THE EFFECTS OF LYSOPHOSPHATIDYLCHOLINE AND LIDOCAINE ON THE ELECTROCARDIOGRAPHIC CHANGES AND ACTION POTENTIAL CHARACTERISTICS OF THE CANINE HEART.

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

DEDICATED TO:

MY FAMILY

AND

MY HUSBAND, PROKOPIS

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Abstract

Lysophosphoglycerides (LPG) accumulate in ischemic myocardial tissue of animals. These metabolites have been shown to be potential mediators of arrthymogenesis induced by ischemia. Lysophosphoglycerides have been shown in several studies to cause marked electrophysiological derangements *in vitro* closely resembling changes seen in the ischemic myocardium *in vivo*. The concentrations of endogenous LPGs in ischemic myocardium are sufficient to elicit membrane dysfunction and cause electrophysiological derangements. The electrophysiological effects are exacerbated by concomitants of ischemia. In this study, lysophosphatidylcholine (LPC) is infused into the left anterior descending coronary artery of canine hearts. The arrhythmogenic effects are observed electrocardiographically. Bolus infusion of LPC into the left anterior descending (LAD) coronary artery leads to arrhythmias. The incidence of ventricular tachycardia produced by bolus LPC infusion is reduced with the administration of lidocaine. Continuous infusion of LPC for one hour (1 mM) and 5 hours (0.5 mM) produces arrhythmias. Concomitant infusion of lidocaine decreases the arrhythmia scores. Histological examination under light microscopy of myocardial samples showed that LPC infusion causes similar changes to those seen in experimental coronary artery occlusion. Lidocaine did not alter these histological changes. The action potentials of Purkinje fibers are recorded by the microelectrode technique. The superfusion of the Purkinje fibers with Tyrode's solution containing LPC (40 μM) changes the characteristics of the action potentials. LPC causes significant decreases in resting membrane potential (RMP) amplitude (AMP), action potential duration at the 50% level of membrane repolarization (APD_{50}),

action potential duration at the 90% level of membrane repolarization (APD_{90}) and maximum rate of rise of voltage of phase 0 ($\dot{V}max$). Lidocaine (18 μM) delays the electrophysiological alterations seen in Purkinje fibers superfused with LPC containing solution. Abnormal automaticity is present in LPC superfused Purkinje fibers and absent in LPC and lidocaine superfused Purkinje fibers. LPC causes arrhythmias in the canine heart in the in vivo experiments. Electrophysiological abnormalities of Purkinje fiber action potentials are seen in the presence of LPC. In this way the accumulation of LPC in ischemic myocardium may predispose to the development of arrhythmias. Lidocaine showed no effect on the histological characteristics of the myocardium. Lidocaine delays both the production of arrhythmias as well as the changes of the action potential characteristics caused by LPC.

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LIST OF ABBREVIATIONS

AMP	amplitude
APD_{50}	action potential duration at the 50% level of membrane repolarization
APD_{90}	action potential duration at the 90% level of membrane repolarization
ECG	electrocardiogram
LAD	left anterior descending
LPC	lysophosphatidylcholine
LPG	lysophosphoglyceride
RMP	resting membrane potential
$\dot{V}_{max}$	maximum rate of rise of voltage of phase 0

INTRODUCTION

A. CARDIOVASCULAR SYSTEM

The cardiovascular system is a unique system in the body in that all other systems depend on its proper and constant functioning. The heart and blood vessels offer a unique transportation system that delivers to all cells of the body the materials needed for their proper function and removes the wastes produced by their metabolism. It serves as a communication system between all cells in the body as well as a transportation system. What is referred to as the cardiovascular system includes the heart, arteries, capillaries and veins, all of which differ in structure and function. The heart acts as two united pumps each of which has its own special function.

Both of the pumps are involved in a circular motion where blood is carried away from the heart by the arteries to the tissues of the body and returned to the heart via the veins. This work is divided so that the right heart is responsible for blood flow to the lungs and the left heart for blood flow to the rest of the tissues in the body. The heart maintains a spontaneous rhythmicity or automaticity which is an intrinsic property of embryonic heart cells that develops before cardiac innervation is present. In the adult heart this pacemaker activity is usually in the sinus node. The normal sequence of the transmission of the electrical activation originating in the sinoatrial node is to the atrial myocardium, atrioventricular node, atrioventricular bundle, bundle branches, Purkinje network, and finally to the ventricular myocardium.

Disorders of cardiac rhythm are attributed either to abnormal impulse generation, abnormal impulse conduction, or both. Presently the predominant form of heart disease is found in ischemic heart disease, and its signs are manifested primarily as ventricular arrhythmias.

B. MYOCARDIAL ISCHEMIA

Myocardial ischemia refers to decreased blood flow to any region of the heart. Ischemic heart disease is the clinical syndrome produced when blood flow to a region of the heart is reduced, it has also been referred to as coronary heart disease and arteriosclerotic heart disease. Ischemic heart disease remains one of the primary public health problems in modern society (Karpannen, 1984; Parmley and Chatterjee, 1984). With ischemic heart disease are associated a number of abnormalities, that include narrowing of the coronary arteries, abnormalities of lipid metabolism, blood coagulation, blood flow patterns and coronary arterial structure. There are a variety of causes that are responsible for the above conditions. The normal cell electrophysiology as well as the various changes seen in the presence of myocardial ischemia are described briefly, in the sections below.

1. Normal cell electrophysiology

Normal resting potentials as well as action potentials result from transmembrane ionic gradients that are established by pumps as well as selective permeability of the membrane to various ions. In a normal cell the intracellular K^+ concentration is 150 mM and the extracellular K^+ concentration is approximately 5 mM. Corresponding values for Na^+ are 10 mM and 140 mM. The resting

membrane potential is established by the gradient of K^+, since the resting membrane is primarily permeable to K^+. Given the gradient of K^+ concentrations intracellularly and extracellularly the resting potential is -90 mV (the interior being negative to the exterior). The action potential begins by an increase in the membrane permeability to Na^+ which allows Na^+ to enter the cell according to its electrochemical gradient. Depolarization of the cell occurs with the Na^+ entering the cell and causes increased permeability to Ca^{2+}. At the same time the K^+ permeability is decreased thus allowing a delay in repolarization of the membrane for approximately 250-350 msec and forming what is called the plateau. Repolarization occurs when K^+ permeability increases and Ca^{2+} permeability decreases.

Conduction of action potentials occurs by local current flow along cells both in the interior and exterior. A depolarized cell that has undergone an action potential causes the depolarization of the adjacent cell and this process continues.

2. Altered cell metabolism in ischemia

Coronary occlusion has an immediate effect on ventricular muscle cells since these cells are dependent upon coronary flow for oxygen and substrates (Jennings and Ganote 1974). Coronary occlusion results in decreased production of high energy phosphates as well as a decrease in ATP content of the affected cells (Braasch et al., 1968). This decrease in ATP content will affect the proper functioning of the ATP dependent Na^+-K^+ ATPase in the cell

membrane, therefore Na^+ will accumulate inside the cell and K^+ will leak out. Since there is decreased coronary flow the K^+ will accumulate extracellularily. Since the energy metabolism is now anaerobic lactate will accumulate inside the cell, decrease the pH and eventually H^+ leaves the cell and decreases the pH in the extracellular space. The resting membrane potential of these cells will now decrease and eventually lead to the inactivation of the fast response. Slow response action potentials will result. These are dependent on Na^+ entering through the inactivated Na^+ (depressed fast response) or on a small inward Ca^{2+} which flows through the slow channel.

Within minutes of coronary artery occlusion there occurs a rise in extracellular K^+ concentration which leads to cell membrane depolarization (Harris, 1966; Hill and Gettes, 1980). This depolarization changes the excitability of cells and may allow the eventual development of arrhythmias. Clearly the loss of oxygen and nutrient supply, as well as the accumulation of the cell's metabolites into the extracellular space are the two factors that can be observed as a consequence of ischemia.

Normal cell metabolism acquires energy from oxidative metabolism of fatty acids and glucose. Glycogen stores are limited and anaerobic glycolysis offers a minor contribution. In the ischemic cell the lack of oxygen stops the tricarboxylic acid cycle and thus to produce energy, anaerobic glycolysis is required. Consequent to the breakdown of glycogen, lactic acid is produced

and accumulates. Anaerobic glycolysis will cease when the intracellular space becomes sufficiently acidic. Phosphocreatine breakdown will allow for the maintenance of ATP levels. Studies have shown that electrophysiological changes and decrease in force of contraction precede the low levels of ATP (Jennings et al. 1981; Allen et al. 1983) so that, insufficient high energy phosphates are not responsible for the early changes seen in ischemia. However, it is possible that compartmentation of ATP exists and when total ATP levels are normal, the ATP pool responsible for the contractile mechanism may be seriously compromised.

3. Effects of high extracellular potassium caused by ischemia

The increase in extracellular K^+ during ischemia is important electrophysiologically. In animal models studies have shown a progressive increase in extracellular K^+ over the initial few minutes to levels of 10-15 mM (Allen et al. 1983; Kleber, 1983). The resting membrane potential is maintained by the K^+ concentration gradient. When this gradient is reduced during ischemia since K^+ accumulates extracellularly the transmembrane potential becomes less negative. This depolarization produces a large diastolic voltage difference between the ischemic region and the rest of the heart (Fozzard and Makielski, 1985).

The repolarization phase of the cardiac action potential in a normal cell is due to the efflux of K^+. That is approximately equal to the influx of Na^+ and Ca^{2+} that depolarized the cell. An increase in K^+ permeability was demonstrated in in vivo hypoxia

studies (Vleugels et al. 1980). The K^+ efflux would not be altered greatly by this permeability increase since the diastolic potential is close to E_K. The K^+ efflux could be affected during the plateau of the action potential since the driving force is large. This efflux may contribute to the accumulation of extracellular K^+ and favor early repolarization of the action potential. The cell is found with a net loss of the cellular cation K^+ and no gain of Na^+ intracellularly. In an effort to maintain electroneutrality anions are also lost.

4. Effects of ischemia on the action potential and conduction

Several changes in cell electrophysiology are the basis of the ECG changes and the early ischemic arrhythmias. Three changes of the action potential that are associated with ischemia are the following: reduction of the upstroke velocity of the depolarization phase, as well as reduced conduction; the alteration of inward Na^+ current which is responsible for excitation; and the shortening of the action potential due to a net increase in outward current.

The ischemic depolarization of the cell inactivates the Na^+ channels and thus reduces the number of channels available to be opened. This therefore reduces the excitatory inward Na^+ current which decreases the upstroke velocity of the depolarization phase and conduction velocity. The action potentials generated after the onset of ischemia are slowly conducted. These abnormal action potentials are found to be blocked by drugs such as lidocaine (Lazzara et al. 1978). The action potential duration during

ischemia is shortened (Janse and Kleber, 1981). The shortening of the action potential must occur if there is a reduction in the inward current or an increase in the outward current. It has been suggested that the increased K^+ current is a consequence of increased intracellular Ca^{2+} which causes the activation of the K^+ channels (Isenberg et al. 1983). Although this mechanism has not been clarified it is still important to note that the shortening of the action potential removes the safe period found in cardiac cells during which reexcitation is impossible. With the plateau shortened reexcitation is possible without full recovery of the cell from the action potential.

5. Effect of ischemia on contraction

Ischemia causes a rapid and dramatic fall in contraction strength. ATP levels, as mentioned previously, remain sufficiently high therefore this fall in contraction is not due to low ATP levels. Cardiac contraction is triggered and controlled by the action potential. The Ca^{2+} current triggers the release of Ca^{2+} from the sarcoplasmic reticulum (Fozzard 1977). In ischemia it is proposed that there is a change in the sensitivity of the contractile proteins to Ca^{2+}.

A decline in contractile function in patients with ischemic heart disease is also referred to as pump failure (Hearse et al., 1977). After the initial loss of functional myocardial tissue places an abnormal burden on the remaining regions of the left ventricle, the overload can be tolerated for a lesser or greater

period of time. In some cases so little normal myocardium remains that ischemic heart disease causes either acute or chronic heart failure. The loss of functional myocardium is great enough to lead to impairment of cardiac pumping. The chronic heart failure that results from the ischemic heart disease can be understood as being due to the death of large number of myocardial cells that were deprived of their blood supply. However, there seems to be more involved in the mechanism of early pump failure.

When coronary arterial flow is interrupted the ischemic regions lose their ability to contract almost immediately. Complete interruption of coronary blood flow to a region of the canine heart is followed within a few seconds by the shortening of the cardiac systole and by the loss of contractility. A marked lowering in contractility causes the tension generated by the ischemic portions of the ventricle to be unable to overcome the intraventricular pressure generated by the normally perfused myocardium.

The rapid decline in myocardial contractility that occurs after coronary arterial occlusion could be due to, the lack of a substrate or substrates normally supplied by coronary circulation or to the accumulation of one or more metabolites. The substrate whose absence is felt most by the ischemic myocardium is oxygen. The role of O_2 as being the critical substrate responsible for early pump failure is supported by the finding that anoxia, like ischemia, causes a precipitous decline in cardiac contractile function. The rapid decline in myocardial contractility that

follows interruption of coronary blood flow is accompanied by an increased rate of glycolysis in which glycogen breakdown is associated with increased cellular levels of cyclic AMP (Steinberg and Khoo, 1977) and the conversion of phosphorylase b to phosphorylase a. Of greater importance in accelerating glycolysis is the release of the inhibitory effects of ATP and glucose-6-P which in the normal heart maintain phosphorylase b activity at a low level. Oxidative metabolism causes ATP levels to decrease along with glucose-6-P levels while at the same time AMP and inorganic phosphate levels rise. The latter also contribute to the acceleration of glycogenolysis in the ischemic heart as both of these substances increase phosphorylase b activity. Glucose flux through the glycolytic pathway is increased in the ischemic heart as a result of stimulation of the reaction catalyzed by phosphofructokinase (PFK). As in the case for phosphorylase b the activity of this enzyme is enhanced when oxidative ATP production ceases. The increased rate of glycolysis in the ischemic heart is transient. Eventual cessation of anaerobic ATP production is probably due to the accumulation of NADH and $FADH_2$ and lack of NAD (Liedtke, 1978) which cause the glycolytic pathway to be inhibited at the step where G-3-P is reduced. Also the increased rate of lactate production is accompanied by the accumulation of H^+ so that the rapid development of acidosis inhibits the step in the glycolytic cycle that is catalyzed by PFK.

Necrosis of the cells occurs after 40-60 minutes of total myocardial ischemia (Jennings, 1974). The mechanism of this cell

death is probably related to a breakdown in membrane function caused when ATP concentrations fall below levels that are needed to maintain key ion pumps ($\leq$0.1 mM). Both cellular acidosis and alterations in ATP dependent regulatory processes may participate in the sequence of steps which leads to a loss of contractility. Phasic control of myocardial contractility is affected mainly by changes in the amount of Ca^{2+} made available to and also bound to the contractile proteins of the heart. Thus, the myocardial weakness in the ischemic heart can involve any mechanism that either reduces the amount of Ca^{2+} released during excitation-contraction coupling or prevents Ca^{2+} from being bound to troponin.

6. Anoxia

When a region of the heart is perfused with blood that does not contain O_2 the initial fall in myocardial contractility is generally similar to that seen in the ischemic myocardium which receives no blood flow. In the case of myocardial anoxia, the glycolytic rate remains elevated. This persistence of active glycolysis in the perfused anoxic heart is probably due to washout of both H^+ and lactate which is not possible in the ischemic heart. As the anoxic heart remains perfused the removal of H^+ can partially release the inhibition of PFK that is caused by acidosis. Washout of lactate in the perfused anoxic heart facilitates pyruvate reduction and thereby allows partial renewal of the supply of oxidized NAD.

7. Effects of free fatty acid (FFA) accumulation during ischemia

Although O_2 is almost certainly the major substrate whose lack sets into motion the series of events that leads to decreased myocardial contractility and ultimately to cell death, the nature of other substrates available to the myocardium appears able to modify the response to ischemia. Kurien et al. (1971) observed that production of coronary artery occlusion in intact dogs produces a marked elevation of free fatty acids (FFA). High levels of circulating FFA have been observed in patients after myocardial infarction (Kurien and Oliver, 1966; Oliver 1972; Vetter et al., 1974; Opie, 1972; Opie, 1975). Support for the suggestion that lipids alter the electrophysiological behaviour and exert an arrhythmogenic effect on humans (Kurien and Oliver, 1966; Gupta et al., 1969; Kurien et al., 1971; Rowe and Oliver, 1974) and experimental animals (Sevareid et al., 1969; Kurien et al., 1969; Willebrands et al., 1973, Wasilewska-Dziubinska, 1975; Cowan and Vaughan-Williams, 1977; Coraboeuf et al., 1978; Simonsen and Kjekshus, 1978; Athias et al., 1979; Vik-Mo et al., 1979). This increase of FFA was associated with the development of frequent ventricular ectopic systoles, ventricular tachycardia (VT) and ventricular fibrillation (VF) (Kurien et al., 1971, Opie, 1975). The observed arrhythmias occurred 10-30 minutes after the maximum recorded level of FFA. Fatty acids may be detrimental as increased concentrations of these substrates have an O_2 wasting effect (Challoner and Steinberg, 1966; Mjos, 1971; Simonsen and Kjeksus, 1978) that arises from their ability to increase O_2 consumption without a corresponding increase in the rate of ATP production.

Free fatty acids (FFA) have been found to inhibit subcellular enzymes and to uncouple mitochondrial respiration (Pande and Mead, 1968). When FFA levels are elevated in ischemic tissue, long chain acyl CoA and acyl carnitine are increased (Liedtke et al., 1978) along with lysophosphoglycerides (Sobel et al., 1978). Acyl-CoA has been found to inhibit nucleotide translocases in vitro and thus may impair energy production in the ischemic myocardium in vivo (Ho and Pande, 1979). Acyl carnitine will inhibit Na^+-K^+ dependent ATPase (Adams et al., 1979a, 1979b), as will lysophosphoglycerides (Karli et al., 1979).

C. RATE AND RHYTHM DISORDERS

It has been found that one half the deaths from heart disease result from disordered cardiac rate and rhythm (Bigger et al., 1977; Prystowsky et al., 1983). Disordered cardiac rhythm can affect the mechanical activity of the heart and if it is affected severely the result may be fatal. The arrhythmias themselves may become life threatening events if they are severe and persistent. In patients who sustain an acute myocardial infarction changes in cardiac rate and rhythm are common (Armstrong et al., 1972; Fozzard, 1983). These arrhythmias are grouped into various classes including sinus tachycardia, sinus bradycardia and AV block, and premature systoles and ventricular tachycardia. A number of mechanisms exist where an interruption of coronary blood flow can lead to premature systoles and arrhythmia. Some of these arise from reflex changes such as sympathetic activity but most arise from abnormalities in the ischemic areas of the myocardium.

The dependence of the cardiac cell on a continuing supply of oxygen and the resulting marked curtailment of ATP production when coronary blood flow is interrupted, can inhibit the Na^+ pump and is probably directly responsible for a fall in intracellular K^+. The depolarizing effects of low intracellular potassium are magnified by the accumulation of potassium in the extracellular space around the ischemic cell, so the resting potential falls toward zero. In the severely affected regions of the ischemic heart, the membrane potential decreases to levels that completely abolish any ability to either initiate or propagate a sodium dependent action potential. In contrast the slow inward current is inactivated less by partial membrane depolarization so that slow responses can arise in ischemic heart regions (Schneider and Sperelakis, 1974; Sperelakis and Schneider, 1975). In this way the less severely ischemic areas around the region of complete myocardial necrosis are the most likely to give rise to premature systoles and tachycardias.

For reentry to occur conduction must be slowed and a strategically located region of unidirectional block must be present. Slow conduction may occur when the loop of fibers is in a diseased region of the heart. In this case both the rate of phase 0 depolarization and the overshoot of the action potential may be reduced as a result of decreased resting membrane potential. Depression of the resting membrane potential and action potential upstroke is rarely uniform in diseased regions and unidirectional block may occur in areas where the action potential is depressed. These marginal areas exhibit slowly rising, low amplitude action potentials (slow responses) because

of the effects of partial depolarization which inactivate the opening of the fast channel. Since the marginal areas around an infarcted area of the ventricles may remain in a viable but depressed state for long periods of time after a single occlusive event in the coronary arteries, the risk of serious arrhythmia can persist.

The inactivation of the fast channel when there is partial membrane depolarization and the presence of the slow response when the membrane potential falls to approximately -50 mV lead to decremental conduction and unidirectional block. These conduction abnormalities aid in the development of re-entrant premature systoles and tachycardias. Ischemia may further slow conduction by increasing the resistance of the intercalated discs. These mechanisms operate primarily in the marginal areas around a myocardial infarction because membrane potential falls to zero in the central regions of the infarct which are therefore inexcitable. The effects of ischemia on the cardiac sarcolemma and the ionic currents have not been clarified.

D. MECHANISM OF VENTRICULAR ARRHYTHMIAS IN MYOCARDIAL ISCHEMIA AND INFARCTION

Ischemic heart disease is the most prevalent form of heart disease and an important set of problems surrounding this disease is centered around ventricular arrhythmias (Bigger et al., 1977). Although a variety of pathologic and electrophysiologic mechanisms are involved in ventricular arrhythmias seen in myocardial ischemia two factors are generally important. One of these factors involves changes in the cellular electrophysiology of cardiac cells, the other involves the

geometry of the cardiac musculature. Changes in cellular electrophysiology may be due to changes in the extracellular environment, or to alterations of the sarcolemma or intracellular composition of the cell. The size of the ischemic area as well as the types of cells affected are also critical factors in predisposing to ventricular arrhythmias (Chapman, 1972; Nielsen, 1973). It is not known how the size of the infarct increases the predisposition to cardiac arrhythmias but it may be assumed that size and shape of ischemic region may interact with the rentrant pathways and allow an arrhythmia to be produced and maintained (Wit et al., 1972).

1. Two phases of arrhythmia after experimental infarction

Most of the evidence regarding myocardial infarction in animals has been obtained from the model of coronary artery occlusion introduced by Harris in 1950. This model has shown that arrhythmias occur in two distinct phases, the early arrhythmic phase and the late arrhythmic phase.

a) Early arrhythmic phase

Reentrant activation and ventricular fibrillation shown in the early moments after coronary occlusion are a result of the presence of slow response action potential or severely depressed fast response actions potentials (Bigger et al., 1977). Bipolar electrograms recorded immediately after coronary artery ligation in the dog show decrease in amplitude and increase in duration (Scherlag et al., 1970; Waldo and Kaiser, 1973). The ischemic region shows slow conduction (Scherlag et al., 1974) and delays in

activation between endocardium and epicardium (Cox et al., 1973). When the artery is occluded for a longer period of time more and more disordered electrical activity is evident. When this asynchronous activity persists it results in ectopic beats, ventricular tachycardia, and ventricular fibrillation (Waldo and Kaiser 1973; Boineau and Cox, 1973). The low amplitude electrical activity that is present in ischemic ventricular tissue may be due to slow conduction between cells and to inexcitability of cells or to abnormal automaticity. Conduction is slowed in the ischemic area due to the inhomogeneity of injury that is found in the affected cells. Some cells may have undergone extensive biochemical and ultrastructural changes and are thus inexcitable. Other cells in the ischemic region may be capable of generating action potentials (whether they be generated by the depressed fast response or the slow response). Reentrant activation may result from the slowed conduction and the barriers to conduction caused by inexcitable cells. The impulse may then move slowly through the infarcted region and emerge to re-excite normal myocardium and elicit arrhythmias.

A prolonged refractory period is important for the occurrence of reentry. A prolonged refractory period allows for the impulse to be conducted slowly enough so that it enters a region of the myocardium that is able to fire an action potential. A self perpetuating cycle of action potentials is started when an impulse is conducted slowly enough between two patches of myocardium so that the returning impulse finds the patch able to fire an action

potential. Such continuous electrical activity was recorded in ischemic animal models (Janse et al., 1980). Premature ventricular beats occur if the impulse escapes after one circuit. If the circuit continues and keeps exiting to the ventricle as a whole, ventricular tachycardia or ventricular fibrillation occurs. On the other hand if the refractory period is prolonged longer than the action potential duration the reentry beat will encounter refractory tissue and will be lost. The necessary conditions for reentry are found in ischemia due to the patchy dispersion of excitability, reduced conduction velocities, and varying refractory periods.

The low amplitude electrical activity that persists in the infarcting zone may also be due to abnormal automaticity. Automaticity is also enhanced during ischemia. The flow of current from the ischemic tissue to healthy tissue during diastole can cause tissue near the ischemic region to come to threshold earlier than it would otherwise have, and produces a premature ventricular beat from that focus (Janse et al., 1980). This premature beat could enter a reentrant circuit and cause tachycardia.

Other mechanisms of ectopic beats such as alteration of pacemaker mechanisms in Purkinje cells and early and late after depolarizations (triggered automaticity) may play a role in early ischemia, but are probably more important is chronic ischemia (Wellens et al., 1974).

b) The late arrhythmic phase

The subendocardial Purkinje fibers are affected by coronary artery occlusion at a later time. Substrates and oxygen are derived to a large extent from the blood in the ventricular cavity, and Purkinje fibers contain rich supplies of intracellular glycogen. Lipid droplets accumulate in the cytoplasm of Purkinje fibers over the infarcted area after a 6-10 hour coronary occlusion in the dog (Wit and Friedman, 1975). Electrophysiologic changes greatly parallel the lipid droplet accumulation in Purkinje fibers. Twenty-four hours after the coronary artery occlusion there is a pronounced decrease in maximum diastolic potential, amplitude, and rate of phase 0 depolarization (Wit and Friedman, 1975; Friedman et al., 1973; Man et al., 1983). The action potentials of some Purkinje cells exhibit slow response characteristics, as well as spontaneous diastolic depolarizations and spontaneous activation (Wit and Friedman, 1975).

Spontaneous diastolic depolarization leads to automatic impulse initiation by the Purkinje cells under the infarct. Ventricular ectopic beats result when an impulse that has originated in the Purkinje cells propagates to ventricular muscle adjacent to the infarct (Bigger et al., 1977). Reentry is promoted by the large variation in action potential duration between the infarcted region Purkinje fibers and those in the non-infarcted region (Friedman et al., 1973). Conduction may block in regions with very long action potentials and may propagate slowly in areas with shorter action potential duration. Reentrant activation may be

established and become continuous when the appropriate conduction time and path length are present. The arrhythmias that can occur in the late phase of acute infarction using the Harris model vary. Surviving Purkinje fibers exhibit abnormal automaticity from which premature action potentials may arise and initiate a rapid reentrant rhythm in the Purkinje fibers of the infarct. This reentry pattern is responsible for ventricular tachycardia that is seen in acute myocardial infarction.

It is well accepted that cardiac arrhythmias arise from abnormal impulse initiation, abnormal impulse conduction, or both acting simultaneously in the same location or different locations in the heart (Wit et al., 1975; Bigger et al., 1977; Elharrar et al., 1977; Lazzara et al., 1978). Abnormal impulse initiation results from either automaticity or triggered activity. Enhanced automaticity may be due to a normal automatic mechanism or to an abnormal mechanism such as automaticity in depolarized fibers. Triggered activity is caused by after depolarizations that occur either during repolarization or after repolarization is complete. Triggered activity due to delayed afterdepolarizations is dependent on critical heart rates. Abnormal impulse conduction results in reentrant excitation. Unidirectional block and slowed conduction are necessary for the occurrence of reentry. A reentrant mechanism can be determined anatomically or functionally in the tissue. Initiation and perpetuation of a reentry mechanism depends on a relationship between conduction velocity and duration of the functional refractory period in the reentry pathway.

2. Prevention of arrhythmias

a) Prevention of arrhythmias originating from abnormal impulse initiation

To alleviate arrhythmias that originate from abnormal impulse initiation the antiarrhythmic drugs may do one of several things: the automatic mechanism may be suppressed by affecting the ionic current directly; reduce conduction in tissue surrounding an automatic focus; and/or modify refractory periods in tissue containing an automatic focus as well as tissue surrounding an automatic focus.

b) Prevention of arrhythmias due to reentrant mechanisms

To prevent reentry mechanisms the drugs may do the following: eliminate premature beats that initiate the mechanism; reduce the disparity in effective refractory periods; improve conduction, thereby preventing unidirectional block; depress conduction to produce bidirectional block; and/or lengthen the refractory period in the tachycardia circuit more than the total circuit time.

E. LIDOCAINE

Cardiac arrhythmias have a high morbidity and mortality (Armstrong et al., 1972; Bigger et al., 1977). Antiarrhythmic drugs have been developed for the prevention and treatment of cardiac rhythm disorders. One of the most widely used antiarrhythmic drugs is lidocaine (Figure 1). It was introduced as a local anesthetic in 1943, and still used as such today. In the late 1940's and 1950's it was used for the

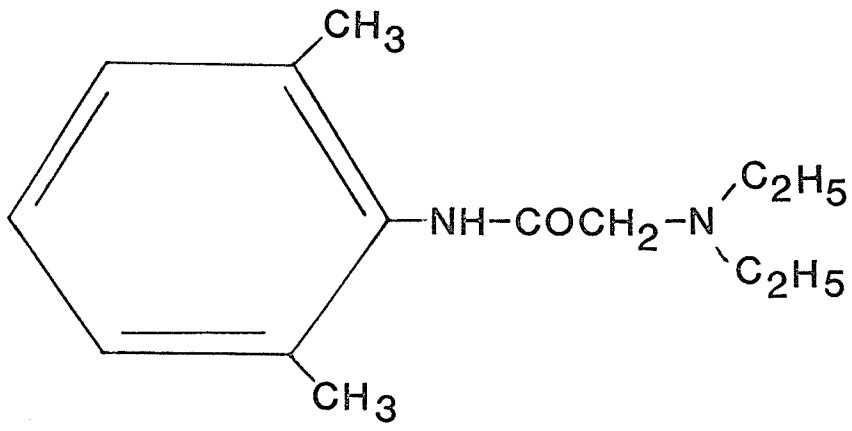


Figure 1. The chemical structure of lidocaine.

treatment of arrhythmias occurring during cardiac catheterization (Garden and Steinhaus, 1956; Hitchcock and Keown, 1958). In the 1960's it gained popularity for the treatment of ventricular arrhythmias associated with cardiac surgery, digitalis intoxication, and acute myocardial infarction (Weiss, 1960). It has been demonstrated to be an effective drug in treating and preventing ventricular ectopic activity associated with myocardial infarction.

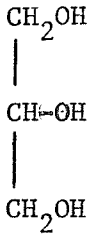
Lidocaine has the properties of shortening the action potential duration as well as the refractory period (Davis and Temte, 1969), while not changing the conduction velocity in the Purkinje system (Josephson et al., 1972). It depresses automaticity and phase-4 depolarization of action potentials (Davis and Temte, 1969; Allen et al., 1978; Lazzara et al., 1978). The ability of lidocaine to suppress automaticity in damaged myocardium may be of importance in its antiarrhythmic action in addition to its effects on conduction velocity and refractory periods (Allen et al., 1978; Lazzara et al., 1978). The therapeutic usefulness of lidocaine is due primarily to the fact that it acts on disturbances of ventricular origin. Lidocaine selectively depresses cells within the infarcted region of the myocardium while having a small effect on phase 0 characteristics in normal Purkinje fibers (Sasyniuk, 1978). The degree of depression of the upstroke velocity of action potentials in an infarcted area is related to the initial resting membrane potential. The lower the initial resting membrane potential the greater the depression produced by lidocaine. In Purkinje fibers surviving infarction the maximum upstroke velocity is not reached at full repolarization. In these cases lidocaine slows responsiveness and further prolongs the time

of recovery of maximum upstroke velocity. The refractory period of cells within an infarcted region is extended even though the action potential duration is shortened by the drug. The net result of the lidocaine effects within an infarcted region is to slow conduction of premature beats. It may not abolish reentry but it is effective in abolishing enhanced spontaneous activity within an infarcted zone.

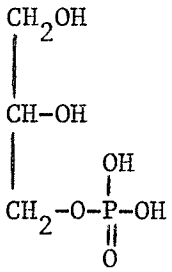
Lidocaine treatment has been found to promptly suppress 80-90% of ventricular arrhythmias after myocardial infarction (Jewitt et al., 1968; Bigger and Heissenbuttel, 1969). An initial intravenous loading dose is given to produce an antiarrhythmic effect within minutes and the effective concentration is maintained by constant i.v. infusion.

F. LYSOPHOSPHOGLYCERIDES

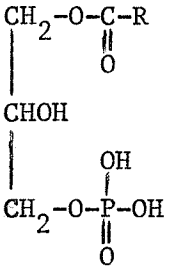
Animal cells contain three types of lipids: phospholipids, glycolipids, and sterols (usually cholesterol). Of the phospholipid group there are two subgroups the glycerol phospholipids and the sphingophospholipids. The glycerol phospholipids are the immediate concern. The simplest glycerol phospholipid is phosphatidic acid, a phosphorylated 1,2-diglyceride (Figure 2). The other glycerol phospholipids are most commonly named using "phosphatidyl" as a general term to indicate derivatives of phosphatidic acid regardless of length, degree of unsaturation, or mode of linkage of the 2-carbon chains. So that phosphatidyl choline is the choline ester of phosphatidic acid. When only one hydrocarbon chain is present and one hydroxyl group of the glycerol is free the prefix "lyso" is used, as in lysophosphatidylcholine (LPC). Biological membranes are made up of



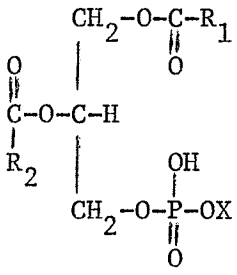
GLYCEROL



L-GLYCEROL 3-PHOSPHORIC ACID



L-PHOSPHATIDIC ACID



PHOSPHOGLYCERIDES

Figure 2. Phosphoglyceride structure.

Phosphoglycerides are also known as glycerol phosphatides or phospholipids. The parent compound is glycerol.

R = fatty acid chain

X = alcohol

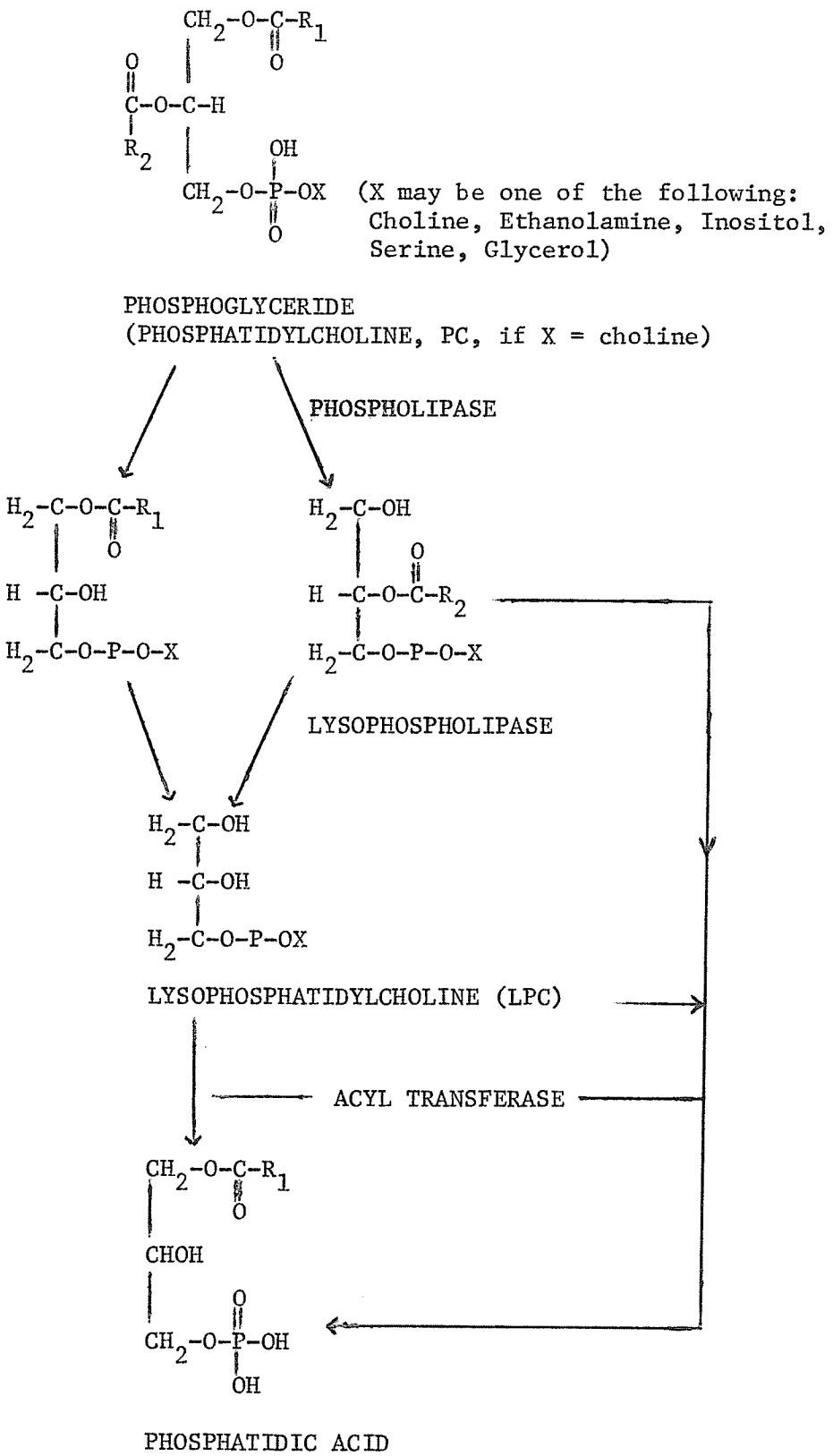
phospholipids, the two most abundant are phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Together these make up approximately 90% of the membrane phospholipids (Figure 3). The term lysophosphoglyceride is frequently interchanged in the literature with lysophospholipid or lysolipid. Since lysophosphoglyceride is the most descriptive of the three terms it will be the one used here.

Three general sources of these lipid materials may be identified. They may be brought to the heart via the arterial circulation as a result of mobilization of extra-cardiac lipid pools, they may appear within myocardial cells as a result of ischemia-induced abnormalities in lipid metabolism or they may be derived from membranes of ischemic cells as a result of enzymatic digestion of membrane phospholipids.

In the ischemic heart many biochemical changes are present such as changes in pH, K^+, lactate, O_2, as well as accumulation of metabolites, the one of specific interest being LPC. Taking into consideration the environment of the ischemic heart as well as the existence of lysophosphoglycerides leads one to consider LPC and its implication in the pathogenesis of ischemic myocardial damage. Lysophosphoglycerides (LPG) are products of membrane phospholipid catabolism (Van Golde and van den Bergh, 1977; Gross, R.W. et al., 1982) accumulated in perfused hearts and myocardium which was made ischemic (Sobel et al., 1978; Vasdev et al., 1979; Chien et al., 1981; Corr et al., 1982). Ischemic injury is represented by functional alterations in the myocardial sarcolemma seen as electrophysiological derangements and

Figure 3. Lysophosphoglyceride metabolism.

LPGs are produced by phosphoglyceride breakdown catalyzed by phospholipase, and lysophospholipase, and reacylated to phosphoglycerides by acyl transferase.


(X may be one of the following: Choline, Ethanolamine, Inositol, Serine, Glycerol)
PHOSPHOGLYCERIDE
(PHOSPHATIDYLCHOLINE, PC, if X = choline)
PHOSPHOLIPASE
LYSOPHOSPHOLIPASE
LYSOPHOSPHATIDYLCHOLINE (LPC)
ACYL TRANSFERASE
PHOSPHATIDIC ACID

(Sobel, 1979)

ultrastructural discontinuity of the plasma membrane (Herdson et al., 1965).

1. Metabolism of lysophosphoglycerides (LPGs)

Cardiac muscle has an extensive capacity to metabolize LPC to either FFA and glycerophosphoryl choline (GPC) by lysophospholipase (Corr et al., 1984), to PC and GPC by lysophospholipase, to PC and GPC by cytosolic lysophospholipase-transacylase, or to PC by acyl transferase. Weglicki et al. (1980) have reported enhanced activity of phospholipase A_2 in sarcolemmal preparations by beta-adrenergic stimulation with isoproterenol potentially explaining increased synthesis of LPGs in ischemic myocardium exposed to neuroadrenergic stimulation in vivo. Nevertheless, accumulation of LPGs in ischemic myocardium probably requires concomitant inhibition of their metabolism. Several metabolic pathways potentially responsible for accumulation of LPGs in ischemic myocardium have been characterized. Microsomal lysophospholipase, active in normal myocardium, is inhibited by 81% when pH is reduced to 6.5, a value comparable to that seen within minutes in ischemic myocardium in vivo. Also, a long chain acyl carnitine (itself an amphiphile with arrhythmogenic properties) markedly inhibits cytosolic lysophospholipase and cytosolic lysophosphatidylcholine transacylase (Gross and Sobel, 1980, 1981). So that inhibition of lysophospholipase and transacylase by concomitants of ischemia may result in decreased catabolism of LPG and its consequent accumulation.

Lysophosphoglycerides have exhibited a marked effect on membrane integrity on many systems (van den Bosch, 1974). Phospholipase A_2 activation accompanies myocardial ischemia (Needleman et al., 1975). Phospholipase activity has been found in cardiac membranes (Weglicki et al., 1971, 1972) as well as in cardiac lysosomes (Franson et al., 1972) and lysosomal enzyme activity increases in the ischemic heart (Kennett and Weglicki, 1978). Accumulation of LPGs under physiological conditions is prevented since reacylation and hydrolysis are occurring. In the ischemic situation accumulation of LPGs may occur due to augmented phospholipase A_2 activity, inhibition of reacylation and decreased washout of the region (van den Bosch, 1974).

2. Effects of lysophosphoglycerides (LPGs) during myocardial ischemia

In 1978, Sobel et al. reported that during myocardial ischemia lysophosphoglycerides accumulated that were arrhythmogenic. This group reported that LPGs bound to albumin altered action potentials of isolated Purkinje fibers in vitro. The effects seen were the following: reduction of the maximum diastolic potential, peak $\dot{V}_{max}$ of phase 0, amplitude, and action potential duration. All of these alterations are seen in ischemic myocardium in vivo. Ischemic tissue in vivo shows enhanced automaticity, triangulation of the action potential, increased threshold for stimulation, conduction delay as well as fractionation of the action potential (Ten Eick et al., 1976; Downar et al., 1977b; Lazzara et al., 1978; Corr et al., 1979). These effects are

mimicked by high LPC concentrations (100-300 μM) and exhibited as electrical alternans, refractoriness to external stimulation, post-repolarization refractoriness, and eventual total abolition of action potentials (Corr et al., 1981). Bergman (1981) has reported dysrhythmia in isolated hearts with LPC concentrations as low as 6 μM. Arnsdorf (1979) has reported effects on passive and active membrane properties with 20-40 μM LPC concentrations. In Sobel's study (1978) the two major LPGs, LPE and LPC both show more than a 50% increase as compared to control values. The maximum diastolic potential, peak $\dot{V}_{max}$ amplitude, overshoot of phase 0, and the action potential duration were all decreased in a dose-dependent fashion by LPC. Man et al. (1983) reported a two-fold increase in the levels LPC and LPE in 24 hour ischemic canine myocardial tissue.

Functional alterations in cardiac cell membrane, shown by electrophysiological changes and ultrastructural discontinuity of the plasma membrane itself are usually seen in ischemic injury. Under physiological conditions lysophosphoglycerides cannot accumulate because reacylation and hydrolysis are always preventing the build up. In the ischemic condition LPGs do accumulate due to stimulation of phospholipase A_2 activity, inhibition of reacylation, and decreased washout from that particular region of the heart (Bagdonas et al., 1961; Snyder et al., 1981). Studies have been done *in vitro* and *in vivo* to check the accumulation of LPGs. Results have shown that accumulation of LPE and LPC was evident from ischemic zones of hearts sampled *in situ*, both

increased compared to control values by more than 50% (Shaikh and Downar, 1981; Man et al., 1983). Similarly in isolated hearts perfused at low flow (0.2 ml/min) LPG and LPC increased significantly compared to values in hearts perfused at 20 ml/min. In ischemic liver tissue lysophosphatide levels were also increased (Boime et al., 1970). Sobel's (1978) observations indicate that LPC and LPE accumulate in myocardium early after the onset of ischemia in situ and in vivo. In canine heart sarcolemma it has been shown that LPC inhibits Na^+ and K^+ stimulated ATPase activity (Karli et al., 1979). The accumulated LPGs are likely to be catabolites of membrane constituents. In extracts of ischemic myocardium, the overall concentrations of LPGs are similar to those found to exert deleterious effects on the isolated Purkinje fibers in vitro.

After exposure of ventricular muscle to exogenous, radiolabelled LPC, introduction of LPG into the sarcolemma in quantities as low as 1% of total cellular phospholipid is sufficient to induce electrophysiological derangement simulating those of ischemia. E-M autoradiography with ^{14}C and ^{3}H-LPC indicates that selective sarcolemmal incorporation comprising less than 5% of sarcolemmal phospholipid is sufficient (Corr et al., 1982). Reversal of the induced electrophysiological derangements is associated with a decrease in the concentration of sarcolemmal LPC. This decrease is associated with the metabolism of LPC to both PC and FFA, further supporting the specificity of LPG as a mediator. Thus, exposure of normoxic myocardium to the concentration of exogenous LPG comparable to those in effluents from ischemic tissue

in vivo results in sarcolemmal incorporation sufficient to produce electrophysiological derangements associated with ischemia.

Sudden death associated with coronary artery disease is due to large cardiac rhythm disturbances (Armstrong et al., 1972) and alterations leading to ventricular fibrillation (Downar et al., 1977). Malignant ventricular dysrhythmias that occur after experimental coronary artery occlusion seem to be due to reentrant mechanisms (Corr et al., 1979). The reentrant mechanism depends on the heterogeneity of cellular electrical activity within the ischemic zone. The factors that facilitate initiation and maintenance of reentry are changes in conduction properties of the myocardium as well as altered recovery times, in other words changes in phase 0 and repolarization of the action potential. In the study conducted by Corr et al. (1979) LPC and LPE bound to albumin were both found to induce dose and time dependent decreases in the maximum diastolic potential overshoot of phase 0 and $\dot{V}_{max}$ of phase 0. The membrane response curve showed a rightward and downward shift. Action potentials were converted to the slow response type with frequent unresponsiveness to external stimulation. The conduction time was prolonged in isolated Purkinje fibers.

Because early malignant arrhythmia induced by ischemia appears to be a result of reentrant mechanisms, and because several of the effects elicited by LPG have been identified as factors predisposing to reentry, it appears likely that the increased

concentrations observed in ischemic myocardium may contribute to arrhythmia induced by ischemia. The suggestion that lysophosphoglycerides contribute to ischemic damage in the heart was originally put forward by Hadju (1957). Liberated lysophosphatides have been implicated in playing a role in the pathogenesis of cardiac abnormalities in the ischemic heart (Bruce and Myers, 1972, Sobel et al., 1978, Vasdev et al., 1979, Shaikh and Downar, 1981). Also the action potential alterations induced by LPC resemble those seen in subepicardial action potentials from ischemic myocardium in situ.

3. Detergent actions of lysophosphoglycerides (LPGs)

Damage to cardiac membranes during prolonged ischemia and reperfusion of the ischemic myocardium plays an important role in the impaired function and cell death that follow even temporary obstruction of a coronary artery. Evidence that lipid metabolism abnormalities may be involved in the ischemic myocardium has been accumulating for a number of years. Detergent actions on the membranes may also play a role in myocardial damage (Wood, 1982; Man et al., 1983). Sobel proposed that the accumulation of lysophosphoglycerides is due to a mechanism by which lipid abnormalities cause membrane damage. These and other observations lead to the hypothesis that a variety of lipids or lipid derived substances can through their detergent effects cause either reversible or irreversible membrane damage. Lysophosphoglycerides have been shown to exert detergent-like effects in several systems including red blood cells (Gazitt et al., 1975; Weltzien, 1979),

bacteria (Ray et al., 1970; Weltzien, 1979) and a variety of normal and transformed cell and membrane systems (Weltzien, 1979). The detergent effects are largely due to the amphiphilic nature of the lipids and are a result of three general mechanisms. At low concentrations amphiphiles are incorporated into biological membranes thus altering the membrane bilayer, but protecting the cell from lysis (Schramm et al., 1967; Kwant and Seeman, 1969; Seeman, 1972; Raz and Livne, 1973). At high amphiphile concentrations the formation of micelles occurs. The amphiphile will form aggregates of lipids that have been pulled out of the membrane, resulting in loss of membrane integrity with the loss of phospholipids (Helenius and Simons, 1975). Although these detergent-like effects have been found to be slow reactions (Roseman and Thompson, 1980) they have been shown to be facilitated by a number of phospholipid exchange proteins (Di Corleto et al., 1979). At still higher concentrations of amphiphiles there occurs a further loss of membrane phospholipid which can lead to actual physical disruption of the membrane (Lucy, 1970). In the heart and other tissues (Helenius and Simons, 1975) this allows for uncontrolled Ca^{2+} entry and thus cell death (Katz and Messineo, 1981; Chien et al., 1977; Chien et al., 1978; Mittnacht et al., 1979).

G. STATEMENT OF THE PROBLEM

Lysophosphoglycerides have been shown to be potential mediators of arrhythmogenesis induced by ischemia (Corr et al., 1978; Sobel et al., 1978; Man and Choy, 1982; Clarkson et al., 1983; Man et al., 1983a;

Man et al., 1983b) since significantly higher concentrations are found in ischemic myocardium. Lysophosphoglycerides have been shown in several studies to cause marked electrophysiological derangements closely resembling changes seen in ischemic myocardium in vivo. Findings from several laboratories using various systems from several species support the view that the concentration of endogenous LPG observed in ischemic myocardium are sufficient to elicit membrane dysfunction and cause electrophysiological derangements. Furthermore, the concentrations observed in vivo early after the onset of ischemia exceed those required to induce comparable membrane dysfunction with exogenous LPG. Also, their electrophysiological effects are exacerbated by concomitants of ischemia such as H^+ accumulation. Therefore the LPGs appear to be metabolic mediators of arrhythmia induced by ischemia.

LPC is established as the most abundant lysophosphoglyceride and its arrhythmogenic properties are evident in the ischemic heart. Lidocaine is also established as the effective treatment for ventricular arrhythmias. Although isolated perfused hearts have been used in determining the arrythmogenic effects of LPC, in vivo studies have not been done to delineate the effects of the presence of LPC as well as the influence of lidocaine on the entire heart. Therefore, the whole animal LPC infusion studies are proposed. Along with being a very effective antiarrhythmic drug lidocaine has the advantages of having a short half-life in the circulation and shows an immediate antiarrhythmic action. To delineate further the mechanism of action of LPC and lidocaine the electrophysiological studies are proposed. Purkinje fibers will be superfused with Tyrode's solution containing LPC and the action

potential characteristics will be studied. The action potential characteristics of the Purkinje fibers will also be studied in the presence of both LPC and lidocaine. These will aid in defining the mechanism through which lidocaine exerts its action on the canine heart. The purpose of this study is to observe the effect of both LPC and lidocaine in the canine heart using in vivo and in vitro preparations. The in vivo preparations involve the infusion of LPC into the LAD coronary artery along with i.v. lidocaine. The in vitro studies involve superfusion of canine Purkinje fibers with both LPC and lidocaine.

METHODS

A. WHOLE ANIMAL STUDIES

1. Surgical preparation of the animals

Mongrel dogs of either sex were anesthetized with sodium pentobarbital 30 mg/kg i.v. An endotracheal tube was placed in the trachea and the dogs were ventilated with room air using a Harvard respirator pump. The heart rhythm was monitored with a lead II EKG and recorded on a Gould Brush 2400 recorder. A left thoracotomy was performed in the intercostal space between the fourth and fifth ribs. The pericardium was opened and used to cradle the heart. The femoral artery was cannulated with polyethylene tubing. Arterial blood from the femoral artery was subsequently used to supply the left anterior descending (LAD) coronary artery. The LAD was isolated at a point 0.5 to 1 cm from the origin and cannulated within three minutes using polyethylene tubing (Clay Adams). Immediately after cannulation of the LAD coronary artery the cannula was connected via a three-way valve to the femoral artery cannula in order to restore arterial blood flow to the portion of the heart located distally to the cannula inserted in the LAD. This experimental set-up allowed LPC infusion to affect only a portion of the heart supplied by the LAD coronary artery which later could be distinguished from the region receiving the normal blood supply from the other coronary arteries. A side arm of the cannula allowed for the injection or continuous infusion of lysophosphatidylcholine (prepared from egg yolk, Sigma Chemical Company) or saline. The perfusion pressure was also monitored and the mean perfusion pressure was obtained from a digital display. Cannulation of the femoral vein allowed for infusion of drugs as necessary.

To determine the effect of infusing a constant amount of LPC into the LAD coronary artery a calibrated pump was used for delivering arterial blood. Blood flow from the femoral artery was passed through a roller pump and the rate of the pump was adjusted to maintain the same perfusion pressure as the pressure during the rest period. This flow rate was maintained constant for the rest of the experiment. The blood flow was then determined from the calibration curve of the roller pump and the rate of LPC infusion adjusted so that the blood in the cannula entering the LAD coronary artery contained 1 mM LPC for the length of the perfusion. LPC (1 mM) was chosen after preliminary experiments were done with various concentrations and time periods.

In order to assess the effects of LPC infusion into the LAD coronary artery for a longer time, a five hour time period of infusion with 0.5 mM LPC was chosen. This choice was a result of experimentation with varying concentrations and time periods. In the five hour infusion experiments the pump was not used to deliver arterial blood from the femoral artery to the LAD coronary artery. Experience from the one hour experiments showed that when the pump was discontinued the animal could be monitored up to three hours reliably after which deterioration of the blood pressure was evident. The pump was eliminated to avoid this complication and any possible hemolysis due to the roller pump. In order to maintain the animal in a stable state for a minimum five hour period a needle tip connected to polyethylene tubing was used. An estimation was made of the flow rate in the LAD coronary artery based on body

weight of the animal and the flow rates obtained from the one hour experiments. LPC was infused into the LAD coronary artery based on the estimated flow value to provide a concentration of 0.5 mM. Thus, in order to obtain a more stable preparation it was necessary to sacrifice the exact concentration of LPC. A 26 gauge needle tip was inserted into the LAD coronary artery in a horizontal position to the direction of the normal blood flow. The needle tip was connected to polyethylene tubing. To verify that blood flow was not obstructed and that the LPC being infused was reaching its intended destination methylene blue biological stain was used. A sufficient stabilization period of at least one hour was allowed so that any temporary effects of the surgery could be overcome, and the ECG showed no abnormalities.

2. Bolus injection of LPC

After a sufficient stabilization period, a bolus injection (<10 sec) of a 20 mM LPC solution was given through the LAD cannula. The presence of any arrhythmias was recorded and the rhythm was analyzed further. The animal was allowed a rest period after which an increasing amount of LPC was injected until the occurence of sustained ventricular tachycardia was achieved. The animals were then randomly assigned to receive 4 mg/kg lidocaine or saline which served as controls. The amount of LPC that produced sustained ventricular tachycardia was injected again after the administration of either lidocaine or saline. The arrhythmias produced by LPC with or without lidocaine were assessed.

3. Continuous one hour infusion of LPC

Prior to LPC infusion animals were given 2 mg/kg lidocaine or the equivalent volume of saline. Five minutes after this treatment 70 μg/kg/min lidocaine or the same volume of saline was infused for a one hour period. Lidocaine was given in two stages. The initial loading dose of 2 mg/kg served to raise the level of the drug in the blood. Since lidocaine has a short-half-life a continuous dose was required during the LPC infusion period to maintain a steady level of the drug. LPC was infused into the LAD coronary artery cannula to provide a final concentration of 1 mM for the duration of one hour. Concurrent to the LPC infusion, lidocaine (70 μg/kg/min) or saline was given i.v. The cardiac rhythm was recorded for the duration of the continuous LPC infusion as well as subsequent to the infusion. A saline infusion instead of LPC was used as baseline comparison.

4. Continuous five hour infusion of LPC

After the stabilization period, 2 mg/kg plus 70 μg/kg/min lidocaine or the equivalent volume of saline was infused into the femoral vein for 30 minutes. LPC was then infused in the LAD coronary artery for a maximum of five hours or until ventricular fibrillation was observed on the ECG. Lidocaine (70 μg/kg/min) or saline infusions through the femoral vein accompanied the LPC that was being infused into the LAD coronary artery. Equivalent volume of saline infusion into the LAD coronary artery served as control in another group of animals. The cardiac rhythm was recorded throughout the infusions and also subsequent to them. At the end of

the experiments methylene blue was again injected into the IAD cannula to ensure that the LPC was being infused into the intended region of the heart and that it cleared within a short period of time (under 30 seconds).

B. ARRHYTHMIA CLASSIFICATION

In order to describe what was occurring to the heart rhythm in the whole animal LPC infusion studies the following classification scheme was used.

Ventricular arrhythmias were classified as follows:

Types of arrhythmias	Score
Normal ECG pattern (no arrhythmia present)	0
Infrequent ectopic beats (less than 15 in 10 seconds)	1
Frequent ectopics (more than 15 in 10 seconds)	2
Non-sustained ventricular tachycardia (a run of ectopic beats, 3 or more in less than 10 seconds)	3
Sustained ventricular tachycardia (lasting more than 10 seconds)	4
Ventricular fibrillation	5

C. HISTOLOGY

In all the LPC infusion experiments, samples of the perfused and non-perfused regions of the myocardium were fixed in a buffered 10% formaldehyde solution, and subsequently embedded in paraffin. The tissue was then sectioned and stained with eosin and hematoxylin. The slides were examined with a light microscope.

D. ELECTROPHYSIOLOGICAL STUDIES

Experiments were performed using isolated canine Purkinje fibers exposed to 40 μM LPC in one group, and 40 μM LPC as well as 18 μM lidocaine in another group. Adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg) the heart was quickly removed and placed in oxygenated modified Tyrode's solution of the following composition:

Normal Tyrode's solution

NaCl	125 mM
$NaHCO_3$	24 mM
Dextrose	5.5 mM
$MgCl_2 \cdot 6H_2O$	0.5 mM
$NaH_2PO_4 \cdot H_2O$	1.0 mM
KCl	4.0 mM
$CaCl_2 \cdot H_2O$	2.0 mM

pH 7.3

Distal portions of the left bundle (anterior as well as posterior divisions) were dissected out. The dissected Purkinje fibers were pinned through the attached endocardial muscle to the bottom of a 10 ml wax

lined bath. The tissue was superfused constantly with modified Tyrode's solution at a rate of 14 ml/min, with flow calibrated and held constant throughout each experiment. The temperature of the solution in the tissue bath was held constant at 37.0°C by circulating water from a constant temperature water bath. All solutions were aerated with $95\%O_2$-$5\%CO_2$. The measured pO_2 was 700 mm Hg.

All chemicals were reagent grade from Fisher Chemical Company.

Purkinje fibers were stimulated with the use of a Pulsar 4 digital pulse generator, through the use of a bipolar Teflon-coated (except at the tips) platinum electrode. The Purkinje fibers were stimulated at twice the threshold voltage, with square wave pulses of 2 msec duration and at a cycle length of 1000 msec. Intracellular action potentials were recorded with glass microelectrodes (Frederick Haer & Company) filled with 3M KCl (resistance 10-30 MOhm), connected with Ag/AgCl wire to a high input impedance preamplifier with capacitance neutralization. Signals were amplified and visualized on an oscilloscope and recorded on a Tanberg FM tape recorder.

Once a stable action potential was obtained LPC was added to the Tyrode's to achieve a concentration of 40 μM. The tissue was superfused with LPC containing solution and subsequently the LPC was washed out with Tyrode's solution for a minimum of one hour or for the same duration as the LPC superfusion.

To test the effect of lidocaine on LPC superfused Purkinje fibers once the action potential was obtained and had stabilized the tissue was superfused with 18 μM lidocaine (therapeutic concentration range 5-20 μM) for a 30 minute period. Subsequent superfusion followed with both LPC and lidocaine. Recordings were taken initially, and at frequent time periods during the superfusions. The initial recordings with Tyrode's solution served as the control for each cell. Resting membrane potential (RMP), overshoot of phase 0, action potential duration at 50% and 90% of full recovery (APD_{50}, APD_{90}) and the maximum rate of rise of voltage of phase 0, ($\dot{V}_{max}$) as well as amplitude (AMP) of the action potentials were recorded on the tape. The stored signals were analyzed and the changes were compared.

E. STATISTICAL ANALYSES

Results from the bolus LPC infusion experiments were analyzed using paired or non-paired Student's t-tests. The results from the experiments where LPC was infused into the LAD coronary artery for one hour or five hours were analyzed using ANOVA as well as non-paired t-tests. The histological results were analyzed by Chi-Squared statistics. Analysis of the electrophysiologic parameters was made using paired or non-paired Students t-tests. The criteria for ANOVA were not fulfilled by the electrophysiology experiments since the loss of cells during the superfusion period was not a random event, but rather a result of the treatment. Hence, multiple t-tests were used. In order to correct for error in alpha due to multiple comparisons the Bonferonni correction was used. With alpha set at 0.05 and five or less (at times 10, 20, 30, 45, 60 minutes) comparisons being made for each parameter

statistical significance was accepted at alpha <0.05/number of comparisons. Statistical significance was accepted at $p<0.01$. Analysis of the automaticity data was made using Chi-Squared statistics. Slopes of each individual cell over time were obtained using linear regression analysis.

RESULTS

A. RESULTS OF WHOLE ANIMAL STUDIES

1. Results of bolus LPC infusion experiments

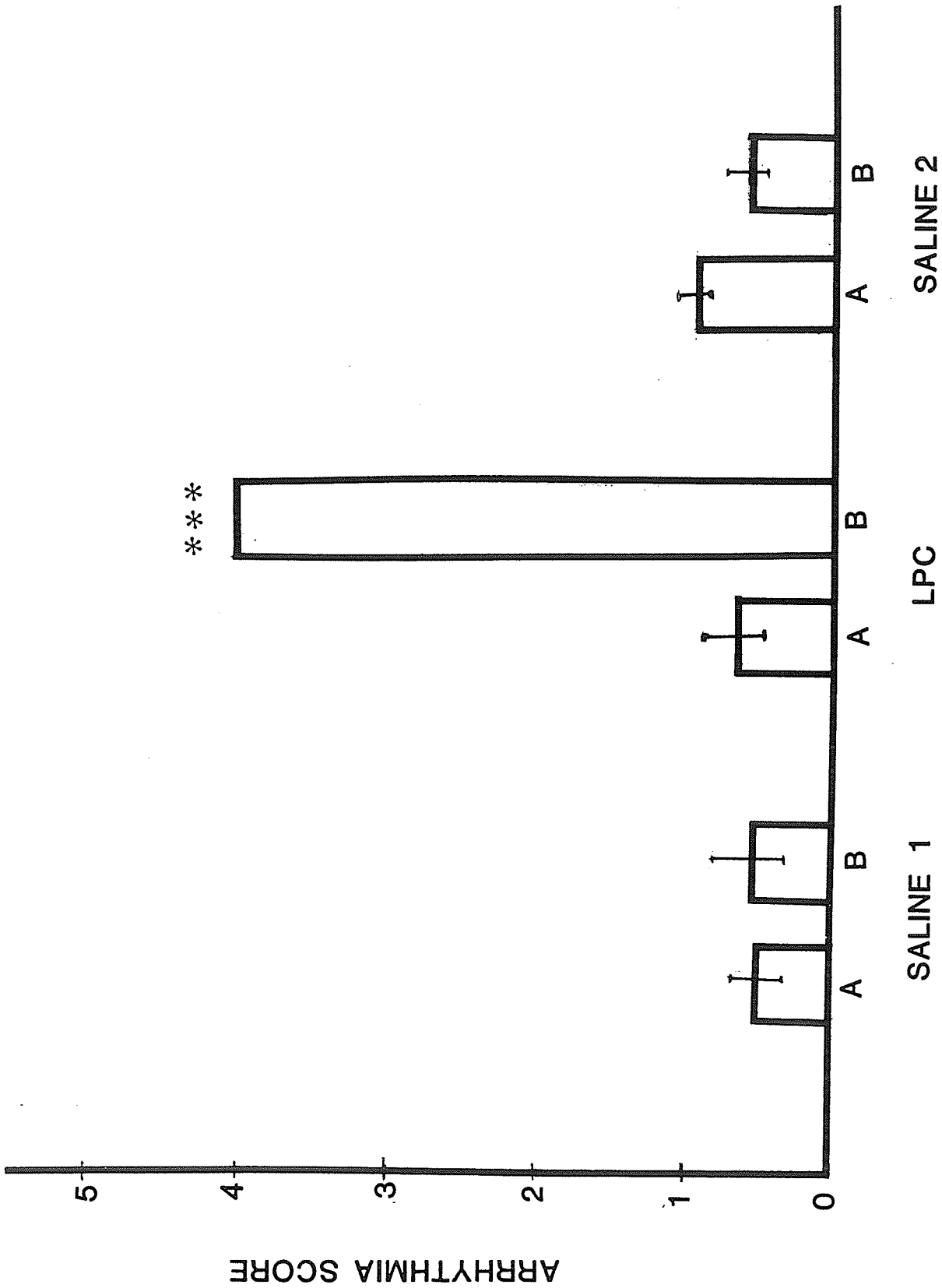
The purpose of these experiments was to determine the arrhythmogenic action of a bolus LPC infusion into the LAD coronary artery. The arrhythmogenicity was established by varying the amount of LPC infused into the LAD coronary artery. LPC was infused into the LAD coronary artery and the volume increased each time ventricular tachycardia (VT) was not observed. A corresponding volume of saline was also infused. The decision to inject LPC or saline first was random. Figure (4) shows the arrhythmias obtained from the bolus LPC infusion experiments. The average volume of LPC required to produce VT was 0.77 $\pm$ 0.28 ml ($\pm$ standard error) of a 20 mM LPC solution. This corresponds to 15 $\pm$ 6 μmoles LPC.

The arrhythmia scores prior to saline injection were 0.50 $\pm$ 0.17 and after saline injection 0.56 $\pm$ 0.22 ($\pm$ standard error). There was no significant difference in the two groups. The arrhythmia score prior to LPC was 0.63 $\pm$ 0.21 and 4.0 after LPC injection. The difference between these two comparisons was statistically significant ($P < 0.001$). Saline infusion after LPC shows an arrhythmia score of 0.56 $\pm$ 0.18. All the saline infusions showed no significant difference between them.

After establishing the amount of LPC required to produce VT (by bolus injection in <10 secs), the animals were pretreated with

Figure 4. The effect on cardiac arrhythmias of bolus LPC injection into the coronary artery.

Animals were randomly assigned to receive either a 20 mM LPC solution or the same volume of saline. The amount of LPC injected was increased until the appearance of ventricular tachycardia. The final concentrations of LPC given range from 0.3 to 1.0 ml (mean volume = 0.77 $\pm$ 0.28 ml, n=19). This represents 15 $\pm$ 6 µmoles of LPC. The arrhythmias are classified as described earlier. A and B represent the arrhythmia score before and after the injection into the coronary artery. Bars represent standard errors (S.E.). Saline 1 represents prior to LPC infusion (n=10) and saline 2 represents after LPC infusion (n=9). * Represents $P < 0.05$, ** represents $P < 0.01$, *** represents $P < 0.001$ and N.S. represents $P > 0.05$.


ARRHYTHMIA SCORE
0
1
2
3
4
5
A
B
A
B
A
B
A
B
SALINE 1
LPC
SALINE 2
***

either lidocaine (4 mg/kg) or the corresponding volume of saline. The results are summarized in Figure 5. This figure represents the series of 19 experiments where VT was produced by LPC infusion into the LAD coronary artery. The lidocaine treated animals showed a significant ($P < 0.001$) reduction in the arrhythmia score to 1.75 ± 0.35 from the 3.9 ± 0.14 arrhythmia score shown in the saline pretreated group.

2. Results of one hour of continuous LPC (1 mM) infusion

In order to quantify the concentration of LPC being infused into the LAD coronary artery as well as to determine the effect of LPC infusion for a longer period of time a calibrated rotary pump was used. In the experiments where LPC was being infused by a bolus the amount of LPC entering the LAD coronary artery could be determined but the concentration was not known. Since the blood flow could be determined from the calibrated rotary pump, this procedure would allow for the determination of the concentration of LPC that the LAD coronary artery was receiving. LPC (1 mM) was chosen after preliminary experiments were done with various concentrations.

Infusion of LPC (1 mM) continuously for one hour (Figure 6) into the LAD with saline pretreatment resulted in an arrhythmia score of 3.5 ± 0.4 (n=11). Lidocaine pretreated animals (n=14) resulted in an arrhythmia score of 2.4 ± 0.5. Lidocaine significantly ($P < 0.05$) reduced the arrhythmia score. The control group received a saline pretreatment prior to the infusion of

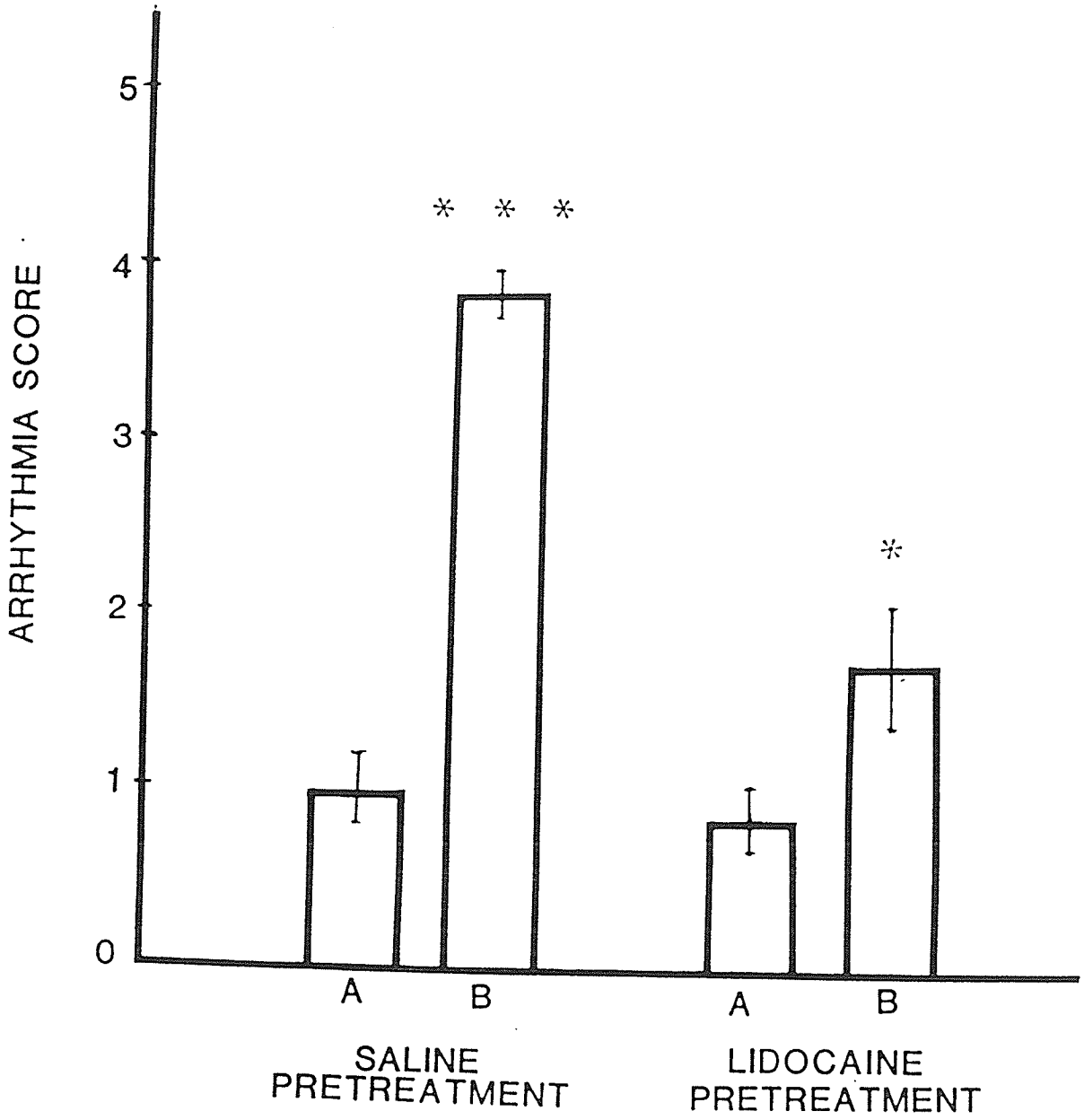
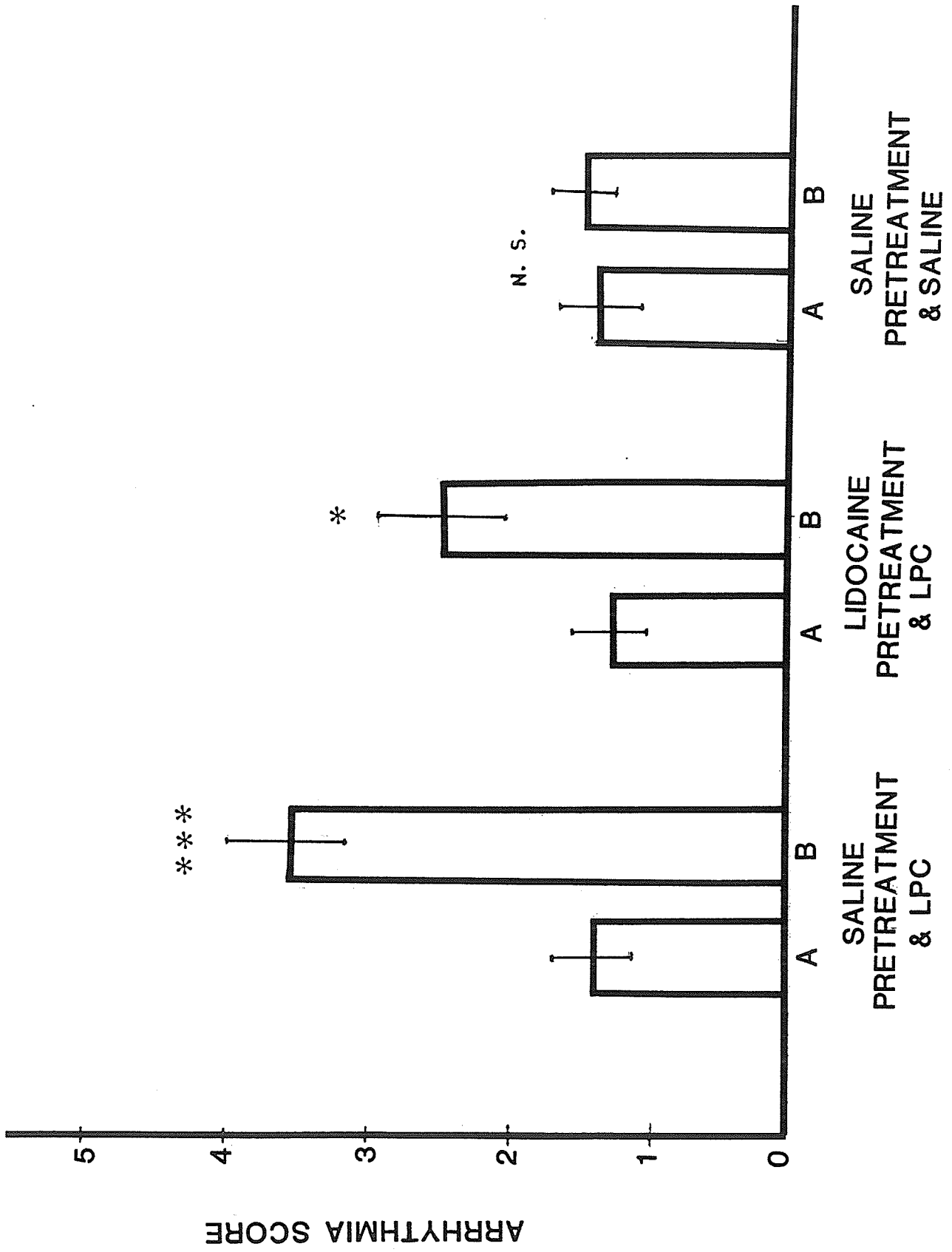


Figure 5. The effect of lidocaine pretreatment on LPC-induced arrhythmias.

Animals were randomly assigned to receive 4 mg/kg lidocaine (n=12) or saline (n=7) pretreatment prior to the same amount of LPC that produced ventricular tachycardia as shown in Figure 4. Symbols are the same as in Figure 4.

Figure 6. The effect of infusion of arterial blood containing 1 mM LPC into the coronary artery and the effect of lidocaine.

The blood flow into the LAD cannula was estimated with a calibrated roller pump to provide the same mean perfusion pressure. LPC was infused into the cannula to provide a final concentration of 1 mM for 1 hour. Animals were divided into 3 groups. The first group (n=11) received a saline pretreatment before the infusion of LPC. The second group (n=14) received 2 mg/kg + 70 μg/kg/min lidocaine pretreatment prior to the infusion of LPC. The third group (n=11) received a saline pretreatment prior to the infusion of saline instead of LPC. This group served as time controls. Symbols are the same as Figure 4.


ARRHYTHMIA SCORE
0
1
2
3
4
5
A
B
SALINE PRETREATMENT & LPC
LIDOCAINE PRETREATMENT & LPC
SALINE PRETREATMENT & SALINE
***
*
N. S.

saline instead of LPC. This group served as time controls for comparison to the other two groups. The arrhythmia scores showed no time effect for this experimental protocol. The pretreatment scores in all three groups showed no significant difference between them.

Figure 7 shows ECG recordings of a representative experiment where the LAD coronary artery was infused continuously with 1 mM LPC solution for one hour.

3. Results of five hours of continuous LPC (0.5 mM) infusion

To test the effects of LPC infusion over a longer period of time the concentration of 0.5 mM was chosen for a five hour infusion period. This concentration was chosen after preliminary experiments were done with various LPC concentrations. The arrhythmia scores during LPC infusion showed a significant increase ($P < 0.01$) from the rest period at the first hour until the end of the five hour LPC infusion. Table I shows the individual arrhythmia scores during LPC (0.5 mM) infusion.

To test the effect of lidocaine on the arrhythmia score during the five hour infusion of LPC into the LAD coronary artery, i.v. lidocaine 2 mg/kg and subsequently 70 µg/kg/min was infused concomitantly with the LPC infusion period. A modest increase in arrhythmia scores occurred. Table II shows arrhythmia scores for the individual experiments during LPC and lidocaine infusion. A significant ($P < 0.01$) increase from the rest period was observed during the fifth hour of LPC and lidocaine infusion. This is

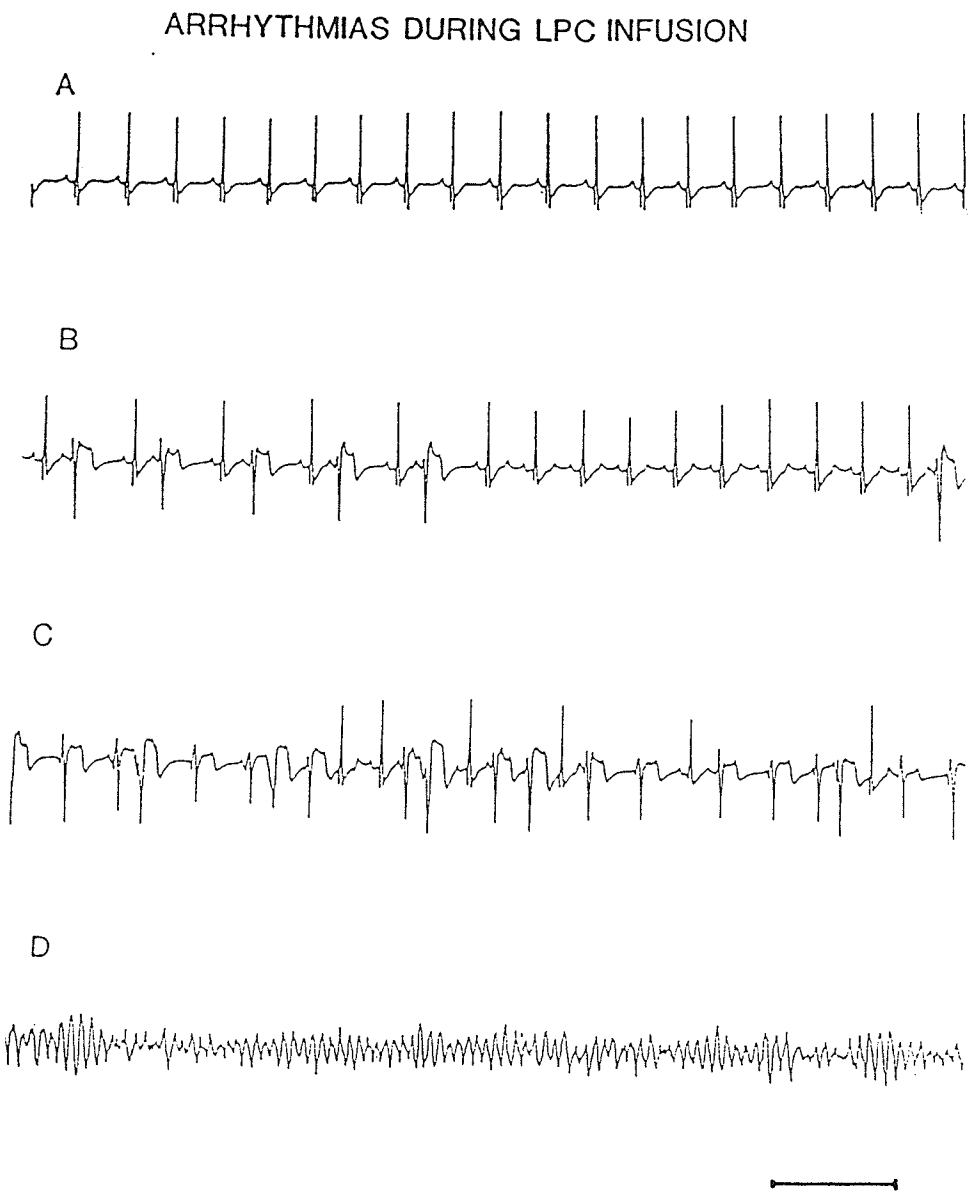


Figure 7. Ventricular arrhythmias during LPC infusion.

This figure shows a representative experiment where LPC (1 mM) was infused into the LAD coronary artery. Electrocardiac recording was obtained from Lead II. A represents the normal cardiac rhythm at time zero. B, C and D are the arrhythmias recorded at 17, 24 and 27 minutes respectively into the infusion period. Time scale represents 1 second.

TABLE I.

Arrhythmia scores for five hour LPC (0.5 mM) infusion

EXPT. NO.	REST PERIOD	HOURS OF LPC INFUSION 1	2	3	4	5
1	0	0	0	0	0	0
2	0	5	5	5	5	5
3	1	5	5	5	5	5
4	0	4	4	4	3	3
5	0	0	0	2	1	2
6	1	2	2	5	5	5
7	0	1	1	1	1	1
MEAN	0.3 ± 0.2	2.4 ± 0.8**	2.4 ± 0.8**	3.1 ± 0.8**	2.9 ± 0.8**	3.0 ± 0.8**

** $P < 0.01$, significantly different from the rest period.

TABLE II.

Arrhythmia scores for five hour LPC (0.5 mM) and lidocaine infusion

		HOURS OF LPC AND LIDOCAINE INFUSION				
EXPT. NO.	REST PERIOD	1	2	3	4	5
1	1	0	1	1	1	1
2	0	0	0	0	2	5
3	1	1	1	1	1	3
4	0	1	1	2	2	3
5	1	1	1	1	3	1
6	1	3	1	2	2	3
MEAN	0.7±0.2	1.0±0.4	0.8±0.1	1.2±0.3	1.8±0.3	2.7±0.6**

** $P < 0.01$ significantly different from the rest period.

contrasted to a significant ($P < 0.01$) increase from rest seen in the LPC group which occurred during the first hour of infusion.

The five hour saline arrhythmia scores (Table III) showed no increase in arrhythmia during the five hour infusion period. The arrhythmia scores remained at rest level showing no time effect during the length of the infusion.

Figure 8 shows the comparison of the arrhythmia scores in the three groups. It is evident that even though the arrhythmia scores during the LPC and lidocaine infusion period were lower than the LPC infusion these scores did not differ significantly. The saline and LPC comparison showed a significant ($P < 0.05$) difference during the first hour of infusion. There was no significant difference in arrhythmia scores between saline and LPC with lidocaine for the first two hours. At the third hour to the end of the infusion, LPC and lidocaine showed a significantly ($P < 0.01$) higher arrhythmia score than the corresponding saline values.

The appearance of a significantly higher arrhythmia score was delayed with lidocaine. In LPC and lidocaine group a significant arrhythmia score of 2.7 ± 0.6 was observed only after five hours of infusion compared to the first hour of infusion in the LPC group (2.4 ± 0.84).

TABLE III.

Arrhythmia scores for time control experiments (five hour saline infusion)

		HOURS OF SALINE INFUSION				
EXPT. NO.	REST PERIOD	1	2	3	4	5
1	1	1	1	0	0	1
2	1	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	1	0	0	0	0	0
7	1	1	1	1	2	2
MEAN	0.6±0.2	0.3±0.2	0.3±0.2	0.1±0.1	0.3±0.3	0.4±0.3

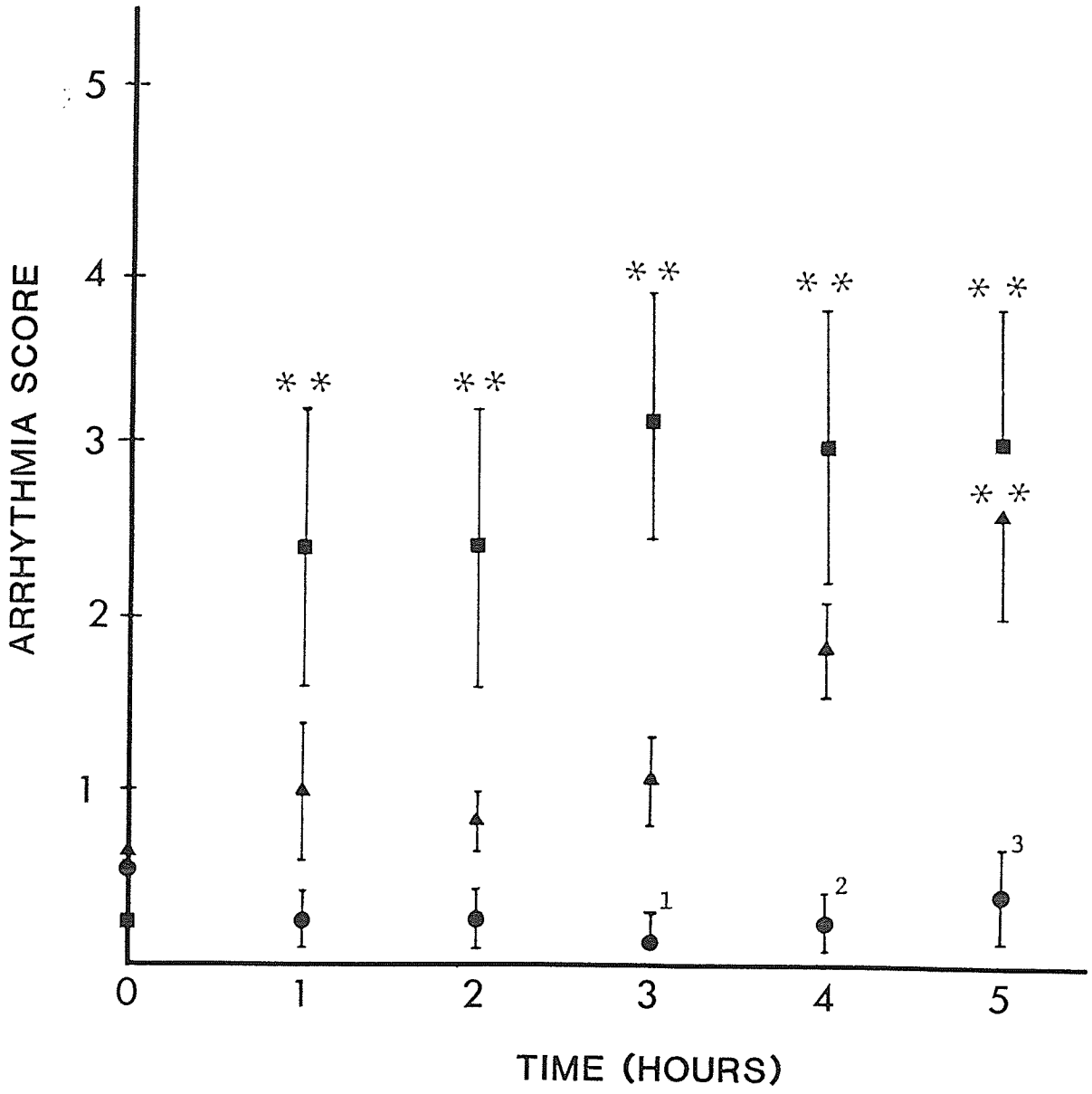
Figure 8. Comparison of arrhythmia scores during five hours of LPC (0.5 mM) infusion, five hours LPC (0.5 mM) and lidocaine infusion and time controls.

In the first group of animals (n=7) LPC was infused into the LAD coronary artery for a five hour time period. The second group (n=6) received LPC as well as i.v. lidocaine for the same time period. The third group (n=7) received saline instead of LPC as well as i.v. saline to test for time effects. Bars represent standard errors. ** Represents $P<0.01$, for comparisons made between rest period and time of LPC infusion. Comparisons between LPC and LPC with lidocaine infusion groups at all time periods showed no significant differences although arrhythmia scores in the LPC infusion group were consistently higher. The comparisons at each time period between saline and LPC infusion groups showed a significantly higher difference ($P < 0.05$) for the LPC group.

1 3 hours saline vs 3 hours LPC and lidocaine $P < 0.01$

2 4 hours saline vs 4 hours LPC and lidocaine $P < 0.01$

3 5 hours saline vs 5 hours LPC and lidocaine $P < 0.01$


ARRHYTHMIA SCORE
5
4
3
2
1
0
1
2
3
4
5
TIME (HOURS)
* *
* *
* *
* *
* *
1
2
3

4. Histology

Comparisons were made on the histological aspects of perfused and non-perfused regions of the myocardium. The comparisons were based on regularity or irregularity of the cardiac fibers, presence or absence of nuclei, and size of interstitial spaces. In the experiments where LPC was given as a bolus infusion and 1 hour infusion there occurred a significantly higher ($P < 0.001$) incidence of irregularities in the region receiving LPC than in the region that did not receive LPC whether lidocaine was present or not (TABLE IV). In the experiments receiving saline infusion, no differences were observed between the myocardial regions that received saline and those that did not.

In the five hour LPC infusion and five hour LPC infusion with lidocaine experiments the trend was the same. Both of these groups showed a significant difference between the two regions. The myocardial regions receiving the LPC had the higher number of histological irregularities. When comparing the regions that received LPC in all of the experimental groups in all cases the ones receiving LPC and LPC with lidocaine exhibit a significantly higher number of irregularities. The presence of lidocaine did not significantly decrease the number of experiments showing irregularities.

TABLE IV

Experiments showing histological irregularities of myocardial regions that receive LPC and those that do not.

	Region receiving LPC	Region not receiving LPC	
Bolus Saline	3/10	3/12	N.S.
Bolus LPC	18/18[1]	3/19	P<0.001
One Hour Saline	1/5	0/5	N.S.
One Hour LPC (1 mM)	9/12[2]	0/12	P<0.001
One Hour LPC (1 mM) and Lidocaine	10/13[3]	1/15	P<0.001
Five Hours Saline	0/5	0/5	N.S.
Five Hours LPC (0.5 mM)	3/3[4]	0/3	P<0.05
Five Hours LPC and Lidocaine	4/4[5]	1/6	P<0.05

1. Bolus Saline vs Bolus LPC P<0.001

2. One Hour Saline vs One Hour LPC (1.0 mM) P<0.05

3. One Hour Saline vs One Hour LPC (1.0 mM) and Lidocaine P<0.05.
One Hour LPC (1.0 mM) vs One Hour LPC (1.0 mM) and Lidocaine N.S.

4. Five Hours Saline vs Five Hours LPC (0.5 mM) P<0.05

5. Five Hours Saline vs Five Hours LPC (0.5 mM) and Lidocaine P<0.05.
Five Hours LPC (0.5 mM) vs Five Hours LPC (0.5 mM) and Lidocaine N.S.

B. RESULTS OF ELECTROPHYSIOLOGICAL PARAMETERS OF SUPERFUSED CANINE PURKINJE FIBERS

The arrhythmogenic action of LPC infusion on the heart was established in the whole animal studies. These also showed that the arrhythmias induced by LPC could be decreased by lidocaine. In order to investigate the cellular action of LPC as well as the effect of lidocaine on the LPC-induced arrhythmias electrophysiological studies using intracellular recordings were performed.

1. LPC (40 μM) superfusion of canine Purkinje fibers

As seen in Figure (9) there occurs a decrease from control in the resting membrane potential (RMP) over time as the canine Purkinje fibers were superfused with LPC (40 μM). During the superfusion some of the Purkinje fibers became inexcitable due to the LPC treatment. At 20 minutes of LPC superfusion the RMP is significantly different ($P < 0.01$) from control.

Figure 10 shows a similar trend for the amplitudes (AMP) of the action potentials. The AMP decreased over time with a significant difference ($P < 0.01$) from the control at 10 minutes of LPC superfusion.

The action potential duration at the 50% level of membrane repolarization (APD_{50}), shortened during the LPC superfusion. A significant decrease ($P < 0.01$) of the APD_{50} from control was seen at 30 minutes of superfusion (Figure 11). A similar decrease was evident when considering the action potential duration at the 90%

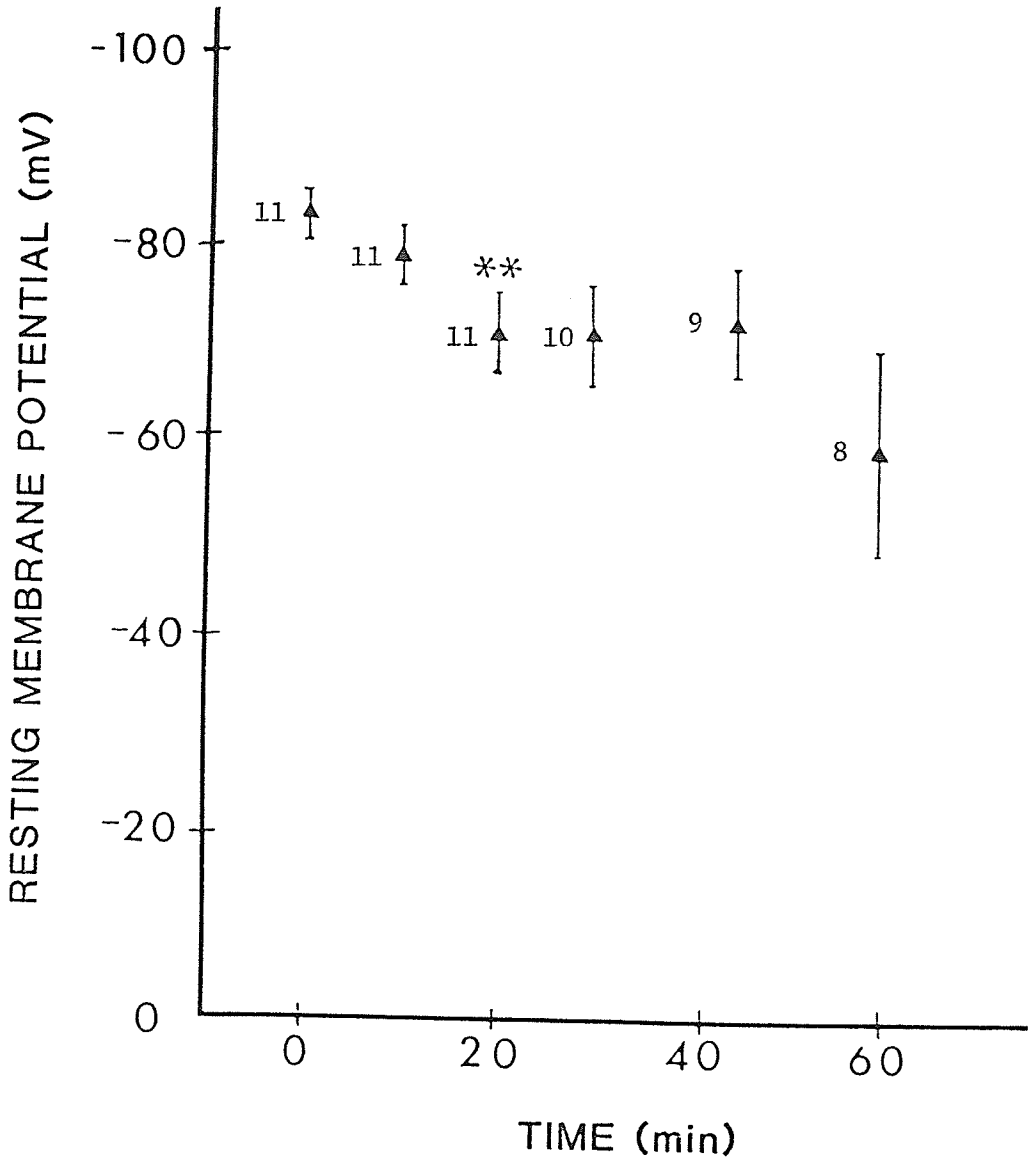


Figure 9. Resting membrane potentials (RMP) of canine Purkinje fibers during LPC (40 μM) superfusion.

The Purkinje fibers were superfused initially with modified Tyrode's solution. Action potentials were obtained and allowed to stabilize. An initial recording during superfusion with modified Tyrode's served as control (time 0). LPC (40 μM) superfusion was started and recordings made at various time intervals. Numbers by each point refer to number of cells.

** $P < 0.01$.

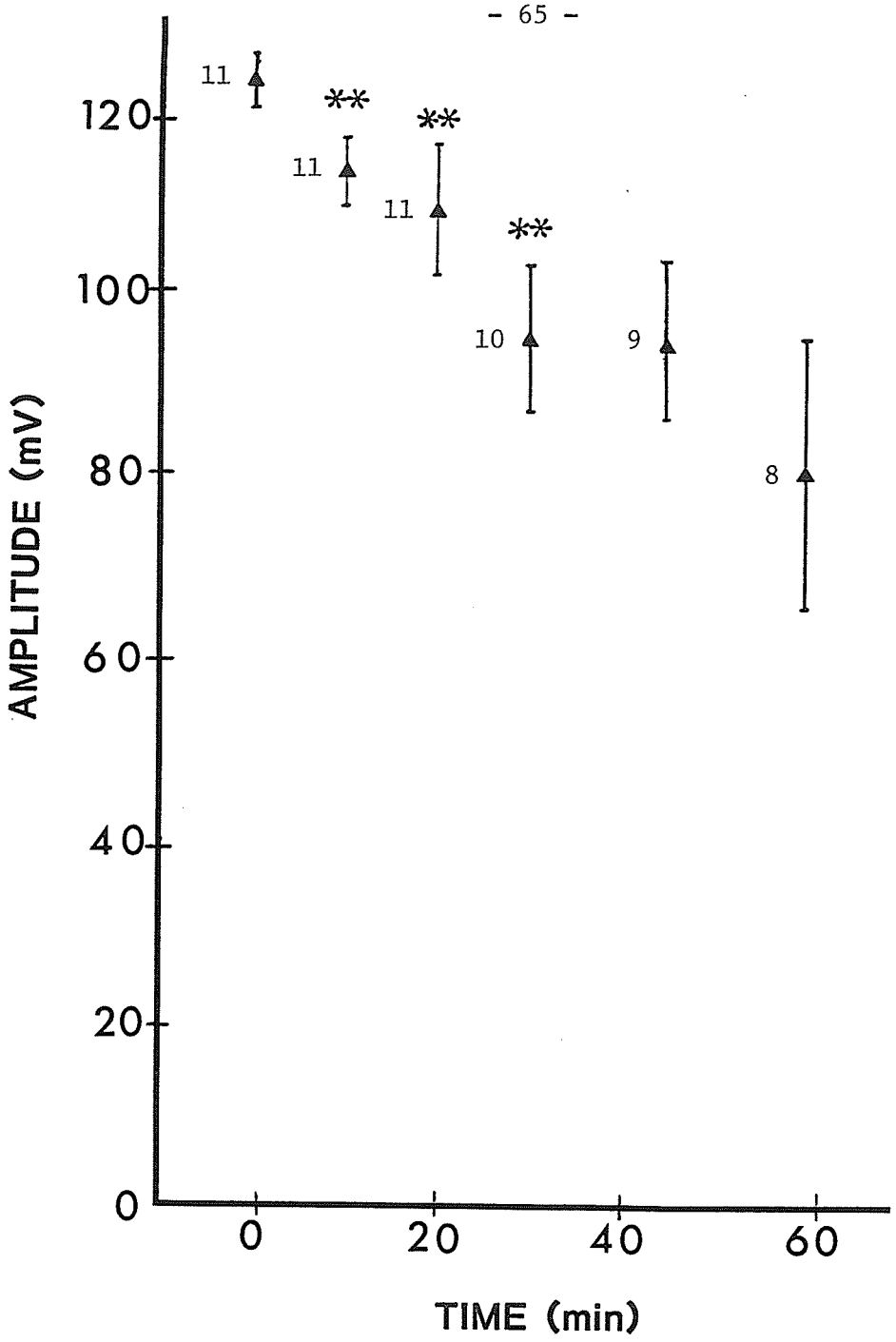


Figure 10. Amplitudes (AMP) of canine Purkinje fibers during LPC (40 μM) superfusion.

Protocol and symbols as described in Figure 9.

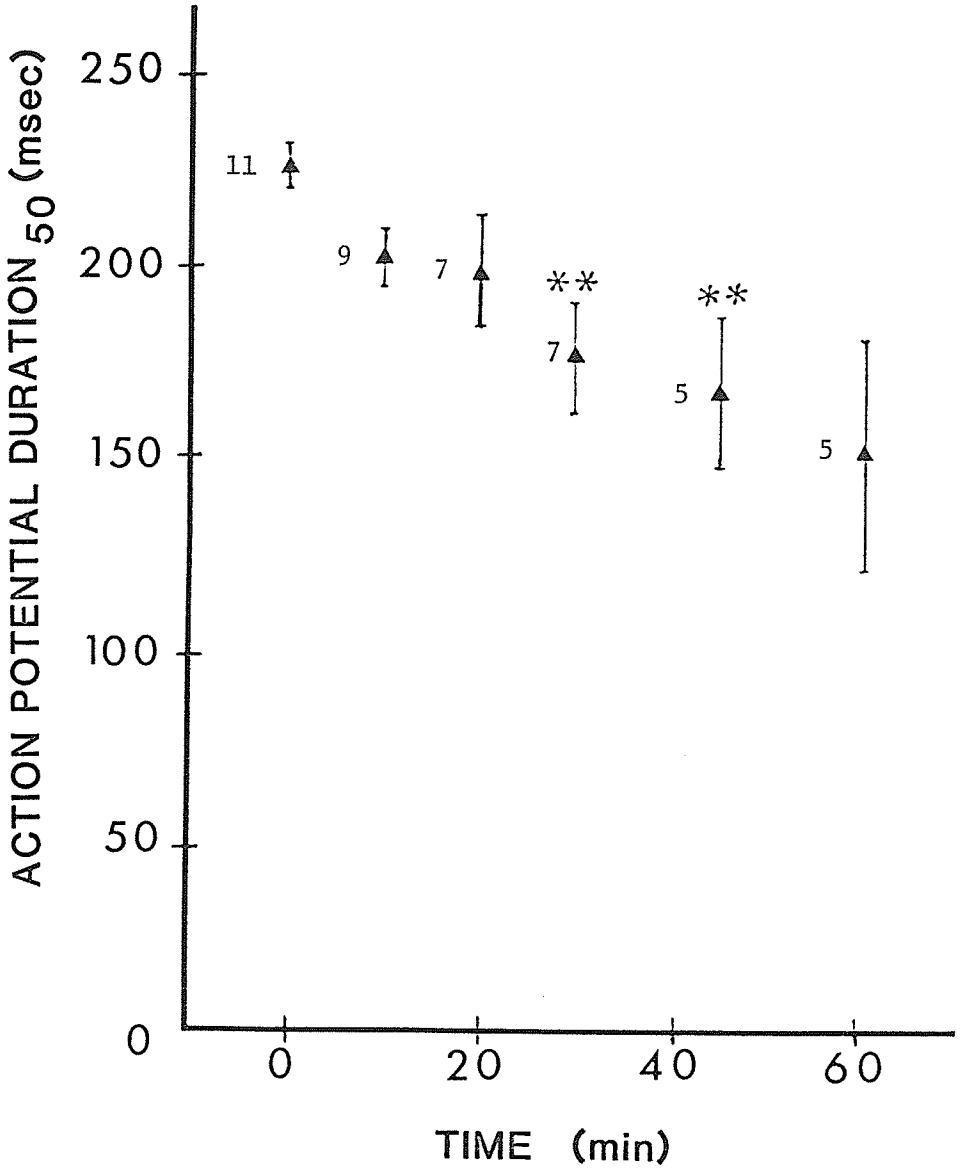


Figure 11. Action potential duration at the 50% level of membrane repolarization (APD_{50}) of canine Purkinje fibers during LPC superfusion.

Protocol and symbols as described in Figure 9.

level of membrane repolarization (APD_{90}). A statistically significant difference here was seen at 30 minutes of LPC superfusion (Figure 12). Thus both the APD_{50} and APD_{90} of the canine Purkinje fibers decreased significantly from the control at 30 minutes of LPC superfusion.

The $\dot{V}_{max}$ values differ significantly from the control values at 30 minutes of LPC superfusion (Figure 13).

In contrast to the decrease seen with time in the above parameters of the canine Purkinje fiber action potentials, the conduction times during LPC superfusion increased (Figure 14). A significant increase from the control values was evident at 45 minutes of LPC superfusion.

2. LPC (40 μM) and lidocaine (18 μM) superfusion of canine Purkinje fibers

Thirty minutes of superfusion with 18 μM lidocaine resulted in a significant reduction of APD_{50} and APD_{90} without any significant changes in RMP and AMP.

The RMP during LPC and lidocaine superfusion are shown in Figure (15). A significant difference ($P < 0.01$) from control RMP was seen at 60 minutes.

The amplitudes of the action potentials during LPC and lidocaine superfusion showed a decrease over time but a significant

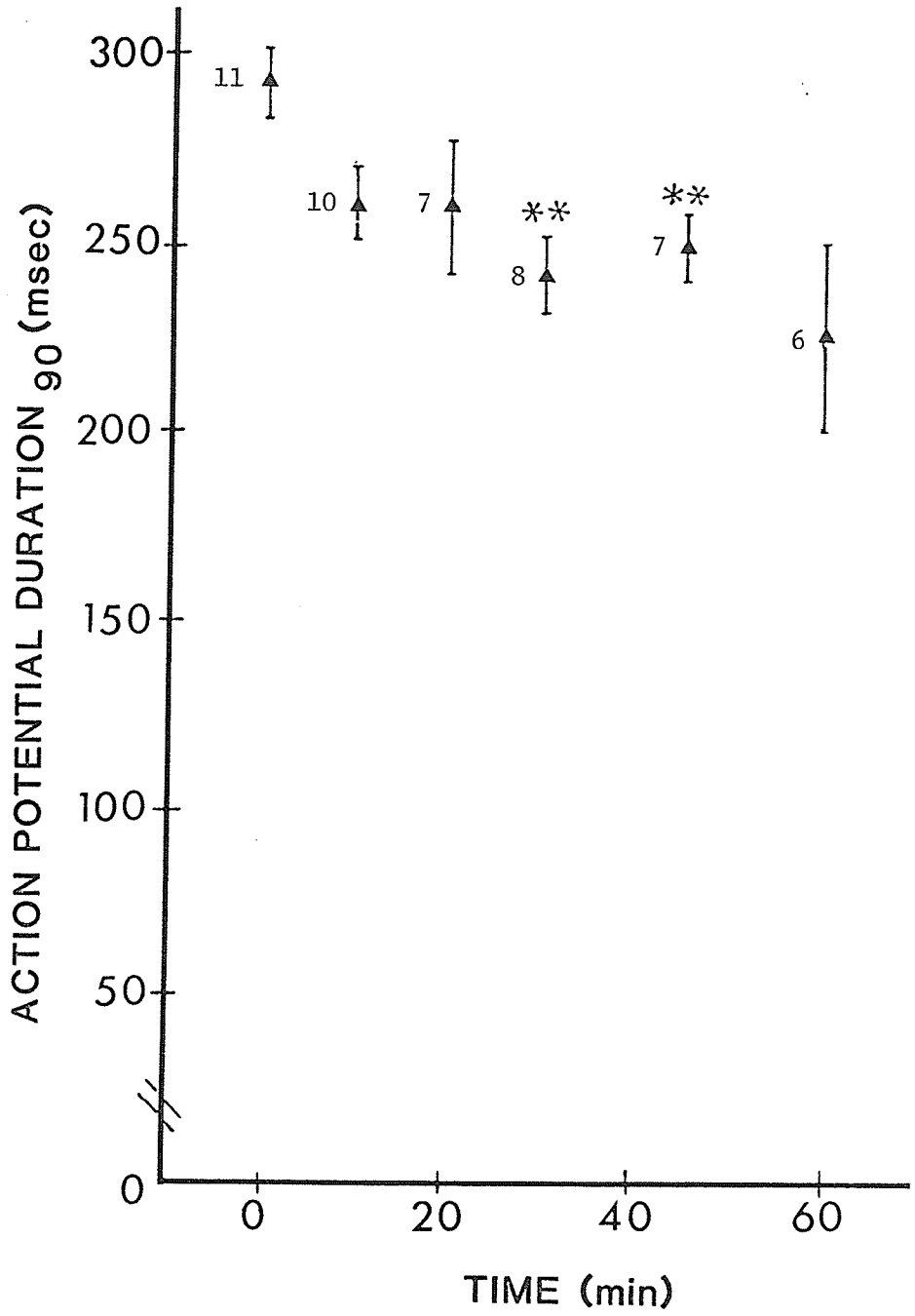


Figure 12. Action potential duration at the 90% level of membrane repolarization (APD_{90}) of canine Purkinje fibers during LPC superfusion.

Protocol and symbols as described in Figure 9.

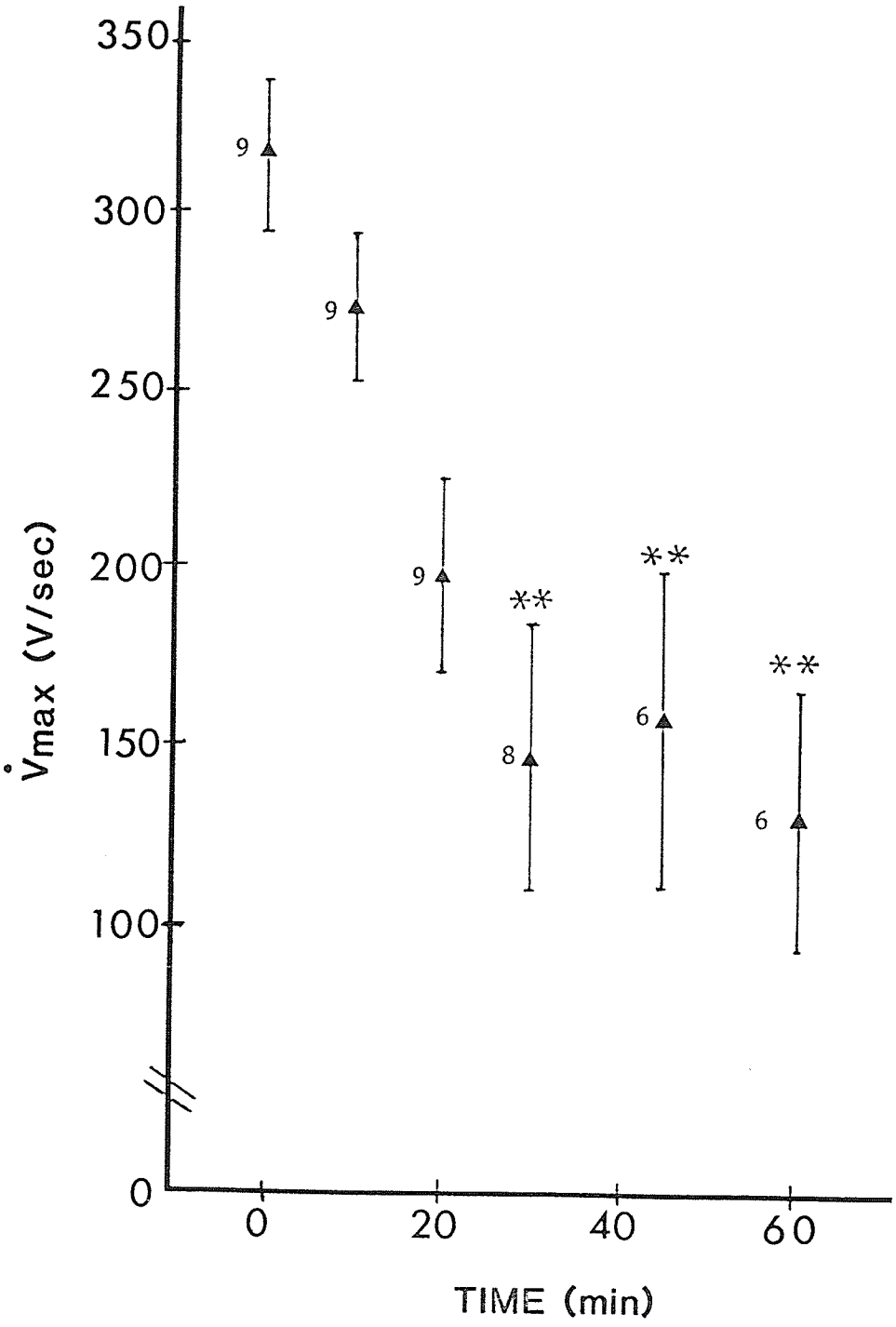


Figure 13. $\dot{V}_{max}$ of canine Purkinje fibers during LPC superfusion.

Protocol and symbols as described in Figure 9.

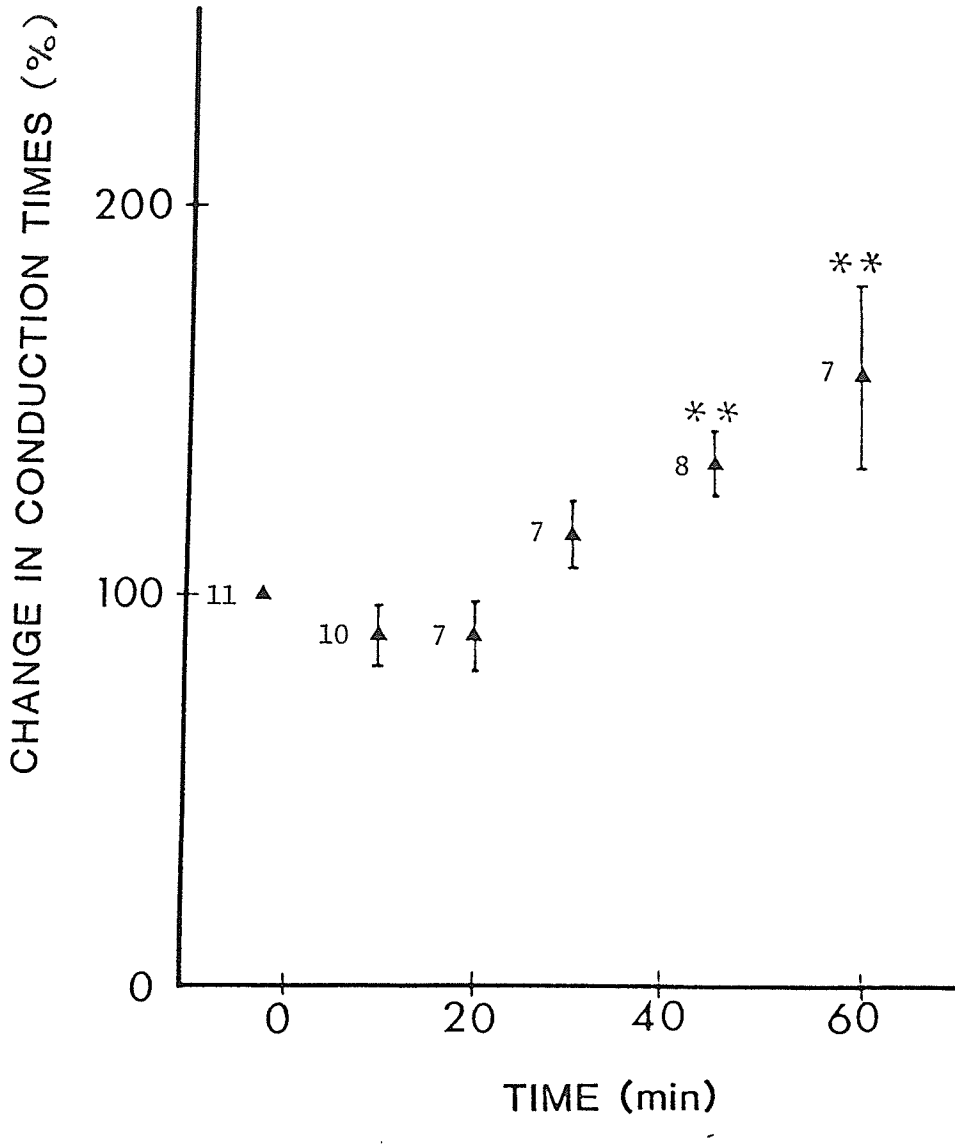


Figure 14. Changes in conduction times in canine Purkinje fibers during LPC superfusion.

Since conduction time varies with the distance of the microelectrodes from the stimulator and this distance was different for every cell it was necessary to normalize the values. In this way all conduction times started at 100 and all changes from that value at various time periods are shown in this figure.

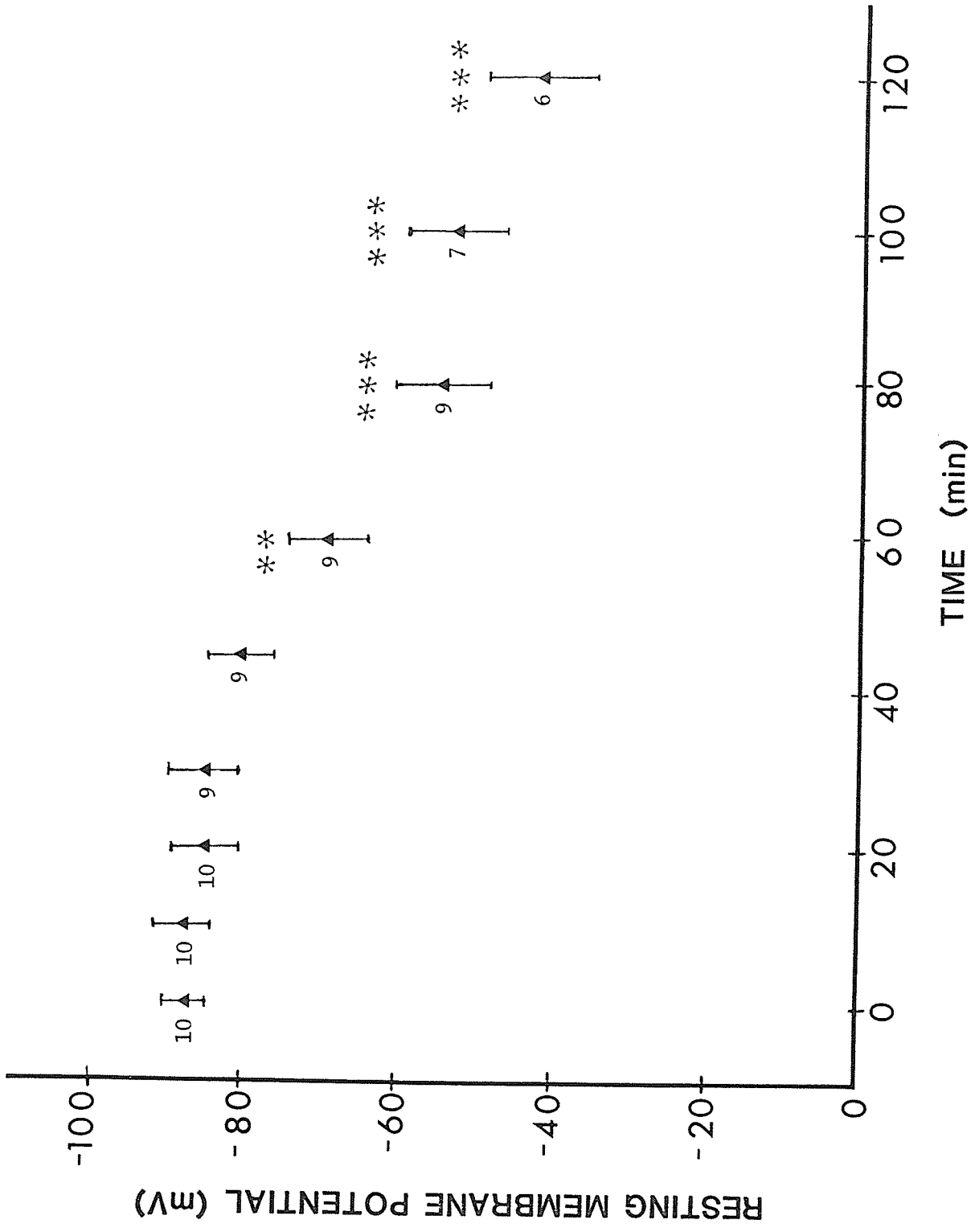
Protocol and symbols as described in Figure 9.

Figure 15. Resting membrane potentials (RMP) of canine Purkinje fibers during LPC and lidocaine superfusion.

The Purkinje fibers were superfused initially with modified Tyrode's solution. Action potentials were obtained, allowed to stabilize and recorded. Superfusion continued for a minimum of 30 minutes with modified Tyrode's solution containing lidocaine (18 μM). At the end of lidocaine superfusion another recording was made. Immediately following this recording superfusion continued with an LPC (40 μM) and lidocaine (18 μM) containing modified Tyrode's solution. Recordings were made at various time intervals. Numbers by each point refer to number of cells.

** $P < 0.01$.

*** $P < 0.001$.


RESTING MEMBRANE POTENTIAL (mV)
-100
-80
-60
-40
-20
0
0
20
40
60
80
100
120
TIME (min)
10
10
10
9
9
9
9
7
6
**
****
****
***

difference from the control amplitude was seen at 45 minutes of LPC lidocaine superfusion (Figure 16).

The APD_{50} for LPC and lidocaine superfusion is shown in Figure (17). Although a decrease of the APD_{50} of the cell is occurring a significant decrease ($P < 0.01$) from the lidocaine value is not seen. Lidocaine reduces the control APD_{50} from 210 $\pm$ 7.5 milliseconds to 140 $\pm$ 6.8 milliseconds. Therefore to compare any change of APD_{50} values were compared to the end of the lidocaine pretreatment. This was used as the control and compared to the various time periods during LPC and lidocaine superfusion. Given the above there was no significant difference between APD_{50} values at lidocaine and at any of the other time periods. The same trend was true for the APD_{90} values (Figure 18) during LPC and lidocaine superfusion.

The $\dot{V}_{max}$ values (Figure 19) showed a significant decrease ($P < 0.01$) from the control values at 60 minutes.

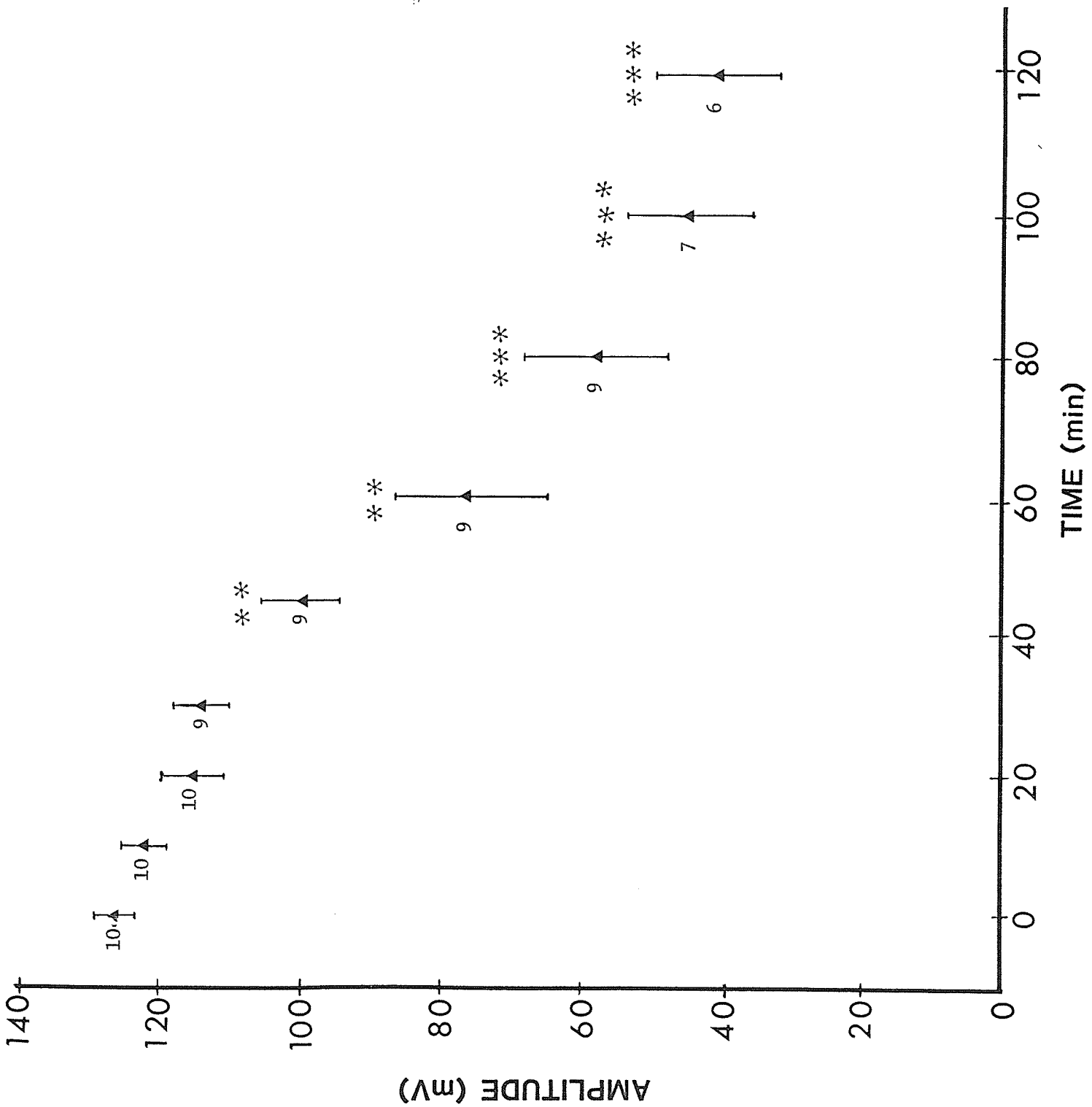
An increase in conduction values is found during LPC and lidocaine superfusion (Figure 20). This increase in conduction is significantly different ($P < 0.01$) from the control at 80 minutes of superfusion.

3. Comparison of LPC with LPC and lidocaine superfusion

The values of the action potential parameters that differ from control as well as the time at which they are statistically different are tabulated in Table V.

Figure 16. Amplitudes (AMP) of canine Purkinje fibers during IPC and lidocaine superfusion.

Protocols and symbols as described in Figure 15.


AMPLITUDE (mV)
TIME (min)
0
20
40
60
80
100
120
140
0
20
40
60
80
100
120
10
10
10
9
**
9
**
9
***
9
***
7
***
6

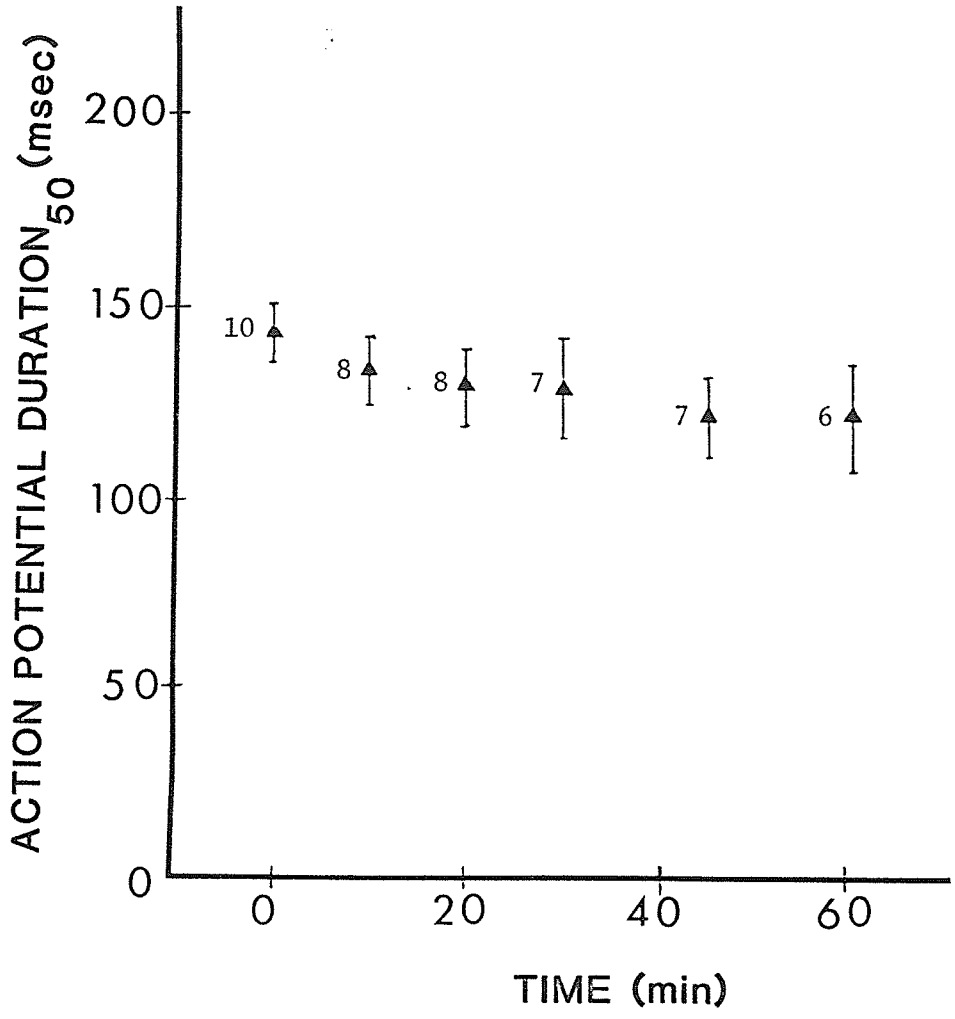


Figure 17. Action potential duration at the 50% level of membrane repolarization (APD_{50}) of canine Purkinje fibers during LPC and lidocaine superfusion.

Protocols and symbols as described in Figure 15.

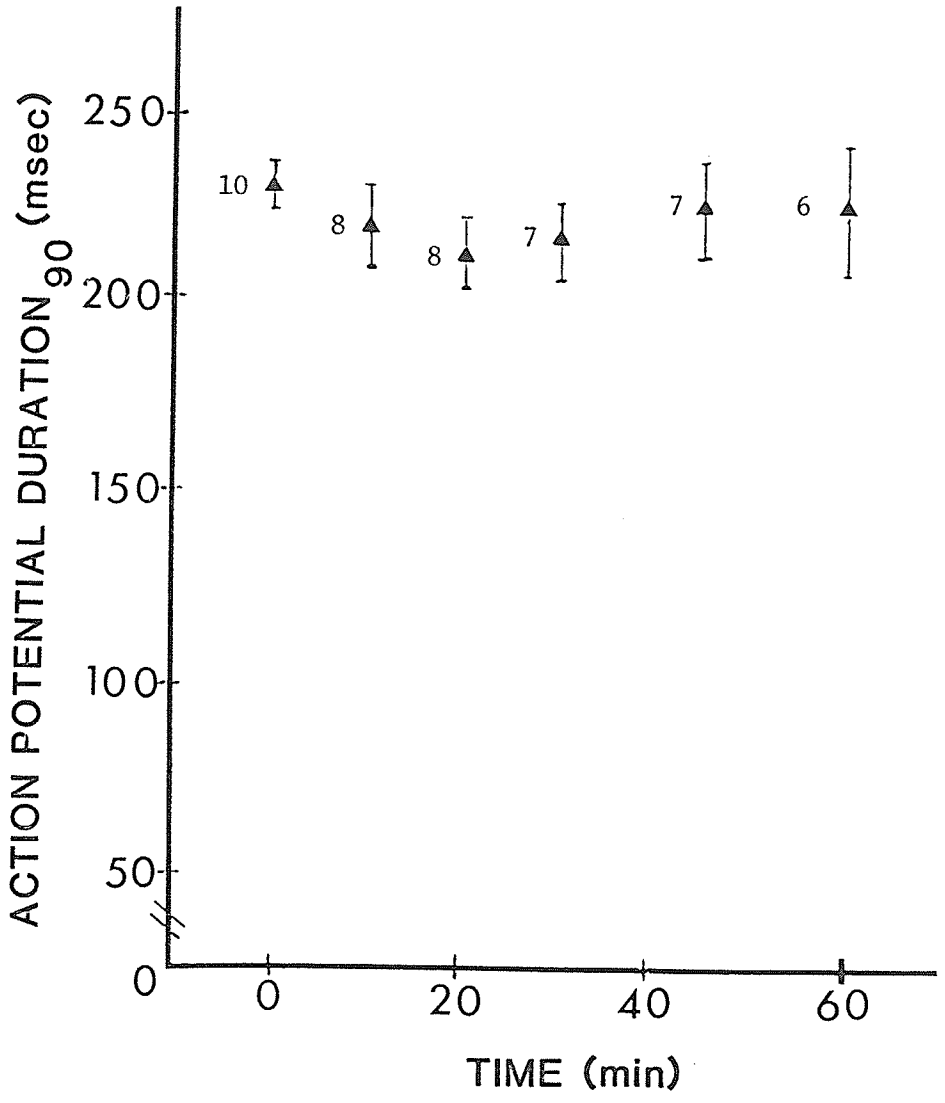


Figure 18. Action potential duration at the 90% level of membrane repolarization (APD_{90}) of canine Purkinje fibers during LPC and lidocaine superfusion.

Protocol and symbols as described in Figure 15.

Figure 19. $\dot{V}_{max}$ of canine Purkinje fibers during IPC and lidocaine superfusion.

Protocol and symbols as described in Figure 15.

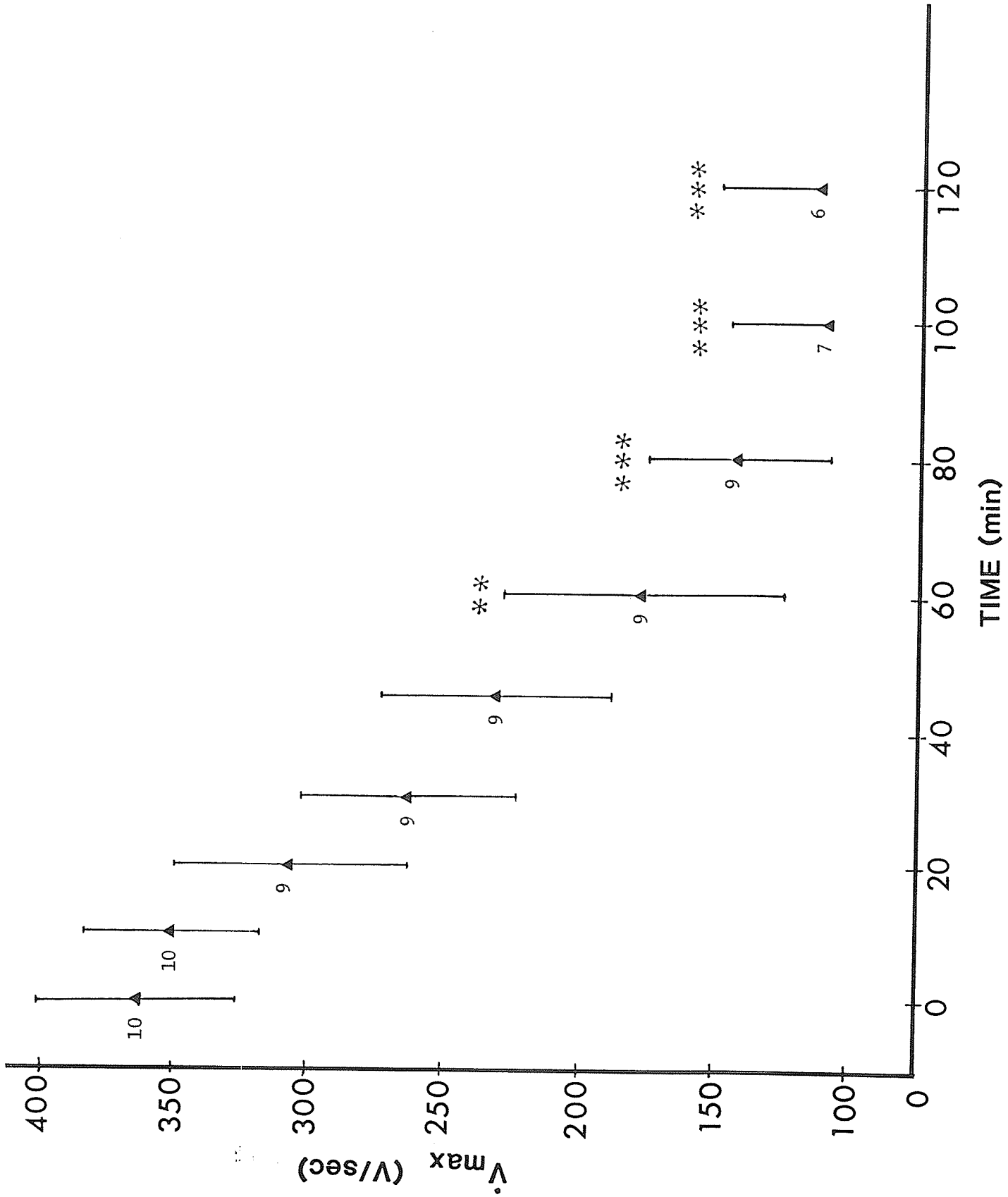

400
350
300
250
200
150
100
0
V̇max (V/sec)
0
20
40
60
80
100
120
TIME (min)
10
10
9
9
9
9
7
6
**
***
***
***

Figure 20. Changes in conduction times in canine Purkinje fibers during IPC and lidocaine superfusion.

Since conduction time varies with the distance of the microelectrode from the stimulator and this distance was different for every cell it was necessary to normalize the values. In this way all conduction times started at 100 and all changes from that value at various time periods are shown in this figure.

Protocol and symbols as described in Figure 15.

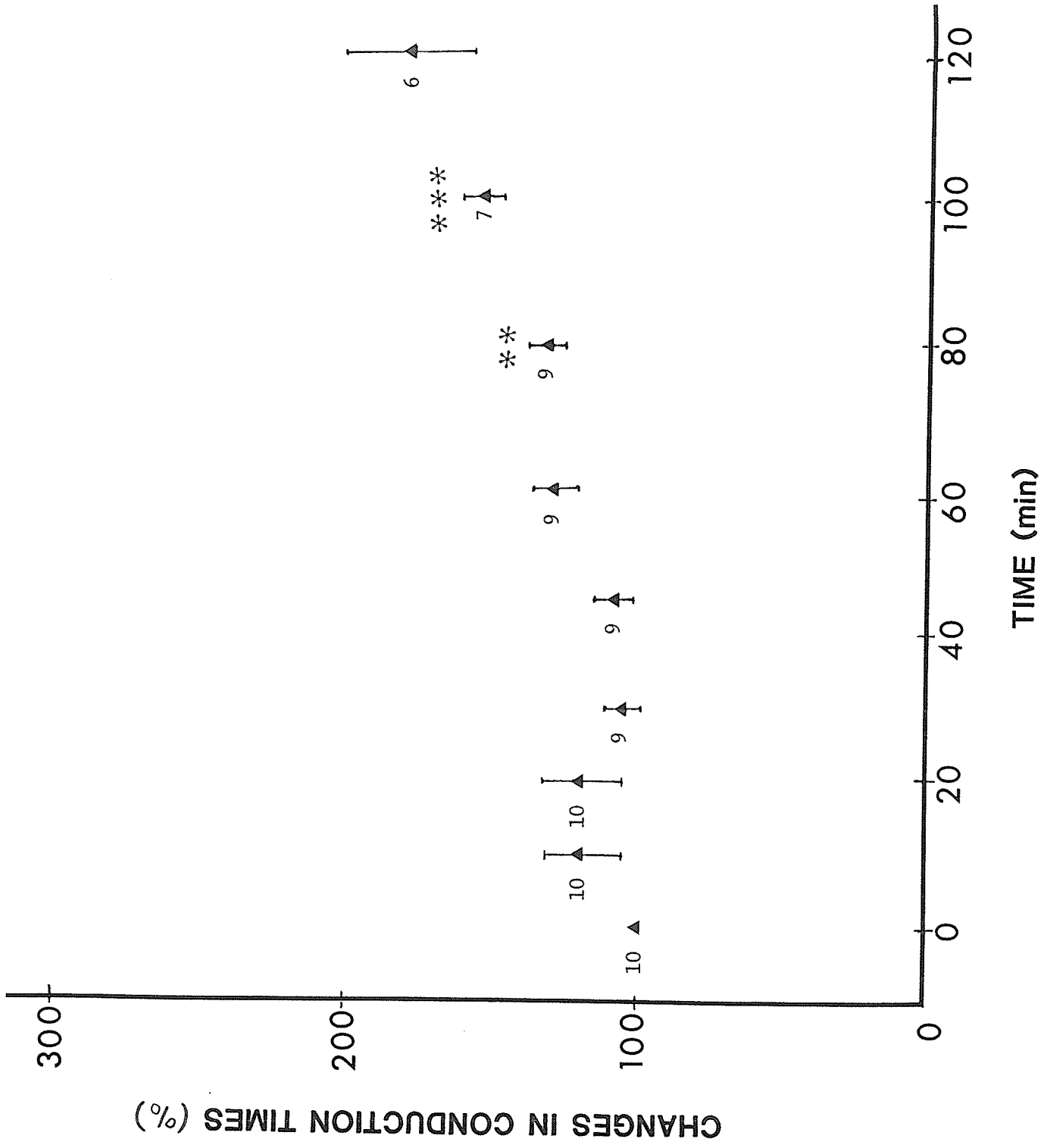

CHANGES IN CONDUCTION TIMES (%)
300
200
100
0
0
20
40
60
80
100
120
TIME (min)
10
10
10
9
9
9
6
**
***
7
6

TABLE V

Summary of action potential parameters during LPC and LPC with lidocaine superfusion.

A. Action potential parameters that differ significantly ($P<0.01$) from control and the time during which significance occurs (Time) during LPC superfusion.

	Control	LPC superfusion	Time
RMP (-mV)	83.1± 2.3 (11)	71.1± 4.5 (11)	20
AMP (mV)	123.1± 1.6 (11)	113.6± 2.9 (11)	10
APD_{50} (msec)	224.5± 8.6 (11)	174.3±15.6 (7)	30
APD_{90} (msec)	292.7± 8.5 (11)	243.8±11.1 (8)	30
$\dot{V}_{max}$ (v/s)	315.6±23.4 (9)	147.2±36.1 (8)	30
Conduction	100* (11)	134.9± 8.8 (8)	45

B. Action potential parameters that differ significantly ($P<0.01$) from control and the time during which significance occurs (Time) during LPC and lidocaine superfusion.

	Control	LPC with lidocaine superfusion	Time
RMP (-mV)	86.8± 2.8 (10)	69.1± 4.8 (9)	60
AMP (mV)	125.1± 2.8 (10)	100.2± 6.4 (9)	45
APD_{50} (msec)	140.0± 6.8^{L} (10)	NS	--
APD_{90} (msec)	229.7± 6.2^{L} (10)	NS	--
$\dot{V}_{max}$ (v/s)	362.5±37.9 (10)	177.8±49.1 (9)	60
Conduction	100* (10)	130.4± 6.4 (9)	80

( ) numbers in brackets represent number of cells

L = values at lidocaine superfusion

* Conduction times are normalized in order to see change from control values

A significant ($P < .01$) decrease from control in RMP values occurs at 20 minutes in the LPC group, whereas it is not seen until 60 minutes in the LPC and lidocaine superfused group. A delay is also seen in the amplitude comparisons, at 10 minutes LPC in one case, compared to 45 minutes LPC and lidocaine in the other. The trend continues with the APD_{50} values. A significant difference ($P < 0.01$) is found at 30 minutes in the LPC group in comparison to no significant difference from control in the LPC and lidocaine group. Similarly, there is no significant difference from control in APD_{90} values in the LPC and lidocaine group, whereas a significant difference ($P < 0.01$) from control is found in the LPC group at 30 minutes into the superfusion.

The delay is not as prolonged when comparing $\dot{V}_{max}$ values although it still exists with LPC and lidocaine. A significant difference from the control is seen at 30 minutes whereas in the LPC and lidocaine group the significant decrease is seen at 60 minutes.

Conduction increases from control are seen at 45 minutes in LPC and at 80 minutes in LPC and lidocaine. So that in all the parameters measured the LPC and lidocaine group showed a delay in reaching a significant difference from the control values.

When comparing LPC with LPC and lidocaine parameters at the same time periods a trend is evident. In the RMP, AMP and $\dot{V}_{max}$ one sees that generally throughout the superfusion period the LPC and lidocaine group maintains higher values than the LPC group.

The time to reach one half of the control RMP was calculated in both the LPC and LPC with lidocaine groups and found to be significantly different. To reach a mean RMP of -41.8 $\pm$ 1.5 mV, 61 $\pm$ 15.8 minutes of LPC superfusion was required compared to 113.4 $\pm$ 11.2 minutes of LPC and lidocaine superfusion. These times were statistically different from each other ($P < 0.01$).

Table VI shows the slopes of individual cells over time during the superfusion period. It is evident from this table that the different parameters have varying ranges during LPC and LPC with lidocaine superfusion. The mean slopes for each parameter were compared and in all cases the LPC slopes were larger. The variation of slopes between the two groups (Table VII) shows an almost four fold decrease in the RMP and AMP of the LPC with lidocaine group. The other values exhibit a 1.3 to 2.5 fold decrease of the LPC and lidocaine group from the LPC group. These data confirm that the LPC and lidocaine group show less scatter than those in the LPC group. This correlates with the large inhomogeneity of variance seen in the LPC group compared to the LPC and lidocaine group. Lidocaine tends to make the variance more homogeneous. The range between the highest and lowest slope is also decreased in the LPC and lidocaine group compared to the LPC group (see Table VII).

4. Automaticity

The canine Purkinje fibers were stimulated at a basic cycle length of 1000 msec during the superfusion with LPC containing solution. It became apparent that even though the cells were being

TABLE VI

Slopes of action potential parameters of individual cells over time during LPC, and LPC with lidocaine superfusion.

CELL NO.	RMP	AMP	APD_{50}	APD_{90}	$\dot{V}_{max}$
LPC SUPERFUSION					
1	-1.82	-3.71	-1.5	-2.0	-15.9
2	-1.02	-1.81		-1.65	- 7.58
3	-0.15		-0.42	-0.11	
4	-0.03		-0.29	-0.11	
5	0	-0.31	-1.09	-0.005	- 0.91
6	-0.35		-0.88	-1.28	- 2.71
7	-0.51	-1.02	-1.12	-0.50	- 2.75
8	-0.59	-1.52	-1.37	-1.50	- 5.93
9	-0.97		-3.50	-4.0	-11.0
10	-1.19	-1.92	-2.97	-2.73	- 6.33
11	-0.19		-0.30	-0.37	- 0.16
MEAN	-0.62 $\pm$0.17	-1.7 $\pm$0.5	-1.34 $\pm$0.35	-1.30 $\pm$0.38	- 5.9 $\pm$ 1.7
LPC AND LIDOCAINE SUPERFUSION					
12	-0.43	-0.75			- 2.69
13	-0.15		-0.36		- 1.22
14	-0.59	-0.64	-0.59	-0.06	- 1.31
15	-0.50	-0.89	-0.49	+0.03	- 2.06
16	-0.47	-1.50	-1.5	-1.03	-12.9
17	-0.33	-0.88	-0.17	+0.07	- 0.96
18	-0.45	-1.15	+0.53	+0.46	- 2.56
19	-0.49	-0.98	-0.46	-0.34	- 3.70
20	-0.26		-0.21	-0.42	- 1.22
21	-0.43	-0.84	-0.13	-0.08	- 3.66
MEAN	-0.41 $\pm$0.04	-0.95 $\pm$0.09	-0.37 $\pm$0.18	-0.18 $\pm$0.16	- 3.20 $\pm$ 1.10

TABLE VII

Comparison of slopes of action potential parameters in LPC and LPC with lidocaine groups

LPC		RMP	AMP	APD_{50}	APD_{90}	$\dot{V}_{max}$
	Lowest	-1.8	-3.7	-3.0	-4.0	-15.9
	Highest	0	-0.3	-0.30	- .005	- 0.2
	Difference	1.8	3.4	2.7	3.99	15.7
LPC AND LIDOCAINE						
	Lowest	-0.59	-0.6	-1.5	-1.0	-12.9
	Highest	-0.15	-1.5	+0.5	+0.5	-0.96
	Difference	.44	0.9	2.0	1.5	11.9
LPC : LPC AND LIDOCAINE		4.1 X	4.0 X	1.3 X	2.5 X	1.3 X

stimulated at a constant rate some cells were initiating action potentials that did not correspond to the rate of the stimulator. Thus it was decided to determine the rates of these cells. The times were noted that stimulation resulted in a rate other than the one of the stimulator. The stimulator was also turned off at various intervals during superfusion in order to determine whether cells were firing spontaneously or not and these times were also recorded.

During LPC superfusion 10/11 cells were not responsive to stimulation at one per second (Table VIII). Two of these exhibited a slow spontaneous rate of 0.4 Hz. Hence the lack of a one to one response to electrical stimulation may be the result of conduction block from the site of stimulation to the recording site. Three of the ten cells not responsive to stimulation showed a rate of 1.0 action potential/second but this did not follow the stimulus. This may be due to triggered activity or conduction block leading to reentry. At some point during LPC superfusion no stimulation led to a response in all 11 cells (Table IX). This may imply the formation of an automatic site during LPC superfusion.

During LPC and lidocaine superfusion (Table X) one cell showed conduction block (0.4 Hz) and was not responsive to stimulation for the rest of the LPC and lidocaine superfusion period. Two cells exhibited a faster rate than that of the stimulator. It may be possible that in these triggered activity or reentry is involved. The experimental set-up did not allow for the

TABLE VIII

Automaticity during LPC superfusion:
First time during stimulation that rate
is not equal to one action potential per second

Cell	Control Rate	Time	Rate
1		12	1.4
2	1	10	1.2
3	1	82	2.0
4	1	82	1.0*
5	1	28	1.2
6	1	15	1.2
7	1	26	1.0*
8	1	18	.4
9	1	20	.4
10	1	--	1.0
11	1	89	1.0*

Rates are expressed as action potentials per second.

Time is in minutes.

* Rate is 1 action potential per second but does not follow the stimulator.

10/11 do not follow stimulator rate during LPC superfusion.

Mean time of LPC superfusion where rate is not equal to one action potential per second during stimulation is 38.2 $\pm$ 10.2 minutes.

TABLE IX

Time at which no stimulation results in a signal during LPC superfusion

Cell	Rate at control with no stimulation	Time during superfusion	Rate
1	NS	10	1.2
2	NS	10	1.2
3	NS	70	0.4
4	NS	70	0.4
5	NS	17	0.8
6	NS	17	0.8
7	NS	56	0.2
8	NS	18	0.4
9	NS	20	0.4
10	NS	24	0.6
11	NS	24	0.6

Rates are expressed as action potentials per second.

NS represents no signal.

Time is in minutes.

11/11 cells elicit a response without stimulation during LPC superfusion.

Mean time required for no stimulation to elicit a response during LPC superfusion is 30.5 ± 7.0 mins.

TABLE X

Automaticity during LPC and lidocaine superfusion:
First time during stimulation that rate is not equal
to one action potential per second

Cell	Control rate	Lidocaine rate	Time	Rate
1	1	1	150	1.4
2	1	1	132	1.2
3	1	1	-	1
4	1	1	-	1
5	1	1	24	0.4
6	1	1	-	1
7	1	1	-	1
8	1	1	-	1
9	1	1	-	1
10	1	1	-	1

Rates are expressed as action potentials per second.

Time is in minutes.

- represents entire superfusion period.

3/10 do not follow stimulator rate during LPC and lidocaine superfusion.

distinction to be made. When the cells of the LPC and lidocaine superfusion were not stimulated eight resulted in no signal (Table XI). This may imply that the presence of lidocaine removes the automatic focus present in the LPC perfused cells. Lidocaine inhibited the appearance of LPC induced spontaneous activity.

At some point during superfusion with LPC (Table VIII) 10/11 cells did not follow the stimulator while only 3/10 did not follow the stimulator in the group superfused with LPC and lidocaine containing solution (Table X). This difference was found to be statistically significant ($P < 0.05$). The mean time for the above to occur in the LPC group was 38.2 ± 10.2 minutes. At intervals during the superfusions no stimulation resulted in an action potential response. In the LPC superfused group 11/11 cells elicited a response without stimulation (Table IX). In the group superfused with LPC and lidocaine only 2/10 cells elicited a response (Table XI). This difference was found to be statistically significant between the two groups ($P < 0.001$).

TABLE XI

Times at which no stimulation results in a signal during LPC and lidocaine superfusion

Cell	Rate at control with no stimulation	Time during superfusion	Rate
1	NS	141	0.2
2	NS	141	0.2
3	NS	-	NS
4	NS	-	NS
5	NS	-	NS
6	NS	-	NS
7	NS	-	NS
8	NS	-	NS
9	NS	-	NS
10	NS	-	NS

Rates are expressed as action potentials per second.

NS represents no signal.

Time is in minutes.

2/10 cells elicit a response without stimulation during LPC superfusion.

DISCUSSION

Degradation of myocardial phospholipids has been demonstrated in the ischemic heart (Sobel et al., 1978; Chien et al., 1981). Within a few minutes of the onset of ischemia lysophosphoglycerides increase in the myocardium (Corr et al., 1982). Accumulation of these metabolites may occur if there exists increased production due to phospholipase A_1 or A_2 activation, decreased catabolism due to decreased lysophospholipase activity, increased production due to a reversal of the reaction catalyzed by lysophospholipase transacylase; or combinations of these reactions. Various metabolic determinants have been found to result in inhibition of lysophosphoglyceride catabolism in ischemic tissue, thus accumulation of these substances may result. However, the exact mechanisms are not well characterized.

The whole animal model used in these studies served to determine whether or not lysophosphoglyceride infusion into an otherwise healthy heart would result in arrhythmia. In the experiments where bolus LPC was infused into the LAD coronary artery it was determined that 15 $\pm$ 6 μmoles of LPC caused ventricular tachycardia. This arrhythmia was decreased with the use of i.v. lidocaine (2 mg/kg). Since LPC was found to be arrhythmogenic it was necessary to determine the concentration of it being infused into the coronary circulation rather than knowing only the amount. Although it is stated that LPC (1 mM) was infused into the coronary circulation this may not be the concentration of "free" LPC present in the blood. LPC binds to albumin and other proteins present in the blood and therefore is not available for interaction into the myocardial cell membrane. As has been shown by Man and Choy (1982) that

a much higher concentration of albumin bound LPC is necessary to produce arrhythmia in hamster hearts in vitro. The infusion of LPC is continuous for one hour. Unfortunately the experimental set-up did not allow for the determination of the amount of LPC that accumulates or the amount that is broken down by endogenous enzymes. The LPC was infused into the LAD coronary artery to a region which perfused the apical portion of the left anterior free wall and part of the left anterior septum of the ventricle. It is clear that the infusion of LPC into the LAD coronary artery did have an arrhythmogenic effect. In comparison the infusion of saline did not have any arrhythmogenic effect. Therefore the arrhythmias originated from an area that received blood containing LPC. However, the exact location of the arrhythmogenic site is not determined. The advantage of using the experimental animal model is that LPC is infused into the healthy heart of an animal. Hence, these experiments were performed under physiological conditions rather than in the presence of hypoxia, acidosis, elevated K^+ or features of ischemia that might obscure the alterations initiated specifically by LPC. LPC by itself was shown to be arrhythmogenic without the other concomitant changes found in ischemic myocardium. It has been shown that a pH decrease would exacerbate the LPC effects in vitro (Corr and Sobel, 1983).

Since ischemia in vivo is in most cases a gradual event one would expect that LPC accumulates and exerts its effects over a longer period of time. In order to determine the effect of a continuous infusion of LPC over a longer period of time LPC (0.5 mM) was infused into the LAD coronary artery for a continuous five hour period. Consideration was given to the protocol to allow the animal to receive

LPC and yet maintain its physiological stability. As mentioned earlier it was necessary to alter the surgical procedure for the experiments where LPC was being infused into the LAD coronary artery continuously for one hour. A calibrated rotary pump was used continuously during the one hour LPC infusion. Once the infusion time ended the animal was monitored electrocardiographically for another three hours. In some animals there occurred a general deterioration and the blood pressure was not maintained. In addition to the blood pressure decrease seen in some animals the concern arose regarding the use of the rotary pump. Blood passing through that pump continuously for five hours would be subject to hemolysis. In order to avoid the occurrence of hemolysis a different set-up which did not require the use of the pump was employed. The needle tip inserted into the LAD coronary artery allowed for LPC to be mixed with blood in the coronary circulation. The concentration was actually an estimate based on the body weight of the animal as well as the flow rate determined from the experiments where LPC was infused continuously for one hour.

Regardless of the duration of the LPC infusion (bolus, one hour, or five hours) arrhythmias were produced. In the experiments where bolus LPC was infused into the LAD coronary artery the smaller amounts of LPC produced ectopic beats. With an increase in the amount of LPC infused the ectopic beats were seen more frequently. In these experiments the amount of LPC infused was increased until ventricular tachycardia was produced. Initially, when LPC was infused continuously for one hour or five hours ectopic beats were seen. As the LPC infusion continued ectopic beats were evident more frequently, followed by episodes of

ventricular tachycardia. Ventricular tachycardia led to ventricular fibrillation in 4 of 11 animals in the group which received continuous LPC infusion for one hour and in 3 of 8 animals in the group which received continuous LPC infusion into the LAD coronary artery for five hours. It is apparent that the arrhythmogenic focus which produced ventricular tachycardia led to ventricular fibrillation.

It was demonstrated by the infusion of LPC into the LAD coronary artery for one hour and five hours that the arrhythmias produced were concentration and time dependent. Production of arrhythmias was decreased in the presence of lidocaine. The appearance of significantly higher arrhythmia scores was delayed in the presence of lidocaine. In the present studies, the arrhythmia score was reduced in all three groups of animals that received i.v. lidocaine pretreatment in addition to LPC. In the experiments where LPC was given by a bolus infusion lidocaine treatment suppressed the formation of ventricular tachycardia. The LPC in these experiments was infused into the LAD coronary artery for short durations (<10 seconds). The effect produced could be due to the presence of an ectopic focus. Lidocaine reduced the arrhythmia scores when LPC was infused continuously for one and five hours. It was observed that when infusing LPC continuously for one hour the arrhythmia score was significantly reduced in the presence of lidocaine. Lidocaine may provide a membrane stabilizing effect on the ventricular myocardium and thus not allow for the development of arrhythmias. ECG changes showing cardiac arrhythmias manifest by ectopic beats were also evident in the perfusion of rat hearts with LPC (Man and Choy, 1982; Man and Lederman, 1985). Ventricular fibrillation was also present in these studies during perfusion with LPC.

Another interesting aspect regarding the production of arrhythmias in the presence of LPC and LPC with lidocaine lies in the analysis of the time curves. The LPC time curves (Figure 8) appear to consist of at least two exponential functions. The first one being a much faster one. When comparing the LPC curve to the LPC and lidocaine curve the effect of lidocaine appears to be exerted on the fast component. The rate of deterioration of the myocardium seen with lidocaine is faster than with LPC alone, at the time of operation of the slow component. It would be interesting to extend these observations past a five hour period and compare the LPC curve to the LPC with lidocaine curve. Curve fitting could be applied to this data. If best fit is achieved by a two-exponential term one could speculate that two different populations of ventricular myocytes exist which may have differing susceptibilities to the toxic effects of LPC.

Histological changes are evident in ischemic myocardium and such changes were also produced by LPC infusion into the LAD coronary artery. Histological examination of the myocardial regions receiving IPC resulted in finding similarities to those that have been made experimentally ischemic (Man et al., 1983). Given the limitations of light microscopy it was determined that myocardial regions which received LPC exhibited unorganized myocardial fibers' many of them losing their distinct striations and becoming undefined. Nuclei were absent in many cells and not well defined in others. Large spaces were also evident interstitially.

Histological examination of myocardial regions receiving LPC in the presence of i.v. lidocaine showed the same characteristics described for the myocardial regions receiving LPC. Although the presence of lidocaine was shown to decrease the arrhythmia scores it did not prevent any of the histological abnormalities seen with LPC. This phenomenon agrees with other studies done on the arrhythmogenicity of LPC and the abolition of this by lidocaine. Lidocaine was shown to be effective in abolishing lysophosphoglyceride-induced arrhythmias in rat hearts as well as protecting red blood cells from hemolysis (Neufeld et al., 1985). This study showed that hemolysis was inhibited in the presence of lidocaine but binding of LPC to the cell membrane was not prevented. Lidocaine produced a membrane stabilizing effect on the red blood cells and protected against membrane dysfunction. It is possible to postulate that LPC was bound to the cardiac sarcolemma and produced the histological changes seen in the myocardial region exposed to LPC. At the same time lidocaine may stabilize the cell membrane and thus reduce the arrhythmia score, yet lidocaine would not affect the histological changes. This observation would correlate with the earlier period of LPC infusion and i.v. lidocaine where arrhythmia is not present or where very few ectopic beats are evident. In the latter part of the infusion period it appears that the LPC-induced effects have overcome the membrane stabilizing effects of lidocaine or that the membrane stabilizing effect is not sufficient and arrhythmia ensues.

Membrane fractions prepared from ischemic hearts and liver show that LPC may have a nonspecific effect on membrane permeability to Ca^{2+} (Lee and Chang, 1977; Chien, 1978; Chien et al., 1981; Van Echteld et

al., 1981). LPC has cytolytic properties (Weltzien, 1979) therefore Ca^{2+} entry may be facilitated. LPC incorporation into the sarcolemma and change in Ca^{2+} permeability seen in ischemia may be related (Chien, 1981). Irreversible injury in myocardium is associated with impaired ionic fluxes and the resultant accumulation of Ca^{2+}. Irreversible cell injury is associated with the accumulation of Ca^{2+} (Shen and Jennings, 1972). The Ca^{2+} accumulation by mitochondria at the expense of oxidative phosphorylation, results in the depletion of high energy phosphates contributing to cell death (Kloner et al., 1979). Mitochondria from ischemic tissue have been found to sequester Ca^{2+}. This sequestration of Ca^{2+} as well as the myofibrillar contracture can be prevented by Ca^{2+} antagonists (Henry et al., 1977). Thus the accumulation of lysophosphoglycerides may lead to derangements in calcium homeostasis (Sedlis et al., 1983).

Prophylactic treatment with lidocaine suppresses ectopics in patients with acute myocardial infarction. In this way it may decrease the incidence of ventricular fibrillation and hence decrease mortality (Harrison and Berte, 1982). It is also apparent from these studies that lidocaine aids in reducing LPC induced arrhythmias. Lidocaine may be acting in a similar manner in both of these situations. It was of interest to further delineate the mechanism of action of LPC in conjunction with lidocaine, thus the electrophysiological studies were pursued. The source of the increased levels of lysophosphoglycerides in ischemic myocardium is not known. LPC has been found to increase in venous effluents from ischemic regions (Snyder et al., 1981). It has been speculated that an extramyocytic source such as endothelial cells

may be involved, or an intracellular source with movement to the extracellular space since LPC exhibits an affinity for albumin binding sites (Stein et al., 1965). Reduction of the pH to 6.5, which is comparable to the pH seen in ischemic myocardium in vivo, inhibits microsomal lysophospholipase (Gross and Sobel, 1982). Cytosolic lysophospholipase is inhibited by long-chain acyl carnitine which accumulates in ischemic myocardium (Liedtke et al., 1978; Gross and Sobel, 1982). Thus it appears, catabolism of LPC may be inhibited by metabolite accumulation during ischemia.

Action potential changes induced by LPC resemble those seen in action potentials from ischemic myocardium (Sobel et al., 1978). The concentration of LPC (40 μM) used in the superfusion of canine Purkinje fibers was a considerably lower concentration than the LPC concentration (0.75 mM) reported by Corr et al. (1979) to produce the same type of electrophysiological derangements. The major difference between the two studies is that LPC in the present study was not bound to protein in the modified Tyrode's solution whereas in the former experiments LPC was albumin bound. It has been demonstrated when comparing LPC effects to the effects of albumin bound LPC, that the albumin-bound LPC elliminates the arrhythmogenic effects of LPC (Man and Choy, 1982).

Changes seen in the present studies with LPC superfusion of Purkinje fibers are consistent with other studies (Sobel and Corr, 1979; Sobel et al., 1978; Corr et al., 1979; Arnsdorf and Sawick, 1981) that show a decrease RMP, AMP, $\dot{V}_{max}$ and APD. These effects have been identified as predisposing to reentry. LPC also increased the automaticity which potentiates the occurrence of arrhythmias.

RMP in canine Purkinje fibers was also decreased with continued superfusion with LPC containing solution. Comparison of LPC and lidocaine superfusion with LPC superfusion over the same time intervals also shows a decrease, but a significant difference from control is evident at a later time. Low resting potential appears to be a common occurrence in the experimentally infarcted dog heart (Friedman et al., 1973; Lazzara et al., 1973, 1974) and in chronically diseased human myocardium (Spear et al., 1979). Resting membrane potential is lowered by physiologic changes that the diseased heart is exposed to such as ischemia, stretch, changes in pH and altered extracellular ionic concentration. In the present study superfusion with LPC-containing solution also decreased RMP. Impulse propagation in fibers which have a low membrane potential can be the cause of conduction disturbances. The three mechanisms involved are: incomplete repolarization, phase 4 depolarization and low resting membrane potential. Stimulation of Purkinje fibers does not result in a response until the membrane potential has been restored to at least -50 mV. The earliest response is obtained at the very end of the absolute refractory period and the beginning of the relative refractory period. This is a small, slowly rising response which does not propagate. The optimal response is obtained when the membrane potential has returned to the normal potential.

Spontaneous phase 4 depolarizations are usually due to disorders of impulse formation. In order to effectively abolish these spontaneous depolarizations the interval required for phase 4 depolarization to reach threshold must be delayed. In addition this delay may affect the

ectopic focus to a greater extent. Delaying the interval required to reach phase 4 depolarization may be done in the following ways: prolonging the time required to reach threshold by decreasing the pacemaker discharge; prolonging the cycle length by increasing the voltage required to reach threshold potential; increasing the maximum diastolic potential, which also increases cycle length. Lidocaine decreases the pacemaker discharge and thus prolongs the time required to reach threshold. It may be through this way that lidocaine exerts its antiarrhythmic effect.

Conduction velocity is important in the arrhythmias where reentry is involved. Although the mechanism of LPC induced arrhythmias is not clear it is known that LPC incorporates into the cell membrane (Corr et al., 1982). Incorporation may lead to instability of the membrane and hence to conduction disturbances. Unidirectional functional block is a prerequisite for reentry. By increasing or decreasing the conduction velocity the unidirectional block may be converted to a bidirectional or total block and hence the abolition of the resultant arrhythmia. The unidirectional block will be overcome by increasing the conduction velocity in the affected tissue. Decreasing the conduction velocity will produce bidirectional block. It is important for the reciprocal impulse to be timed correctly in order that it arrives late enough to avoid refractoriness in the blocked pathway and early enough to precede the next antegrade impulse. Lidocaine does not change the conduction velocity.

Increases or decreases in refractory period duration may eliminate reentrant arrhythmias. Increasing refractoriness may produce bidirectional block. Decreasing refractoriness may eliminate unidirectional block. In disorders of automaticity an increased refractory period will increase the action potential duration and thus increase the cycle length. This will lead to a decreased discharge rate of an ectopic focus. The durations of repolarization, action potential, and refractory period are decreased in the presence of lidocaine. However, the refractory period is decreased less than the action potential duration. Thus, the net result is an increase in refractoriness relative to the action potential duration.

Conduction velocity of an impulse for any level of membrane potential is a property of membrane responsiveness. Alterations in membrane responsiveness have effects upon reentrant arrhythmias which are the same as changes in conduction velocity.

CONCLUSIONS

1. Infusion of bolus LPC into the LAD coronary artery leads to arrhythmias.

2. Lidocaine reduces the incidence of ventricular tachycardia produced by bolus LPC infusion.

3. Infusion of LPC (1 mM) continuously for one hour produces arrhythmias. (Arrhythmia score = 3.5 ± 0.4).

4. Infusion of i.v. lidocaine concommitantly with LPC significantly decreases the arrhythmia score. (Arrhythmia score = 2.4 ± 0.5).

5. Infusion of LPC (0.5 mM) continuously for five hours increased the arrhythmia score. (Arrhythmia score = 3.0 ± 0.8).

6. Lidocaine prevents the occurrence of a higher arrhythmia score during the first four hours of LPC (0.5 mM) infusion into the LAD coronary artery. (The arrhythmia score at the end of the fourth hours of infusion was 1.8 ± 0.3).

7. Histological analysis shows that LPC infusion causes similar myocardial changes to those seen in experimental coronary artery occlusion.

8. LPC decreases the RMP, AMP, APD_{50}, APD_{90}, $\dot{V}_{max}$ and conduction velocity of canine Purkinje fibers.

9. Lidocaine delays the electrophysiological effects seen in Purkinje fibers superfused with LPC containing solution.

10. Abnormal automaticity is present in LPC superfused Purkinje fibers and absent in LPC and lidocaine superfused Purkinje fibers.

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