The catabolism of plasmenylcholine in the guinea pig heart

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The hydrolysis of the alkenyl bonds of plasmenylcholine and plasmenylethanolamine by plasmalogenase, followed by hydrolysis of the resultant lysophospholipid by lysophospholipase, has been postulated as the major pathway for the catabolism of these plasmalogens. However, the postulation was based solely on the presence of plasmalogenase activity towards plasmenylethanolamine and plasmenylcholine in the brain. In this study we have demonstrated the absence of plasmalogenase activity for plasmenylcholine in the guinea pig heart under a wide range of experimental conditions. Plasmenylcholine was hydrolysed by phospholipase A₂ activities in cardiac microsomal, mitochondrial and cytosolic fractions. Phospholipase A₂ activities in these fractions had an alkaline pH optimum and were enhanced by Ca²⁺. The enzymes also displayed high specificity for plasmenylcholine with linoleoyl or oleoyl at the C-2 position. Lysoplasmalogenase activity for lysoplasmenylcholine was also detected and characterized in the microsomal and mitochondrial fractions. Since the cardiac plasmalogenase is only active towards plasmenylethanolamine but not plasmenylcholine, the catabolism of these two plasmalogens must be different from each other. We postulate that the major pathway for the catabolism of plasmenylcholine involves the hydrolysis of the C-2 fatty acid by phospholipase A₂, and hydrolysis of the vinyl ether group of the resultant lysoplasmenylcholine by lysoplasmalogenase.

INTRODUCTION

Phospholipids with an O-alkenyl group at the C-1 (*sn*-1) position (plasmalogens) are widely distributed in mammalian organs, usually as choline and ethanolamine lipids [1]. In spite of their ubiquitous distribution, not much is known about their physiological function [2,3], although various hypothesis have been proposed. In addition to serving as structural components of cellular membranes, they may be involved in ion transport [2], function as membrane stabilizers [3], or serve as reservoirs for prostaglandin and thromboxane precursors [4].

Although plasmenylethanolamine is the major plasmalogen found in mammalian tissues, a substantial amount of plasmenylcholine is also present in most mammalian hearts [3,5]. The catabolism of plasmalogens is thought to involve the initial hydrolysis of the vinyl ether linkage at the C-1 position of the phospholipid by a plasmalogenase, followed by deacylation of the resultant lysophospholipid by a lysophospholipase [6]. The presence of plasmalogenase activity in the brain [7,8], and heart [9] towards plasmenylethanolamine have been reported. However, the pathway for the catabolism of plasmenylcholine in the heart is a matter of conjecture. It may follow the same pathway as plasmenylethanolamine, or it may be first deacylated by the action of phospholipase A₂, and subsequently by lysoplasmalogenase. The absence of plasmalogenase activity towards plasmenylcholine in the liver [10], and the identification of a lysoplasmalogenase activity [10] support the hypothesis that plasmenylcholine may be catabolized differently from plasmenylethanolamine. Since the heart is the only organ which contains a high

amount of plasmenylcholine [3,5,11], this study was conducted to delineate its major catabolic pathway in cardiac tissue.

MATERIALS AND METHODS

Materials

Oleic, linoleic, stearic, and arachidonic acids were obtained from NuChek Prep, Elysian, MN, U.S.A. [1-¹⁴C]Stearic acid (56.5 mCi/mmol), [1-¹⁴C]linoleic acid (51 mCi/mmol) and [9,10(n)-³H]oleic acid (4.8 Ci/mmol) were obtained from Amersham International. [5,6,8,9,11,14,15(n)-³H]Arachidonic acid (83.8 mCi/mmol) was purchased from NEN Research Products. Pig liver lysophosphatidylcholine was obtained from Serdary Research Laboratories. Anhydrous tetrahydrofuran, metallic sodium, dimethylsulphoxide and 1,1'-carbonyldiimidazole were the product of Aldrich Chemicals. Sucrose, Tris/HCl, Tris/succinate, EDTA, EGTA, NAD⁺, GSH, aldehyde dehydrogenase, sodium deoxycholate, sodium taurocholate, sodium taurodeoxycholate, Tween-20, Triton X-100, Triton QS 15, dicetyl phosphate, dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide were purchased from Sigma. Silicic acid (Bio-Sil A) was obtained from Bio-Rad. All other chemicals and solvents were of reagent grade and were obtained from Fisher Scientific.

Preparation of subcellular fractions

Male guinea pigs were decapitated and their hearts excised and placed on ice. Seven to ten hearts were homogenized with two 30 s bursts at 4 °C in a Polytron homogenizer at a setting of 6 in 0.25 M-sucrose/10 mM-

Abbreviation used: GPC, *sn*-glycero-3-phosphocholine.

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Tris/HCl (pH 7.4)/1 mM-EDTA. The homogenate was centrifuged twice at 3000 *g* for 10 min. The resultant supernatant was centrifuged twice at 20000 *g* for 10 min. The pellet was resuspended in homogenizing buffer without EDTA and formed the mitochondrial fraction. The supernatant collected from the final 20000 *g* centrifugation was recentrifuged at 100000 *g* for 60 min. The pellet (microsomes) was suspended in the same buffer as the mitochondria. The supernatant obtained from the 100000 *g* centrifugation formed the cytosolic fraction. All fractions were stored at -20°C . Protein was estimated in each fraction by the method of Lowry *et al.* [12].

The purity of the subcellular fractions were assessed by marker enzymes. Fumarase [13] and succinate dehydrogenase [14] activities were used as mitochondrial markers. Glucose-6-phosphatase [15], 5'-nucleotidase [16] and K^{+} -stimulated *p*-nitrophenylphosphatase [16] activities were employed as microsomal markers. From the determination of these enzyme activities in the subcellular fractions, the microsomal fraction was contaminated with 5% of mitochondrial material, whereas the mitochondrial fraction contained 9% of microsomal material. The cytosolic fraction contained less than 1% of the mitochondrial or microsomal enzymes.

Preparation of plasmenylcholine

Lipids were extracted from bovine and canine hearts according to the method of Folch *et al.* [17]. The solvent in the lipid sample was evaporated under reduced pressure, the residue was redissolved in chloroform and applied to a silicic acid column. Fractions were eluted as described by Sheltawy & Dawson [18] and they were analysed by t.l.c. as previously described [9]. Fractions containing phosphatidylcholine were pooled and re-purified by preparative t.l.c. using a solvent system of chloroform/methanol/water/acetic acid (35:15:2:1, by vol.). Choline plasmalogens were obtained from the purified choline glycerophospholipids by mild alkaline hydrolysis [19], and the resultant lysoplasmenylcholine was purified by silicic acid column chromatography as described above and the purity was checked by t.l.c. The alkenyl content was measured by the method of Gottfried & Rapport [20] and the phosphorus content was measured by the method of Bartlett [21].

Synthesis of plasmenylcholine containing radiolabelled fatty acids

1-Alkenyl-GPC was prepared from plasmenylcholine by the method of Renkonen [19]. The lysoplasmalogen was purified by silicic acid column chromatography and synthesis of plasmenylcholine was carried out essentially as described by Hermetter & Paltauf [22]. Radiolabelled fatty acids were used as precursors for the synthesis and butylated hydroxytoluene was added to protect the unsaturated double bonds in the acyl groups after the reaction was terminated. The product was extracted three times with chloroform/methanol (2:1, v/v) and 0.9% KCl was added to the combined extracts to give a ratio of chloroform/methanol/water (4:2:3, by vol.) to achieve a clear phase separation. The lower phase was washed again by the addition of methanol and 0.9% KCl. The product in the lower phase was further purified by silicic acid column chromatography and its purity was assessed by t.l.c. The alkenyl and phosphate contents of the products were measured as described above and an

aliquot of the sample was taken for radioactivity determination by scintillation counting.

Enzyme assays

Plasmalogenase activity was measured by three different methods.

Assay A. Plasmalogenase activity was determined by following the rate of substrate disappearance [23]. Purified choline plasmalogen (2.6 μmol) was dispersed in 1 ml of water by sonication until clear. The reaction mixture (1.5 ml) contained 200 mM-Tris buffer, 347 μM -choline plasmalogen and 1 mg of protein (subcellular fraction). The assay was incubated at 37°C for 60 min and the reaction was stopped by the addition of 6 ml of chloroform/methanol (2:1, v/v). The upper and lower phases were obtained by centrifugation and aliquots of the lower layer (750 μl) were removed for the analysis of the alkenyl groups by the method of Gottfried & Rapport [20].

Assay B. Plasmalogenase activity was assayed by indirect determination of the aldehydes formed. Enzyme activity was monitored by the appearance of NADH which was produced from a coupled reaction with aldehyde dehydrogenase as described previously [9]. Plasmenylcholine or plasmenylethanolamine was dispersed by sonication and was used as substrate, and the reaction was initiated by the addition of the enzyme preparation (up to 750 μg) of each subcellular fraction.

Assay C. Plasmalogenase activity was assayed with labelled plasmenylcholine which was synthesized by the method described above. The production of labelled lysophosphatidylcholine in the reaction mixture after incubation was determined; 1.5 mg of protein from the subcellular fractions was used in each assay, and the assay conditions were similar to that described for phospholipase A_2 activity below.

Phospholipase A_2 . Phospholipase A_2 activity was assayed routinely in a mixture containing 100 mM-Tris buffer, 2% deoxycholate or 2 mM-lysophosphatidylcholine, 0.8 mM radiolabelled plasmenylcholine, 1.5 mg of protein and other additives as indicated in the text and Figures. The final volume of the reaction mixture was 0.5 ml, and the reaction was initiated by the addition of the enzyme sample. After 60 min of incubation at 37°C , the reaction was terminated by the addition of 3 ml of chloroform/methanol (2:1, v/v), followed by 1 ml of 0.9% KCl. The mixture was centrifuged, and the labelled fatty acid released from the reaction (in the lower phase) was separated from the remaining substrate by t.l.c. The t.l.c. plates were developed in chloroform/methanol/water/acetic acid (35:15:2:1, by vol.) until the solvent was 10 cm from the origin. The plates removed, dried and rechromatographed in heptane/isopropyl ether/acetic acid (15:10:1, by vol.). Complete separation of neutral and phospholipid classes was obtained by this method. The lipid fractions on the t.l.c. plate were visualized by I_2 vapour and those corresponding to phosphatidylcholine, lysophosphatidylcholine, diacylglycerol and fatty acid were removed from the t.l.c. plate, and the radioactivity associated with these fractions were determined by scintillation counting as described previously [24]. For each assay, a separate assay blank (without the

addition of enzyme) was performed in which the labelled substrate was treated in an identical manner as the actual assay. Phospholipase A₂ activity was calculated from the production of labelled fatty acid.

Lysoplasmalogenase. A spectrophotometric assay similar to that reported for plasmenylethanolamine [9] was developed to measure lysoplasmalogenase activity in subcellular fractions. The enzyme activity was routinely measured at 37 °C with 120 mM-Tris/HCl buffer (pH 8.5), 120 mM-KCl, 7 mM-GSH, 8.3 mM-NAD⁺, 1 unit of aldehyde dehydrogenase, 0.2% deoxycholate, 2 μ M-rotenone, 416 μ M-lysoplasmenylcholine (5.7 μ mol of lysoplasmalogen was sonicated in 1 ml of water to give a stock solution) in a total volume of 1.2 ml. The contents of the reaction mixture were added sequentially and mixed after each addition. The reaction was initiated by addition of the enzyme to the sample cuvette. The increase in A_{340} was followed spectrophotometrically. Controls were set up to monitor the specificity of the reaction as described previously [9].

RESULTS

Our initial approach was to identify the presence of a plasmalogenase activity for plasmenylcholine in the guinea pig heart, similar to that reported for plasmenylethanolamine in the hamster heart. Plasmalogenase activity in guinea pig heart homogenates, cytosol, mitochondria and microsomes was monitored by measuring the loss of substrate (assay A) with up to 3 mg of protein, between pH 5 and 10, in the presence or absence of EGTA and EDTA (2 mM) and Ca²⁺ or Mg²⁺ (0–10 mM). No plasmalogenase activity was detected under any of these experimental conditions. The more sensitive assay B was then utilized for this study under the same conditions with up to 1 mg of protein. Again, no plasmalogenase activity was detected. As a positive control, plasmalogenase activity in the guinea pig heart subcellular fractions towards plasmenylethanolamine was assayed. The results indicated that plasmalogenase activity towards plasmenylethanolamine was indeed present in guinea pig heart microsomes and activity could be detected with as little as 10 μ g of microsomal protein.

Radiolabelled plasmenylcholine with oleic acid at the C-2 position was utilized in a final attempt to confirm the absence of plasmalogenase activity towards plasmenylcholine. The use of this substrate provided a means to study not only plasmalogenase activity but also phospholipase A₂ and C activities towards the substrate. Since the action of plasmalogenase activity would yield labelled lysophosphatidylcholine which would be susceptible to hydrolysis by lysophospholipases, unlabelled lysophosphatidylcholine was initially added to the reaction to prevent the loss of labelled lysophosphatidylcholine [25]. In later experiments deoxycholate was utilized to inhibit the lysophospholipase, since it was found to be equally effective as lysophosphatidylcholine. When subcellular fractions of the guinea pig heart were incubated with plasmenylcholine labelled at the C-2 position with [³H]oleic acid at pH 7.4 and 8.5, with or without chelators and cations, there was no production of labelled lysophosphatidylcholine. However, labelled fatty acid was produced, indicating the presence of phospholipase A₂ activity. In all subcellular fractions, the reaction was found to be most active at pH 8.5 and in the presence of

Fig. 1. Effect of Ca²⁺ and Mg²⁺ on phospholipase A₂ hydrolysis of plasmenylcholine

Phospholipase A₂ activity in guinea pig heart microsomes (●), mitochondria (▲) and cytosol (■) was measured in the presence of Ca²⁺ (closed symbols), and Mg²⁺ (open symbols). The reaction mixture consisted of 100 mM-Tris/HCl buffer, pH 8.5, 2% deoxycholate, 0.8 mM-1-alkenyl-2-[³H]oleoyl-GPC and 1.5 mg of protein. Incubation was at 37 °C for 1 h. Enzyme activity is defined as nmol of fatty acid released/h per 1.5 mg of protein. Each point in the Figure is the mean value of three individual sets of experiments, each of which was determined in duplicate. The S.D. of each point is less than 10% of the mean value.

Ca²⁺. Thus the effect of Ca²⁺ and Mg²⁺ on the phospholipase A₂ activity towards plasmenylcholine in all three subcellular fractions was studied, and the results obtained are shown in Fig. 1. Although phospholipase A₂ activity in all subcellular fractions did not have an absolute requirement for cations, the enzyme activity was greatly enhanced by the addition of cations, especially Ca²⁺. Only an insignificant amount of radioactivity was found in the diacylglycerol fraction under all circumstances. Incubations were also carried out in the presence of 0.2% taurocholate, taurodeoxycholate, Tween-20, Triton X-100, Triton QS-15, dodecyltrimethylammonium bromide and 10 mM-GSH. Plasmalogenase activity was not observed under any of these conditions. This study clearly showed that plasmalogenase for plasmenylcholine was not present in guinea pig heart subcellular fractions. It appears that the majority of plasmenylcholine was catabolized by phospholipase A₂ and not by phospholipase C.

The effects of pH and metallic cations on the catabolism of plasmenylcholine were further studied. Labelled plasmalogen was incubated with the various subcellular fractions at pH 5–9 in the presence of 2 mM-EGTA or 1 mM-Ca²⁺ (Fig. 2). Again there was no production of labelled lysophosphatidylcholine under these conditions. In the presence of EGTA, activities of the mitochondrial and cytosolic phospholipase A₂ activities were not greatly affected by pH, but the microsomal activity showed a significant peak at pH 8.5. In the presence of Ca²⁺, phospholipase A₂ from all three fractions showed a definite pH optimum at alkaline pH

Fig. 2. Effect of pH on phospholipase A_2 hydrolysis of plasmenylcholine

The hydrolysis of 1-alkenyl-2- $[^3H]$ oleoyl-GPC by guinea pig heart microsomes (●), mitochondria (▲) and cytosol (■) was determined at different pH values in the presence of 1 mM- Ca^{2+} (closed symbols) or 2 mM-EGTA (open symbols). The buffers (100 mM) used were Tris/succinate (pH 5–6) and Tris/HCl (pH 7–9). The assay conditions are as described in Fig. 1. Enzyme activity is defined as nmol of fatty acid released/h per 1.5 mg of protein. Each point in the Figure is the mean value of two individual sets of experiment, each of which was determined in duplicate. The S.D. of each point is less than 10% of the mean value.

Fig. 3. Double-reciprocal plots of phospholipase A_2 activities versus 1-alkenyl-2- $[^3H]$ oleoyl-GPC concentration

The double reciprocal plots of microsomal (●), mitochondrial (▲) and cytosolic (■) phospholipase A_2 hydrolysis of plasmenylcholine are shown in the Figure. Conditions for the assay are described in Fig. 1. Each point is the mean value of two individual sets of experiment, each of which was determined in duplicate. The S.D. values of all points in the Figure are less than 10% of the mean values. The lines were fitted by least-square analysis.

Table 1. Acyl specificity of guinea pig heart phospholipase A_2 hydrolysis of plasmenylcholine

The reaction mixture consisted of 100 mM-Tris/HCl buffer, pH 8.5, 2% deoxycholate, 0.8 mM labelled plasmenylcholine, 1 mM- Ca^{2+} and 1.5 mg of protein. Incubation was at 37 °C for 1 h. Labelled fatty acid was separated from the mixture by t.l.c. as described in the Materials and methods section. The results represent the mean $\pm$ S.D. of the values obtained from three individual sets of experiments; each of which was determined in duplicate. Abbreviation: ND, not detectable.

Substrate	Phospholipase A_2 activity (nmol of fatty acid released/h per 1.5 mg of protein)		
	Microsomes	Cytosol	Mitochondria
1-Alkenyl-2- $[^{14}C]$ stearoyl-GPC	1.38 $\pm$ 0.11	ND	ND
1-Alkenyl-2- $[^3H]$ oleoyl-GPC	4.38 $\pm$ 1.24	2.54 $\pm$ 0.28	2.20 $\pm$ 0.46
1-Alkenyl-2- $[^{14}C]$ linoleoyl-GPC	3.80 $\pm$ 1.37	2.07 $\pm$ 0.14	2.18 $\pm$ 0.56
1-Alkenyl-2- $[^3H]$ arachidonyl-GPC	2.85 $\pm$ 0.84	1.20 $\pm$ 0.26	1.41 $\pm$ 0.52

(8.5). The highest activity was associated with the microsomal fraction, followed by the cytosol and lastly the mitochondrial fractions. Phospholipase A_2 activities in the mitochondrial, microsomal and cytosolic fractions were characterized with respect to protein concentration and time. Enzyme activity was linear with up to 2 mg of protein and 60 min of incubation.

The apparent K_m of the phospholipase A_2 activities in the three subcellular fractions towards plasmenylcholine with oleic acid at the C-2 position were determined, and the results shown in Fig. 3. All three enzymes had an identical K_m value of 5 mM. The acyl specificity of the phospholipase A_2 activities towards plasmenylcholine with stearic, oleic, linoleic and arachidonic acid at the C-2 position was also investigated (Table 1). No plasmalogenase activity was detected with any of the substrates employed and the highest phospholipase A_2 activity with

each substrate was always associated with the microsomal fraction. 1-Alkenyl-2-stearoyl-GPC was the least preferred substrate, followed by 1-alkenyl-2-arachidonyl-GPC. The highest enzyme activities were obtained with 1-alkenyl-2-oleoyl-GPC and 1-alkenyl-2-linoleoyl-GPC.

The catabolism of lysoplasmenylcholine formed from the hydrolytic action of phospholipase A_2 on plasmenylcholine was investigated. Lysoplasmalogenase activity has been reported in rat liver [10] and brain [26], but the enzyme has not been described in any mammalian heart. Hence, attempts were made to detect and quantify this enzyme in guinea pig heart subcellular fractions. Lysoplasmalogenase activity was found in the cardiac microsomal and mitochondrial fractions, but not in the cytosol. The enzyme activity was only detected in the presence of certain detergents (Table 2), and the highest enzyme activity was obtained with sodium deoxycholate.

Table 2. Effects of detergents on lysoplasmalogenase activity towards lysoplasmenylcholine

Lysoplasmalogenase activity was measured with 70 μ g of guinea pig microsomal protein in the presence of 0.2% detergent as described in the Materials and methods section. The results represent the mean values from two individual sets of experiment, each of which was determined in duplicate.

Detergent	Charge	Lysoplasmalogenase activity (nmol of substrate/h per mg of protein)
None		0
Deoxycholate	Anionic	640 ± 35
Taurocholate	Anionic	549 ± 21
Taurodeoxycholate	Anionic	0
Dicetyl phosphate	Anionic	375 ± 27
Tween 20	Non-polar	0
Triton X-100	Non-polar	535 ± 23
Triton QS-15	Zwitterionic	145 ± 13
Dodecyltrimethylammonium bromide*	Cationic	0
Hexadecyltrimethylammonium bromide*	Cationic	0

* These detergents inhibited the coupling enzyme (aldehyde dehydrogenase) used in the assay (see the text).

Fig. 4. Effect of deoxycholate concentration on the hydrolysis of lysoplasmenylcholine by guinea pig heart microsomal lysoplasmalogenase

Lysoplasmalogenase activity towards lysoplasmenylcholine was assayed with 70 μ g of microsomal protein as described in the Materials and methods section. Enzyme activity is defined as nmol of substrate hydrolysed/h. Each point is the mean value of three individual sets of experiments, each of which was determined in duplicate. The vertical bars represent the S.D.

Fig. 5. Effect of Ca²⁺ and Mg²⁺ on the hydrolysis of lysoplasmenylcholine by guinea pig heart microsomal lysoplasmalogenase

The hydrolysis of lysoplasmenylcholine by guinea pig heart microsomes (40 μ g of protein) was measured in the presence of Ca²⁺ (●) and Mg²⁺ (○). Enzyme activity is defined as nmol of substrate hydrolysed/h. Each point is the mean value of three individual sets of experiment, each of which was determined in duplicate. The vertical bars represent the S.D.

At present, the effectiveness of cationic detergents in the detection of lysoplasmalogenase activity in guinea pig heart subcellular fraction is not known since these detergents inhibited the coupling enzyme (aldehyde dehydrogenase) used in the assay. The dependence of enzyme activity on deoxycholate concentration is shown in Fig. 4. Further characterization revealed that the enzyme had a pH optimum around 8.5 and enzyme activity was highly temperature-sensitive. Enzyme activity at 37 °C was about 3-fold higher than that at 25 °C, and the temperature effect was shown to be independent of the coupled reaction. Lysoplasmalogenase activity did not have an absolute requirement for cations, although both Ca²⁺ and Mg²⁺ caused an enhancement of activity (Fig. 5).

DISCUSSION

This study is the first attempt to delineate a complete route for the catabolism of plasmenylcholine in a mammalian heart. The results show conclusively that the major pathway for the catabolism of plasmenylcholine in the guinea pig heart proceeds via hydrolysis of the fatty acyl group at the C-2 position, to yield lysoplasmenylcholine, which can be further hydrolysed by a lysoplasmalogenase. The lysoplasmenylcholine formed can also be reacylated to the parent phospholipid by 1-alkenyl-GPC:acyl-CoA acyltransferase, and the identification and characterization of this enzyme is described in the

accompanying paper [27]. The major route for plasmacylcholine catabolism in the guinea pig heart is different from the one proposed for the catabolism of both choline and ethanolamine plasmalogens in the brain, which involves the initial hydrolysis by a plasmalogenase, and subsequently by a lysophospholipase [6]. The absence of plasmalogenase activity towards plasmacylcholine in the canine heart [28] and the hamster heart (G. Arthur & P. C. Choy, unpublished work) lead us to postulate that mammalian hearts do not have the ability to initiate the direct hydrolysis of the alkenyl bond in plasmacylcholine.

Phospholipase A_2 activity in all three subcellular fractions showed some preferences for unsaturated acyl groups at the C-2 position of plasmacylcholine. Plasmacylcholine with oleoyl or linoleoyl moieties at the C-2 position were more readily hydrolysed by the phospholipases than were those with arachidonoyl groups. Similar observations have been reported for phospholipase A_2 in fat cell homogenates [29], rat brain mitochondria and human cerebral cortex [30]. The hydrolysis of plasmacylcholine by cardiac phospholipase A_2 has recently been studied in the canine heart [28]. Comparison between the guinea pig and the canine heart enzymes revealed that they differed substantially in pH profiles, K_m of substrate and response in the presence of Ca^{2+} . The canine heart enzyme displayed a neutral pH optimum and was inhibited by Ca^{2+} , whereas the guinea pig enzyme was most active at alkaline pH, and activity was enhanced by Ca^{2+} . It appears that the control of phospholipase A_2 activity for plasmacylcholine catabolism may be species-specific.

One major requirement to demonstrate that the majority of plasmacylcholine is first catabolized by deacylation is the positive identification of enzymes which can further metabolize the lysoplasmacylcholine formed, since there is no accumulation of lysoplasmacylcholine in the guinea pig heart. In the accompanying paper [27], we have provided evidence for the presence of an acyltransferase which is specific for the reacylation of lysoplasmacylcholine. In the present study, we are the first to show that cardiac tissue has the ability to hydrolyse the vinyl ether bond of lysoplasmacylcholine; the enzyme was located in both microsomal and mitochondrial fractions. It is interesting to note that the enzyme has an absolute requirement for detergent; however, its mode of action on the lysoplasmalogenase activity in the heart is not clear. The type of detergent did not appear to be of fundamental importance in the expression of enzyme activity since activity was obtained with anionic as well as neutral and zwitterionic detergents. It is quite possible that the enzyme was located exclusively on the non-cytoplasmic side of the membrane. Upon homogenization, the enzyme would be found on the inside of the membrane vesicles and enzyme activity was latent until the vesicles were disrupted by detergents. Studies are necessary to determine the topology of the lysoplasmalogenase in guinea pig heart microsomes.

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REFERENCES

1. Mangold, H. C. (1972) in *Ether Lipids: Chemistry and Biology* (Snyder, F., ed.), pp. 157–176, Academic Press, New York
2. Gross, R. W. (1984) *Biochemistry* **23**, 158–165
3. Horrocks, L. A. & Sharma, M. (1982) in *Phospholipids* (Hawthorne, J. N. & Ansell, G. B., eds.), pp. 51–93, Elsevier Biomedical Press, Amsterdam
4. Horrocks, L. A. & Fu, S. C. (1978) *Adv. Exp. Med. Biol.* **101**, 397–406
5. Diagne, A., Fauvel, J., Record, M., Chap, H. & Douste-Blazy, L. (1984) *Biochim. Biophys. Acta* **793**, 221–231
6. Snyder, F. (1985) in *Biochemistry of Lipids and Membranes* (Vance, D. E. & Vance, J. E., eds.), pp. 271–298, Benjamin/Cummings
7. Ansell, G. B. & Spanner, S. (1965) *Biochem. J.* **94**, 252–258
8. D'Amato, R. A., Horrocks, L. A. & Richardson, K. E. (1975) *J. Neurochem.* **24**, 1251–1255
9. Arthur, G., Covic, L., Wientzek, M. & Choy, P. C. (1985) *Biochim. Biophys. Acta* **833**, 189–195
10. Warner, H. R. & Lands, W. E. M. (1961) *J. Biol. Chem.* **236**, 2404–2409
11. Horrocks, L. A. (1972) in *Ether Lipids: Chemistry and Biology* (Snyder, F., ed.), pp. 177–272, Academic Press, New York
12. Lowry, O. H., Roseborough, M. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
13. Hill, R. C. & Bradshaw, R. A. (1969) *Methods Enzymol.* **13**, 96–99
14. Pennington, R. J. (1961) *Biochem. J.* **80**, 649–654
15. Morre, J. D. (1971) *Methods Enzymol.* **22**, 130–148
16. Bers, D. M. (1979) *Biochim. Biophys. Acta* **555**, 131–146
17. Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
18. Sheltawy, A. S. & Dawson, R. M. C. (1969) in *Chromatographic and Electrophoretic Techniques* (Smith, I., ed.), vol. 1, pp. 453–493, Heinmann, London
19. Renkonen, O. (1963) *Acta. Chem. Scand.* **17**, 634–640
20. Gottfried, E. L. & Rapport, M. M. (1962) *J. Biol. Chem.* **237**, 329–333
21. Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
22. Hermetter, A. & Paltauf, F. (1982) *Chem. Phys. Lipids* **30**, 47–53
23. Dorman, R. V., Freysz, L., Mandell, P. & Horrocks, L. A. (1977) *J. Lipid Res.* **18**, 115–117
24. Arthur, G. & Choy, P. C. (1984) *Biochim. Biophys. Acta* **795**, 221–229
25. Tam, S. W., Man, R. Y. K. & Choy, P. C. (1984) *Can. J. Biochem. Cell Biol.* **62**, 1269–1274
26. Gunawan, J. & Debuch, H. (1982) *J. Neurochem.* **39**, 693–699
27. Arthur, G. & Choy, P. C. (1986) *Biochem. J.* **236**, 481–487
28. Wolf, R. A. & Gross, R. W. (1985) *J. Biol. Chem.* **260**, 7295–7303
29. Woelk, H. (1974) *Biochem. Biophys. Res. Commun.* **59**, 1278–1285
30. Woelk, H., Goracci, G. & Porcellati, G. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* **355**, 75–81