unchanged. Thus, digitalis sensitivity of the ischemic tissues was not altered by changes in the intrinsic properties of the enzyme or changes in glycoside binding to the enzyme.

It is possible that since Na,K-ATPase activity or digitalis binding was performed with muscle homogenates, ischemia-induced changes in the ionic milieu to which the enzyme is exposed may have been altered during homogenization. This is indeed likely since it has been demonstrated that early ischemia causes K^+ leakage into the extracellular space (Harris, 1954; Hill and Gettes, 1980; Weiss and Shine, 1981) and intracellular calcium accumulation (Shen et al., 1972; Tomlinson and Dhalla, 1973; Nayler, 1981; Farber et al., 1981). Such changes will alter Na,K-ATPase activity since a rise in extracellular K^+ stimulates, and a rise in intracellular Ca^{++} inhibits

sodium pump activity (Dhalla et al., 1974). Thus, the in vivo enzyme sensitivity to the inhibitory effects of digitalis may be masked. In addition, ischemia may cause an elevation of the intracellular sodium concentration by increasing membrane permeability or by non-specific membrane leakage. Since the sodium concentration in the extracellular space is almost ten-fold higher than that in the cytosol, the gradient itself may cause an influx of sodium. Such increases in the intracellular sodium concentration have been shown to enhance glycoside binding to Na,K-ATPase and to augment the toxic actions of digitalis (Yamamoto et al., 1979). Thus, increases in the intracellular Na^+ concentration enhance and increases in the extracellular K^+ concentration reduce glycoside binding to Na,K-ATPase. Ischemia-induced changes in glycoside binding to the enzyme may or may not change, depending upon the extent of redistribution of the ions.

In intact tissues, the changes in ionic gradients may not be as quickly altered as in the homogenized preparations, since cellular integrity is maintained. Therefore, monovalent cation transport studies using intact ventricular strips and slices were performed. In these studies, the effect of partial and complete ischemia on the sodium pump activity and the reserve capacity of the sodium pump were examined. A moderate inhibition of the sodium pump by the cardiac glycosides or increasing the sodium influx by modest high frequency electrical stimulation does not cause a corresponding increase in the extracellular sodium concentration (Langer, 1972), indicating that the sodium pump has a reserve capacity. However, inhibition of the sodium pump exceeding the reserve capacity causes a progressive increase in

the intracellular sodium concentration and produces electrophysiological imbalance and finally toxicity or arrhythmias. Thus, if ischemia reduces the reserve capacity of the sodium pump, digitalis-induced toxicity will be facilitated. Results of the present study show that ouabain-sensitive (specific) ^{86}Rb and ^{42}K uptakes, estimates of sodium pump activity, to ventricular slices or strips prepared from hearts made partially or completely ischemic for 2 hr are similar to values observed in non-ischemic tissues. These studies further show that the reserve capacity of the sodium pump, reduction of which enhances digitalis toxicity, is not altered by 2 hr of partial ischemia. Thus, even in intact tissue preparations, ischemia failed to alter the sodium pump activity, suggesting that glycoside binding to Na,K-ATPase may be unchanged.

Although these data indicate that 2 hr of ischemia does not affect Na,K-ATPase or sodium pump activity, or the sensitivity of the enzyme to digitalis, it appeared necessary to determine whether ischemia produced by coronary artery occlusion, a situation closer to myocardial ischemic disease in man, affects glycoside binding to Na,K-ATPase or influences Na,K-ATPase activity itself. Since anesthetized cats with LAD artery ligation required less digoxin to produce arrhythmias, possible alterations in glycoside sensitivity of ischemic tissues were examined in this animal species. Partially and completely ischemic tissues from LAD-ligated animals were visually identified by dye staining after a 3 hr period or at the onset of digoxin-induced arrhythmias. It is uncertain whether ischemic tissues obtained by dye staining are in fact ischemic or represent tissues that have an

altered affinity for the dye. However, since the dye was infused intravenously to the LAD artery ligated animal, tissues that are normally not perfused will not be expected to be stained with the dye. Flow distribution studies using radioactive microspheres have indicated that ischemic tissues are considerably less well perfused than non-ischemic tissues (Lubbe et al., 1974). Therefore, it is reasonable to assume that ischemic tissues prepared under the present experimental conditions are indeed obtained from cardiac tissues that have either partially or completely reduced perfusion.

When ischemic tissues, both partial and complete, were prepared and assayed for Na,K-ATPase activity and (^{3}H)ouabain binding, the values were similar to those in non-ischemic tissues. In coronary artery occluded animals infused with digoxin, higher enzyme activity and (^{3}H)ouabain binding were observed in ischemic tissues compared to non-ischemic tissues at the onset of arrhythmias. Therefore, in ischemic hearts, arrhythmias were produced with a lower fractional occupancy of the glycoside binding sites on Na,K-ATPase. It is clear from these studies that at a time when coronary artery occlusion significantly enhanced the arrhythmogenic effects of digitalis, Na,K-ATPase activity, and the number of glycoside binding sites are higher in the ischemic tissues than in non-ischemic tissues. Thus, the mechanism by which digitalis produces enhanced the arrhythmic response in the ischemic heart does not appear to be due to lower Na,K-ATPase activity or greater digoxin binding to the enzyme. It is possible, however, that non-uniform digitalis effects on the heart, observed in coronary artery-occluded cats infused with digoxin, may contribute to

digitalis-induced arrhythmias. Myocardial digoxin uptake was lower in coronary artery-occluded than in non-occluded hearts. Furthermore, in coronary artery-occluded hearts, the myocardial digoxin uptake in ischemic tissues was less than 10% of that in non-ischemic tissues. Myocardial digoxin uptake in partially ischemic tissues was also significantly less than non-ischemic tissue. These results are in agreement with those of other investigators (Thompson et al., 1974; Beller et al., 1976). The possibility that non-uniform effects of digitalis influences arrhythmogenic actions of digoxin is discussed below.

Reperfusion following a 2 hr ischemia, on the other hand, caused a significant reduction in Na,K-ATPase activity without altering the sensitivity of the residual enzyme to digitalis inhibition. This finding is not surprising in view of the fact that reperfusion can cause deleterious effects due to hemorrhage (Bresnahan et al., 1974) and also can alter metabolic function due to intracellular accumulation of calcium early following reperfusion (Kane et al., 1975; Nayler, 1981). Consistent with these findings are the results that sodium pump activity in ventricular slices following 20 min reperfusion after 2 hr of ischemia was significantly reduced. These results are in contrast to those obtained by Ku and Lucchesi (1979). In their study, sodium pump activity in sodium-loaded ventricular slices obtained from border ischemic and completely ischemic tissues in coronary artery-occluded and then reperfused canine heart was significantly increased compared to control tissues. Furthermore, the sensitivity of the sodium pump to the inhibitory effects of ouabain

was increased. However, the in vivo significance of these results are not clear since sodium pump activity estimated in sodium-loaded tissues does not reflect the actual sodium pump activity in the tissues. In the present studies, it was not experimentally feasible to examine whether reperfused hearts were more sensitive to the arrhythmogenic effects of digitalis since reperfusion itself caused the development of arrhythmias. However, reperfusion may potentiate digitalis toxicity by reducing the number of functional Na,K-ATPase molecules, and thus the reserve capcity of the sodium pump.

It is possible that myocardial ischemia may alter factors other than the glycoside-Na,K-ATPase interaction to sensitize the heart to the arrhythmogenic action of digitalis. Such possibilities include changes in glycoside pharmacokinetics such that the glycoside effect on the heart may be altered in a non-uniform manner, or by changes in myocardial glycoside uptake. Though unlikely, changes in glycoside binding to sites other than Na,K-ATPase may affect the cellular response to the toxic effects of digitalis.

Changes in glycoside pharmacokinetics, such as an altered body distribution, may have reduced the dose of digitalis required to produce arrhythmias in intact coronary artery-occluded animals. In these animals, occlusion of the left anterior descending coronary artery reduced cardiac output and left ventricular pressure (Hood et al., 1967, 1969; Banka et al., 1975). Such effects may reduce blood perfusion to body tissues and organs, and elevate the plasma glycoside concentration. Digitalis absorption following oral administration

was further shown to be slower in animals in which the coronary artery was occluded (Korhonen et al., 1979). However, in the present experiments digoxin was infused intravenously. Digoxin infusion at a constant rate caused a linear increase in the plasma glycoside concentration; however, the rate of increase was similar in both occluded and non-occluded animals. Therefore, the plasma digoxin concentrations at any given time were similar in the two groups. The onset of glycoside-induced arrhythmias was earlier in LAD artery occluded than in control animals and therefore, the plasma digoxin concentrations at the onset of arrhythmias were lower in the former than in the latter group of animals. Table 3 shows that the plasma digoxin concentration in dl-propranolol pretreated animals are significantly lower than in the C-1 sectioned animals despite the longer infusion time in the former. This is apparently due to differences in the assay procedure. In the C-1 sectioned animals, a radioimmunoassay was used, whereas in propranolol-pretreated animals plasma samples were assayed for radioactivity following ^{3}H-labeled digoxin infusion.

It can be concluded from above results that a direct action of digitalis on the ischemic heart is an important mechanism for the enhanced arrhythmic response to digitalis. However, it does not appear to be due to changes in Na,K-ATPase activity, the number of glycoside binding sites or in their affinity for the glycosides or changes in sensitivity of the enzyme to the inhibitory effects of digitalis.

C. Role of Sympathetic Nervous System in Ischemia-Induced Enhancement of Arrhythmogenic Effects of Digitalis

The myocardial ischemia produced in this study by coronary artery occlusion in anesthetized cats reduced the dose of digoxin to produce glycoside-induced ventricular arrhythmias. These results are similar to those reported by other investigators who used either cats, dogs or pigs (Bellet et al., 1934; Hood et al., 1967; Balcon et al., 1968; Morris et al., 1969; Kumar et al., 1970; Ku and Lucchesi, 1979). Using the anesthetized cat as a model, therefore, the role of autonomic nervous system on ischemia-induced digitalis-sensitization of the heart was examined.

Several groups of investigators have observed marked elevation of plasma and urine norepinephrine and epinephrine concentrations in patients with myocardial ischemia and in experimental animals with coronary artery occlusion (Forssman et al., 1952; Raab and Gigee, 1954; Valorie et al., 1967; Klein et al., 1968; Staszewska-Barczak and Ceremuzynski, 1968; Siggers et al., 1971). These findings suggest that ischemia increases sympathetic activity either centrally or peripherally and causes catecholamine release from nerve terminals and/or the adrenal medulla.

It is well known that increased sympathetic nerve activity, by either centrally or peripherally-induced, is closely related to the production of digitalis-induced arrhythmias (Gillis and Quest, 1979). Exogenous catecholamines have also been demonstrated to enhance digitalis toxicity (Angelucci, 1966; Morrow, 1967; Raper and Wale, 1969; Tse and Han, 1974; Gillis and Quest, 1979). Studies employing

spinal cord section (Raines et al., 1967; Gills et al., 1972; Somberg et al., 1978), cardiac denervation (Mendez et al., 1961), agents that depress central sympathetic outflow (Gillis et al., 1972), drugs that block either ganglionic transmission (Gillis et al., 1975) or beta-adrenergic receptors (Kelliher and Roberts, 1974; Evans et al., 1976), all clearly demonstrate that sympathetic discharge to the heart can potentiate digitalis toxicity. Therefore, if ischemia enhances sympathetic outflow to the heart or to the adrenal glands to release catecholamines, digitalis toxicity may be potentiated. In the present study, when sympathectomy was performed by sectioning the spinal cord at the C-1 level to abolish the central sympathetic outflow, the dose of digoxin required to produce ventricular arrhythmias was significantly increased in these animals. These results give support to the important role of the sympathetic nervous system in digitalis-induced arrhythmias. S pinal section at C-1, however, failed to abolish the effect of ischemia to enhance the sensitivity of the heart to the arrhythmogenic action of digitalis. These results suggest that the mechanism of increased digitalis sensitivity is by an effect other than a centrally-mediated change in sympathetic activity produced by ischemia.

Several investigators have suggested the importance of the role of a cardio-cardiac sympathetic reflex in the mediation of ischemia-induced elevation of sympathetic discharge to the heart (Malliani et al., 1969; Uchida and Murao, 1974; Bosnjak et al., 1979). Other investigators have disputed these findings based on their observations

that this reflex was not present in neurally-intact animals (Felder and Thames, 1981) or that sympathoinhibition occurs in animals in which coronary artery was occluded (Thoren, 1973; Kedzi et al., 1974). The cardio-cardiac sympathetic reflex system, whether it reduces or elevates cardiac efferent sympathetic nerve activities, would not be removed by C-1 section since brain regions above C-1 level may not be involved in this reflex. Therefore, in order to eliminate the effect of this reflex on the heart, in addition to any central sympathetic effects, dl-propranolol was administered to the anesthetized cats prior to coronary occlusion or digoxin infusion. dl-Propranolol significantly prolonged the time to glycoside-induced arrhythmias in both LAD artery ligated and control animals when digoxin was infused at a constant rate of 60 μg/kg/hr. It failed, however, to abolish the enhanced arrhythmic response observed in LAD artery ligated animals. These results demonstrate the lack of significant involvement of the sympathetic nervous system in the elevated digitalis sensitivity of the ischemic heart. Since the beta-adrenergic blocking agent, dl-propranolol, was used, one can additionally rule out the possibility that ischemia-induced sympathoadrenal activation that elevates plasma catecholamine concentration (Staszewska-Barczak, 1971; Karlsberg et al., 1981) or that locally-mediated increase in norepinephrine release from the nerve terminals in ischemic tissues of the heart (Lammerant et al., 1966; Staszewska-Barczak and Ceremuzynski, 1968; Shahab et al., 1969; Abrahamsson et al., 1981) play an important role.

Studies using nerve activity recordings have shown that coronary artery occlusion or digitalis can elicit either a decrease, increase

or no change in cardiac efferent nerve activity (Lathers et al., 1977, 1978). It was therefore postulated that such non-uniform neural discharges observed in individual nerve filaments within the same nerve bundle may alter myocardial excitability and conduction in a non-uniform manner and cause development of arrhythmias. Weaver and co-workers have additionally demonstrated that coronary artery occlusion alters renal nerve activity (Weaver et al., 1981). Thus, coronary artery occlusion influences nerve activities not only of those related to the heart but also to other organs. It is possible that coronary artery occlusion may affect the activity of the afferent nerves arising from parts of the body other than the heart such as kidney, baroreceptors and carotid sinus and influence the efferent cardiac sympathetic nerve activity. Digitalis action on the heart may thus be altered by such reflex mechanisms. Furthermore, if the above hypothesis is correct, the effects of both ischemia and digitalis to cause non-uniformity in the nerve activities may potentiate each other and enhance digitalis toxicity.

In the present study with anesthetized cats, inferior cardiac efferent nerve activity was monitored during one minute coronary artery occlusions in the presence and absence of digoxin. LAD artery ligation itself caused either a decrease, increase or no change in nerve activity; results that support the findings of Lathers et al. (1978) and others. However, the mean electrical activity of the nerve was not different from the control activity. In the presence of near-toxic or toxic doses of digoxin, however, the mean nerve activity was significantly reduced during part of the occlusion, although increases, decreases and no changes in nerve activity were still

observed. Thus, coronary artery occlusion-induced sympathoinhibition in the presence of digoxin may have a slight protective effect against digitalis toxicity, similar to the effects of spinal-section or propranolol treatment.

The cause of sympathoinhibition during coronary artery occlusion has been identified to be due to activation of vagal afferent nerves which reflexly inhibit sympathetic outflow (Recordati et al., 1971; Thoren, 1973; Kedzi et al., 1974; Felder and Thames, 1979; Thames, 1979). It is possible that a reduction in sympathetic activity in the presence of digitalis-induced vagal activation may cause A-V block and produce ventricular arrhythmias. Bilateral vagotomy, however, failed to affect either the dose of digoxin to produce arrhythmias, or the increased sensitivity of the ischemic heart to the glycoside. Thus, any changes in sympathetic nerve activity caused by coronary artery occlusion do not appear to play a significant role in the increased digitalis sensitivity of the ischemic heart.

From these studies, it appears that removal of autonomic input to the heart does not affect the ischemia-induced enhancement of arrhythmogenic effects of digitalis. Therefore, effects of digitalis on the autonomic nervous system may not be significantly affected by ischemia. These results confirm the previous conclusion that ischemia enhances a direct action of digitalis on the heart.

D. Role of Ischemia-induced Release of Catecholamines, Histamine and K^+ in Digitalis Toxicity

The toxic actions of digitalis on the heart may be enhanced by many factors. Of these factors, effects of norepinephrine, histamine

and K^+ were examined since these substances have been shown, either directly or indirectly, to be released from ischemic hearts, and to produce arrhythmias at high concentrations. The potentiating effects of catecholamines in the arrhythmogenic actions of digitalis have been well demonstrated. Pretreatment of animals with reserpine (Ciafalo et al., 1967), beta-adrenergic receptor blocking agents (Vaughan Williams, 1963; Evans et al., 1976) increased the dose of digitalis required to produce cardiac arrhythmias. Procedures that blocked central sympathetic outflow such as by spinal section (Gillis et al., 1972; Somberg et al., 1978) or central α_2-adrenoceptor stimulation with clonidine (Lechat and Schmitt, 1982) also desensitized the animals to the arrhythmogenic effects of digitalis. In a related study, animals infused with norepinephrine or epinephrine were also sensitized to the toxic effects of digitalis (Morrow, 1967; Lum et al., 1977). Thus, if ischemia increases catecholamine levels in the heart, digitalis toxicity would be expected to be enhanced.

Studies to determine whether ischemia causes release of endogenous catecholamines have indicated that a sustained norepinephrine secretion from the heart occurs following coronary artery occlusion in dogs (Staszewska-Barczak, 1971). Similar studies have also demonstrated that coronary artery occlusion increased the norepinephrine concentration in the coronary effluents (Richardson, 1963; Lammerant et al., 1966; Ceremuzynski, 1969; Shahab et al., 1969). In isolated guinea-pig heart made ischemic and reperfused, the norepinephrine concentration in the effluent was significantly increased (Abrahamsson et al., 1981). These results suggest that ischemia-induced release of

catecholamine in the isolated heart may enhance the arrhythmogenic effects of digitalis.

To study such a possibility, the endogenous norepinephrine from the nerve terminals were depleted by pretreatment of guinea-pigs with reserpine 24 hr prior to sacrifice. Langendorff preparations obtained from these guinea pigs did not respond to a dose of tyramine (10^{-4}M) that caused a marked positive inotropic effect in control preparations. The result that coronary artery occlusion of the heart still reduced the time to the onset of arrhythmias following perfusion with a toxic dose of digoxin signifies that either ischemia did not cause enough catecholamine release to potentiate the digitalis effect or that locally-released catecholamines had no significant effect on the ischemia-induced enhancement of the toxic actions of digitalis. Since norepinephrine release by ischemia was not determined in the present study, neither the former nor the latter possibility can be ruled out. It is clear, however, that ischemia potentiates the arrhythmogenic potential of digitalis even in the absence of catecholamines. Therefore, a factor or factors other than ischemia-induced release of catecholamines seem to be important.

Although not experimentally proven, results from several studies suggest that ischemia may cause the release of endogenous cardiac histamine. During immediate hypersensitivity reactions, histamine was released together with other mediators such as prostaglandins (Levi and Burke, 1980). Since prostaglandins are released during acute myocardial ischemia (Alexander et al., 1975; Kraemer et al., 1976; Berger et al., 1976; Ogletree et al., 1977), histamine may also be

released simultaneously. Histamine was also suggested to be one of the agents responsible for coronary arterial spasms (Ginsburg et al., 1981).

Both H_1 and H_2 antagonists were shown to increase the dose of digitalis required to produce cardiac arrhythmias (Levi and Capurro, 1975; Somberg et al., 1980). In isolated hearts, however, the positive inotropic, chronotropic and arrhythmogenic effects of histamine were demonstrated to be mediated exclusively by H_2-receptors, since the effects were antagonized by H_2-blockers and mimicked by H_2-agonists, whereas H_1-antagonists were without effect (Levi et al., 1976). Therefore, if ischemia causes histamine release, the arrhythmogenic effects of digitalis may be enhanced. Pretreatment with cimetidine, however, failed to abolish the LAD artery ligation-induced reduction in time to onset of arrhythmias during perfusion of isolated hearts with a toxic concentration of digoxin. In hearts obtained from reserpine-treated guinea pigs, cimetidine pretreatment produced similar results. Thus, neither catecholamines or histamine nor the combination both are responsible for the early arrhythmias observed in LAD artery ligated hearts perfused with digitalis.

Studies with coronary artery-occluded isolated guinea-pig hearts and intact, anesthetized cats have indicated that digitalis is not uniformly distributed. Similar unequal digitalis binding in ischemic and non-ischemic tissues were also observed by other investigators (Thompson et al., 1974; Beller et al., 1976). Therefore, such non-uniform glycoside distribution resulting in non-homogeneous glycoside effect may predispose the heart to the development of arrhythmias by

non-uniformly altering the action potential duration and conduction in the myocardium. Such a system was simulated by cannulation of the left anterior descending coronary artery in isolated guinea-pig heart, and by differential perfusion of the heart with digitalis. In this model, the time to the glycoside-induced arrhythmias was, however, not different from that observed in uniformly perfused hearts. Thus, a non-uniform glycoside effect produced under the present experimental conditions did not reduce the tolerance of the heart to the arrhythmogenic effects of digitalis.

On the basis of these data and results that LAD artery ligation enhances the toxic actions of digitalis, the increased sensitivity of an ischemic heart to digitalis appears to be the presence of the glycoside in normal (non-ischemic) tissue adjacent to an ischemic zone. Since the concentration of digitalis in ischemic tissues is low, it may be speculated that electrophysiological changes in ischemic tissues play an important role. Thus, non-uniform alterations in electrical events occurring in border zones may produce arrhythmias.

Ischemic cells have been shown to release K^+ to the extracellular space by increasing the membrane permeability to this ion (Harris et al., 1954; Hill and Gettes, 1980). Since elevation of extracellular K^+ partially depolarizes myocardial cells, it was hypothesized that the local liberation of K^+ is an important factor in ectopic excitation during early ischemia and necrosis (Harris, 1966). It was further speculated that the elevated concentration of K^+ within a

region and the resulting K^+ gradient at the boundary produce an increased excitability to electrical stimulation and injury current within the ischemic region, both of which summate and generate ectopic impulses (Hoffman, 1966; Hirshe et al., 1980; Janse et al., 1980). Therefore, in the ischemic myocardium with no arrhythmic beats, digitalis may enhance these electrical events and produce arrhythmias. The hypothesis that an elevated extracellular K^+ concentration in the ischemic zone augments the arrhythmogenic action of digitalis was tested using a similar separate-perfusion guinea-pig heart model. Instead of a normal solution containing 5.8 mM K^+, a solution containing 10.6 mM K^+ was perfused via the cannulated LAD artery, while the rest of the heart was perfused with a solution containing normal K^+ and digoxin. Since the extracellular K^+ concentration in acutely ischemic tissues was found to be between 8 and 12 mM, 10.6 mM K^+ was chosen as the value to be used for the present experiments. The rationale behind this experimental procedure was to produce a model as close to an intact ischemic heart as possible. In this model, the onset of glycoside-induced arrhythmias was significantly earlier than that in control hearts perfused with normal K^+ and digoxin to the whole heart. Since the differential perfusion by itself did not produce arrhythmias, these results clearly demonstrate that the presence of an area with elevated extracellular K^+ concentrations within the heart, even in the absence of ischemia, is sufficient to augment the toxic action of digitalis. Whether this is due to the proposed mechanism described above is still a question to be answered. However, the present results, despite the well known observation that

K^+ antagonizes glycoside binding to Na,K-ATPase and thus reduces the digitalis action upon the heart, suggest that the mechanism involved is a complex interaction of ionic events occurring in tissue areas in contact with both non-ischemic and ischemic tissues.

Several mechanisms by which the above phenomenon could occur may be proposed. It has been shown that ischemia causes a reduction in the resting membrane potential of myocardial cells (Carmeliet et al., 1969; Wit and Bigger, 1975). It has also been reported that under certain conditions ischemic cardiac cells can generate propagated and automatic action potentials (Carmeliet et al., 1969; Cranefield et al., 1971). These action potentials were due to slow inward currents mainly carried by calcium ions and therefore were named "slow responses". Slow responses were elicited in the ischemic myocardium when a high concentration of catecholamines or K^+ was administered to the ischemic area (Scherlag et al., 1974). This is probably due to catecholamine- or K^+-induced enhancement of slow inward current. Digitalis may cause the appearance of automatic activity by contributing to the depolarization process such that the slow inward current is augmented and slow responses result. Since the completely ischemic areas are not exposed to digitalis, only the border ischemic areas may undergo such electrophysiological events. This concept supports the importance of border ischemic tissues in the genesis of arrhythmias.

Elevation of extracellular K^+ causes a shortening of the action potential duration and a lengthening of conduction time (Weiss and Shine, 1981). Local changes in these parameters have been proposed to be responsible for arrhythmias observed in myocardial ischemia (Han

and Moe, 1964; Wit and Bigger, 1975; El-Sherif et al., 1977). Therefore, in ischemic conditions without arrhythmias, digitalis may enhance the non-uniform changes in refractory period and conduction, and elicit arrhythmias early.

In conclusion, the enhanced arrhythmogenic effects of digitalis in acute myocardial ischemia may be due to regional extracellular K^+ accumulation.

SUMMARY AND CONCLUSIONS

Myocardial ischemia produced by LAD artery ligation enhanced the arrhythmogenic effects of digitalis in both intact animals and in isolated perfused heart preparations. Removal of the autonomic input to the heart failed to modify this effect. The direct action of digitalis on the heart appears to be the primary mechanism for the phenomenon.

Ischemia produced by LAD artery ligation or by reduced perfusion of the entire heart failed to alter Na,K-ATPase activity, glycoside binding to the enzyme or sodium pump activity. Partial ischemia also did not affect the reserve capacity of the sodium pump. Infusion of a high K^+, but not normal K^+, solution to the LAD artery caused an earlier onset of digoxin-induced arrhythmias. It may be speculated that the mechanism by which acute myocardial ischemia predisposes the heart to the toxic effects of digitalis is due to an ischemia-induced elevation of extracellular K^+.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abrahamsson, T., Almgren, O. and Carlsson, L.: Ischemia-induced release of noradrenaline from the isolated perfused rat heart. Influence of perfusion substrate and duration of ischemia. J. Mol. Cell. Cardiol. 13: 1, 1981.

Akera, T. and Brody, T.M.: The role of Na^+,K^+-ATPase in the inotropic action of digitalis. Pharmacol. Rev. 29: 187-220, 1977.

Akera, T. and Brody, T.M.: Myocardial membranes: Regulation and function of the sodium pump. Ann. Rev. Physiol. 44: 375-388, 1982.

Akera, T. and Cheng, V-J.K.: A simple method for the determination of affinity and binding site concentration in receptor binding studies. Biochim. Biophys. Acta 470: 412-423, 1977.

Akera, T., Bennett, R.T., Olgaard , M.K. and Brody, T.M.: Cardiac Na^+,K^+-adenosine triphosphatase inhibition by ouabain and myocardial sodium: A computer simulation. J. Pharmacol. Exp. Ther. 199: 287-297, 1976.

Akera, T., Brody, T.M., So, R.H.-M., Tobin, T. and Baskin, S.I.: Factors and agents that influence cardiac glycoside-Na,K-ATPase interaction. Ann. N.Y. Acad. Sci. 242: 617-634, 1974.

Akera, T., Larsen, F.S. and Brody, T.M.: The effect of ouabain on sodium-and potassium-activated adenosine triphosphatase from the hearts of several mammalian species. J. Pharmacol. Exp. Ther. 170: 17-26, 1969.

Akera, T., Olgaard, M.K., Temma, K. and Brody, T.M.: Development of the positive inotropic action of ouabain: Effects of transmembrane sodium movement. J. Pharmacol. Exp. Ther. 203: 675-684, 1977.

Alexander, R.W., Kent, K.M., Pisano, J.J., Keiser, H.R. and Cooper, T.: Regulation of postocclusive hyperaemia by endogenously synthesized prostaglandins in the dog heart. J. Clin. Invest. 55: 1174-1181, 1975.

Allen, J.C. and Schwartz, A.: A possible biochemical explanation for the insensitivity of the rat to cardiac glycosides. J. Pharmacol. Exp. Ther. 168: 42-46, 1969.

Allen, J.C., Martinez-Maldonado, M., Eknoyan, G., Suki, W.N. and Schwartz, A.: Relation between digitalis binding in vivo and inhibition of sodium, potassium-adenosine triphosphatase in canine kidney. Biochem. Pharmacol. 20: 73-80, 1971.

Angelucci, L., Lorentz, G. and Baldieri, M.: The relation between noradrenaline content of rabbit heart muscle and the amount of K-strophanthin needed to produce arrhythmias. J. Pharm. Pharmacol. 18: 775-782, 1966.

Aronson, R.S. and Cranefield, P.F.: Electrical activity of canine cardiac Purkinje fibers in sodium-free, calcium-rich solution. J. Gen. Physiol. 61: 786-808, 1973.

Aronson, R.S. and Gelles, J.M.: The effect of ouabain, dinitrophenol and lithium on the pacemaker current in sheep cardiac Purkinje fibers. Circ. Res. 40: 517-524, 1977.

Balasubramanian, V., McNamara, D.B., Singh, J.N. and Dhalla, N.S.: Biochemical basis of heart function. X. Reduction in the Na^+,K^+-stimulated ATPase activity in failing rat heart due to hypoxia. Can. J. Physiol. Pharmacol. 51: 504-510, 1973.

Balcon, R., Hay, J. and Sowton, E.: Hemodynamic effects of rapid digitalization following acute myocardial infarction. Brit. Heart J. 30: 373-376, 1968.

Banka, V.S., Chadda, K.D., Bodenheimer, M.M. and Helfant, R.H.: Digitalis in experimental acute myocardial infarction: Differential effects on contractile performance of ischemic, border and nonischemic ventricular zones in the dog. Am. J. Cardiol. 35: 801-808, 1975.

Baum, G.L., Dick, M.M., Blum, A., Kaupe, A. and Carballo, J.: Factors involved in digitalis sensitivity in chronic pulmonary insufficiency. Am. Heart J. 57: 460-642, 1959.

Beller, G.A. and Smith, T.W.: Digitalis toxicity during acute hypoxia in intact conscious dogs. J. Pharmacol. Exp. Ther. 193: 963-968, 1975.

Beller, G.A., Conroy, J. and Smith, T.W.: Ischemia-induced alterations in myocardial Na^+,K^+-ATPase and cardiac glycoside binding. J. Clin. Invest. 57: 341-350, 1976.

Beller, G.A., Smith, T.W., Abelmann, W.H., Haber, E. and Hood, W.B. Jr.: Digitalis intoxication: A prospective clinical study with serum level correlations. N. Engl. J. Med. 284: 989-997, 1971.

Bellet, S., Johnston, C.G. and Schecter, A.: Effect of cardiac infarction on the tolerance of dogs to digitalis. Arch. Int. Med. 54: 509-516, 1934.

Berger, H.J., Zarbet, B.L., Speroff, L., Cohen, L.S. and Wolfson, S.: Regional cardiac prostaglandin release during myocardial ischemia in anesthetized dogs. Circ. Res. 38: 566-571, 1976.

Berti, F. and Shore, P.A.: Interaction of reserpine and ouabain on amine concentrating mechanisms in the adrenergic neurone. Biochem. Pharmacol. 16: 2271-2274, 1967.

Bonting, S.L., Simon, K.A. and Hawkins, N.M.: Studies on sodium-potassium-activated adenosine triphosphatase. I. Quantitative distribution in several tissues of the cat. Arch. Biochem. Biophys. 95: 416-423, 1961.

Bosnjak, Z.J., Zuperku, E.J., Coon, R.L. and Kampine, J.P.: Acute coronary artery occlusion and cardiac sympathetic afferent nerve activity. Proc. Soc. Exp. Biol. Med. 161: 142-148, 1979.

Boyajy, L.D. and Nash, C.B.: Influence of reserpine on arrhythmias, inotropic effects and myocardial potassium balance induced by digitalis materials. J. Pharmacol. Exp. Ther. 147: 193-201, 1965.

Boyajy, L.D. and Nash, C.B.: Alteration of ouabain toxicity by cardiac denervation. Toxicol. Appl. Pharmacol. 9: 199-208, 1966.

Braunwald, E., Maroko, P.R. and Libby, P.: Reduction of infarct size following coronary occlusion. Circ. Res. 35: 192-201, 1974.

Bresnahan, G.F., Roberts, R. and Shell, W.E.: Deleterious effects due to hemorrhage after myocardial reperfusion. Am. J. Cardiol. 33: 82-86, 1974.

Brown, A.M.: Excitation of afferent cardiac sympathetic nerve fibers during myocardial ischemia. J. Physiol. London 190: 35-53, 1967.

Brown, A.M. and Malliani, A.: Spinal sympathetic reflexes initiated by coronary receptors. J. Physiol. London 212: 685-705, 1971.

Cagin, N.A., Somberg, J., Bounous, H., Mittag, T., Raines, A. and Levitt, B.: The influence of spinal cord transection on the capacity of digitoxin to induce cardiotoxicity. Arch. Int. Pharmacodyn. Ther. 207: 340-347, 1974.

Carmeliet, E. and Vereecke, J.: Adrenalin and the plateau phase of the cardiac action potential. Importance of Ca^{++}, Na^{+}, and K^{+} conductance. Pflugers Arch. Ges. Physiol. 313: 300-315, 1969.

Cattell, M. and Gold, H.: The influence of digitalis glycosides on the force of contraction of mammalian cardiac muscle. J. Pharmacol. Exp. Ther. 62: 116-125, 1938.

Ceremuzynski, L., Staszewska-Barczak, J. and Herbaczynaska-Cedro, K.: Cardiac rhythm disturbances and the release of catecholamines after acute coronary occlusion in dogs. Cardiovasc. Res. 3: 190-197, 1969.

Ciofalo, F., Levitt, B. and Roberts, J.: Some factors affecting ouabain-induced arrhythmias in the reserpine-treated cat. Brit. J. Pharmacol. 30: 143-154, 1967.

Clausen, T. and Hansen, O.: Active Na-K transport and the rate of ouabain binding. The effect of insulin and other stimuli on skeletal muscle and adipocytes. J. Physiol. 270: 415-430, 1977.

Cooper, Y., Gilbert, J.W., Bloodwell, R.D. and Crout, R.J.: Chronic extrinsic cardiac denervation by regional neural ablation. Circ. Res. 9: 275-281, 1961.

Corr, P.B., Pearle, D.L., Hinton, J.R., Roberts, W.C. and Gillis, R.A.: Site of myocardial infarction. A determinant of the cardiovascular changes induced in the cat by coronary occlusion. Circ. Res. 39: 840-847, 1976.

Costantin, L.: Extracardiac factors contributing to hypotension during coronary occlusion. Am. J. Cardiol. 11: 205-217, 1963.

Cranefield, P., Klein, H.O. and Hoffman, B.F.: Conduction of the cardiac impulse: I. Delay, block, and one-way block in depressed Purkinje fibers. Circ. Res. 28: 199-208, 1971.

Dengler, H.J., Spiegel, H.E. and Titus, E.O.: Uptake of tritium-labelled norepinephrine in brain and other tissues of cat in vitro. Science 133: 1072-1073, 1962.

Dhalla, N.S., Singh, J.N., Fedelesova, M., Balasubramanian, V. and McNamara, D.B.: Biochemical basis of heart function. XII. Sodium-potassium stimulated adenosine triphosphatase activity in the perfused rat heart made to fail by substrate-lack. Cardiovasc. Res. 8: 227-236, 1974.

Dutta, S., Goswami, S., Datta, D.K., Lindower, J.O. and Marks, B.H.: The uptake and binding of six radiolabeled cardiac glycosides by guinea-pig hearts and by isolated sarcoplasmic reticulum. J. Pharmacol. Exp. Ther. 164: 10-21, 1968.

El-Sherif, N., Scherlag, B.J., Lazzara, R. and Hope, R.R.: Reentrant ventricular arrhythmias in the late myocardial infarction period. II. Patterns of initiation and termination of reentry. Circulation 55: 702-719, 1977.

Evans, D.B., Peschka, M.T., Lee, R.J. and Laffan, R.J.: Anti-arrhythmic action of nadolol, a beta-adrenergic receptor blocking agent. Eur. J. Pharmacol. 35: 17-27, 1976.

Evans, D.E. and Gillis, R.A.: Effect of ouabain and its interaction with diphenylhydantoin on cardiac arrhythmias induced by hypothalamic stimulation. J. Pharmacol. Exp. Ther. 195: 577-586, 1975.

Farber, J.L., Chien, K.R. and Mittnacht, S.: The pathogenesis of irreversible cell injury in ischemia. Am. J. Pathol. 102: 271-281, 1981.

Felder, R.B. and Thames, M.D.: Interaction between cardiac receptors and sinoaortic baroreceptors in the control of efferent cardiac sympathetic nerve activity during myocardial ischemia in dogs. Circ. Res. 45: 728-736, 1979.

Felder, R.B. and Thames, M.D.: The cardiocardiac sympathetic reflex during coronary occlusion in anesthetized dogs. Circ. Res. 48: 685-692, 1981.

Ferrier, G.R.: Digitalis arrhythmias: Role of oscillatory afterpotentials. Progr. Cardiovasc. Dis. 19: 459-474, 1977.

Ferrier, G.R., Saunders, J.H. and Mendez, C.: Cellular mechanism for the generation of ventricular arrhythmias by acetylstrophanthidin. C irc. Res. 32: 600-609, 1973.

Forssman, D., Hansson, G. and Jansen, C.C.: Adrenal function in coronary thrombosis. Acta Medica Scandinavica 142: 441-449, 1952.

Friedman, J.P., Harris, C.N. and Goldman, R.H.: Digitalis toxicity in normal and hypoxemic dogs: Correlation with myocardial Na^+,K^+-activated ATPase activity. Clin. Res. 20: 205, 1972.

Gillis, R.A. and Quest, J.A.: The role of the nervous system in the cardiovascular effects of digitalis. Pharmacol. Rev. 31: 19-97, 1979.

Gillis, R.A., Clancy, M.M. and Anderson, R.J.: Deleterious effects of bretylium in cats with digitalis-induced ventricular tachycardia. Circulation 47: 974-983, 1973.

Gillis, R.A., Jolson, H., Thibodeaux, H. and Levitt, B.: Antagonism of deslanoside-induced cardiotoxicity by combined nicotinic and muscarinic blockade of autonomic ganglia. J. Pharmacol. Exp. Ther. 195: 126-132, 1975.

Gillis, R.A., Raines, A., Sohn, Y.J., Levitt, B. and Standaert, F.G.: Neuroexcitatory effects of digitalis and their role in the development of cardiac arrhythmias. J. Pharmacol. Exp. Ther. 183: 154-168, 1972.

Ginsburg, R., Bristow, M.R., Kantrowitz, N., Baim, D.S. and Harrison, D.C.: Histamine provocation of clinical coronary artery spasm: Implications concerning pathogenesis of variant angina pectoris. Am. Heart J. 102: 819-822, 1981.

Glynn, I.M.: The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol. London 136: 148-173, 1957.

Glynn, I.M.: The action of cardiac glycosides on ion movements. Pharmacol. Rev. 16: 381-407, 1964.

Goldman, R.H., Coltart, D.J. and Friedman, J.P.: The inotropic effects of digitalis in hyperkalemia: Relation to Na,K-ATPase. Circulation 48: 830-839, 1973.

Han, J. and Moe, G.K.: Nonuniform recovery of excitability in ventricular muscle. Circ. Res. 14: 44-60, 1964.

Hansen, O., Jensen, J. and Norby, J.G.: Mutual exclusion of ATP, ADP and g-strophanthin binding to Na^+,K^+-ATPase. Nature 234: 122-123, 1971.

Hargreave, F.E.: Digitalis and cor pulmonale. Brit. Med. J. 2: 943, 1965.

Harken, A.H., Barlow, C.H., Harden, W.R. and Chance, B.: Two and three dimensional display of myocardial ischemic "border zone" in dogs. Am. J. Cardiol. 42: 954-959, 1978.

Harris, A.S.: Potassium and experimental coronary artery occlusion. Am. Heart J. 71: 797-802, 1966.

Harris, A.S., Bisteni, A., Russell, R.A., Brigham, J.C. and Firestone, J.E.: Excitatory factors in ventricular tachycardia resulting from myocardial ischemia: Potassium a major excitant. Science 199: 200-203, 1954.

Harrison, D.C., Robinson, M.D. and Kleiger, R.E.: Role of hypoxia in digitalis toxicity. Am. J. Med. Sci. 256: 352-359, 1968.

Harvey, S.C.: The effects of ouabain and phenytoin on myocardial noradrenaline. Arch. Int., Pharmacodyn. Ther. 213: 222-234, 1975.

Hayashi, K.D., Moss, A.J. and Yu, P.N.: Urinary catecholamine secretion in myocardial infarction. Circulation 40: 473-481, 1969.

Hearse, D.J., Opie, L.H., Katzeff, I.E., Lubbe, W.F., Vanderwerff, T.J., Peisach, M. and Boulle, G.: Characterization of the border zone in acute regional ischemia in the dog. Am. J. Cardiol. 40: 716-726, 1977.

Herrick, J.B.: Clinical features of sudden obstruction of the coronary arteries. J. Am. Med. Assoc. 59: 2015-2020, 1912.

Hill, J.L. and Gettes, L.S.: Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. Circulation 61: 768-778, 1980.

Hill, J.L., Gettes, L.S., Lynch, M.R. and Hebert, N.C.: Flexible valinomycin electrodes for on-line determination of intravascular and myocardial K^+. Am. J. Physiol. 235: H455-H459, 1978.

Hirche, H.J., Franz, C., Bos, L., Bissig, R., Lang, R. and Schramm, M.: Myocardial extracellular K^+ and H^+ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. J. Mol. Cell. Cardiol. 12: 579-593, 1980.

Hoffman, B.F.: The genesis of cardiac arrhythmia. Prog. Cardiovasc. Dis. 8: 319-329, 1966.

Hood, W.B. Jr., Covelli, V.H. and Norman, J.C.: Acute coronary occlusion in pigs: Effects of acetylstrophanthidin. Cardiovasc. Res. 3: 441-446, 1969.

Hood, W.B. Jr., McCarthy, B. and Lown, B.: Myocardial effects of isoproterenol and acetylstrophanthidin. Circ. Res. 21: 195-199, 1967.

Horackova, M. and Vassort, G.: Excitation-contraction coupling in frog heart: Effect of veratrine. Pfluegers Arch. Eur. J. Physiol. 352: 291302, 1974.

Hougen, T.J. and Smith, T.W.: Inhibition of myocardial monovalent cation active transport by subtoxic doses of ouabain in the dog. Circ. Res. 42: 856-863, 1978.

Howard, L.L., Brunschwig, J.P. and Pressman, B.C.: Affect of the ionophore monensin on cultured beating heart cells. Pharmacologist 18: 122, 1976.

Ismail-Beigi, F. and Edelman, I.S.: The mechanism of the calorigenic action of thyroid hormone. Stimulation of Na^+,K^+-activated adenosine triphosphatase activity. J. Gen. Physiol. 57: 710-722, 1971.

Janse, M.J., Cinca, J. and Morena, H.: The border zone in myocardial ischemia. An electrophysiological, metabolic and histochemical correlation in the pig heart. Circ. Res. 44: 576-588, 1979.

Jennings, R.B., Reimer, K.A., Hill, M.L. and Mayer, S.E.: Total ischemia in dog hearts in vitro. 1. Comparison of high energy phosphate production, utilization and depletion, and of adenosine nucleotide catabolism in total ischemia in vitro vs. severe ischemia in vivo. Circ. Res. 49: 892-900, 1981.

Kane, J.J., Murphy, M.L., Bissett, J.K., De Soyza, N., Doherty, J.E. and Straub, K.D.: Mitochondrial function, oxygen extraction, epicardial S-T segment changes in tritiated digoxin distribution after reperfusion of ischemic myocardium. Am. J. Cardiol. 36: 218-224, 1975.

Karliner, J.S. and Braunwald, E.: Present status of digitalis treatment of acute myocardial infarction. Circulation 45: 891-902, 1972.

Karlsberg, R.P., Cryer, P.E. and Roberts, R.: Serial plasma catecholamine responses early in the course of clinical acute myocardial infarction: Relationship to infarct extent and mortality. Am. Heart J. 102: 24-29, 1981.

Kass, R.S., Tsien, R.W. and Weingart, W.: Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibers. J. Physiol. London 281: 209-226, 1978.

Kelliher, G.J. and Roberts, J.: A study of the anti-arrhythmic action of certain beta-blocking agents. Am. Heart J. 87: 458-467, 1974.

Kedzi, P., Kordenat, R. and Misra, S.: Reflex inhibitory effects of vagal afferents in experimental myocardial infarction. Am. J. Cardiol. 33: 853-860, 1974.

Kleber, A.G., Janse, M.J., Capelle, F.J.L. and Durrer, D.: Mechanism and time course of S-T and T-Q segment changes during acute regional myocardial ischemia in the pig heart determined by extracellular and intracellular recordings. Circ. Res. 42: 603-613, 1978.

Klein, R.F., Troyer, W.G. and Thompson, H.K.: Catecholamine excretion in myocardial infarction. Arch. Intern. Med. 122: 476-482, 1968.

Korhonen, U.R., Jounela, A.J., Pakarinen, A.J., Pentikainen, P.J. and Takkunen, J.T.: Pharmacokinetics of digoxin in patients with acute myocardial infarction. Am. J. Cardiol. 44: 1190-1194, 1979.

Korth, C., Marx, H. and Weinberg, S.: Uber die Wirkung des Strophanthins auf des zentralnervensystem. Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol. 185: 42-56, 1937.

Kraemer, R.J., Phernetton, T.M. and Folts, J.D.: Prostaglandin-like substances in coronary venous blood following myocardial ischemia. J. Pharmacol. Exp. Ther. 199: 611-619, 1976.

Kreuger, E. and Unna, K.: Comparative studies on the toxic effects of digitoxin and ouabain in cats. J. Pharmacol. Exp. Ther. 76: 282-294, 1942.

Ku, D. and Lucchesi, B.R.: Ischemic-induced alterations in cardiac sensitivity to digitalis. Europ. J. Pharmacol. 57: 135-147, 1979.

Ku, D., Akera, T., Frank, M., Brody, T.M. and Iwasa, J.: The effects of grayanotoxin I and α-dihydrograyanotoxin II on guinea-pig myocardium. J. Pharmacol. Exp. Ther. 200: 363-372, 1977.

Ku, D., Akera, T., Pew, C.L. and Brody, T.M.: Cardiac glycosides: Correlations among Na^+,K^+-ATPase, sodium pump and contractility in the guinea-pig heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 285: 185-200, 1974.

Kumar, R., Hood, W.B. Jr., Joison, J., Gilmour, D.P., Norman, J.C. and Abelmann, W.H.: Experimental myocardial infarction. VI. Efficacy and toxicity of digitalis in acute and healing phase in intact conscious dogs. J. Clin. Invest. 49: 358-364, 1970.

Lammerant, J., De Herdt, P. and De Schryver, C.: Direct release of myocardial catecholamine into the left heart chambers: The enhancing effect of acute coronary occlusion. Arch. Int. Pharmacodyn. Ther. 163: 219-226, 1966.

Langer, G.A.: Effects of digitalis on myocardial ionic exchange. Circulation 46: 180-187, 1972.

Langer, G.A. and Serena, S.D.: Effects of strophanthidin upon contraction and ionic exchange in rabbit ventricular myocardium: Relation to control of active state. J. Mol. Cell. Cardiol. 65-90, 1970.

Lathers, C.M., Kelliher, G.J., Roberts, J. and Beasley, A.B.: Non-uniform cardiac sympathetic nerve discharge. Mechanism for coronary occlusion and digitalis-induced arrhythmia. Circulation 57: 1058-1065, 1978.

Lechat, P. and Schmitt, H.: Interactions between the autonomic nervous system and the cardiovascular effects of ouabain in guinea-pig. Europ. J. Pharmacol. 78: 21-32, 1982.

Lederer, W.J. and Tsien, R.W.: Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. J. Physiol. London 263: 73-100, 1976.

Lee, K.S. and Klaus, W.: The subcellular basis for the mechanism of inotropic action of cardiac glycosides. Pharmacol. Rev. 23: 193-261, 1971.

Levi, R.: Effects of exogenous and immunologically released histamine on the isolated heart: A quantitative comparison. J. Pharmacol. Exp. Ther. 182: 227-238, 1972.

Levi, R., Allan, G. and Zavecz, J.H.: Cardiac histamine receptors. Fed. Proc. 35: 1942-1947, 1976.

Levi, R. and Burke, J.A.: Cardiac anaphylaxis: SRS-A potentiates and extends the effects of released histamine. Europ. J. Pharmacol. 62: 41-49, 1980.

Levi, R. and Capurro, N.: Cardiac histamine-ouabain interaction: Potentiation by ouabain of the arrhythmogenic effects of histamine. J. Pharmacol. Exp. Ther. 192: 113-119, 1974.

Levitt, B., Cagin, N.A., Somberg, J., Bounous, H., Mittag, T. and Raines, A.: Alteration of the effects and distribution of ouabain by spinal cord transection in the cat. J. Pharmacol. Exp. Ther. 185: 24-28, 1973.

Levitt, B., Gillis, R.A., Roberts, J. and Raines, A.: Influence of the cardiac vagus nerves on the cardiotoxicity of acetylstrophanthidin (AcS), ouabain (O), and digitoxin (D). Pharmacologist 12: 304, 1970.

Lewis, P.J. and Haeusler, G.: Reduction of sympathetic nervous system activity as a mechanism for the hypotensive effect of propranolol. Nature New Biol. 256: 440-441, 1975.

Lindmar, R. and Loffelholz, K.: The neuronal efflux of noradrenaline: Dependency on sodium and facilitation by ouabain. Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol. 284: 93-100, 1974.

Lo Sasso, A.M. and Paradise, R.R.: Influence of the vagus on development of ventricular arrhythmias induced by acetylstrophanthidin in dogs anesthetized with pentobarbital or halothane. Anesth. Analg. Curr. Res. 48: 317-327, 1969.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

Lubbe, W.F., Peisach, M., Pretorius, R., Bryuneel, K.J.J. and Opie, L.H.: Distribution of myocardial blood flow before and after coronary artery ligation in the baboon. Relation to early ventricular fibrillation. Cardiovasc. Res. 8: 478-487, 1974.

Lullmann, H., Timmermans, P.B.M.W.M. and Ziegler, A.: Accumulation of drugs by resting or beating cardiac tissue. Europ. J. Pharmacol. 60: 277-285, 1979.

Lum, B.K.B., Yano, S.S. and Yatsushiro, G.N.: Cardiotoxic interactions between sympathomimetic amines and ouabain. Proc. West Pharmacol. Soc. 20: 275-280, 1977.

Malliani, A., Schwartz, P.J. and Zanchetti, A.: A sympathetic reflex elicited by experimental coronary occlusion. Am. J. Physiol. 217: 703-709, 1969.

Malsky, P.M., Vokonas, P.S., Paul, S.J., Robbins, S.L. and Hood, W.S.: Autoradiographic measurement of regional blood flow in normal and ischemic myocardium. Am. J. Physiol. 232: 576-583, 1977.

Mason, D.T., Zelis, R., Lee, G., Hughes, J.L., Spann, J.F. Jr. and Amsterdam, E.A.: Current concepts and treatment of digitalis toxicity. Am. J. Cardiol. 27: 546-559, 1971.

Matsui, H. and Schwartz, A.: Mechanism of cardiac glycoside inhibition of the (Na^+,K^+)-dependent ATPase from cardiac tissue. Biochem. Biophys. Acta 151: 655-663, 1968.

McDonald, T.F. and MacLeod, D.P.: Maintenance of resting potential in anoxic guinea-pig ventricular muscle: Electrogenic sodium pumping. Science 172: 570-572, 1971.

McDonald, T.F. and MacLeod, D.P.: Anoxic atrial and ventricular muscle electrical activity, cell potassium and metabolism. A comparative study. J. Mol. Cell. Cardiol. 5: 149-159, 1973.

McDonald, T.F. and MacLeod, D.P.: Metabolism and the electrical activity of anoxic ventricular muscle. J. Physiol. London 229: 559-582, 1973.

McLain, P.L.: Effects of cardiac glycosides on spontaneous efferent activity in vagus and sympathetic nerves of cats. Int. J. Neuropharmacol. 8: 379-387, 1969.

McLain, P.L., Kruse, T.K. and Redick, T.F.: The effect of atropine on digitoxin bradycardia in cats. J. Pharmacol. Exp. Ther. 126: 76-81, 1958.

Mendez, C., Aceves, J. and Mendez, R.: Inhibition of adrenergic cardiac acceleration by cardiac glycosides. J. Pharmacol. Exp. Ther. 131: 191-198, 1961.

Morris, J.J., Taft, C.V., Whalen, R.E. and McIntosh, H.D.: Digitalis and experimental myocardial infarction. Am. Heart J. 77: 342-355, 1969.

Moss, A.J., Davis, H.T., Conard, D.L., De Camilla, J.J. and Odoroff, C.L.: Digitalis-associated cardiac mortality after myocardial infarction. Circulation 64: 1150-1155, 1981.

Nadeau, R. and De Champlain, J.: Comparative effects of 6-hydroxydopamine and reserpine on ouabain toxicity. Life Sci. 13: 1753-1761, 1973.

Nayler, W.G.: The role of calcium in the ischemic myocardium. Am. J. Pathol. 102: 262-270.

Nayler, W.G., Poole-Wilson, P.A. and Williams, A.: Hypoxia and calcium. J. Mol. Cell. Cardiol. 11: 683-706, 1979.

Oberg, B. and Thoren, P.: Circulatory responses to stimulation of medullated and non-medullated afferents in the cardiac nerve in the cat. Acta Physiol. Scand. 87: 121-132, 1973.

Ogletree, M.L., Flynn, J.T., Feola, M. and Lefer, A.M.: Early prostaglandin release from the ischemic myocardium in the dog. Surgery, Gynecol. Obstet. 144: 743-740, 1977.

Pace, D.G. and Gillis, R.A.: Neuroexcitatory effects of digoxin in the cat. J. Pharmacol. Exp. Ther. 199: 583-600, 1976.

Papp, J.G. and Vaughan Williams, E.M.: The effect of bretylium on intracellular cardiac action potentials in relation to its anti-arrhythmic and local anesthetic activity. Brit. J. Pharmacol. 37: 380-390, 1969.

Pappano, A.J.: Calcium-dependent action potentials produced by catecholamines in guinea pig atrial muscle fibers depolarized by potassium. Circ. Res. 27: 379-390, 1970.

Penna, M., Linares, F. and Caceres, L.: Mechanism for cardiac stimulation during hypoxia. Am. J. Physiol. 208: 1237-1242, 1965.

Powell, J.R. and Brody, M.J.: Identification and specific blockage of two receptors for histamine in the cardiovascular system. J. Pharmacol. Exp. Ther. 196: 1-14, 1976.

Puri, P.S.: Modification of experimental infarct size by cardiac drugs. Am. J. Cardiol. 33: 521-528, 1974.

Raab, W. and Gigee, W.: Total urinary catechol excretion in cardiovascular and other clinical conditions. Circulation 9: 592-599, 1954.

Raab, W., Van Lith, P., Lepeschkin, E. and Herrlich, H.C.: Catecholamine-induced myocardial hypoxia in the presence of impaired coronary dilatability independent of external cardiac work. Am. J. Cardiol. 9: 455-470, 1962.

Raines, A., Levitt, B. and Standaert, F.G.: The effect of spinal section on ventricular rhythm disorders induced by ouabain. Arch. Int. Pharmacodyn. Ther. 170: 485-490, 1967.

Raines, A., Moros, D. and Levitt, B.: The effect of guanethidine on ouabain-induced ventricular arrhythmia in the cat. Arch. Int. Pharmacodyn. Ther. 174: 373-377, 1968.

Raper, C. and Wale, J.: Cardiac arrhythmias produced by interaction of ouabain and beta-receptor stimulation. Eur. J. Pharmacol. 6: 223-234, 1969.

Rau, E.E., Shine, K.I. and Langer, G.A.: Potassium exchange and mechanical performance in anoxic mammalian myocardium. Am. J. Physiol. 232: H85-H94, 1977.

Recordati, G., Schwartz, P.J., Pagani, M., Malliani, A. and Brown, A.M.: Activation of actiac vagal receptors during myocardial ischemia. Experientia 27: 1423-1424, 1971.

Reimer, K.A., Lowe, J.E., Rasmussen, M.M. and Jennings, R.B.: Wave-front phenomenon of ischemic cell death. 1. Myocardial infarct size vs. duration of coronary occlusion in dogs. Circulation 56: 786-798, 1977.

Repke, K.R.H.: Uber den biochemischen wirkungsmodus von digitalis. Klin. Wochenschr. 42: 157-165, 1964.

Repke, K.R.H., Est, M. and Portius, H.J.: Uber die ursache der spicies-untershiede in der digitalisempfindlichkeit. Biochem. Pharmacol. 14: 1785-1802, 1965.

Richardson, J.A.: Plasma catecholamine concentration in acute infarction. Coronary Heart Diseases (Likoff, W. and Moyer, J.H., eds.), Grune and Strutton, New York, pp. 273-277, 1963.

Robinson, G.C. and Wilson, F.N.: A quantitative study of the effect of digitalis on the heart of the cat. J. Pharmacol. Exp. Ther. 10: 491-507, 1918.

Rocha E Silva, M.: Action of histamine upon the circulatory apparatus. In Handbook of Experimental Pharmacology (Rocha E Silva, M., ed.), 18: part 1, Histamine. Springer-Verlag, New York, 1966.

Rosen, M.R., Wit, A.L. and Hoffmen, B.F.: Electrophysiology and pharmacology of cardiac arrhythmias. IV. Cardiac antiarrhythmic and toxic effects of digitalis. Am. Heart J. 89: 391-399, 1975.

Saito, H., Otani, T., Shudo, I. and Tanabe, T.: Effect of 6-hydroxy-dopamine on cardiotoxicity of ouabain in guinea pigs. Jap. J. Pharmacol. 24: 923-925, 1974.

Sayen, J.J., Sheldon, W.F. and Horwitz, O.: Studies of coronary disease in the experimental animal. II. Polarographic determinations of local oxygen availability in the dogs left ventricle during coronary occlusion and pure oxygen breathing. J. Clin. Invest. 30: 932-940, 1952.

Schatzmann, H.J.: Herzyglykoside als hemmstoffe fur den aktiven kalium- und natriumtransport durch die erythrocytenmembran. Helv. Physiol. Pharmacol. Acta 11: 346-354, 1953.

Scherlag, B.J., El-Sherif, N., Hope, R.R. and Lazzara, R.: Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. Circ. Res. 35: 372-383, 1974.

Schwartz, A.: Is the cell membrane Na^+,K^+-ATPase enzyme system the pharmacological receptor for digitalis? Circ. Res. 39: 2-7, 1976.

Schwartz, A., Lindenmayer, G.E. and Allen, J.C.: The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects. Pharmacol. Rev. 27: 3-134, 1975.

Schwartz, A., Wood, J.M., Allen, J.C., Bornet, E.P., Entman, M.L., Goldstein, M.A., Sordahl, L.A. and Suzuki, M.: Biochemical and morphologic correlates of cardiac ischemia. Am. J. Cardiol. 32: 46-61, 1973.

Selzer, A.: The use of digitalis in acute myocardial infarction. Prog. Cardiovasc. Dis. 10: 518-528, 1965.

Shahab, L., Wollenberger, A., Haase, M. and Schiller, U.: Noradrenlinabgabe aus dem hundeherzen nach vorubergehender okklusion einer korronararterie. Acta Biol. Med. Germ. 22: 135-143, 1969.

Sharma, V.K. and Banerjee, S.P.: The effect of 6-hydroxydopamine on specific (^{3}H)ouabain binding to some sympathetically innervated organs of the cat. Mol. Pharmacol. 13: 796-804, 1977.

Shen, A.C. and Jennings, R.B.: Myocardial calcium and magnesium in acute ischemic injury. Am. J. Pathol. 67: 417-440, 1972.

Shine, K.I.: Ionic events in ischemia and anoxia. Am. J. Pathol. 102: 256-261, 1981.

Siggers, D.C., Salter, C. and Fluck, D.C.: Serial plasma adrenaline and noradrenaline levels in myocardial infarction using a new double isotope technique. Brit. Heart J. 33: 878-883, 1971.

Skou, J.C.: The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochem. Biophys. Acta 23: 394-401, 1957.

Skou, J.C.: Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. Physiol. Rev. 45: 596-617, 1965.

Solti, F., Iskum, M. and Nagy, J.: Studies on the acute cardiac action of strophanthin in the dog by means of cardiac denervation. Acta Physiol. Acad. Sci. Hung. 26: 377-385, 1965.

Somberg, J.C., Bounous, H., Cagin, N. and Levitt, B.: Histamine antagonists as antiarrhythmic agents in ouabain cardiotoxicity in the cat. J. Pharmacol. Exp. Ther. 214: 375-380, 1980.

Somberg, J.C., Risler, T. and Smith, T.W.: Neural factors in digitalis toxicity: Protective effect of C-1 spinal cord transection. Am. J. Physiol. 235: H531-H536, 1978.

Staszewska-Barczak, J.: The reflex stimulation of catecholamine section during the acute stages of myocardial infarction in the dog. Clin. Sci. 41: 419-439, 1971.

Staszewska-Barczak, J. and Ceremuzynski, L.: The continuous estimation of catecholamine release in the early stages of myocardial infarction in the dog. Clin. Sci. 34: 531-539, 1968.

Stickney, J.L.: Inhibition of ^{3}H-l-norepinephrine uptake by ouabain is species dependent. Res. Commun. Chem. Pathol. Pharmacol. 14: 227-236, 1976.

Thames, M.D.: Acetylstrophanthidin-induced reflex inhibition of canine renal sympathetic nerve activity mediated by cardiac receptors with vagal afferents. Circ. Res. 44: 8-15, 1979.

Thompson, A.J., Hargis, J., Murphy, M.L. and Doherty, J.E.: Tritiated digoxin. XX. Tissue distribution in experimental myocardial infarction. Am. Heart J. 88: 319-324, 1974.

Thoren, P.: Evidence for a depressor reflex elicited from left ventricular receptors during occlusion of one coronary artery in the cat. Acta Physiol. Scand. 88: 23-34, 1973.

Tobin, T. and Brody, T.M.: Rates of dissociation of enzyme-ouabain complexes and $K_{0.5}$ values in Na^{+},K^{+}-ATPase from different species. Biochem. Pharmacol. 21: 1553-1560, 1972.

Tomlinson, C.W. and Dhalla, N.S.: Excitation-contraction coupling in heart. IX. Changes in the intracellular stores of calcium in failing hearts due to lack of substrate and oxygen. Cardiovasc. Res. 7: 470-476, 1973.

Travell, J., Gold, H. and Modell, W.: Effect of experimental cardiac infarction of response to digitalis. Arch. Intern. Med. 61: 184-197, 1938.

Tse, W.W. and Han, J.: Interaction of epinephrine and ouabain on automaticity of canine Purkinje fibers. Circ. Res. 34: 777-782, 1974.

Tsien, R.W. and Carpenter, D.O.: Ionic mechanisms of pacemaker activity in cardiac Purkinje fibers. Fed. Proc. 37: 2127-2131, 1978.

Uchida, Y. and Murao, S.: Excitation of afferent cardiac sympathetic nerve fibers during coronary occlusion. Am. J. Physiol. 226: 1094-1099, 1974.

Uchida, Y., Kamisaka, K., Murao, S. and Ueda, H.: Mechanosensitivity of afferent cardiac sympathetic nerve fibers. Am. J. Physiol. 226: 1088-1093, 1974.

Valorie, C., Thomas, M. and Shillingford, J.P.: Urinary excretion of free noradrenaline and adrenaline following acute myocardial infarction. Lancet 1: 127-130, 1967.

Vassalle, M. and Scida, E.E.: The role of sodium in spontaneous discharge in the absence and in the presence of strophanthidin. Fed. Proc. 38: 880, 1979.

Vaughan Williams, E.M.: Prevention of arrhythmias due to cardiac glycosides by block of sympathetic beta-receptors. Lancet 1: 420-421, 1963.

Vokonas, P.S., Malsky, P.M., Paul, S.J., Robbins, S.L. and Hook, W.B.: Radioautographic studies in experimental myocardial infarction: Profiles of ischemic blood flow and quantification of infarct size in relation to magnitude of ischemic zone. Am. J. Cardiol. 42: 67-75, 1978.

Waldenstrom, A.P., Hjalmarson, A.C. and Thornell, L.T.: A possible role of noradrenaline in the development of myocardial infarction: An experimental study in the isolated rat heart. Am. Heart J. 95: 43-51, 1978.

Weaver, L.C.: Cardiopulmonary sympathetic afferent influences on renal nerve activity. Am. J. Physiol. 233: H592-H599, 1977.

Weaver, L.C., Danos, L.M., Oehl, R.S. and Meckler, R.L.: Contrasting reflex influences of cardiac afferent nerves during coronary occlusion. Am. J. Physiol. 240: H620-H629, 1981.

Weiss, J. and Shine, K.I.: Extracellular potassium accumulation during myocardial ischemia: Implications for arrhythmogenesis. J. Mol. Cell. Cardiol. 13: 699-704, 1981.

Williams, J.F., Boyd, D.L. Jr. and Border, J.F.: Effect of acute hypoxia and hypercapneic acidosis on the development of acetyl-strophanthidin-induced arrhythmias. J. Clin. Invest. 47: 1885-1894, 1968.

Wit, A.L. and Bigger, T.J.: Possible electrophysiological mechanisms for lethal arrhythmias accompanying myocardial ischemia and infarction. Circulation 51/52(Suppl. III): 96-115, 1975.

Wit, A.L., Hoffman, B.F. and Cranefield, P.F.: Slow conduction and reentry in the ventricular conduction system. I. Return extrasystole in canine Purkinje fibers. Circ. Res. 30: 1-10, 1972.

Withering, W.: An account of the foxglove, and some of its medical uses: With practical remarks on dropsy, and other diseases. Birmingham. 1785.

Wollenberger, A. and Sahab, L.: Anoxic-induced release of noradrenaline from the isolated perfused heart. Nature 207: 88-89, 1965.

Yamamoto, S., Akera, T. and Brody, T.M.: Sodium influx rate and ouabain-sensitive rubidium uptake in isolated guinea-pig atria. Biochem. Biophys. Acta 555: 270-284, 1979.

Yazaki, Y. and Fujii, J.: Depressed Na-K-ATPase activity in the failing rabbit heart. Jap. Heart J. 13: 73-83, 1972.

Zipes, D.P., Besch, H.R. and Watanabe, A.M.: Role of the slow current in cardiac electrophysiology. Circulation 51: 761-766, 1975.