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Lysophosphatidylcholine-induced arrhythmias and its accumulation in the rat perfused heart

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1 The tissue level of lysophosphatidylcholine (LPC) was determined in rat hearts perfused with a solution containing 5 μM LPC. The relationship between LPC accumulation and the severity of arrhythmias produced was examined.

2 The accumulation of LPC was dependent on the perfusion time and this accumulation was associated with the occurrence of severe arrhythmias. A positive correlation between the tissue LPC content and the arrhythmia score was found ($P < 0.01$).

3 No consistent alteration in total phospholipid, phosphatidylcholine or cholesterol content was found. This suggests that LPC-induced arrhythmias are not associated with alterations of major lipid components in the heart.

4 When severe arrhythmias occurred in the presence of LPC in the rat perfused heart, less than 2% of total tissue phospholipid was in the form of LPC.

5 The positive correlation between LPC accumulation and the occurrence of arrhythmias suggests a cause and effect relationship of LPC with cardiac arrhythmias in the rat perfused heart. However, in the ischaemic heart, other biochemical factors can contribute, to different degrees, to ischaemia-induced cardiac arrhythmias.

Introduction

In recent years, amphipathic metabolites such as lysophospholipids have been suggested to have an important role in membrane dysfunction in the ischaemic myocardium (Katz & Messineo, 1981; Corr *et al.*, 1984). Lysophosphatidylcholine (LPC), a major lysophospholipid, has been shown to accumulate during myocardial ischaemia (Shaikh & Downar, 1981; Corr *et al.*, 1982; Man *et al.*, 1983; Steenbergen & Jennings, 1984). Exogenous LPC caused a variety of electrophysiological alterations in isolated cardiac tissues (Corr *et al.*, 1979; Arnsdorf & Sawicki, 1981). In perfused hearts of the hamster and rat, LPC produced cardiac arrhythmias (Man & Choy, 1982). The incorporation of a small amount of exogenous LPC (using radiolabelled LPC) in canine Purkinje fibres caused marked electrophysiological abnormalities (Gross *et al.*, 1982). However, the time course of accumulation of LPC under their experimental conditions and the relationship with the occurrence of electrophysiological alterations was not known. Since LPC-induced arrhythmias in the rat perfused heart were concentration- and time-dependent (Man & Lederman, 1985), the present study was designed to examine the time course of accumulation of exogenous LPC and the relationship between LPC accumulation and the occurrence of cardiac arrhythmias in the rat perfused heart.

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Methods

Perfusion of the rat heart

Long Evans rats (250–300 g) were anaesthetized with ether. The heart was rapidly excised and placed in cool modified Krebs-Henseleit solution aerated with 95% O_2:5% CO_2. The solution had the following composition (in mM): NaCl 118, NaH_2PO_4 1.18, $MgSO_4$ 1.18, KCl 4.7, $CaCl_2$ 1.8, $NaHCO_3$ 26.3 and glucose 11.2. The aorta was cannulated for coronary perfusion. The heart was allowed to beat spontaneously. The perfusion flow rate was adjusted to provide a mean pressure of 60–70 mmHg, and once stabilized, the flow rate was maintained constant for the duration of

the experiment. The temperature of the perfusate was kept at 37 ± 0.5°C. The electrocardiographic recording and perfusion pressure were monitored continuously. The detailed methodology of the isolated heart perfusion system has been described previously (Man & Lederman, 1985).

Hearts were perfused for 10, 15, 20 or 30 min with a solution containing 5 μM LPC. After the appropriate perfusion time, 1 ml of normal buffer solution was perfused via the aortic cannula to eliminate LPC in the vascular space. The heart was then blotted dry and weighed. The heart was used immediately for lipid extraction. To serve as controls, hearts were excised and used for lipid extraction (0 perfusion time) or perfused for 15 or 30 min using normal oxygenated Krebs-Henseleit solution.

In order to categorize changes observed in the electrocardiographic recordings, a scoring system was devised as follows: 0 = no arrhythmia, 1 = occasional ectopic beats, 2 = frequent ectopic beats and/or decrease in signal amplitude and rise in perfusion pressure, 3 = ventricular fibrillation or cessation of contraction. A 20 s interval was used to assess the arrhythmia score. The numerical score just before the end of the perfusion time for each heart was used to represent the severity of the arrhythmias.

Tissue analysis

Lipids were extracted from the tissue with a neutral solvent system. This extraction procedure has been shown to provide 95–96% recovery of LPC (Mock *et al.*, 1984). The tissue was homogenized in 10 vol (w/v) of chloroform/methanol (1:2, v/v) and extracted twice with the same solvent. The extracts were pooled and a biphasic mixture was obtained by the addition of 0.12 M KCl to provide a solvent mixture of $CHCl_3$/CH_3OH/H_2O (2:1:0.8, v/v). The lower phase was removed and evaporated under reduced pressure. The lipid extract was reconstituted in a small volume of chloroform/methanol (2:1) and was used immediately for lipid analysis. The phospholipids in the lipid extract were separated by thin layer chromatography in a solvent containing chloroform/methanol/water/acetic acid (75:60:8:10, v/v). The phospholipids on the thin layer chromatographic plates were visualized by iodine vapour and identified by comparison with phospholipid standards. Lipid phosphorus was determined by the method of Bartlett (1958).

The dry weight of the tissue was determined by complete dehydration in an oven at 100°C under reduced pressure. Total phospholipid content was assessed by the method of Raheja *et al.* (1973) and total cholesterol was measured by enzymatic assay with cholesterol oxidase (Allain *et al.*, 1974). Protein concentration was determined according to the method of Lowry *et al.* (1951).

Materials

LPC prepared from egg yolk was obtained from Sigma Chemical Company. Phosphatidylcholine and LPC standards were products of Serdary Research Laboratories. Thin-layer chromatographic plates (SIL-G25) were obtained from Brinkmann. All other chemicals were of reagent grade and were obtained from Fisher Chemical Company.

Statistical analysis

Values are expressed as mean ± s.d. Analysis of variance and Student's t test for paired or unpaired data were used where appropriate. A P value <0.05 was considered significant.

Results

The changes in LPC content of the rat perfused hearts are summarized in Table 1. The LPC content increased after 10, 15, 20 and 30 min of perfusion with 5 μM LPC. However, we noticed an increase in tissue water content in hearts perfused with LPC. The results of the tissue water determinations of hearts perfused with normal buffer and LPC containing buffer are summarized in Table 2. There was no significant change in the tissue water content (expressed as dry to wet weight ratio and tissue water to dry weight ratio) of control rat hearts after different perfusion durations. After 15 min of perfusion in the presence of 5 μM LPC, the tissue water content significantly increased as compared to the control groups. At 20 min, the tissue water was significantly higher than the control groups as well as after 10 min in the presence of LPC. By 30 min of perfusion with LPC, the tissue water content was significantly higher than that in all other groups.

In order to correct for the changes in tissue water content, the results in Table 1 are also expressed as μmol LPC g^{-1} dry wt. There was no significant change in the LPC content of control hearts which were perfused up to 30 min with normal perfusate. After 10 min of perfusion with 5 μM LPC, the increase in LPC content was significantly different from the 0 and 15 min control groups. After 15 min perfusion with LPC, the increase in LPC content significantly surpassed all control groups. There were further significant increases in LPC content after 20 and 30 min of perfusion with 5 μM LPC.

The severity of arrhythmias was scored according to the criteria described in the Methods section. No arrhythmia was observed in the control groups. In Figure 1, it is evident that the severity of the arrhythmias, expressed as the arrhythmia score, increased with the elevation in LPC content of the rat hearts. Regression analysis showed a positive correlation

Table 1 Lysophosphatidylcholine (LPC) content of rat perfused hearts

Time (min)	*LPC* (μmol g^{-1} wet wt)	*LPC* (μmol g^{-1} dry wt)	n
Control			
0	0.124 ± 0.038	0.58 ± 0.18	6
15	0.150 ± 0.051	0.68 ± 0.23	6
30	0.205 ± 0.088	0.92 ± 0.46	10
Perfusion with 5 μM LPC			
10	0.278 ± 0.063**	1.33 ± 0.30*	5
15	0.332 ± 0.076**§	1.68 ± 0.38**§	6
20	0.415 ± 0.076**	2.24 ± 0.41**	7
30	0.410 ± 0.126**¶	2.53 ± 0.78**¶	8

*$P < 0.05$, **$P < 0.01$ when compared with control value at 0 time.
§ $P < 0.01$ when compared with control value at 15 min.
¶ $P < 0.01$ when compared with control value at 30 min.

between the arrhythmia score and the tissue level of LPC in all hearts perfused for various time periods with 5 μM LPC ($P < 0.01$).

In addition, the effect of 1 μg ml^{-1} timolol (3.5×10^{-6} M), a concentration known to block β-adrenoceptors, on LPC-induced arrhythmias was tested. The presence of timolol in the perfusate 10 min before and during the perfusion with a buffer containing 5 μM LPC did not affect the arrhythmia scores after 10, 20 and 30 min of perfusion (arrhythmia scores 1.2 ± 1.0, 2.4 ± 0.6 and 3.0 ± 0 respectively, $n = 5$).

The alterations in total phospholipid, phosphatidylcholine and cholesterol contents of rat perfused hearts are summarized in Table 3. After 30 min of perfusion in the presence of 5 μM LPC, there was a small but significant increase in total phospholipid content when compared to all other groups perfused with LPC. The increase in phosphatidylcholine after 30 min perfusion with 5 μM LPC was not significantly different from the 30 min control group. However, the increase in total cholesterol content of 30 min LPC-perfused hearts was significantly different from the 30 min control group. In addition, there was a decrease in cholesterol content of the 15 min control group.

Discussion

Lysophospholipids have been implicated in the pathogenesis of cardiac arrhythmias in the ischaemic myocardium (Corr *et al.*, 1984). This is based on the findings that lysophospholipids such as LPC have been shown to accumulate during myocardial ischaemia (Shaikh & Downar, 1981; Corr *et al.*, 1982; Man *et al.*, 1983; Steenbergen & Jennings, 1984) and exogenous addition of LPC can produce electrophysiological abnormalities (Corr *et al.*, 1979, Arnsdorf & Sawicki, 1981). However, little information is

Table 2 Tissue water contents of rat perfused hearts

Time (min)	*g dry wt:g wet wt*	*g H_2O:g wet wt*	n
Control			
0	0.213 ± 0.004	3.70 ± 0.12	6
15	0.219 ± 0.004	3.56 ± 0.17	6
30	0.223 ± 0.005	3.52 ± 0.37	4
Perfusion with 5 μM LPC			
10	0.209 ± 0.005	3.79 ± 0.16	4
15	0.198 ± 0.004*§	4.08 ± 0.35*§	6
20	0.185 ± 0.005**	4.41 ± 0.17**	4
30	0.162 ± 0.003**¶	5.19 ± 0.32**¶	10

*$P < 0.05$, **$P < 0.01$ when compared with control value at 0 time.
§ $P < 0.01$ when compared with control value at 15 min.
¶ $P < 0.01$ when compared with control value at 30 min.

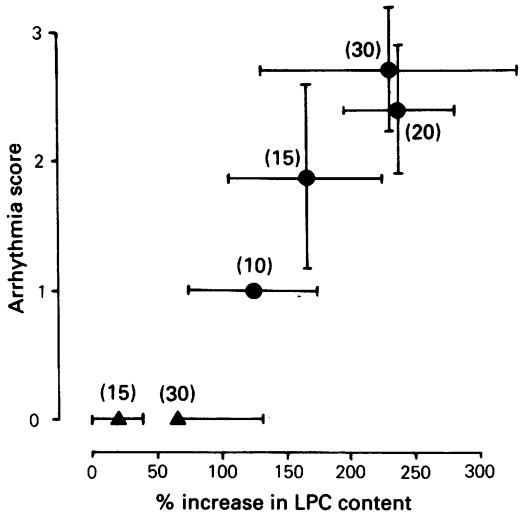

Figure 1 The relationship between arrhythmia score and the percentage increase in lysophosphatidylcholine (LPC) content of control and LPC perfused hearts of the rat. The percentage increase in LPC was determined by comparison with the LPC content of the control group with 0 perfusion time. (▲) Represents control hearts and (●) represents hearts perfused with a buffer containing 5 μM LPC. Numbers in parentheses represent perfusion time in min. Values are presented as mean ± s.d.

available regarding a direct relationship between LPC accumulation and cardiac arrhythmias. The present study explores the temporal relationship between the accumulation of LPC and the severity of cardiac arrhythmias. Our results showed a positive correlation between the tissue level of LPC and the arrhythmia score.

It has been found that electrophysiological derangements observed in superfused canine Purkinje fibres correlated with the incorporation of radiolabelled LPC (Gross *et al.*, 1982). Furthermore, the recovery of action potential characteristics was associated with a decrease in radiolabelled LPC due to the further metabolism of LPC. The incorporation of LPC and the electrophysiological derangements occurred within 10 min. However, other time points were not investigated in this study (Gross *et al.*, 1982). In the present study, substantial accumulation of LPC from the perfusate was detected in the rat heart after 10 min. The concentration of LPC in our perfusate was 5 μM compared to 100–200 μM used by Gross *et al.*, (1982). In spite of the 20–40 times lower concentration of LPC in our study, a significant accumulation of LPC was observed after 10 min of perfusion in the rat heart. This suggests a faster rate of incorporation of LPC must have occurred in the rat perfused heart. This is most likely due to the faster delivery of LPC in the rat heart compared to the delivery of LPC in superfused Purkinje fibres. Alternatively, species differences may also account for some of the discrepancies.

Lysophospholipids such as LPC can be reacylated in the mammalian heart to form phosphatidylcholine, the parent compound of LPC. Alternatively, LPC can be hydrolyzed by lysophospholipase into glycerol phosphocholine and fatty acids (Savard & Choy, 1982). Hence the accumulation of LPC in the present study represented the total uptake of LPC reduced by the amount of LPC metabolized. The simple procedure of measuring the uptake of radiolabelled LPC to represent the tissue accumulation of LPC was therefore not used. Instead, the various lipid fractions

Table 3 Total phospholipid, phosphatidylcholine and cholesterol content of rat perfused hearts

Time (min)	*Total phospholipid* (μmol g^{-1} dry wt)	*Phosphatidylcholine* (μmol g^{-1} dry wt)	*Total cholesterol* (mg g^{-1} dry wt)
Control			
0	130.6 ± 6.1	51.7 ± 7.5	9.45 ± 0.20
15	128.5 ± 2.3	47.4 ± 8.7	6.20 ± 1.05§
30	121.7 ± 14.4	48.1 ± 9.4	8.63 ± 1.26
Perfusion with 5μM LPC			
10	142.2 ± 27.3	42.1 ± 4.3	10.48 ± 4.79
15	130.0 ± 6.8	53.6 ± 9.1	10.58 ± 0.76
20	134.1 ± 5.4	47.6 ± 7.6	9.41 ± 1.73
30	162.8 ± 13.0*	56.8 ± 6.2	12.04 ± 2.66§

Values were obtained from 4 to 8 separate experiments.
* $P < 0.05$ when compared with all other groups perfused with LPC.
§ $P < 0.05$ when compared with all groups.

were separated by thin layer chromotography after extraction. The appropriate phospholipids were identified with phospholipid standards before quantification.

Although there is a positive correlation between LPC accumulation and the occurrence of cardiac arrhythmias in the rat perfused heart, it is premature to infer that the same relationship exists during myocardial ischaemia. There are many well documented biochemical alterations, especially the accumulation of long chain acyl carnitine in the ischaemic tissue, that can interact with the effect of LPC (Corr *et al.*, 1984). All these factors will ultimately contribute, to different degrees, to ischaemia-induced cardiac arrhythmias. It is also possible that LPC-induced arrhythmias may involve the release of noradrenaline in the perfused heart. This possibility was investigated by using timolol, a β-adrenoceptor blocker that does not possess membrane stabilizing properties. Our results suggest that LPC-induced arrhythmias in the perfused heart do not require the stimulation of β-adrenoceptors.

In the present study, no consistent alteration in total phospholipid, phosphatidylcholine and cholesterol contents were found after various periods of perfusion with a perfusate containing 5 μM LPC. Hence, the results indicate that LPC-induced arrhythmias do not require any alteration of major lipid components in the heart in order for them to be elicited. It is also clear that LPC in less than 2% of total tissue phospholipid in the rat heart is associated with the production of severe arrhythmias. In this regard, it has also been shown that the incorporation of as little LPC as 1% of tissue phospholipid (calculated by the incorporation of [^{14}C]-LPC) in canine Purkinje fibres and ventricular muscles induced marked electrophysiological alterations (Gross *et al.*, 1984).

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