Potential Arrhythmogenic Electrophysiological Derangements in Canine Purkinje Fibers Induced by Lysophosphoglycerides

PETER B. CORR, MICHAEL E. CAIN, FRANCIS X. WITKOWSKI, DAVID A. PRICE, AND BURTON E. SOBEL

SUMMARY We have recently detected accumulation of lysophosphoglycerides, catabolites of phospholipids, in ischemic myocardium early after coronary occlusion. In the present study we delineated effects of selected concentrations of albumin-bound lysophosphatidyl choline (LPC) comparable to those accompanying ischemia in vivo on action potentials of isolated canine Purkinje fibers. Lysophosphoglycerides induced concentration-dependent (0.75-3.0 mM) decreases in resting membrane potential, overshoot of phase 0, maximal velocity of upstroke ($V_{max}$) of phase 0, and action potential duration. The highest concentrations (2.0-3.0 mM) induced fractionation of the action potential into several components, unresponsiveness to external stimulation, and enhanced automaticity at normal and reduced membrane potentials. LPC induced a rightward shift in the membrane response curve, a 40-fold prolongation of conduction time, and an increase in the ratio of effective refractory period to action potential duration such that the effective refractory period persisted beyond action potential duration, resulting in postpolarization refractoriness. These electrophysiological alterations were entirely reversible after 70 minutes of perfusion without LPC, with the exception of a persistent depression in the $V_{max}$ of phase 0. Lysophosphatidyl ethanolamine (LPE) elicited alterations in action potentials identical to those elicited by LPC. Furthermore, LPC (3.0 mM) induced comparable alterations in action potentials recorded from isolated rabbit papillary muscles. Since lysophospholipids accumulate early after myocardial ischemia, and since concentrations equivalent to those occurring in vivo induce electrophysiological alterations resembling those seen in ischemic myocardium in vivo, lysophosphoglycerides may be of major importance as biochemical mediators of malignant dysrhythmia induced by ischemia.

SUDDEN DEATH associated with coronary artery disease is usually due to profound, often unheralded disturbances in cardiac rhythm (Adgey et al., 1969; Lovell and Prineas, 1971). Ultrastructural and functional alterations of myocardial sarcolemma with associated electrocardiographic changes are among the early manifestations of ischemic injury (Herdson et al., 1965). Although several factors may be responsible for the perpetuation of malignant dysrhythmias early after the onset of ischemia, including alterations in regional myocardial adrenergic stimulation (Corr et al., 1978), the specific factors affecting myocardial sarcolemma that are responsible for the well recognized changes in ionic conductances and resulting disturbances of cardiac rhythm have not yet been elucidated. We have previously reported that lysophosphoglycerides accumulate in ischemic tissue in vivo (Sobel et al., 1978), possibly due to activation of phospholipase A$_2$ in response to ischemia (Needleman et al., 1975). Since lysophosphoglycerides exhibit marked effects on membrane integrity in many systems (van den Bosch, 1974), their accumulation may be of major importance in the genesis of the dysrhythmias associated with ischemia.

Under normal physiological conditions, accumulation of lysophosphoglycerides is precluded by both reacylation and hydrolysis (van den Bosch, 1974). However, these mechanisms appear to be insufficient to preclude an increase in the concentrations of lysophosphoglycerides resulting from enhanced activity of phospholipases, possibly decreased lysophospholipase and acyl transferase activity, and decreased washout in ischemic tissue. The present study was designed to characterize the electrophysiological alterations in normally oxygenated isolated canine Purkinje fibers induced by lysophosphoglycerides at concentrations equivalent to those found in ischemic tissue in vivo. Since the concentrations of lysophosphoglycerides found in extracts of ischemic myocardium averaged 21 µmol/g dry weight (Sobel et al., 1978), or approximately 4 mM, similar concentrations (0.75-3.0 mM) were used in the present study. The findings in vitro
ARRHYTHMOGENIC EFFECTS OF LYSOPHOSPHOGLYCERIDES/Corr et al.

were compared to electrophysiological alterations typical of ischemic tissue in vivo to determine whether accumulation of these catabolites could account for changes implicated in the genesis of the early malignant dysrhythmias associated with acute myocardial ischemia.

Methods

Adult mongrel dogs (10–31 kg) were anesthetized with sodium thiopental (10 mg/kg), and the hearts were removed quickly and placed in oxygenated Krebs’ solution. The distal portions of the right or left bundle branches with attached ventricular muscle were removed, pinned to the bottom of a 7.5-ml wax-lined bath, and continuously superfused with a modified Krebs’ solution at 37.5°C, pH = 7.4, gassed with 95% O₂-5% CO₂ and containing the following (in mEq/liter): Na⁺ = 150.0, K⁺ = 4.0, Mg²⁺ = 2.0, Ca²⁺ = 2.4, Cl⁻ = 136.0, PO₄³⁻ = 0.9, HCO₃⁻ = 22.0, and glucose (5.0 mmol/liter). The tissues were stimulated with pulses obtained from a four-channel, programmable, phototically isolated stimulator via bipolar Teflon-coated stainless steel electrodes with pulses of 2.0-msec duration at twice the diastolic current threshold. Unless otherwise noted, a basic cycle length of 800 msec was used throughout. Intracellular potentials were recorded with glass microelectrodes filled with 3 M KCl (6–20 MΩ DC resistance), and all experiments were performed with the use of only single, maintained impalements. In four experiments, rabbit papillary muscles were excised from the hearts of rabbits (2.1–2.7 kg) after cervical dislocation. Small right ventricular papillary muscles were used to ensure adequate oxygenation, and the preparations were superfused by the same system, to compare results with lysophosphatidyl choline (LPC, 3.0 mm) on muscle tissue and to clarify possible species differences. Signals were processed first through a high-impedance unity gain electrometer (model M4A, W-P Instruments), a constant gain operational amplifier (50x), and a variable gain amplifier prior to storage on FM analog tape (15 ips, frequency response 1.6 kHz at 3 dB). A specially designed Vmax analyzer was used for electronic differentiation to obtain the maximal rate of rise (Vmax) of phase 0 of each action potential with which the peak level of the differentiator was held for 300 msec to allow accurate computer analysis. The output of the differentiator and the peak-held level of the differentiator were stored on separate channels of FM analog tape. In the presence of external stimulation of the fiber, the differentiator was triggered from the initial stimulation artifact with the peak-held circuit closed for 3 msec. Subsequently, the circuit was opened automatically, and the next differentiated signal (phase 0 depolarization) was allowed to trigger the peak-held circuit; this held value (Vmax) was stored on FM analog tape. Calibration signals (0 and −100 mV) were stored on the action potential recording channel, and the differentiation of linear ramps with slopes of 0 and 1000 V/sec were stored on the Vmax recording channel prior to cell impalement.

Action potential recordings were analyzed off-line with a completely automated system facilitating statistical analyses and verification of the reproducibility of consecutive signals (Witkowski and Corr, 1978). The system used comprised a PDP-12 computer with disc and converters permitting analog-to-digital conversion of each action potential at a sampling frequency of 10 kHz (100 µsec between samples) throughout the first 100 msec, and at 2.5 kHz throughout the next 300 msec of each action potential. With this system, up to 200 consecutive action potentials can be analyzed in a single sequence, with subsequent incremental plotting of each action potential (Houston Instruments DP1-5H plotter). Parameters measured were maximal diastolic potential (−mV), overshoot of phase 0 (+mV), action potential duration at 50%, 70%, and 95% (APD₅₀, ₇₀, and ₉₀) of full recovery (msec), and Vmax of phase 0 (in V/sec). At Vmax values > 350 V/sec, the held level of Vmax stored on analog tape was sampled at 2.5 kHz during the 100- to 200-msec interval during analog-to-digital conversion of the entire action potential. At Vmax levels < 350 V/sec, the rate of rise of voltage was calculated by the computer directly from the digital reconstruction of the action potential, since at these lower levels the frequency response of the tape recorder and the number of digital points were sufficient for accurate determination. At least 25 consecutive action potentials were used for statistical analysis of data at each selected interval, and continuous analysis was performed during intervals in which parameters changed rapidly.

Measurements of effective refractory period (ERP) were determined by initiating a premature stimulus (S₂) after the basic driving stimulus (S₁) during the absolute refractory period, and then gradually and progressively delaying S₂ until the first impulse that propagated to a second microelectrode in the same Purkinje strand was obtained. The S₁ was of the same pulse duration (2 msec) and current intensity as S₁ and was introduced after every 8 to 12 S₁. In six experiments performed to assess relative conduction time, two intracellular microelectrodes were located at opposite ends of the fiber, with the greatest separation possible in a single Purkinje fiber strand. A third bipolar extracellular electrode was used for stimulation at one end of the fiber. The time between initiation of the action potential recorded at each of the two recording electrodes was taken as the relative conduction between their sites under control and experimental conditions. All signals were monitored on a storage oscilloscope and photographed with a Polaroid camera.

The effects of lysophosphoglycerides on membrane responsiveness, defined as the relationship between the rate of rise of the premature impulse
(V\textsubscript{max}) and the membrane potential from which the impulse arose (takeoff potential), were determined by inserting S\textsubscript{2} premature stimuli at selected intervals during the repolarization phase of the action potential. At least three curves characterizing membrane responsiveness were obtained prior to implementation of any experimental intervention to verify reproducibility during the control period. A lack of variability during control assured that alterations during experimental intervention were not due to variations in the preparation per se.

To facilitate evaluation of automaticity, either low K\textsuperscript{+} or isoproterenol was used to enhance and stabilize the variable intrinsic rate of spontaneous depolarization of different fibers. In selected experiments, spontaneous depolarization was enhanced by either constant infusion of isoproterenol (10\textsuperscript{-6} M) via a separate input into the bath or by perfusion with Krebs' solution containing K\textsuperscript{+} = 2.4 mM (Carmeliet and Verdonck, 1974). In experiments in which low concentrations of [K\textsuperscript{+}]\textsubscript{b} were used to enhance automaticity, the albumin solution was first dialyzed against buffer containing the selected concentration of K\textsuperscript{+}. Enhanced automaticity was maintained constant for at least 30 minutes before implementation of any other intervention.

Superfusion of the tissue was at a rate of 6–8 ml/min, with the rate calibrated and maintained constant during each experiment. Changing the superfusion medium from a control medium to one containing one of several lipids was accomplished by turning a stopcock and injecting the lipid-containing solution at a flow rate identical to the rate during the control interval. To prevent bubbling, oxygenation was achieved by passing each solution through Silastic tubing (o.d. = 0.125; i.d. = 0.062) in an oxygen atmosphere. The measured PO\textsubscript{2} of the perfusates was consistently > 350 mm Hg. Bovine serum albumin (BSA-fraction V) in H\textsubscript{2}O was defatted by the addition of deactivated charcoal and was adjusted to pH = 3.0 by the addition of 0.2 N HCl, centrifugation, filtration of the supernatant fraction through a series of 0.45-µm Millipore filters, and adjustment of the pH to 7.0 with 0.2 N NaOH, with final BSA concentration determined by the biuret method. We added palmitic acid in equimolar amounts to the defatted albumin solution by first dissolving the palmitic acid in absolute ethanol, slowly combining it with K\textsubscript{2}CO\textsubscript{3} dissolved in distilled water, heating the palmitate salt to dryness in an N\textsubscript{2} atmosphere, and redissolving potassium palmitate in H\textsubscript{2}O with gentle heating. The potassium palmitate was then added slowly to the defatted albumin solution at 40°C, and the mixture was dialyzed against Krebs' buffer for 18 hours and diluted to yield equimolar (0.4 mM) BSA and bound palmitate.

Synthetic lyso phosphoglycerides [1-palmitoyl-glycerol-3-phosphorylcholine (LPC) or 1-palmitoyl-glycerol-3-phosphorylethanolamine (LPE), Sigma Chemical] were bound to equimolar albumin and palmitate by first dissolving the lysophosphoglycerides in ethanol and slowly adding the dissolved lipids to the albumin solution with constant stirring at 40°C in an N\textsubscript{2} atmosphere. Since LPE does not bind readily to albumin in the absence of LPC, in experiments involving LPE, equimolar LPE and LPC were dissolved in ethanol and combined with albumin. Purity of the LPC and LPE was determined by two-dimensional thin-layer chromatography (Sobel et al., 1978) both before and after binding of the lipids to albumin to verify the absence of chemical contaminants. Although both synthetic LPC and LPE were approximately 98% pure, gas-liquid chromatography (GLC) after transsterification using fatty acid markers, as well as two-dimensional thin-layer chromatography, revealed the following contaminants based on combined analysis of multiple samples: contaminants of LPC were stearic acid (0.7%), myristic acid (0.2%), margaric (0.4%), and phosphatidyl choline dipalmitoyl (1.0%); LPE contaminants were oleic acid (0.7%), stearic acid (1.4%), and phosphatidyl ethanolamine dipalmitoyl (1.0%).

The experimental protocol was performed with single maintained impalements throughout all controls, experimental interventions, and recovery periods. Experiments were disregarded if the maintained impalement criteria were not met. In all experiments, perfusion of lipid constituents was performed for 10 minutes, with the exception of studies on automaticity, in which superfusion with LPC was maintained for 5 minutes. Solutions with selected constituents were introduced simply by turning a stopcock and providing injectate, with total flow maintained constant. Oxygenation was provided by equilibrating the perfusate through Silastic tubing, thereby avoiding foaming and providing a measured oxygen tension in the albumin solutions consistently exceeding 350 mm Hg. Cumulative dose-response curves were not used, and each dose was evaluated in a different Purkinje fiber or ventricular muscle preparation. All measured parameters are reported as means ± SE, with significance determined by paired or unpaired Student's t-test or analysis of variance as noted; P values of < 0.01 were considered significant for differences.

**Results**

In experiments in which Purkinje fibers were perfused with equimolar (0.4 mM) albumin and palmitate dialyzed against Krebs' buffer, no significant changes in overshoot of phase 0 or V\textsubscript{max} of phase 0 occurred. However, the action potentials recorded were slightly prolonged at 70% and 95% of full recovery and demonstrated a slight reduction in maximal diastolic potential of 4 mV (Table 1). Increasing the concentration of albumin-bound palmitate to 2.92 mM did not significantly alter any
TABLE 1 Influence of GPC and/or Palmitate Bound to Albumin (0.4 mm) Dialyzed in Krebs' Solution on the Transmembrane Action Potential

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Albumin plus palmitate (0.4 mM)</th>
<th>Albumin plus palmitate (2.92 mM)</th>
<th>Albumin-palmitate (0.4 mM) plus GPC (3.0 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDP (mV)</td>
<td>-97 ± 1</td>
<td>-93 ± 1*</td>
<td>-95 ± 1</td>
<td>-92 ± 1*</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Overshoot (mV)</td>
<td>34 ± 2</td>
<td>34 ± 1.2</td>
<td>40 ± 3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>V max (V/sec)</td>
<td>503 ± 22</td>
<td>475 ± 35</td>
<td>507 ± 15</td>
<td>425 ± 46*</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD m (msec)</td>
<td>232 ± 10</td>
<td>279 ± 9</td>
<td>226 ± 22</td>
<td>362 ± 22*</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD T (msec)</td>
<td>253 ± 12</td>
<td>302 ± 9*</td>
<td>248 ± 27</td>
<td>452 ± 27*</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD m (msec)</td>
<td>299 ± 8</td>
<td>351 ± 12*</td>
<td>293 ± 18</td>
<td>530 ± 19*</td>
</tr>
<tr>
<td>(n)</td>
<td>(13)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± se. MDP = maximum diastolic potential. Numbers in parentheses indicate number of experiments.

* Significant difference from control values (P < 0.01) determined by analysis of variance

action potential parameter (Table 1). Thus, although the vehicle used to deliver the lysophosphoglycerides induced some lengthening in APD (an effect opposite to that seen with LPC) and a slight decrease in maximal diastolic potential, it had no discernible effects on any other action potential parameters. Glycerophosphorylcholine (GPC) bound to albumin, a phosphoglyceride product with both fatty acids removed, induced a slight (5 mV) decrease in maximal diastolic potential and V max (78 V/sec) and significantly prolonged APD (Table 1). However, the effects of GPC (3.0 mM) were far less profound than those induced with LPC (see below). Effects of phosphatidyl choline (1,2-dipalmitoylglycerol-3-phosphorylcholine), a possible parent compound of LPC in vivo, could not be defined because of the insolubility of this compound in solutions of albumin.

Effects of Lysophosphoglycerides on Action Potentials in Canine Purkinje Fibers

LPC bound to albumin induced consistent and concentration-dependent (0.75–3.0 mM) alterations in the recorded transmembrane action potentials of canine Purkinje fibers. Representative alterations in action potential parameters are shown for four concentrations of LPC in Figure 1. The combined data from 31 experiments illustrating the peak al-
terations induced by LPC at each concentration are shown in Figure 2 and demonstrate marked decreases in maximal diastolic potential, overshoot of phase 0, $V_{max}$ of phase 0, and $APD_{50,70}$ and $90$. Since LPC shortens action potentials at 50% of full repolarization most markedly, the configuration of recorded action potentials becomes somewhat triangular (Fig. 1).

With concentrations of LPC of 2.0 and 3.0 mM, the cells became inexcitable to external stimulation even with maximally intense stimuli (Fig. 3, panel A). Furthermore, fractionation of the action potential into several components during repolarization and depolarization is observed consistently (Fig. 3, panels B and C), often with electrical alternans (panel D, Fig. 3). Thus LPC induced changes in membrane function resulting in abnormal depolarization and heterogeneous repolarization, with fractionation of the action potential and refractoriness to external stimuli.

Since, in addition to LPC, LPE accumulates in ischemic tissue in vivo (Sobel et al., 1978), experiments were performed to examine the relative effects of LPE compared to LPC on the transmembrane action potential. Since LPE does not readily bind to albumin in the absence of LPC, an equimolar complex of LPC (1.0 mM) and LPE (1.0 mM) reduced the maximal diastolic potential to $-52.5 \pm 7.6$ mV, the overshoot of phase 0 to $8.1 \pm 7.5$ mV, the $V_{max}$ to $186.3 \pm 130.0$ V/sec, and the $APD_{50}$ to $178.6 \pm 38.0$ msec. Thus LPE and LPC (both in concentrations of 1.0 mM) induced changes in action potential parameters ($n = 6$) comparable to those induced by 2.0 mM LPC and substantially greater than those induced by 1.0 mM LPC. Therefore, LPE appears to be at least as potent as LPC.

The time course and reversibility of effects of LPC on the maximal diastolic potential are shown in Figure 4. With the lowest concentrations of LPC (0.75 mM) employed, no significant changes in diastolic potential were seen. With progressively higher

**Figure 2** Dose-response effect of LPC on action potential parameters including the maximal diastolic potential ($-mV$), the overshoot of phase 0 ($+mV$), the $V_{max}$ of phase 0 (V/sec) and $APD_{50,70}$ and $90$. Each point represents mean values from at least six individual experiments, and the vertical bars represent SE.

**Figure 3** Representative action potential tracings illustrating the following effects induced by LPC. Panel A: inability to externally depolarize the fiber ($\times$ indicates stimulation artifact at maximal current strength of 5 mA); panels B and C: fractionation of the action potential into several components during depolarization and repolarization; panel D: electrical alternans.

With concentrations of LPC of 2.0 and 3.0 mM, the cells became inexcitable to external stimulation even with maximally intense stimuli (Fig. 3, panel A). Furthermore, fractionation of the action potential into several components during repolarization and depolarization is observed consistently (Fig. 3, panels B and C), often with electrical alternans (panel D, Fig. 3). Thus LPC induced changes in membrane function resulting in abnormal depolarization and heterogeneous repolarization, with fractionation of the action potential and refractoriness to external stimuli.

Since, in addition to LPC, LPE accumulates in ischemic tissue in vivo (Sobel et al., 1978), experiments were performed to examine the relative effects of LPE compared to LPC on the transmembrane action potential. Since LPE does not readily bind to albumin in the absence of LPC, an equimolar complex of LPC (1.0 mM) and LPE (1.0 mM) reduced the maximal diastolic potential to $-52.5 \pm 7.6$ mV, the overshoot of phase 0 to $8.1 \pm 7.5$ mV, the $V_{max}$ to $186.3 \pm 130.0$ V/sec, and the $APD_{50}$ to $178.6 \pm 38.0$ msec. Thus LPE and LPC (both in concentrations of 1.0 mM) induced changes in action potential parameters ($n = 6$) comparable to those induced by 2.0 mM LPC and substantially greater than those induced by 1.0 mM LPC. Therefore, LPE appears to be at least as potent as LPC.

The time course and reversibility of effects of LPC on the maximal diastolic potential are shown in Figure 4. With the lowest concentrations of LPC (0.75 mM) employed, no significant changes in diastolic potential were seen. With progressively higher...
muscles. LPC (3.0 mM) induced decreases in resting membrane potential and overshoot of phase 0 on isolated rabbit papillary muscles comparable to those alterations seen in canine Purkinje fibers (Table 2). APD and V_{max} of phase 0 in rabbit papillary muscles decreased by nearly 50% with LPC (3.0 mM), although absolute changes were less than that seen with canine Purkinje fibers, due to the considerably lower values of APD and V_{max} of phase 0 in ventricular muscle. Moreover, unresponsiveness to external stimulation was also apparent with LPC (3.0 mM) in isolated rabbit papillary muscles. Additional experiments also demonstrated that action potentials obtained from canine, feline, and porcine endocardium responded in a similar manner to LPC. All of the following electrophysiological studies were performed in the canine Purkinje fiber preparation, due to the relative ease of these measurements compared to ventricular muscle.

**Effects on Membrane Responsiveness in Canine Purkinje Fibers**

Since the higher concentrations of LPC frequently resulted in marked alteration of action potential configuration and unresponsiveness to external stimulation, 1.5 mM concentrations were used to assess the influence of 10 minutes of superfusion of LPC on the membrane response curve. Results obtained in three of five experiments are shown in Figure 5. As can be seen, LPC induced a large shift to the right in the membrane response relationship, with the maximal effects being seen at 10 minutes, at the end of the superfusion period. After 30 minutes of perfusion without LPC, membrane function nonuniformly returned toward control levels. During the interval of 30-70 minutes of perfusion without LPC, after initial exposure to the lysosphoglyceride.

**TABLE 2 Influence of LPC (3.0 mM) Bound to Albumin on the Transmembrane Action Potential of Isolated Rabbit Papillary Muscle**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>-89 ± 3</td>
<td>-56 ± 4*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Overshoot (mV)</td>
<td>+16 ± 1</td>
<td>-5.0 ± 5*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>V_{max} (V/sec)</td>
<td>157 ± 24</td>
<td>82 ± 17*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD_{D0} (maec)</td>
<td>55 ± 12</td>
<td>24 ± 9*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD_{D0} (maec)</td>
<td>66 ± 8</td>
<td>33 ± 8*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>APD_{D0} (maec)</td>
<td>106 ± 21</td>
<td>60 ± 18*</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.E. RMP = resting membrane potential. Numbers in parentheses indicate number of experiments. * Significant difference from control values (P < 0.01) determined by analysis of variance.
glyceride, membrane function improved serially in all five experiments. However, in only one experiment (Fig. 5, panel C) did the membrane response curve return fully to control levels. Thus, as is the case for recovery of $V_{\text{mmax}}$, the alterations in membrane response indicate that LPC may induce prolonged alterations in the inward sodium current.

**Effects on Relative Conduction Time in Canine Purkinje Fibers**

The influence of LPC on relative conduction time was assessed by recording the time of activation at two sites with two separate microelectrodes impaled in a single Purkinje fiber strand. As shown in Figure 6, 3.0 mM LPC induced large and consistent increases in relative conduction time. The combined data from six experiments performed with 3.0 mM LPC indicated that conduction time increased to $4000 \pm 460\%$ of control ($P < 0.001$). Although the peak percent increase in conduction time ranged from 750\% to 8500\% of control, values returned to normal within 70 minutes after subsequent perfusion without LPC (122 ± 21\% of control, where control = 100\%). Thus, although membrane responsiveness was still depressed after 70 minutes of perfusion without LPC, as noted previously, there was no concomitant persistent increase of conduction time.

**Effects of LPC on the Ratio of ERP to APD in Canine Purkinje Fibers**

Since 3.0 mM concentrations of LPC usually led to unresponsiveness of isolated Purkinje fibers to electrical stimulation, effects of LPC on the ERP/APD ratio were evaluated with 2.5 mM LPC. With initial exposure to LPC, ERP decreased from 265.2 ± 5.3 msec to 236 ± 8.4 msec ($P < 0.01$), and APD at 100\% of recovery (APD$_{100}$) also decreased in a parallel fashion, from 331 ± 6.5 msec to 290 ± 4.0 msec ($P < 0.01$), resulting in no change in the ERP/ADP$_{100}$ ratio (0.80 ± 0.02 to 0.81 ± 0.01). Subsequently, when the LPC effect was maximal, ERP increased somewhat and APD$_{100}$ continued to shorten, resulting in persistence of ERP beyond APD$_{100}$. This phenomenon of postrepolarization refractoriness is similar to that seen in slow-response
fibers in vitro (Wit et al., 1974; Zipes et al., 1975) and recently in ischemic tissue in vivo (Elharrar et al., 1977). Changes in ERP and APD\textsubscript{100} occurring at the time of the peak response to LPC and the corresponding ratios of ERP/APD\textsubscript{100} observed in each of five experiments are summarized in Figure 7.

Effects of LPC on Automaticity in Canine Purkinje Fibers

Baseline automaticity of isolated Purkinje fibers was enhanced by perfusion with Krebs' solution containing low concentrations of K\textsuperscript+ (2.4 mEq/liter) or by the addition of isoproterenol to the bath (10\textsuperscript{-7}M). LPC (2.0 and 3.0 mM) induced a consistent additional increase in automaticity, reflected by a decrease in the basic cycle length in fibers perfused with low concentrations of K\textsuperscript+ (Fig. 8). The enhancement of automaticity persisted well after the onset of subsequent perfusion without LPC before regressing. LPC at a concentration of 1.0 mM failed to alter automaticity substantially, although a non-significant increase in basic cycle length was seen during the 20- to 40-minute interval after subsequent perfusion without LPC (Fig. 8). Results of a representative experiment illustrating the effect of 3.0 mM LPC on automaticity are depicted in Figure 9. Within 1 minute after exposure of the fiber to LPC, automaticity was enhanced markedly, with only a small decrease in the maximal diastolic potential. One minute later, automaticity was still enhanced, but the maximal diastolic potential had not been significantly altered.

![Figure 7](image-url)  
**Figure 7** Effect of LPC, 2.5 mM, on ERP and APD\textsubscript{100} (left side of figure) and the corresponding ratio of ERP/ APD\textsubscript{100} (right side of figure). The points represent mean values for control, LPC, and total recovery, and the vertical bars represent SE. The asterisk indicates a significant (P < 0.01) difference from control values.

![Figure 8](image-url)  
**Figure 8** Effect of the time course of LPC at 1.0, 2.0, and 3.0 mM on basic cycle length of spontaneous action potentials, expressed as percent of control. In the top portion of the figure, baseline automaticity was enhanced by superfusion with Krebs' buffer with low K\textsuperscript+ (2.4 mEq/liter), whereas in the bottom portion of the figure, baseline automaticity was enhanced with simultaneous infusion of isoproterenol (10\textsuperscript{-7}M). Values are mean points from five different experiments for each concentration of LPC, and the vertical bars represent SE. The asterisk denotes a significant difference from control (P < 0.01).

![Figure 9](image-url)  
**Figure 9** A representative experiment illustrating the effect of LPC, 3.0 mM, on baseline automaticity (enhanced by low K\textsuperscript+ = 2.4 mEq/liter). The time base is constant for all three panels and at 1 second as shown.
fallen to —45 mV, suggesting that the persistently enhanced automaticity may be of an abnormal type (Elharrar et al., 1977) and not dependent on the normal outward potassium current (iKo) current.

Similar but less dramatic effects of 2.0 mM LPC were seen when baseline automaticity was first enhanced with 10^{-7} M isoproterenol. The less striking response under these conditions may be due to opposing effects of LPC with direct effects increasing automaticity opposed by attenuation of the effects of isoproterenol by LPC due to interference with the function of membrane receptors for the catecholamine (Ahumada et al., 1978).

**Discussion**

Malignant ventricular dysrhythmia occurring early after experimental coronary occlusion appears to be due to a reentrant mechanism within ventricular muscle tissue dependent on heterogeneity of cellular electrical activity within the ischemic zone (Bigger et al., 1977; Corr and Sobel, 1977; Elharrar and Zipes, 1977). Electrophysiological factors facilitating initiation and maintenance of reentrant dysrhythmias include alterations in myocardial conduction properties and dispersion of recovery times (Moe, 1975), with cellular counterparts being altered phase 0 and altered repolarization of action potentials.

We have recently reported that lysophosphoglycerides accumulate in myocardium early after ischemia in vitro and in vivo (Sobel et al., 1978), with the predominant species being 2-deacyl LPC and LPE. The concentrations of LPC and LPE found in extracts of ischemic myocardium in vivo averaged 21 μmol/g dry weight, or approximately 4 mM. In the present study, perfusion of isolated canine Purkinje fibers with LPC and LPE in similar concentrations (0.75-3 mM) induced marked, reversible, and concentration-dependent alterations of the cardiac action potential, implicating these compounds as possible biochemical mediators of malignant dysrhythmia induced by ischemia. Contaminants (~2%) in the synthetic LPC or LPE could not account for the marked electrophysiological alterations, since these contaminants consisted of either fatty acids or the parent compounds phosphatidyl choline or phosphatidyl ethanolamine. Fatty acid concentrations of 2.92 mM bound to albumin, nearly 100 times the peak level due to contaminants, failed to induce any significant electrophysiological effects (Table 1).

Both albumin-bound LPC and LPE in concentrations comparable to those found in ischemic tissue in vivo induced: (1) dose- and time-dependent decreases in maximal diastolic potential, overshoot of phase 0, and V_{max} of phase 0; (2) a rightward and downward shift in the membrane response curve; (3) conversion of action potentials to slow-response type potentials, with frequent unresponsiveness of the fibers to external stimulation; and (4) marked prolongation of conduction time through isolated Purkinje fibers. These findings suggest that lysophosphoglycerides markedly alter sarcolemmal function governing phase 0 depolarization and, by implication, fast sodium conductance (g_{Na}). The slow-current response, which may be mediated by either Na+ or Ca^{2+}, appears to be less affected by lysophosphoglycerides, even at high concentrations (3.0 mM), judging from the persistence of depolarization at resting membrane potentials less than ~60 mV.

Under normal physiological conditions, phase 0 depolarization is dependent primarily on an increase in the inward sodium current (i_{Na}) through the "fast" channels, and V_{max} is proportional to the maximal i_{Na} during phase 0 (Wit and Bigger, 1975). Any reduction in i_{Na} due to a decrease in maximal diastolic potential or sodium conductance can produce changes in phase 0 of the action potential, with consequent abnormalities of impulse propagation and conduction time, both progenitors of reentrant dysrhythmias (Moe, 1975; Wit and Bigger, 1975). Failure of V_{max} and membrane responsiveness to return completely to control values during recovery after exposure of Purkinje fibers to lysophosphoglycerides, despite normalization of other phase 0 components (maximal diastolic potential and overshoot), suggests that the lysophosphoglycerides may induce persistent alterations in the fast inward sodium current. Although V_{max} and membrane responsiveness were still depressed after 70 minutes of subsequent perfusion without LPC, the depression was not manifest by prolongation of conduction time. This paradox may be explained by the insensitivity of the overall conduction time measurements to alterations of responsiveness affecting the characteristics of individual action potentials. In addition, effects of the lysophosphoglycerides on conduction time may have been due in part to conduction block, persisting during recovery, in selected regions of the Purkinje fiber, with conduction time restored during recovery due to impulse transmission via alternate conduction pathways in adjacent Purkinje strands.

Lysophosphoglycerides induced heterogeneous alterations in action potential repolarization manifested by asymmetric shortening of APD_{max} and APD_{100}, resulting in triangularization and fractionation of the action potential configuration into several components. Alterations in APD induced by the higher concentrations of LPC (>2.5 mM) were accompanied by biphasic changes in the ERP of the Purkinje fiber with exposure of the fibers to LPC. ERP and APD_{100} decreased initially in a parallel fashion. Later, at a time corresponding to the time of occurrence of peak LPC effect, ERP lengthened slightly whereas APD_{100} continued to shorten. Consequently, ERP exceeded APD_{100}, resulting in postrepolarization refractoriness. Although the ionic alterations responsible were not delineated in this
study, the abbreviation of APD induced by lysophosphoglycerides occurred to a greater extent at 50% of full repolarization, suggesting that attenuation of the slow inward current during the plateau may be responsible (Trautwein et al., 1954).

In Purkinje fibers with intrinsic rates of depolarization increased by exposure either to low concentrations of K+ or to isoproterenol, LPC at concentrations significantly increased automaticity. Increased automaticity was seen in fibers with normal resting membrane potentials as well as after sequential decreases in resting membrane potential induced by LPC, suggesting that mechanisms involving both normal and abnormal automaticity may be operative (Wit et al., 1974). Inhibition of the io may be responsible for the initial changes (Noble and Tsien, 1968), although later, abnormal automatic activity similar to that induced by other interventions reducing membrane potential may depend on entirely different alterations in ionic conductances (Wit et al., 1974). Abnormal automaticity is characterized by oscillatory diastolic potentials, which increase in amplitude until threshold is attained and an action potential ensues. Underlying ionic mechanisms responsible for abnormal automaticity may involve (1) slow inward current carried by Na+ and Ca2+, (2) slow-channel repolarization current (io), or (3) an increase in background inward current carried by Ca2+ or Na+ seen at normal resting membrane potentials (Wit and Bigger, 1975).

Similarities of Electrophysiological Alterations Accompanying Ischemia to Those Induced by Lysophosphoglycerides

Electrophysiological alterations induced by lysophosphoglycerides in vitro in concentrations comparable to those seen in ischemic tissue in vivo (Sobel et al., 1978) closely resemble several electrophysiological derangements characteristic of ischemic tissue in vivo (Elharrar et al., 1977; Bigger et al., 1977; Corr and Sobel, 1977; Elharrar and Zipes, 1977; Wit and Bigger, 1975; Downar et al., 1977a; Russell et al., 1977b). The similarity suggests that accumulating lysophosphoglycerides may be important mediators of the early electrophysiological changes contributing to malignant ventricular dysrhythmias accompanying myocardial ischemia. This interpretation is supported by: (1) similar time courses, with a decrease in APD evident within 1 minute after acute coronary occlusion in vivo (Downar et al., 1977a; Russell et al., 1977), and a similar response time in the present study with both LPC and LPE in vitro; (2) parameters affected, with a reduction in membrane potential, overall amplitude of phase 0, and the Vmax of phase 0, as well as conversion to slow-response type potentials evident in ischemic myocardium in vivo (Wit and Bigger, 1975; Downar et al., 1977a; Russell et al., 1977) and also in response to lysophosphoglycerides in vitro; (3) parallel effects on responsiveness to external stimuli, with a loss of response at maximal stimulation levels in ischemic myocardium in vivo (Downar et al., 1977a), with similar refractoriness induced by LPC in vitro; (4) occurrence of electrical alternans in both the intracellular action potentials of ischemic myocardium in vivo, often associated with the development of ventricular fibrillation (Downar et al., 1977a; Russell et al., 1977), and in isolated Purkinje fibers superfused with LPC; (5) similar obliteration of action potentials in severely ischemic tissue in vivo (Downar et al., 1977a) and in normal Purkinje fibers exposed to LPC in vitro; and (6) postrepolarization refractoriness, with similar evolution in ischemic tissue in vivo and Purkinje fibers exposed to LPC in vitro. Both the refractory period and APD initially decrease in parallel during ischemia in vivo, a response similar to that seen initially with LPC. However, with continued ischemia in vivo or prolonged exposure of Purkinje fibers to high concentrations of LPC in vitro, the refractory period lengthens while the APD continues to decrease, resulting in postrepolarization refractoriness (Downar et al., 1977a).

Prolongation of refractoriness beyond the basic cycle length of stimulation may explain the 2:1 conduction block recently demonstrated with recordings of intracellular action potentials in ischemic myocardium in vivo (Downar et al., 1977a; Russell et al., 1977) and may explain the unresponsiveness of ischemic tissue in vivo to external stimuli applied late in diastole (Williams et al., 1974).

Mediation by biochemical factors of the electrophysiological derangements accompanying ischemia is suggested by recent observations of Downar et al. (1977b), who demonstrated that venous effluents from myocardium rendered ischemic in situ elicited electrophysiological derangements in normal myocardium similar to those exhibited by ischemic myocardium in vivo. The factor responsible does not appear to be potassium, lactate, glucose, or pH, since manipulation of normal venous blood with respect to these variables does not result in blood with the same properties (Downar et al., 1977b). Unresponsiveness to external stimulation and fractionation of the action potential are two characteristic findings induced by lysophosphoglycerides in the present study at concentrations comparable to those found in ischemic myocardium in vivo (Sobel et al., 1978). Thus it appears likely that accumulation of lysophosphoglycerides in ischemic myocardium may be a major factor responsible for the electrophysiological changes seen in ischemic myocardium, and in turn may be a major precipitant of the early malignant ventricular dysrhythmia. The presence of a biochemical mediator is suggested also by recent findings indicating that, after 35 minutes of coronary occlusion in vivo, subsequent perfusion of the occluded artery with saline gassed with N2 temporarily restored depressed ac-
tion potentials to near normal within 24 seconds (Downar et al. 1977a).

Although lysophosphoglycerides increase automaticity of both the normal and abnormal types in canine Purkinje fibers, effects on automaticity may not be important during the interval early after ischemia, since ventricular automaticity does not appear to be enhanced in vivo under these conditions (Bigger et al., 1977; Corr and Sobel, 1977; Wit and Bigger, 1975; Penkoske et al., 1978; Lazzara et al., 1974). This apparent discrepancy may be due to the relative lack of accumulation of lysophosphoglycerides in endocardial Purkinje fibers because of washout by intracavitary blood, an interpretation supported by results of several studies demonstrating a relative lack of electrophysiological effects of early ischemia in vivo on endocardial compared to myocardial and epicardial regions (Corr et al., 1978; Bigger et al., 1977; Wit and Bigger, 1975). Although accumulation of lysophosphoglycerides might otherwise enhance automatic firing in the Purkinje conduction system, their regional accumulation in subendocardial zones may be of importance only later, when Purkinje cell automaticity is enhanced (Lazzara et al., 1974; Corr and Sobel, 1977).

The present study does not address the question of whether or not incorporation of lysophosphoglycerides into sarcolemma is a requirement for induction of the electrophysiological alterations. However, since these compounds are present in effluents from hypoperfused, isolated hearts (Sobel et al., 1978), and since extracellular lysophosphoglycerides are sufficient to induce the electrophysiological abnormalities observed in the present study, it appears likely that their accumulation in vivo elicits deleterious electrophysiological effects. Accordingly, clarification of factors affecting lysophosphoglyceride accumulation in ischemic tissue may be useful in developing new and effective approaches for the control of early malignant ventricular dysrhythmias associated with myocardial ischemia.

Acknowledgments

We appreciate the technical assistance of Diana Rankin and Gerard Clarke and the Secretarial help of Linda Binns. We also appreciate the expert statistical analyses performed by Dr. J. Philip Miller.

References

Wit AL, Bigger JT Jr: Possible electrophysiological mechanisms for lethal arrhythmias accompanying myocardial ischemia and infarction. Circulation 52 (suppl III): 96-115, 1975
Zipes DP, Beach HR, Watanabe AM: Role of the slow current in cardiac electrophysiology. Circulation 61: 761-766, 1975
Potential arrhythmogenic electrophysiological derangements in canine Purkinje fibers
induced by lysophosphoglycerides.
P B Corr, M E Cain, F X Witkowski, D A Price and B E Sobel

_Circ Res._ 1979;44:822-832
doi: 10.1161/01.RES.44.6.822

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/44/6/822

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
_Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the
Editorial Office. Once the online version of the published article for which permission is being requested is
located, click Request Permissions in the middle column of the Web page under Services. Further information
about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/