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2-Acyl-*sn*-glycero-3-phosphoethanolamine lysophospholipase A_2 activity in guinea-pig heart microsomes

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We have recently described a lysophospholipase A_2 activity in guinea-pig heart microsomes that hydrolyses 2-acyl-*sn*-glycero-3-phosphocholine (2-acyl-GPC). The presence of a similar activity that hydrolyses 2-acyl-*sn*-glycero-3-phosphoethanolamine (2-acyl-GPE) was not known. In this study, a lysophospholipase A_2 activity in guinea-pig heart microsomes that hydrolyses 2-acyl-GPE has been characterized. The enzyme did not require Ca^{2+} for activity and exhibited a high specificity for 2-arachidonoyl-GPE and 2-linoleoyl-GPE over 2-oleoyl-GPE and 2-palmitoyl-GPE. The specificity for these unsaturated substrates was observed in the presence and absence of detergents. Selective hydrolysis of 2-arachidonoyl-GPE over 2-palmitoyl-GPE was observed when equimolar quantities of the two substrates were incubated with the enzyme. There was no preferential hydrolysis of either 2-linoleoyl- or 2-arachidonoyl-GPE when presented individually or as a mixture. Significant differences in the characteristics of 2-acyl-GPE-hydrolysing and 2-acyl-GPC-hydrolysing activities included differences in their optimum pH, the effect of Ca^{2+} and their acyl specificities. Taken together, these results suggest that the two activities are catalysed by different enzymes. 2-Acyl-GPE lysophospholipase activity with a preference for 2-arachidonoyl-GPE over 2-oleoyl-GPE was observed in guinea-pig brain, liver, kidney and lung microsomes. Lysophospholipase A_1 activity that catalyses the hydrolysis of 1-acyl-GPE was also present in guinea-pig heart microsomes and had different characteristics from the 2-acyl-GPE-hydrolysing activity, including a preference for saturated over unsaturated substrates. The 2-acyl-GPE lysophospholipase A_2 activity appeared to be distinct from Ca^{2+}-independent phospholipase A_2. The characteristics of the 2-acyl-GPE lysophospholipase A_2 suggest it could play a role in the selective release of arachidonic and linoleic acids for further metabolism in cells.

INTRODUCTION

Many cells and tissues respond to physiological and pharmacological stimuli by metabolizing arachidonic acid and other fatty acids via the cyclo-oxygenase, lipoxygenase and epoxygenase pathways. The rate-limiting step in these metabolic responses is the selective release of the precursor fatty acids from the glycerophospholipids to which they are esterified [1]. The involvement of both Ca^{2+}-dependent and Ca^{2+}-independent pathways in the release mechanism has been implicated [1–6]. The Ca^{2+}-dependent pathways are generally accepted to involve phospholipase A_2 activity. The nature of the Ca^{2+}-independent routes are not known. We have suggested that one possible Ca^{2+}-independent pathway could be the initial hydrolysis of a phospholipid to form a 2-acyl-glycerophospholipid which is subsequently hydrolysed by a lysophospholipase A_2 to release the fatty acid [7]. We recently reported the presence of a lysophospholipse A_2 in guinea-pig heart microsomes that hydrolysed 2-acyl-*sn*-glycero-3-phospholcholine (2-acyl-GPC) with a high specificity for 2-linoleoyl- and 2-arachidonoyl-GPE [7]. The 2-acyl-GPEs are expected to be produced from phospholipase A_1 hydrolysis of phosphatidylcholine. In the guinea-pig heart, the majority of arachidonic acid is esterified at the C-2 position of the ethanolamine phospholipids, phosphatidylethanolamine and ethanolamine plasmalogen [8]. 2-Arachidonoyl-*sn*-glycero-3-phosphoethanolamine (2-arachidonoyl-GPE) can therefore be produced from phospholipase A_1 hydrolysis of phosphatidylethanolamine or plasmalogenase hydrolysis of ethanolamine plasmalogen. The presence of a 2-acyl-GPE-hydrolysing lysophospholipase A_2 activity has not been reported in the heart or any other tissue. The presence of such an activity is essential if the proposed pathway is involved in the selective release of fatty acids from ethanolamine phospholipids. In view of the high content of arachidonate in the ethanolamine glycerophospholipid fraction, in guinea-pig heart microsomes, such an enzyme if present may be expected to show a high specificity for arachidonate. In this study we report the presence of 2-acyl-GPE-hydrolysing activity in guinea-pig heart microsomes with characteristics distinct from those of other lysophospholipases in the tissue.

MATERIALS AND METHODS

1-Acyl-2-[^{14}C]arachidonoyl-GPE, 1,2-di[^{14}C]palmitoyl-GPE, 1-palmitoyl-2-[^{14}C]linoleoyl-GPE, 1-palmitoyl-2-[^{14}C]linoleoyl-GPE, 1,2-di[^{14}C]oleoyl-GPC and [1-^{14}C]oleoyl-CoA were obtained from Amersham International. [1-^{14}C]Arachidonoyl-CoA was purchased from Du Pont, Mississauga, Canada. 1,2-Dioleoyl-GPE, 1,2-dipalmitoyl-GPE, Triton QS-15, sodium taurocholate, Triton X-100 and phospholipase A_2 (*Crotalus adamanteus*) were from Sigma Chemical Co., St. Louis, MO, U.S.A. 1,2-Diarachidonoyl-GPE and 1,2-dilinoleoyl-GPE were from Avanti Polar Lipids, Birmingham, AL, U.S.A. 1-Acyl-2-lyso-GPC, 1,2-dioleoyl-GPC and 1-palmitoyl-2-lyso-GPE were from Serdary Research Laboratories, London, Ontario, Canada. Guinea pigs and Sprague–Dawley rats were purchased from Charles River, Quebec, Canada. Ion-exchange resins CM-52 and DEAE-Sepharose were products of Whatman and Pharmacia respectively. Silicic acid (Bio Sil A) was purchased from Bio-Rad, Montreal, Canada. Triacylglycerol lipase from *Rhizopus arrhizus* was purchased from Boehringer Mannheim. Arachi-

Abbreviations used: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine.
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donoyl chloride and linoleoyl chloride were purchased from Nu Chek Prep, Elysian, MN, U.S.A. All other chemicals, t.l.c. plates and solvents (reagent grade) were from Baxter–Canlab, Winnipeg, Canada.

Subcellular fractionation

Guinea pigs and rats (250–300 g) were killed and the necessary organs were removed and placed on ice. Microsomes were prepared by differential centrifugation and the purity of the fractions was monitored as previously described [8]. Six to eight guinea-pig hearts were combined for the preparation of microsomes for use in each experiment. The protein content of each microsomal fraction was determined by the method of Lowry, with bovine serum albumin as standard [9].

Preparation of 2-acyl-GPE

Radiolabelled 1,2-dioleoyl-GPE was synthesized from 1,2-dioloeyl-GPC by transphosphatidation using the method of Comfurius & Zwaal [10]. Other labelled phosphatidylethanolamines were purchased and the lysophospholipids were prepared by selective hydrolysis of the parent phosphatidylethanolamine with triacylglycerol lipase (*Rhizopus arrhizus*). The reaction mixture contained 4 μmol of PE dissolved in 1.2 mM-sodium deoxycholate, 2 mmol of Tris/HCl buffer, pH 7.4, and 30 μmol of $CaCl_2$ in a total volume of 6 ml. The reaction was initiated with the addition of 5 units of lipase, and the incubation was carried out at 37 °C for 30 min. The reaction was terminated by the addition of 12 ml of chloroform/methanol (2:1, v/v). After mixing, the two phases were separated and the solvents in the lower layer were evaporated. The extracted lipids were redissolved in chloroform and applied to a CM-cellulose column. The phospholipids were eluted from the column according to the procedures described by Comfurius & Zwaal [10]. The purified substrates were characterized as previously described [7,11] using guinea-pig-pancreas phospholipase A_1 and also by acylating the substrates and selectively hydrolysing the C-2 acyl group with phospholipase A_2 (*Crotalus adamanteus*). The results demonstrated conclusively that the substrates used in these studies were 2-acyl-GPE. The substrates were stored at −20 °C and used within 24 h of preparation.

Preparation of 1-acyl-GPE

1-Oleoyl-GPE and 1-arachidonoyl-GPE were prepared by a procedure similar to that of Waite & Van Deenen [12]. 2-Acyl-GPE prepared by the procedures described above was acylated with radiolabelled acyl-CoA using the rat-liver microsomal acyltransferases to catalyse the reaction. The assay consisted of 0.02 M-KH_2PO_4 buffer, pH 7.1, 6 mM-ATP, 0.01 M-NaF, 5 mM-$MgCl_2$, 0.92 mM radiolabelled acyl-CoA and 0.6 mM-2-acyl-GPE. Incubation was carried out at 37 °C for 1 h. The reaction products were extracted by phase separation and the phosphatidylethanolamine was separated by CM-cellulose column chromatography. The fractions containing phosphatidylethanolamine were combined and hydrolysed by phospholipase A_2 (*Crotalus adamanteus*). Radiolabelled 1-acyl-GPE was then purified by column chromatography on CM-cellulose columns. [1-^{14}C]Palmitoyl-GPE was prepared from 1,2-di[^{14}C]palmitoyl-GPE as described by Hanahan *et al.* [13].

Synthesis of arachidonoyl-CoA and linoleoyl-CoA

Arachidonoyl-CoA and linoleoyl-CoA were synthesized from the respective acyl chlorides by the method of Reitz & Lands [14].

Lysophospholipase assays

Lysophospholipase activity was measured by monitoring the release of radiolabelled fatty acids from either 1-acyl-GPE or 2-acyl-GPE. Assay mixtures to determine lysophospholipase A_2 activity consisted of 100 mM-Tris-HCl, pH 8.5, 90 μg of guinea-pig heart microsomal protein and 200 μM-2-acyl-GPE in a total reaction volume of 0.5 ml. Lysophospholipase A_1 activity was measured in a reaction mixture of 100 mM-Tris/HCl, pH 8.0, 100 μg of guinea-pig heart microsomal protein and 125 μM-1-acyl-GPE in a total volume of 500 μl. Incubation tubes without any microsomal fraction were set up for each substrate under identical incubation conditions to monitor non-enzymic hydrolysis of the substrates. Incubation was at 37 °C for 10 min for lysophospholipase A_2 and 15 min for lysosphospholipase A_1. The reaction was terminated by the addition of 3 ml of chloroform/methanol (2:1, v/v) followed by the addition of 0.9% KCl (1 ml). The reaction products in the lower phase were separated by t.l.c. using heptane/isopropyl ether/acetic acid (60:40:4, by vol.). The bands were revealed with I_2 vapour and the radioactivity associated with the fatty acid band was determined by scintillation counting on a Beckman LS 3801 counter. Significant differences in the specific activity of the lysophospholipase A_2 were sometimes obtained with different microsomal preparations. The reasons for this are not apparent and we have presented the results as is, without any normalization.

Phospholipase A_2 assays

Phospholipase A_2 activity was measured by determination of the release of radiolabelled fatty acids from 1-acyl-2-[^{14}C]arachidonoyl-GPE. The reaction mixture contained 200 μM of the substrate and 200 μg of guinea-pig heart microsomal protein, and incubation was for 10 min at 37 °C. The total reaction volume was 500 μl and the reaction was stopped by the addition of 3 ml of chloroform/methanol (2:1, v/v) followed by the addition of 1 ml of 0.9% KCl. The reaction products were analysed by t.l.c., as described for the lysophospholipase A_2 assays.

RESULTS

The incubation of guinea-pig heart microsomes with 2-[^{14}C]arachidonoyl-GPE resulted in the rapid release of [^{14}C]arachidonic acid. An optimum pH of 9.0 and an optimum substrate concentration of 150–300 μM were established for the assay of this activity, whereas experiments with 2-linoleoyl-GPE revealed an optimum substrate concentration of 50 μM. Higher concentrations of the substrate resulted in severe inhibition of the activity: with 100 μM-2-linoleoyl-GPE, the specific activity was 60% of that obtained with 50 μM. Other substrates did not exhibit any inhibition at higher concentrations. The rate of reaction was linear for up to 15 min with 90 μg of guinea-pig heart microsomal protein for all of the substrates. Hydrolysis of 2-arachidonoyl-GPE occurred in the presence of EDTA/EGTA, indicating that the lysophospholipase A_2 did not require Ca^{2+} for activity. On the contrary, as can be seen in Fig. 1, the activity was moderately inhibited by the presence of Ca^{2+} in the assay.

Since we were interested in determining the specificity of the enzyme for different molecular species of 2-acyl-GPE, we wanted to eliminate differences due to any differential solubilities of the substrates. The effect of the detergents Triton X-100, miranol DS, deoxycholate, taurocholate and Triton QS-15 on the hydrolysis of 2-arachidonoyl-GPE was therefore investigated (Table 1). All of the detergents (0.5%) except Triton QS-15 severely inhibited the hydrolysis of the substrate by the lysophospholipase A_2. Even at a concentration of 1% this detergent did not inhibit the enzyme activity (results not shown). A concentration of 0.5% Triton QS-15 was selected for subsequent assays.

Table 1. Effect of detergents on guinea-pig heart lysophospholipase A_1 and A_2 activities

Guinea-pig heart microsomal lysophospholipase A_1 and lysophospholipase A_2 activities were assayed with 1-palmitoyl-GPE and 2-arachidonoyl-GPE respectively. Detergents were added to the incubation mixture to give a final concentration of 0.5%. Other reaction conditions are described in the Materials and methods section. Identical incubations without any detergents served as controls. The values represent the means ± S.D. of two experiments, each of which was conducted in triplicate. The specific activity of lysophospholipase A_1 in the absence of detergent was 82 nmol of fatty acid/h per mg of protein, and the specific activity of lysophospholipase A_2 was 452 nmol of fatty acid/h per mg of protein.

Detergent	Lysophospholipase activity (% of control)	
	A_1	A_2
None	100	100
Miranol DS	84±20	26±4
Triton X-100	33±12	7±0.1
Taurocholate	29±6	18±2
Triton QS-15	430±62	184±22

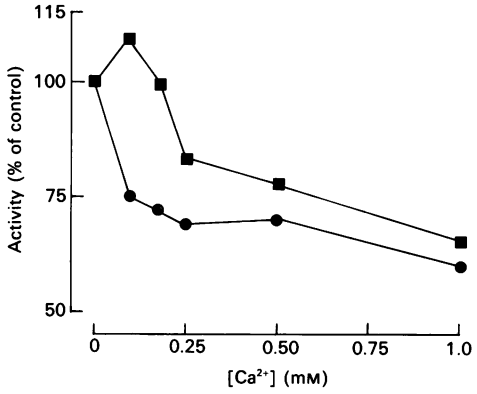

Fig. 1. Effect of Ca^{2+} on the 1-acyl-GPE- and 2-acyl-GPE-hydrolysing activities in guinea-pig heart microsomes

Lysophospholipase A_1 activity (■) was assayed with 1-palmitoyl-GPE (62.5 μM) and lysophospholipase A_2 activity (●) was assayed with 2-arachidonoyl-GPE (200 μM). The reaction conditions used for each activity are described in the Materials and methods section. The results are expressed as a percentage of the activities obtained in the presence of 2 mM-EGTA (control). The values are the means of two separate experiments, each done in triplicate. The control value for lysophopholipase A_1 activity was 85 nmol of fatty acid/h per mg of protein, and the control value for lysophospholipase A_2 was 401 nmol of fatty acid/h per mg of protein.

The specificity of guinea-pig heart microsomal lysophospholipase A_2 for different molecular species was determined next. Because of the substrate inhibition displayed with 2-linoleoyl-GPE, these experiments were conducted at two different concentrations, one at an optimum concentration for 2-arachidonoyl-GPE (200 μM), and the other at the optimum concentration for 2-linoleoyl-GPE (50 μM). With 200 μM of each substrate in the absence of Triton QS-15, the rate of hydrolysis of 2-arachidonoyl-GPE was 10- and 4-fold greater than the hydrolysis of 2-palmitoyl- and 2-oleoyl-GPE respectively (Table 2). When the experiments were repeated in the presence of 0.5% Triton QS-15, 2-arachidonoyl-GPE was hydrolysed 20- and 7-fold faster than were 2-palmitoyl and 2-oleoyl-GPE respectively. The hydrolysis of 2-palmitoyl-GPT and 2-oleoyl-GPE were not significantly affected by the presence of the detergent. Due to the severe inhibition of hydrolysis obtained with 2-linoleoyl-GPE at 200 μM (20% of activity obtained at 50 μM), it was inappropriate to compare the specific activity obtained with this molecular species with that of 2-arachidonoyl-GPE, since this would give a mistaken impression of high selectivity of the lysophospholipase for the latter substrate. Incidentally the substrate inhibition was also observed in the presence of Triton QS-15. When the acyl specificity was determined with 50 μM substrate in the absence of detergent, 2-arachidonoyl-GPE and 2-linoleoyl-GPE were hydrolysed at similar rates that were 6-fold greater than the rate of hydrolysis of 2-palmitoyl-GPE (Table 3). In the presence of 0.5% Triton QS-15, the hydrolysis rates of 2-linoleoyl-GPE and 2-arachidonoyl-GPE were again similar, and about 10-fold greater than the hydrolysis of 2-palmitoyl-GPE.

In order to determine if the enzyme exhibited any selectivity, we compared the hydrolysis of single substrates with the hydrolysis of the substrates when presented as mixtures. Two pairs of substrates were used in these experiments, 2-linoleoyl-GPE/2-arachidonoyl-GPE and 2-palmitoyl-GPE/2-arachidonoyl-GPE. The results obtained are displayed in Table 4. Incubation of the microsomes with a mixture of 2-palmitoyl-GPE and 2-arachidonoyl-GPE resulted in 46% and 56% inhibition of hydrolysis of each substrate respectively, compared with hydrolysis of the individual substrates. Although the inhibition of 2-palmitoyl-GPE hydrolysis of 2-arachidonoyl-GPE was not surprising, the inhibition of 2-arachidonoyl-GPE hydrolysis to a similar extent by 2-palmitoyl-GPE was unexpected, in view of the high specificity displayed by the enzyme for 2-arachidonoyl-GPE over 2-palmitoyl-GPE when the substrates were presented singly. We

Table 2. Acyl specificity of guinea-pig heart microsomal lysophospholipase A_2

Lysophospholipase A_2 activity in guinea-pig heart microsomes (90 μg) was assayed with different 2-acyl-GPEs (200 μM; sp. radioactivity 0.15–0.2 Ci/mol) in the presence and absence of Triton QS-15 (0.5%) in 100 mM-Tris/HCl, pH 8.5 (total volume 500 μl). In experiments with detergent, the substrate was prepared by sonication in detergent solution. Incubation was for 10 min at 37 °C and the reaction was terminated with chloroform/methanol (2:1, v/v). The reaction products were isolated by t.l.c. as described in the Materials and methods section. Values are the means ± S.D. of three experiments, each of which was done in triplicate.

Substrate	Specific activity (nmol of fatty acid/h per mg of protein)		Activity relative to 2-palmitoyl-GPE hydrolysis	
	−Triton QS-15	+Triton QS-15	−Triton QS-15	+Triton QS-15
2-Palmitoyl-GPE	56±7	51±5	1	1
2-Oleoyl-GPE	135±8	151±3	2.4	3
2-Arachidonoyl-GPE	566±19	1039±47	10	20

Table 3. Acyl specificity of guinea-pig heart microsomal lysophospholipase A_2 with 50 μM substrate

Lysophospholipase A_2 activity was assayed with 50 μM of various 2-acyl-GPEs. Other reaction conditions are as described in Table 2. The values are the means ± S.D. of three experiments, each of which was done in triplicate.

Substrate	Specific activity (nmol of fatty acid/h per mg of protein)		Activity relative to 2-palmitoyl-GPE hydrolysis	
	−Triton QS-15	+Triton QS-15	−Triton QS-15	+Triton QS-15
2-Palmitoyl-GPE	65±26	71±13	1	1
2-Linoleoyl-GPE	370±18	734±38	6	10
2-Arachidonoyl-GPE	377±42	694±15	6	10

Table 4. Effect of presenting mixtures of 2-acyl-GPEs on guinea-pig heart microsomal lysophospholipase A_2 activity

Lysophospholipase A_2 activity was assayed with 90 μg of guinea-pig microsomal protein and the following pairs of 2-acyl-GPEs: 2-[^{14}C]palmitoyl-GPE/2-arachidonoyl-GPE, 2-palmitoyl-GPE/2-[^{14}C]arachidonoyl-GPE, 2-[^{14}C]linoleoyl-GPE/2-arachidonoyl-GPE and 2-linoleoyl-GPE/2-[^{14}C]arachidonoyl-GPE. Equimolar amounts of each substrate were sonicated together, and aliquots containing 50 μM of each substrate were used in the assays. Experiments with 50 μM of each individual substrate were used as controls and the specific activities are reported in Table 3. The reaction conditions for the assay are described in the Materials and methods section. The values represent the means ± S.D. of two separate experiments, each of which was carried out in triplicate.

Pair of substrates	Specific activity (nmol fatty acid/h per mg of protein)	(% of control)
2-Palmitoyl-GPE and 2-Arachidonoyl-GPE	35±6	54
	165±14	44
2-Linoleoyl-GPE and 2-Arachidonoyl-GPE	207±9	56
	219±23	58

currently do not have any reasonable explanation for this observation. In spite of the mutual inhibition, hydrolysis of 2-arachidonoyl-GPE was 5-fold greater than that of 2-palmitoyl-GPE. The enzyme therefore displayed a selectivity for arachidonic acid over palmitic acid. With respect to the mixture of 2-linoleoyl-GPE and 2-arachidonoyl-GPE, both subtrates were inhibited to similar extents and therefore there was no selective hydrolysis of either substrate.

Preliminary experiments had indicated that the lysophospholipase A_2 activity was ubiquitously distributed in guinea-pig tissues. We therefore compared the rate of hydrolysis of 2-oleoyl-GPE with that of 2-arachidonoyl-GPE in the presence and absence of 0.5% Triton QS-15 (Table 5), having previously established that Triton QS-15 (0.5%) did not inhibit the activity of the enzyme in any of the tissues. In all of the tissues examined, the hydrolysis of 2-arachidonoyl-GPE was greater than the hydrolysis of 2-oleoyl-GPE in the presence and the absence of Triton QS-15. The highest activity was observed with brain microsomes. As was the case with the heart microsomes, the rate of hydrolysis of 2-arachidonoyl-GPE was increased by the detergent, whereas the hydrolysis of 2-oleoyl-GPE was not significantly affected.

Lysophospholipases catalysing the hydrolysis of 1-acyl-*sn*-glycero-3-phospholipids (lysophospholipase A_1) are present in the heart [7,15–17]. It was therefore of interest to determine whether this activity was distinct from lysophospholipase A_2. The guinea-pig heart microsomal lysophospholipase A_1 activity was characterized with 1-[^{14}C]palmitoyl-GPE as substrate. The pH optimum was 8 and maximum activity was obtained with substrate concentration between 45 and 150 μM. Substrate concentrations greater than 150 μM inhibited the activity. The rate of hydrolysis of the substrate was linear for 10 min with 100 μg of microsomal protein. The lysophospholipase A_1 activity was inhibited by Ca^{2+} (Fig. 1), taurocholate, deoxycholate, miranol DS and Triton X-100 (Table 1). At the same concentration of detergent, inhibition of the lysophospholipase A_1 by miranol DS, taurocholate and Triton X-100 was less severe than the inhibition of the lysophospholipase A_2. Activation of lysophospholipase A_1 activity by Triton QS-15 was much greater than the activation of

Table 5. Acyl specificity of lysophospholipase A_2 in different guinea-pig tissues

Lysophospholipase A_2 activity in the microsomal fractions prepared from guinea-pig heart, liver, brain, lung and kidney were measured with different species of 2-acyl-GPEs. The reaction conditions were identical to those described in Table 2. The values represent the means ± S.D. of three experiments, each of which was carried out in duplicate. QS-15, Triton QS-15.

Tissue	Substrate...	Specific activity (nmol of fatty acid/h per mg of protein)			
		2-Oleoyl-GPE		2-Arachidonoyl-GPE	
		−QS-15	+QS-15	−QS-15	+QS-15
Lung		63±14	61±38	267±78	487±98
Liver		53±28	55±16	180±28	219±29
Brain		165±129	220±87	650±15	1192±236
Kidney		54±16	50±14	141±32	155±15

Table 6. Acyl specificity of guinea-pig heart microsomal lysopohospholipase A_1

Guinea-pig heart microsomal lysophospholipase A_1 activity was measured with different species of 1-acyl-GPE (125 μM; sp. radioactivity 0.1 Ci/mol) with Triton QS-15 (0.5 %). All the other reaction conditions are as described in the Material and methods section. The values are the means ± S.D. of three experiments, each done in triplicate.

Substrate	Specific activity (nmol of fatty acid/h per mg of protein)	Activity relative to 2-arachidonoyl-GPE hydrolysis
Palmitoyl-1-GPE	394 ± 9	16
1-Oleoyl-GPE	45 ± 6	2
1-Arachidonoyl-GPE	24 ± 14	1

Table 7. Effect of Ca^{2+} and trifluoperazine on guinea-pig heart microsomal lysophospholipase A_2 and phospholipase A_2 activities

Lysophospholipase A_2 and phospholipase A_2 activities in guinea-pig heart microsomes were determined as described in the Materials and methods section with 2-arachidonoyl-GPE (200 μM) and 1-acyl-2-arachidonoyl-GPE (200 μM) as substrates. In experiments with trifluoperazine (TFP), the microsomes were incubated with the compound for 5 min at room temperature before the initiation of the assay. The results represent the means ± S.D. of two separate experiments conducted in triplicate. The activities are expressed as percentages of the control. In experiments with Ca^{2+} the controls had 2 mM each of EDTA and EGTA, while in experiments with TFP the controls were incubated without TFP.

Additive	Activity (% of control)	
	Phospholipase A_2	Lysophospholipase A_2
None (control)	100	100
Ca^{2+} (0.5 mM)	88 ± 3	65 ± 7
Ca^{2+} (1.0 mM)	94 ± 4	60 ± 10
TFP (100 μM)	101 ± 13	135 ± 37
TFP (500 μM)	76 ± 11	228 ± 27

lysophospholipase A_2. The acyl specificity of the lysophospholipase A_1 was determined in the presence of 0.5 % Triton QS-15 (Table 6). The results showed quite conclusively that the acyl specificity of the lysophospholipase A_1 was very distinct from that of the lysophospholipase A_2. The hydrolysis of 1-palmitoyl-GPE was 16 times greater than the hydrolysis of 1-arachidonoyl-GPE, and 8.7 times that of 1-oleoyl-GPE. The rate of hydrolysis of 1-palmitoyl-GPE by lysophospholipase A_1 was greater than the rate of hydrolysis of 2-palmitoyl-GPE by the lysophospholipase A_2.

Finally, it was important to provide evidence that the lysophospholipase A_2 activity was not due to unspecific activity of a Ca^{2+}-independent phospholipase A_2. We compared the effects of Ca^{2+} and trifluoperazine on the hydrolysis of 1-acyl-2-arachidonoyl-GPE by phospholipase A_2 and on the hydrolysis of 2-arachidonoyl-GPE by lysophospholipase A_2 (Table 7). Under the optimum conditions for lysophospholipase A_2 activity, the specific activity of the phospholipase A_2 activity was 33 nmol of fatty acid released/h per mg of protein. Phospholipase A_2 activity was not affected by 1 mM-Ca^{2+}, whereas the lysophospholipase A_2 activity was inhibited by 40 %. Trifluoperazine (500 μM) activated the lysophospholipase A_2 activity by 100 %, whereas the phospholipase A_2 activity was inhibited by 25 %. These data suggest that lysophospholipase A_2 and phospholipase A_2 in the guinea-pig heart microsomes are independent of each other.

DISCUSSION

The results of this study have demonstrated the presence of a lysophospholipase A_2 activity that hydrolyses 2-acyl-GPE in guinea-pig heart microsomes and which has a preference for substrates with unsaturated fatty acids. This activity was also present in guinea-pig brain, lung, kidney and liver. The lysophospholipase A_2 activity appears to be distinct from phospholipase A_2 and lysophospholipase A_1 activities in the guinea-pig heart microsomes. The preference of the lysophospholipase A_1 activity for 1-acyl-GPE substrates with saturated fatty acids has not been previously demonstrated as far as we are aware. This preference of lysophospholipase A_1 for substrates with saturated fatty acids and that of lysophospholipase A_2 for polyunsaturated fatty acids is consistent with the expected molecular composition of their intracellular substrates.

The characteristics of the 2-acyl-GPE lysophospholipase A_2 were significantly different from those of the recently described 2-acyl-GPC lysophospholipase A_2 in the same subcellular fraction [7]. The hydrolysis of 2-acyl-GPE was inhibited by Ca^{2+} and had a pH optimum of 9, whereas the hydrolysis of 2-acyl-GPC was not affected by Ca^{2+} and had a pH optimum of 8 [7]. Even though both enzymes had a preference for unsaturated substrates, the acyl specificities of the two activities were also significantly different. 2-Acyl-GPC lysophospholipase A_2 exhibited higher activities with 2-linoleoyl-GPC compared with 2-arachidonoyl-GPC at all concentrations examined [7]. In contrast, the 2-acyl-GPE lysophospholipase A_2 exhibited different optimal substrate concentrations with 2-linoleoyl-GPE and 2-arachidonoyl-GPE. At 50 μM, which was the optimum concentration where hydrolysis of both 2-linoleoyl-GPE and 2-arachidonoyl-GPE could be compared without any substrate inhibition, both substrates were hydrolysed at similar rates. Differences were also observed in the 2-acyl-GPE and 2-acyl-GPC-hydrolysing activities when mixed substrates were presented to the enzymes. The inhibition of 2-arachidonoyl-GPC hydrolysis by 2-palmitoyl-GPC was more severe than vice versa. Thus the selectivity for 2-arachidonoyl-GPC which would be predicted from the specific activities obtained with the individual substrates was abolished. In contrast, the inhibition of 2-arachidonoyl-GPE hydrolysis by 2-palmitoyl-GPE, and vice versa, were similar and relatively moderate. The end result was a selective release of arachidonic acid which was 5 times that of palmitic acid, as opposed to the 7-fold increase that would be predicted from the relative hydrolysis of the individual substrates. With mixtures of 2-linoleoyl-GPC and 2-arachidonyl-GPC, the expected selectively for 2-linoleoyl-GPC by the 2-acyl-GPC lysophospholipase A_2 was lost because of a greater inhibition of 2-linoleoyl-GPC hydrolysis by 2-arachidonoyl-GPC than vice versa. These results differed from those observed with the 2-acyl-GPE lysophospholipase A_2, which did not display a significant preference for either substrate whether presented singly or together.

The cellular role of lysophospholipases A_2 is as yet unclear, but we do not think that their major function is to regulate the levels of lysophospholipids in membranes. The fatty acids produced endogenously by lysophospholipase A_2 will be unsaturated, and their release could result in the synthesis of bioactive fatty acid metabolites such as eicosanoids, independent of the normal receptor-mediated mechanisms that control the synthesis of these compounds. It is well established that the rate-limiting factor in the synthesis of eicosanoids is the concentration

of non-esterified fatty acids [1]. Other lysophospholipid-metabolizing enzymes, such as acyltransferases and transacylases, are present in mammalian membranes [18].

Based on the characteristics of the 2-acyl-GPC lysophospolipase hydrolysing enzyme in guinea pig heart microsomes, we have previously suggested that lysophospholipases A_2 could act sequentially with phospholipase A_1 to release specific unsaturated fatty acids, independent of an increase in the cellular Ca^{2+} concentration, from cholineglycerophospholipids [7]). Our observations that 2-acyl-GPE lysophospholipase A_2 has a preference for 2-linoleoyl-GPE and 2-arachidonoyl-GPE in guinea-pig heart as well as a preference for 2-arachidonoyl-GPE over 2-oleoyl-GPE in other tissues are consistent with a role for this enzyme in the selective release of fatty acids from ethanolamineglycerophospholipids. Since 2-acyl-GPEs may be produced by both phospholipase A_1 and plasmalogenase hydrolysis of their respective substrates, such a pathway could allow for the selective release of fatty acids exclusively from one subclass. The characteristics of both the 2-acyl-GPC and 2-acyl-GPE lysophospholipases lead us to postulate that, if they are involved in a pathway to selectively release fatty acids, then they do not exclusively release one fatty acid at all times, but rather they act to release either arachidonate or linoleate and possibly other polyunsaturated fatty acids. The specificity of the release would be dependent on the selectivity of the phospholipase A_1 and plasmalogenase that generate the 2-acyl-glycerophospholipid from choline- or ethanolamine-glycerophospholipids. Ca^{2+}-independent phospholipase A_2 activities have been purified from the myocardium [19]; however, these have been cytosolic enzymes and their role in the selective release of unsaturated fatty acids for eicosanoid synthesis in normal tissue is uncertain.

In conclusion, we have demonstrated, in guinea-pig heart microsomes as well as the microsomes of other tissues, the presence of a lysophospholipase A_2 that hydrolyses 2-acyl-GPE with a preference for arachidonic and linoleic acids. The enzyme appears to be distinct from the 1-acyl-GPE- and 2-acyl-GPC-hydrolysing activities as well as ethanolamineglycero-phospholipid-hydrolysing Ca^{2+}-independent phospholipase A_2 activity in the same subcellular fraction, but unambiguous proof can only be obtained after purification and characterization of the various hydrolysing activities.

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