Accumulation of Unesterified Arachidonic Acid in Ischemic Canine Myocardium

Relationship to a Phosphatidylcholine Deacylation-Reacylation Cycle and the Depletion of Membrane Phospholipids

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SUMMARY. Studies in ischemic canine left ventricle have shown that the depletion of membrane phospholipids is a critical event in the development of a sarcolemmal calcium permeability defect and associated irreversible myocyte injury. The mechanism of phospholipid loss is unclear, but may be due to the activation of endogenous phospholipases. Since arachidonic acid is a fatty acid found almost entirely in phospholipid, increases in arachidonate provide evidence for increased phospholipase activity. The present study was designed to examine the temporal relationship of the accumulation of free arachidonate with the onset of phospholipid depletion during fixed ligation of the left anterior descending coronary artery in canine myocardium. The following results were demonstrated in ischemic canine myocardium: (1) the accumulation of unesterified arachidonate is minimal during 10–30 minutes of ischemia, but is significantly increased after prolonging the duration of ischemia to 1–3 hours; (2) significant increases in arachidonate precede the development of a significant decrease in total phospholipid content; (3) the decrease in the arachidonate content of phosphatidylcholine is accompanied by similar decreases in all of the fatty acyl moieties; (4) the arachidonate content of lysophosphatidylcholine and diacylglycerol are unchanged during myocardial ischemia; (5) there is evidence of a deacylation-reacylation cycle in phosphatidylcholine prior to the accumulation of free arachidonate; (6) the fatty acyl specificity of the lysophosphatidylcholine acyltransferase corresponds to the pattern of fatty acyl remodeling of phosphatidylcholine during early myocardial ischemia. These data suggest that the accumulation of arachidonate may be a more sensitive measure of phospholipid degradation than the decrease in total phospholipid content in ischemic canine myocardium. It is postulated that the defective reacylation of arachidonate into phosphatidylcholine may contribute to the net loss of membrane phospholipid during myocardial ischemia. (Circ Res 54: 313–322, 1984)

PREVIOUS studies in an ischemic rat liver model have suggested that the depletion of membrane phospholipids is causally related to the loss of cell viability during ischemia (Chien and Farber, 1977; Chien et al., 1978). In this model, the degradation of membrane phospholipids was related temporally to the development of a membrane Ca++ permeability defect, a several-fold increase in tissue Ca++ content, and the development of irreversible damage in ischemically injured cells (Chien et al., 1978, 1980). Pharmacological inhibition of the phospholipid degradation resulted in a protection against the alterations in Ca++ homeostasis and the development of irreversible cell injury (Chien et al., 1977).

Recently, these initial studies have been confirmed by other investigators (Wattiaux et al., 1980; Matsumoto et al., 1981). There are now several reports of phospholipid degradation during ischemia in the kidney (Smith et al., 1980, Patel et al., 1982), brain (Bazan, 1970), and myocardium (Hsueh et al., 1977; Chien et al., 1979; Vasdev et al., 1979; Weglicki, 1980; Chien et al., 1981; Shaikh and Downar, 1981; Corr et al., 1982).

Whereas there is increasing evidence that the degradation of membrane phospholipids is one of the critical events during ischemic cell injury, the exact biochemical mechanisms responsible for the loss of membrane phospholipid are not clear. Myocardial cells contain phospholipase A and C activities (Weglicki et al., 1971; Franson et al., 1972, 1978; Hostetler and Hall, 1980), as well as a lysophosphatidylcholine acyltransferase activity in cardiac membranes (Gross and Sobel, 1982). In an ischemic canine model, Van der Vusse et al. (1982) demonstrated that unesterified arachidonate can accumulate after 2 hours of myocardial ischemia. However, the time course of the accumulation of arachidonate and its temporal relationship to the depletion of membrane phospholipids is not known. In addition, it is not clear whether there is evidence of a phospholipid deacylation-reacylation cycle during myocardial ischemia.

The present study was designed to examine the temporal relationship of the accumulation of free arachidonate with the onset of phospholipid depletion during fixed ligation of the left anterior de-
ascending coronary artery in canine myocardium. The presence of a phospholipid deacylation-reacylation cycle was examined by analyzing the fatty acid composition of phosphatidylcholine, the major phospholipid class which is depleted during prolonged myocardial ischemia. To facilitate a rapid and quantitative analysis of fatty acid composition, a high pressure liquid chromatography method was adapted for these studies. The following results are demonstrated in ischemic canine myocardium: (1) the accumulation of unesterified arachidonate is minimal during 10–30 minutes of ischemia, but is significantly increased after prolonging the duration of ischemia to 1–3 hours; (2) significant increases in arachidonate precede the development of significant decreases in total phospholipid content; (3) the decrease in the arachidonate content of phosphatidylcholine (PC) is accompanied by similar decreases in all of the fatty acyl moieties of PC; (4) the arachidonate content and fatty acid composition of lysophosphatidylcholine (LPC) and diacylglycerol are unchanged during myocardial ischemia; (5) there is evidence of a deacylation-reacylation cycle in PC prior to the accumulation of free arachidonate; (6) the fatty acyl specificity of the lysophosphatidylcholine acyltransferase corresponds to the pattern of fatty acyl remodeling of phosphatidylcholine during early myocardial ischemia. These data suggest that the accumulation of arachidonate may be a more sensitive measure of phospholipid degradation than the decrease in total phospholipid content in ischemic myocardium. It is postulated that defective reacylation of arachidonate into PC may contribute to the net loss of membrane phospholipid during myocardial ischemia.

**Methods**

**Animal Preparation**

Dogs weighing 30–35 kg and of either sex were used in these experiments. The dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv), intubated, and ventilated with oxygen on a Harvard respirator (Harvard Apparatus). A left thoracotomy was performed, the pericardium opened, and the proximal LAD ligated approximately one-third to one-half the distance from its origin to the apex and distal to the first diagonal branch so as to create a region of intense cyanosis involving approximately 30% of the anterior left ventricle (LV).

To obtain an adequate amount of tissue for lipid analyses, transmural sections of both the ischemic and nonischemic regions were frozen immediately in liquid N₂ and used for biochemical analysis in these studies.

**Lipid Analysis**

Myocardial tissue was rinsed in ice cold saline and homogenized in 2.5 volumes of 0.25 M sucrose-10 mM MOPS buffer, pH 7.4. Lipid extraction was performed by the method of Bligh and Dyer (1959), concentrated under N₂, and resuspended in 200 µl of chloroform:methanol (1:1). The concentrated lipid extract was separated into various lipid fractions by thin layer chromatography. For the quantitative determination of the various phospholipid species, 25 µl of the extract was chromatographed on silica gel H plates (Analtech) and developed with a solvent of chloroform:methanol:acetic acid:H₂O (50:25:8:3, vol/vol). To achieve optimum separation of LPC, the solvent system of CHCl₃:methanol:acetic acid:HzO (75:60:3:2) was used (Shaikh and Palmer, 1976). Using this chromatographic system, recovery of LPC as an internal standard exceeded 85%. Neutral lipids were separated by spotting 50 µl of total lipid extract on silica gel H plates (Analtech) with a solvent of hexane:diethyl ether:methanol:acetic acid (80:21:3:2; vol/vol). The spots corresponding to known lipid standards were identified by spraying with dichrofluorescein (0.02% in methanol) under ultraviolet detection.

The phospholipid content was quantified by acid digestion without prior elution, by the method of Rouser et al. (1969). To identify the fatty acid composition of the phospholipid species, the phospholipids were eluted by successive washing of the silica gel spots with methanol (Skipski and Barclay, 1969). The neutral lipids were eluted by repeated diethyl ether washings, concentrated under nitrogen, and quantified by high pressure liquid chromatography of the derivatized fatty acids, as described below.

The fatty acid composition of PC was determined by high pressure liquid chromatography of the bromophenacyl fatty acid derivatives. PC was separated from the total lipid extract by HPLC with a Whatman Partisil PX 10/25 SCX column. A 25-µl aliquot of filtered, total lipid extract was injected directly into a Waters HPLC with a solvent of acetonitrile:methanol:water (400:35:64; vol/vol) under isocratic conditions, and a flow rate of 2 ml/min by a modification of previously described methods; (Gross and Sobel, 1980). Peaks were identified by detection at 206 nm, and recovery of total phospholipid exceeded 95% (Fig. 1). The pooled PC fractions were concentrated under nitrogen, and subjected to alkaline hydrolysis. The free fatty acids were recovered by three successive ether extractions. The pooled ether fraction was dried under nitrogen and resuspended in 2 ml of acetonitrile. The free fatty acids were converted to their bromophenacyl esters with dibromooctophenone and crown ether catalysis in the presence of K₂CO₃, and boiled at 80°C for 50 minutes (Durst et al., 1975; Pei et al., 1977). The derivatized fatty acids were filtered, dried under nitrogen, and resuspended with acetonitrile. The fatty acyl derivatives were separated by reverse phase HPLC with a Waters C18, 10 µm Bondapak column, a flow rate of 1 ml/min, and with an isocratic solvent system of methanol:water (90:10; vol/vol) (Fig. 2). The derivatives were detected at 254 nm, and quantification was performed by peak integration and by comparison with a known amount of C17 fatty acid added as an internal standard to each sample. Recovery of fatty acids as internal standards exceeded 90–95%.

**Membrane Fractionation**

Cardiac microsomal membranes were prepared from canine tissue by the method of Mukherjee et al. (1982). Myocardial tissue was rinsed in ice cold saline, mixed for 5 seconds with a 4-fold volume of 0.25 M sucrose-10 mM MOPS, and centrifuged for 10 minutes at 3,000 rpm in a Beckman J2B centrifuge. This initial centrifugation step was repeated three times. The washed tissue was homogenized in a 3-fold volume of buffer with a polytron setting 7 for 30 seconds, three times, and centrifuged at 9,500 rpm for 10 minutes. The supernatant extract was filtered through four layers of cheesecloth and was centrifuged at
1. Separation of myocardial phospholipids by high pressure liquid chromatography. Aliquots of myocardial tissue homogenates were extracted for lipids as described in the Methods. The lipid extract was filtered and directly injected onto a Waters HPLC with a Whatman Partisil SCX cation exchange column with a solvent of acetonitrile: methanol: water (40:100:34, vol/vol) and detection at 206 nm. Peaks were identified by comparison with retention times of authentic phospholipid standards: 1 = neutral lipids and solvent front; 2 = phosphatidylethanolamine; 3 = lysophosphatidylethanolamine; 4 = phosphatidylcholine; 5 and 6 = sphingomyelin; 7 = lysophosphatidylcholine.

20,000 rpm for 30 minutes. The pellet was resuspended in the appropriate assay buffer.

Lysolecithin Acyltransferase Assay

Lysolecithin acyltransferase was assayed by a modification of the method of Okuyama et al. (1975). The assay mixture contained 50 nmol of 1-palmitoyl-glycerophosphocholine, 0.05 μCi of 14C-lysolecithin (Amersham), 40 nmol of fatty acyl-CoA, 10 μmol of MgCl2, 50 μmol of Tris-HCl buffer (pH 7.2), and up to 80 μg of microsomal protein in a final volume of 0.4 ml. After a 10-minute incubation period at 37°C in a shaking water bath, the reaction was terminated with 3 ml of methanol, and the lipids were extracted by the method of Bligh and Dyer (1959). PC and LPC were separated by thin layer chromatography, as described above, and counted for 14C activity.

Statistical Analysis

Students's t-test was used for statistical analysis of the data. A two-tailed t-test was used, and results considered significant when P < 0.05.

Results

Parameters of Myocardial Ischemia

In this animal model, myocardial ischemia has been documented by several independent hemodynamic, functional, blood flow, and electrocardiographic criteria (Karlsson et al., 1973; Willerson et al., 1977; Roan et al., 1979). There was a 5-fold increase in tissue lactate content in the ischemic vs. the corresponding nonischemic tissue at each time point examined.

Phospholipid Content

Myocardial ischemia resulted in a time-dependent decrease in total phospholipid content. After 10–30 minutes of ischemia, there was no significant decrease in total phospholipid content in the ischemic vs. the corresponding nonischemic tissue. However, increasing the duration of ischemia to 3 hours resulted in a 15–16% decrease of myocardial phospholipid content. This decrease was not due to an artifact of normalization to increased tissue per gram wet weight due to cellular edema. As previously described, the mg DNA/g-wet weight ratio did not change during ischemia (1.7 mg DNA/g wet weight), and the extent of phospholipid loss was nearly identical when the data were normalized to
Phosphatidylcholine Content of Ischemic and Nonischemic Myocardium

<table>
<thead>
<tr>
<th>Duration of ischemia</th>
<th>Group</th>
<th>Mean phosphatidylcholine content (μmol/g wet wt)</th>
<th>Paired nonischemic vs ischemic (% difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>NI</td>
<td>9.9</td>
<td>4.7 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>NI</td>
<td>9.6</td>
<td>3.5 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>NI</td>
<td>10.1</td>
<td>-18.6 ± 3.0*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

Procedures are as described in Methods. The nmol measurement of lipid PO₄⁻⁻ in each phospholipid species was determined by the method of Rouser, without prior elution, and the data were normalized to the determined mg DNA content of the corresponding ischemic (I) and nonischemic (NI) tissue. Data are expressed as the mean μmol lipid PO₄⁻⁻/g wet weight in the ischemic vs. the nonischemic region of all myocardial preparations, and, also, ischemic vs. nonischemic in paired analyses of each experiment. Results are the mean ± SEM.

* P < 0.05 (nonischemic vs. ischemic); n = 4, or greater, at each time point

either parameter (Mukherjee et al., 1982). This decrease in phospholipid content was similar to that reported in previous studies (Chien et al., 1981).

Chromatography of the lipid extract revealed that PC and phosphatidylethanolamine (PE) were the main phospholipid species affected during ischemia. Short durations of ischemia resulted in little or no decrease in either PC or PE, but by 3 hours there was a decrease of over 15% in each of these species in the ischemic myocardium (Table 1). The decrease in PC at 3 hours presented an absolute mean difference of 1.3 μmol lipid PO₄⁻⁻/g wet weight in the ischemic vs. the nonischemic region of the left ventricle. The decreases in PC and PE accounted for over 70% of the decrease in total phospholipid content. Since PC accounted for most of the phospholipid decrease during myocardial ischemia, the present study examined the fatty acyl composition of this particular phospholipid class as a function of the duration of ischemia.

Fatty Acid Composition of PC

To examine the possibility of remodeling of the fatty acyl composition of phospholipids during myocardial ischemia, a rapid, quantitative, and highly reproducible HPLC analysis of myocardial lipids was employed. Routine fatty acid analysis of PC revealed that palmitate, stearate, oleate, linoleate, and arachidonate accounted for over 95% of the total fatty acid content in PC (Table 2). For these studies, each animal was used as its own control, with the nonischemic region serving as the internal control for each preparation. As depicted in Figure 3, after 10 minutes of ischemia, there was no statistically significant difference in either 18:1 or 18:2 content between the ischemic and nonischemic regions. After 30 minutes of ischemia, there was approximately a 10% decrease in oleate content and a corresponding increase in linoleate content of PC. Other fatty acid moieties were not significantly affected (Table 2). This remodeling process cannot be

<table>
<thead>
<tr>
<th>Time of ischemia (min)</th>
<th>Group</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>NI</td>
<td>18.08 ± 1.20 (2.24)</td>
<td>10.92 ± 0.61 (1.35)</td>
<td>27.30 ± 1.60 (3.38)</td>
<td>21.60 ± 1.00 (2.67)</td>
<td>22.76 ± 2.25 (2.74)</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>I</td>
<td>16.41 ± 0.81 (2.05)</td>
<td>10.53 ± 1.0 (1.49)</td>
<td>27.50 ± 1.33 (3.43)</td>
<td>21.51 ± 0.91 (2.69)</td>
<td>22.91 ± 2.24 (2.86)</td>
</tr>
<tr>
<td>30</td>
<td>NI</td>
<td>16.95 ± 0.68 (2.03)</td>
<td>10.80 ± 0.65 (1.30)</td>
<td>28.20 ± 1.12 (3.39)</td>
<td>17.40 ± 0.57 (2.08)</td>
<td>26.62 ± 0.82 (3.20)</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>I</td>
<td>16.37 ± 0.67 (1.96)</td>
<td>11.19 ± 0.6 (1.34)</td>
<td>26.12 ± 1.08 (3.14)</td>
<td>19.21 ± 0.42 (2.31)</td>
<td>27.09 ± 0.91 (3.25)</td>
</tr>
<tr>
<td>180</td>
<td>NI</td>
<td>16.11 ± 0.78 (2.03)</td>
<td>7.80 ± 0.50 (0.98)</td>
<td>30.20 ± 1.33 (3.31)</td>
<td>19.52 ± 0.64 (2.46)</td>
<td>26.32 ± 0.71 (3.32)</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>I</td>
<td>16.04 ± 0.64 (1.77)</td>
<td>7.82 ± 0.45 (0.86)</td>
<td>27.92 ± 0.72 (3.08)</td>
<td>20.86 ± 0.83 (3.20)</td>
<td>27.42 ± 0.92 (3.00)</td>
</tr>
</tbody>
</table>

After 10, 30, and 180 minutes of left anterior descending coronary artery occlusion, myocardial samples were taken from the ischemic and nonischemic portions of the left ventricle, and lipids were extracted as described in Methods. Aliquots of the lipid extracts were taken directly for measurement of absolute phosphatidylcholine content by phosphate analysis, while another portion was taken for measurement of the fatty acyl composition by high pressure liquid chromatography. Data are expressed as percent composition that each fatty acyl species comprises of the total fatty acyl content of phosphatidylcholine. The μmol of fatty acid/mg DNA present in phosphatidylcholine was obtained by multiplying the percent composition by the mean total phosphatidylcholine content, and is given in parentheses. NI = nonischemic; I = ischemic.
explained solely by a direct change in the available fatty acid pool, since there was a larger amount of unesterified oleate than linoleate after 30 minutes of ischemia (Fig. 4). After longer durations of ischemia of 1 and 3 hours, there was no further change in the relative composition of oleate and linoleate, even though the total PC content was decreased at these times. As shown in Table 2, after 3 hours of ischemia, there was no significant difference in the relative fatty acid composition of PC of the ischemic region vs. the nonischemic with respect to palmitate, stearate, or arachidonate content. Thus, as the PC content decreased, arachidonate, and the other fatty acids esterified in PC, were decreased to similar extents. It should be noted that there was a marked alteration in the saturated:unsaturated ratio of C-16 and C-18 fatty acids after 10 minutes vs. 180 minutes of either ischemic or nonischemic conditions. These changes may be related to neural or humoral factors occurring during the surgical procedures.

Unesterified Fatty Acid Content

The decrease in PC content was associated with a corresponding increase in unesterified free fatty acids (Fig. 4). There was a time-dependent increase in free fatty acid content in ischemic canine myocardium. As displayed in Figure 5, the time course of accumulation of unesterified arachidonate paralleled the increases in the total free fatty acid content in the ischemic tissue. At 10–20 minutes, there was no significant increase in free arachidonate. However, extending the duration of ischemia to 1 hour resulted in a greater than 3-fold increase in free arachidonate content.

Previous investigators have demonstrated that the method of tissue lipid extraction can result in artificial increases in myocardial unesterified fatty acid content (Van der Vusse et al., 1980; Hunneman and Schweickhardt, 1982). To optimize the measurement of free fatty acid content, quick-frozen myocardial samples from sham-operated myocardium were homogenized by three separate methods and extracted by the method of Bligh and Dyer (1959): Method 1—polytron homogenization in a Tris-buffer and extraction with chloroform and methanol; Method 2—pulverization of tissue in a stainless steel percussion mortar under liquid N\textsubscript{2} and subsequent direct extraction of the frozen tissue powder; Method 3—pulverization of tissue in a stainless steel percussion mortar under liquid N\textsubscript{2} followed by gentle homogenization of the tissue powder in methanol-water at 4°C with a motorized...
FIGURE 5. Time course of the accumulation of unesterified arachidonate in ischemic canine myocardium. Details of this figure are similar to those of Figure 3. \( P < 0.05 \) for 30 and 60 minutes of ischemia.

Teflon pestle and glass homogenizer. By means of these three separate procedures, the following values of unesterified fatty acid content were obtained: Method 1: 683 ± 72.3 (n = 5); Method 2: 141.2 ± 9.9 nmol/g wet wt (n = 3); Method 3: 342 ± 34.4 nmol/g wet wt (n = 3). Since Method 3 assured complete tissue extraction by gentle homogenization, this procedure was utilized for the determination of free fatty acid content. To ensure that the recorded time course of the increase in unesterified fatty acid content in ischemic myocardium was real, the myocardial content of free fatty acids was evaluated after 1 hour of ischemia, by these three methods of tissue lipid extraction. Regardless of the method used, there was a >70% increase in free fatty acid content which was statistically significant (\( P < 0.05 \)), compared with sham values. Similarly, after 1 hour of ischemia, there was a >70% increase in free arachidonate content in the ischemic vs. the nonischemic myocardium with each of these methods.

Potential Hydrolysis Products

Since myocardial cells have been demonstrated to contain both phospholipase A and phospholipase C activities (Weglicki et al., 1971; Franson et al., 1972, 1978; Hostetler and Hall, 1980), the respective hydrolysis products of these enzymes, LPC and di-glyceride, were quantified as a function of the duration of ischemia. As seen in Table 3, there was no significant increase in LPC content at all time periods examined. HPLC analysis of the fatty acid composition revealed that there was no significant change in the relative fatty acid composition of LPC (Table 3). Total diacylglycerol content was observed to decrease in both the nonischemic and the ischemic tissue (Table 4). Fatty acid analysis revealed no change in fatty acid composition of diglyceride and a low content of arachidonic acid (<10%) (Table 5).

**Lysophosphatidylcholine Acyltransferase**

In order to determine whether the oleate decylation-linoleate reacylation was related to the fatty acyl CoA preference of the lysophosphatidylcholine acyltransferase, we isolated cardiac microsomal membranes and examined the activity of the transferase with various fatty acyl CoA donors. As mentioned by Okuyama and his colleagues, due to the micellar nature of the substrates, the calculation of an accurate \( K_m \) and \( V_{\text{max}} \) is difficult to quantify precisely (Okuyama et al., 1975). However, as seen in Figures 6 and 7, there was approximately a 2-fold greater increase in the reacylation rate of exoge-

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Content and Fatty Acid Composition of Lysophosphatidylcholine in Nonischemic (NI) and Ischemic (I) Canine Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of ischemia (min)</td>
<td>Percent fatty acid composition</td>
</tr>
<tr>
<td></td>
<td>16:0</td>
</tr>
<tr>
<td>10</td>
<td>NI</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>I</td>
</tr>
<tr>
<td>30</td>
<td>NI</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>I</td>
</tr>
<tr>
<td>60</td>
<td>NI</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>I</td>
</tr>
<tr>
<td>180</td>
<td>NI</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>I</td>
</tr>
</tbody>
</table>

Procedures are as described in Methods. Percent fatty acid composition was calculated as the percent that each fatty acid moiety comprises of the total fatty acid content. \( P > 0.05 \) for all fatty acid species (nonischemic vs. ischemic).
Diglyceride content was measured by thin layer chromatography of the total lipid extract as described in Methods. The spot corresponding to diglyceride was located by comparison with the retention time of authentic diglyceride standards and was scraped and eluted by successive ether washings. The lipid was saponified and the fatty acid content was quantified by HPLC of the bromophenacyl derivatives. The data are expressed as nmol fatty acid/mg DNA, and were the mean ± SEM.

P > 0.05 (nonischemic vs ischemic).

Discussion

Previous studies in ischemic canine myocardium have demonstrated that there is a time-dependent degradation of membrane phospholipids which is associated with the production of a sarcolemmal Ca**+** permeability defect (Chien et al., 1981). In our earlier study, short durations of ischemia of 10–30 minutes result in little or no loss of total membrane phospholipid, no change in tissue Ca**+** content, and no morphological evidence of irreversible injury (Chien et al., 1981). Prolonging the duration of ischemia to 3 hours resulted in a 15% decrease in total phospholipid content which was reflected predominantly in PC and PE. The net loss of membrane phospholipid is dependent upon the rate of phospholipid deacylation by endogenous phospholipases and the rate of reacylation by lysolipid acyltransferase.
A major objective of the present study was to examine systematically the accumulation of the potential hydrolysis products as a function of the duration of ischemia, and in relation to the time course of phospholipid depletion and the accumulation of free arachidonate. Previous studies have demonstrated the presence of a myocardial phospholipase A and phospholipase C activities (Weglicki et al., 1971; Hostetler et al., 1980; Weglicki, 1980). In the present study, there was no significant increase in either diglyceride or LPC in ischemic canine myocardium at any timepoint examined. An increase in arachidonate content of diacylglycerol during ischemia might indicate an increased hydrolysis of phospholipids by a phospholipase C. However, there was no significant change in the relative fatty acid composition of diacylglycerol or LPC in ischemic myocardium. These results suggest that the initial degradation of PC proceeded rapidly through these intermediates due to the subsequent action of diglyceride lipases and/or lysophospholipases.

Studies by Gross et al. have demonstrated the presence of a cytosolic and membrane-bound lysophospholipase activity in myocardial cells (Gross and Sobel, 1982).

As suggested by the work of Bilheimer et al. (1978), the source of the increase in unesterified fatty acids during myocardial ischemia was not clear. However, at least a portion of this increase was due to the hydrolysis of phospholipids. The time course of accumulation of arachidonate, an ester of a fatty acid found almost entirely in phospholipid, paralleled that found for the entire free fatty acid fraction. Since there is no detectable difference in the arterial-coronary sinus arachidonate levels during ischemia in canine myocardium (Van der Vusse et al., 1982), the accumulation of free arachidonate cannot be explained simply by increased uptake of arachidonate. In addition, arachidonate is known to be a poor substrate for β-oxidation, and thus the accumulation of arachidonate may not be due solely to decreased utilization (Chien et al., in press). It should be noted that significant increases in free arachidonate are detectable after 1 hour of ischemia, although there is no significant decrease in total phospholipid at this time (Chien et al., 1981). This discrepancy is probably due to the inherent difficulty in detecting nmol per gram wet weight quantities of membrane phospholipid degradation by simply measuring the total phospholipid content which is present in μmol per gram wet weight amounts. Thus, increases in tissue free arachidonate may be a more sensitive indicator of phospholipid degradation during myocardial ischemia. Interestingly, the time course of the accumulation of arachidonate paralleled the time course of irreversible injury, which has been described previously in this model (Chien et al., 1981). However, the causal relationship between these two events during myocardial ischemia remains to be established. It should be noted that the definitive study to elucidate the source of the increased arachidonate will require incorporation of a known amount of radiolabeled arachidonate into the myocardial cell lipids and the subsequent quantitative recovery of the label into the various lipid fractions during myocardial ischemia. In particular, it will be necessary to examine the conversion of arachidonate to prostaglandin and leukotrienes during myocardial ischemia.

Analysis of the fatty acid composition of PC as a function of the duration of ischemia suggested the presence of a deacylation-reacylation cycle prior to the depletion of PC and the accumulation of free arachidonate. After 30 minutes of ischemia, there was a 10% decrease in oleate and a corresponding increase in linoleate content of PC in ischemic myocardium. These results suggest that the initial degradation of PC proceeded rapidly through these intermediates due to the subsequent action of diglyceride lipases and/or lysophospholipases.
replacement with linoleate, as all fatty acids of PC appeared to be decreased during 1–3 hours of ischemia. These results suggest the presence of a deacylation-reacylation cycle involving oleate and linoleate during 10–30 minutes of ischemia. Such a deacylation-reacylation cycle could be the combined result of initial deacylation by a phospholipase A and subsequent reacylation by a lysophospholipid acyltransferase activity (Fig. 8). Both of these activities have been demonstrated previously in myocardial cell membranes (Weglicki et al., 1971; Franson et al., 1972, 1978; Gross and Sobel, 1982). In the present study, it was demonstrated that the LPC acyltransferase has a 2-fold higher rate of activity with arachidonoyl CoA and linoleoyl-CoA than with oleoyl-CoA. This fatty acid preference is similar to that reported in liver microsomes under conditions of low exogenous LPC concentrations in the assay (Okuyama et al., 1975). If all three fatty acids were to be released from the Sn-2 position, the fatty acyl CoA preference of the LPC acyltransferase would result in the preferential re-esterification of the 20:4 and 18:2 moieties. Thus, the in vitro fatty acyl CoA specificity of the LPC acyltransferase may account for the preferential reacylation of linoleate over oleate into PC and the low unesterified arachidonate content observed during the initial 10–30 minutes of in vivo ischemia.

A similar deacylation-reacylation cycle has been demonstrated during the stimulation of prostaglandin synthesis in several different cell types. In human amnion cells, the release of arachidonate from phospholipid during the initiation of human parturition is not accompanied by the accumulation of lysophosphatidylethanolamine or by a net decrease in total phospholipid content (Okita et al., 1982). As the arachidonate content is decreased, there is a corresponding increase in the content of more saturated fatty acids in phospholipid. A similar phospholipid deacylation-reacylation cycle has also been observed in Madin-Darby canine kidney cells stimulated with O-tetradecanoylphorbol-13-acetate (TPA) (Daniel et al., 1982).

The relative biological importance of the reacylation step in these models is not clear at the present time. However, one important result of this cycle is the release of arachidonate from membrane phospholipids without the net loss of total phospholipid content. As described previously, the depletion of membrane phospholipids can induce marked changes in cell membrane permeability to Ca++ (Chien et al., 1978, 1980) and is associated with the development of irreversible cell injury (Chien et al., 1978). In the present study, the temporal correlation between the net loss of PC content, the lack of continued fatty acid remodeling, and the accumulation of arachidonate, a fatty acid which usually is predominantly esterified into phospholipid, suggests that defective reacylation may contribute to a net decrease in phospholipid content with phospholipase A2 activation during myocardial ischemia. However, the present study does not quantitatively determine the relative contribution of defective reacylation to the degradation of phospholipids, and the accumulation of unesterified arachidonate during myocardial ischemia. Since the de novo synthesis of phospholipids is an ATP-dependent process, other biochemical mechanisms, apart from degradation of phospholipid, may contribute to the net loss of phospholipid during myocardial ischemia. Further work will be necessary to define the regulatory mechanisms of the PC deacylation-reacylation cycle in ischemic myocardium and its ultimate relationship to oxygen and substrate deprivation.

Note added in proof: Since the completion of this study, Shaikh et al. have also described the presence of a phosphatidylcholine deacylation-reacylation cycle in ischemic myocardium (J Mol Cell Cardiol 15: 171, 1983).

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Accumulation of unesterified arachidonic acid in ischemic canine myocardium. Relationship to a phosphatidylcholine deacylation-reacylation cycle and the depletion of membrane phospholipids.

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