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INFLUENCE OF ALKYL CHAIN LENGTHS IN DIALKYLGLYCEROPHOSPHOCHOLINES TOWARDS PHOSPHOLIPASE A_2 INHIBITION IN MACROPHAGES

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Rat peritoneal macrophages were cultured with a specific and potent phospholipase A_2 activator A 23187, with 1-stearoyl-2-[^{3}H]arachidonoyl-sn-GPC as source of [^{3}H] arachidonic acid, and with a dialkyl-GPC, at 2, 10 or 20 μM. Four dialkyl-GPCs were prepared by chemical synthesis. Position 2 of rac-glycerol was alkylated with an alkane chain of 8 carbons and position 1 was alkylated with various alkane chains (8, 10, 12, or 16 carbons).

[^{3}H] arachidonic acid was split, then recovered with cell nonesterified fatty acids and nonphosphorous glycerolipids after endocellular phospholipase A_2 activity. It was also recovered with fatty acids and eicosanoids isolated from culture medium.

Inhibition of fatty acid release and eicosanoid synthesis depended on mixed chain dialkyl-GPC structures. The highest inhibitory effect on arachidonic acid release was reached with 1-decyl-2octyl-GPC and was practically as high in culture medium (IC_{50} at 5 μM) as in cells (IC_{50} at 4 μM). 1,2-di-octyl-GPC and 1-dodecyl-2-octyl-GPC had weaker inhibitory effects (but higher in culture medium than in cells). The asymmetrical 1-hexadecyl-2-octyl-GPC poorly affected enzyme activity.

KEY WORDS: Phospholipase A_2 inhibitors; macrophages; dialkylglycerophospho-cholines, chemical modifications

INTRODUCTION

Macrophages and other cells which are involved in inflammation, have active phospholipase A_2.[1–8] In acute inflammation, it is always necessary to lower arachidonic

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acid release from position 2 of phospholipids, since, once released, arachidonic acid becomes a substrate for eicosanoid synthesis. This acid is also generated from the molecular species 1-hexadecyl (or octadecyl)-2-arachidonoyl-GPC which, once in lyso structure, is acetylated to form the pro-inflammatory mediator Platelet Activating Factor.[9–11]

Unfortunately, various phospholipase inhibitors are toxic to cells and most of the irreversible but powerful inhibitors are eliminated from pharmacological applications.[12,13] Dialkyl-GPC which are analogues of diacyl-GPC and -GPE were found early inhibitors of secreted phospholipase A_2 of snake venoms and pancreatic juices.[14–18] In contrast, inhibition of phospholipase A_2 from macrophages by these molecules was found much latter.[19]

Phospholipid subclasses, in inflammatory cells including macrophages, are present in high amounts of 1-alkyl (alk-1′-enyl)-2-acyl-GPC or GPE.[20–23] About forty per cent of arachidonic acid is split by phospholipase A_2 from these ether linked subclasses, in ionophore A 23187 stimulated macrophages, lymphocytes or neutrophils.[24–27] For these reasons, it was necessary to define the structure of non or poorly hydrolysable phospholipids in order to inhibit the enzyme. Snake venoms and pancreatic juice phospholipase A_2 were strongly inhibited by various 1,2-di-alkyl-GPC and 1-alkyl 2-amido-GPC molecules with long alkane chains.[15–18] Macrophage endocellular phospholipase A_2 was found to be more inhibited with 1,2-di-hexadecyl-GPC than bromophenacylbromide.[19]

In this study four dialkyl-GPCs were synthesized with 8 carbons chain at position 2 of -rac-glycerol and, with chains with various length in position 1 i.e., 1-hexadecyl-2-octyl-GPC, 1-dodecyl 2-octyl-GPC, or 1-decyl-2-octyl-GPC, or 1,2-di-octyl-GPC to observe the influence of alkyl linked chain length. These amphipaths were water insoluble and suitable for recognition by phospholipase A_2, according to previously defined essential rules.[28] Enzyme inhibition by dialkyl-GPCs was followed by measures of [^{3}H] arachidonic acid release in rat peritoneal macrophages which were labelled with the molecular species 1-stearoyl 2-[^{3}H]arachidonoyl-GPC.

MATERIALS AND METHODS

Materials

Ionophore A 23187 and standard lipids were obtained from Sigma, USA. All other chemicals were provided from Aldrich, France. 1-Stearoyl-2-[5,6,8,9,11,12,14,15-^{3}H] arachidonoyl-sn-glycerophosphocholine, specific activity 5.22 T Bq/mmol, with a radiochemical purity of 98% was purchased from Amersham International, GB.

Melting points were measured on a Mettler FP 52 and are uncorrected. The IR spectra were obtained using a Bomem MB-100. Nuclear magnetic resonance (^{1}HNMR) spectra were recorded on a Bruker AC 250 spectrometer. High-resolution mass spectra were recorded on a HP 5889A quadripolar. Analytical thin layer (TLC) was performed on Merck 60F-254 silica gel plates. Preparative column chromatography was performed using Merck silica gel 60 (230–400 mesh).

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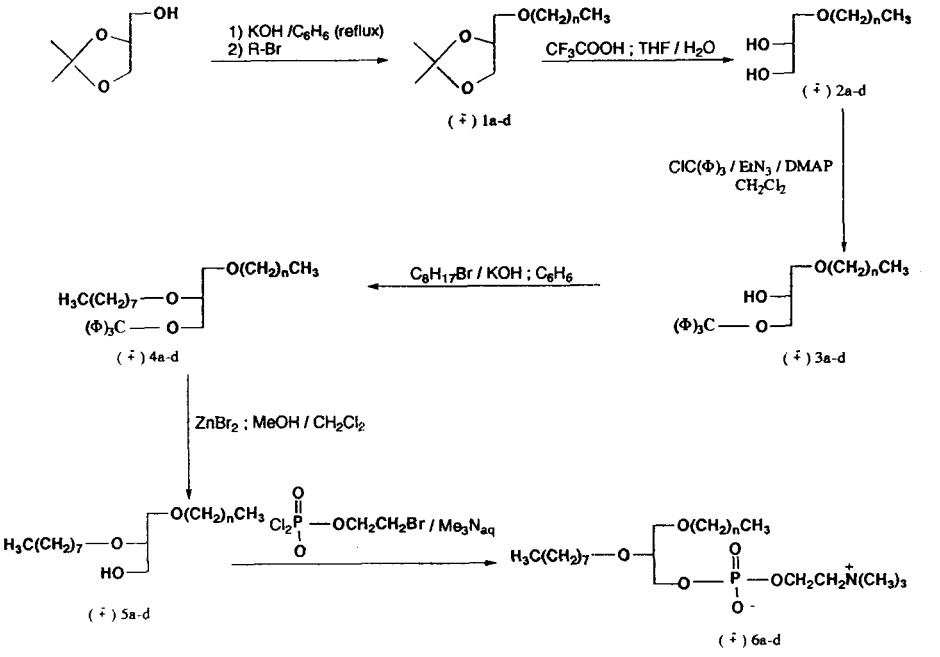

FIGURE 1 Synthesis.

Chemicals

Dialkylglycerophospholipid synthesis (Figure 1) A mixture of rac-1,2-isopropylideneglycerol (1 eq), alkylbromide (1.2 eq) and KOH (5 eq), in refluxing benzene, was stirred for 8h to generate[29] in quantitative yield 1-alkyl-2,3-isopropylideneglycerol (**1a–d**). The isopropylidene protecting group was removed by trifluoroacetic acid in tetrahydrofuran-water (1/1 v/v) at room temperature for 12h to obtain[30] (**2a–d**). Tritylation of these derivatives with an equal mixture of trityl chloride, triethylamine and dimethylaminopyridine in a stirring dichloromethane solution at room temperature for 12h gave (**3a–d**) (yield 88 %).[31] The 2-O-alkylation of glycerol occurred, after 12h with an excess of octylbromide and NaH in a refluxing tetrahydrofuran solution, to give (**4a–d.**) The trityl protecting group was removed with $ZnBr_2$-CH_3OH in dichloromethane, at room temperature for 5h to obtain 1,2-dialkylglycerol (**5a–d**) in quantitative yields.[32] These compounds were phosphorylated with a stirring mixture of 2-bromoethyldichlorophosphate (1.5 eq), triethylamine (4 eq) in dry chloroform at room temperature for 18h.[33,34] After hydrolysis of the resulting bromoethylphospholipid, the product was extracted with ether, washed with water, then with a sodium

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carbonate solution to give, after evaporation, the phosphate bromoethylester sodium salt. Subsequent treatment with 7 ml of an aqueous trimethylamine solution (45%) and 13 ml of chloroform-dimethylformamide-isopropanol (3:5:5) for 12h at 50°C, gave ^{35}phosphorylcholine derivatives (**6a–d**).

Cell isolation and cultures Macrophages were isolated after peritoneal lavages with 50 ml of sterile saline buffer, from male Wistar rats (250 g). After centrifugation, (350×g, 10 min), cell pellets were placed in Dulbecco's phosphate buffer pH 7.2 containing glucose (1 g/1) and after gentle agitation, in order to separate cells, aliquots were distributed in glass tubes. One hour incubation at 37°C was necessary to obtain adherent macrophages (2×10^6 cells/2 ml). Nonadherent cells were removed after 3 washings with Dulbecco's buffer.

Macrophages were cultured for 90 min either with 1-stearoyl 2-[^{3}H]-arachidonoyl-GPC alone (1000 Bq; concentration: 0.01 picomol.), dissolved in ethanol (5 μl) or with radioactive diacyl-GPC and a dialky-GPC at 2 or 10 or 20 μM, dissolved in ethanol (5 μl). During the last 30 min of incubation (60 to 90 min), cells received 10 μM of ionophore A 23187 (dissolved in 10 μl dimethylsulfoxide), in the medium. After incubation, a low speed centrifugation (350×g, 10 min) was used to separate medium from cell pellets.

Lipid analysis

Lipids from cells and medium were extracted with chloroform-methanol.[36] Before extraction, extracellular fluids (medium) were acidified at pH 3.5 with diluted formic acid. Aliquots were directly counted for radioactivity or applied on TLC plates. Lipid classes were separated after a run in toluene-dioxan-acetic acid-formic acid (85:15:0.5:0.5, v/v). Lipids from cells were phospholipids which remained at Rf 0.00, while diglycerides (DG) and monoglycerides (MG) ran from Rf 0.15 to 0.50, non-esterified fatty acids at Rf 0.80 and triglycerides (TG) at Rf 0.90. Lipids from the medium were phospholipids, nonesterified fatty acids and eicosanoids (Rf 0.05 to 0.60 : leukotrienes reached higher Rf values than prostaglandins but these molecules were not separated and were entitled "eicosanoids"). Triglycerides were not found except in traces and are not described in Table 3.

Lipid classes were visualized with iodine vapors which were then blown out and spots containing the various lipid classes were placed in flasks with 4 ml of scintillation liquid and 0.4 ml of distilled water for measures of radioactivity in the scintillation spectrophotometer LKB. Student's test was applied to the data.

RESULTS

2×10^6 macrophages were labelled with tritiated diacyl-GPC (1000 Bq, at 0.01 picomolar concentration in medium). About 90% of radioactivity was recovered in macrophage and medium lipids after 90 min of incubation. Total radioactive lipids in macrophages are listed on Tables 1 and 2. In Table 3, phospholipids, fatty acids and eicosanoids represent 99.5 to 99.7% of radioactivity. Triglyceride traces are not listed.

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TABLE 1
Radioactivity (Bq) in lipid classes of non activated macrophages after hydrolysis of 1-stearoyl-2-[^{3}H]arachidonoyl-GPC

	μM	n	total phospholipids	non esterified fatty acids	TG	DG and MG
Controls		21	534±27	42±5	31±4	8±2
1 -hexadecyl-2-octyl-GPC	2	3	538±21	40±7	29±7	7±2
	10	3	449±8	30±6	33±13	5±1
	20	3	296±17**	15±4	9±4	3.5±1.3
1-dodecyl-2-octyl-GPC	2	3	478±10	40±6	30±6	8±1
	10	3	454±14	25±8	22±4	4±1
	20	3	349±12*	13±2*	5±1**	4±0.5
1-decyl-2-octyl-GPC	2	3	547±23	32±4	29±4	8±1.5
	10	3	388±15*	12±1*	6±1.3*	3±0.7
	20	3	338±23**	9±1*	2±0.3**	3±0.2
1,2-dioctyl-GPC	2	3	424±25	34±6	33±6	9±2
	103	3	183±25**	17±2	8±0.2**	6±0.4
	20	4	242±44**	14±7*	9±2*	4±0.5

Means ± SEM (n= number of samples). *P < 0.05 compared to controls. **P < 0.01 compared to controls. 2×10^6 adherent macrophages/tube, in 2 ml Dulbecco's buffer were incubated (90 min) with the tritiated diacyl-GPC (1000 Bq) and with a dialkyl-GPC at 2 or 10 or 20 μM. The cells were not activated.

1-Tritiated diacyl-GPC hydrolysis in macrophages

Non activated macrophages (Table 1). More than half of the tritiated phospholipid (534 Bq) had reached cell lipids. Its hydrolysis was observed mainly in non esterified fatty acids (42 Bq), then in TG (31 Bq) and DG + MG (8 Bq) and reached 15.5%, as established by comparison with 534 Bq.

All dialkyl-GPCs lowered tritiated phospholipid uptake which was correlated with their micromolar concentration although effects depended of the molecule. 1-hexadecyl,2-octyl-GPC and 1-dodecyl 2-octyl-GPC were the less active and lowered uptakes only at 20 μM (respectively 296 and 349 Bq). These were followed by 1-decyl-2-octyl-GPC. The most efficent molecule was 1,2-di-octyl-GPC with 183 and 242 Bq at 10 and 20 μM respectively which were more than 50% lower compared to controls (534 Bq). In addition, tritiated non esterified fatty acids and triglycerides but not DG + MG were lowered in the presence of 3 dialkyl-GPC. The highest inhibitory effect on phospholipase activity was found with 1-decyl 2-octyl-GPC, at 10 and 20 μM : respectively 12 and 9 Bq on fatty acids and 6 and 2 Bq on triglycerides.

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TABLE 2
Radioactivity (Bq) in lipid classes of the macrophages after hydrolysis of 1-stearoyl-2-[^{3}H]arachidonoyl-GPC. The cells were activated with A 23187

	μM	n	total phospholipids	non esterified fatty acids	TG	DG and MG
A 23187		25	565±19	97±7	18±2	12±1
A 23187 +	2	3	549±21	101±4	19±1	12±2
1-hexadecyl-	10	3	414±36**	63±6	16±0.8	14±3
2-octyl-GPC	20	4	328±11**	37±6**	6±2**	5±2.3**
A 23187 +	2	3	508±38	76±5	15±1	12±2
1-dodecyl-2-	10	3	384±34**	35±4**	8±2*	10±1.5
octyl-GPC	20	3	413±33**	19±2**	5±0.6**	6±0.7*
A 23187 +	2	3	547±19	83±5	16±2	9±1.5
1-decyl-2-	10	3	317±51**	11±2**	3.5±0.8**	3.5±1**
octyl-GPC	20	3	335±7**	8±2**	1.5±0.5**	3±0.8**
A23187 +	2	3	468±36*	58±10	14±2	8±0.8
1,2-Dioctyl-GPC	10	3	216±6**	28±4**	7±1**	5±0.6*
	20	6	168±7**	16±3**	7±1**	9±1.3**

Means ± SEM (n= number of sample Δ). * $P < 0.05$ compared to A 23187. ** $P < 0.01$ compared to A 23187.

Macrophages activated by A 23187 (Table 2). Phospholipid uptake (565 Bq) was not different from control values reported in Table 1 (534 Bq). Hydrolysis of diacyl-GPC concerned mainly fatty acids (97 Bq), a value which was higher than 42 Bq in control experiments ($P < 0.01$, data not shown in Table 2) and to a lesser extent TG: 18 Bq ($P < 0.01$, data not shown in Table 2) or DG + MG 12 Bq (not different from controls in Table 1). Together, they reached 22.5% compared to 565 Bq. As observed in Table 2, all dialkyl-GPC lowered diacyl-GPC uptakes. The more efficient was 1,2-di-octyl-GPC at 10 and 20 μM (216 and 168 Bq compared to 565 Bq; this is about one third). Diacyl-GPC hydrolysis was lowered by dialkyl-GPC : at a low extent for DG + MG (except 1-decyl 2-octyl-GPC) but not for fatty acids and TG. The more efficient molecules were 1-decyl-2-octyl-GPC and 1,2-di-octyl-GPC. For example, the former, at 10 and 20 μM lowered fatty acids to, respectively, 11 and 8 Bq (compared to 97 Bq) and TG to 3.5 and 1.5 Bq (compared to 18 Bq).

2–Tritiated diacyl-GPC hydrolysis in culture medium (Table 3)

Culture medium from non activated macrophages. 155 Bq was found on phospholipids (1/4 of phospholipids found in macrophages), 33 Bq on fatty acids and 31 Bq on eicosanoids and their addition represented a little less than 50% of 155 Bq.

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TABLE 3
Radioactivity (Bq), after hydrolysis of 1-stearoyl-2-[^{3}H]arachidonoyl-GPC, in lipid classes of the medium from non activated macrophages and for macrophages which were activated with A 23187

	μM	n	Total phospholipids	Non esterified fatty acids	eicosanoids
Controls		21	155±11	33±4	31±3
	2	3	201±27	35±6	33±4
1-hexadecyl-2-octyl-	10	3	401±33**	32±7	34±8
GPC	20	3	550±21**	24±6	18±6
	2	3	248±17	33±6	32±4
1-dodecyl-2-octyl-GPC	10	3	418±30**	20±4	28±3
	20	3	454±28**	11±2*	19±2
	2	3	216±42	24±9	35±4
1-decyl-2-octyl-GPC	10	3	648±29**	9±2*	34±1
	20	3	457±23**	13±1*	30±5
	2	3	284±25**	29±7	29±6
1,2-di-octyl-GPC	10	3	612±64**	19±2	23±3
	20	4	529±62**	14±5	25±10
A23187		24	122±6	58±4	46±2
A23187 +	2	3	162±16	57±7	50±6
1-hexadecyl-2-octyl	10	3	366±20**	51±4	49±14
GPC	20	4	505±15**	49±8	32±5
A23187 +	2	3	222±10*	44±3	47±7
1-dodecyl-2-octyl-GPC	10	3	450±14**	28±4*	38±5
	20	3	462±23**	17±6*	23±2**
A23187 +	2	3	243±15**	44±2	43 ±6
1-decyl-2-octyl-GPC	10	3	620±42**	17±1**	40±5
	20	3	543±18**	9±1**	29±3*
A23187 +	2	3	290±20**	49±8	41±5
1,2-di-octyl-GPC	10	3	578±25**	36±2	37±2
	20	6	630±54**	13±4**	25±4**

Means ± SEM (n = number of samples). *$P < 0.05$ and **$P < 0.01$ for dialkyl-GPC compared to controls or A 23187 + dialkyl-GPC compared to A23187 in Tables 1 and 2. Experimental procedures as Tables 1 and 2.

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Diacyl-GPC which did not reach cells was found in greater proportions in the medium in the presence of the four dialkyl-GPCs at a concentration as low as 10 μM. Synthesis of eicosanoids were not modified by dialkyl-GPC. Fatty acids were lowered: 11 Bq with 1-dodecyl 2-octyl-GPC at 20 μM and, respectively 9 and 13 Bq with 1-decyl 2-octyl-GPC at 10 and 20 μM. The two other dialkyl-GPC were ineffective.

Culture medium from macrophages activated by A 23187. 122 Bq was recovered on phospholipids (not different from 155 Bq) and represented twenty percent of phospholipids found in macrophages. 58 Bq was on fatty acids ($P < 0.01$ with 33 Bq, data not shown in Table 3) 46 Bq on eicosanoids ($P < 0.01$ with 31 Bq, data not shown on Table 3). The values together reached 104 Bq, a value just a little lower than 122 Bq.

Diacyl-GPC which did not reach cells was found in greater proportions in the medium in the presence of dialkyl-GPC and as early as 2 μM. Eicosanoid synthesis was inhibited at 20 μM with 1-dodecyl-2-octyl-GPC, 1-decyl-2-octyl-GPC and 1,2-di-octyl-GPC but never with 1-hexadecyl-2-octyl-GPC. Fatty acid releases were also lowered wih these three dialkyl-GPCs but not with 1-hexadecyl-2-octyl-GPC. The highest inhibition was with 1-decyl-2-octyl-GPC, 17 and 9 Bq, at 10 and 20 μM respectively.

DISCUSSION

Uptakes of 1-stearoyl-2-[^{3}H]-arachidonoyl-GPC by macrophages were associated with remarkably active endocellular phospholipase A_2 on this molecular species containing arachidonic acid. Macrophages which have been activated by A 23187, or other molecules, secrete lysosomes which contain a second phospholipase A_2.[37-39] This secreted enzyme is known to hydrolyse phospholipids without molecular species specificity. It was active on diacyl-GPC which remained in the culture medium.

This study reproduced exactly experimental procedures used previously which showed that 1,2-di-hexadecyl-GPC was more efficient than bromophenacyl bromide in inhibiting phospholipases A_2. The dialkyl-GPCs have inhibited these enzymes (**Tables 1, 2, and 3**) but had different inhibitory capacities. They were not labeled and it is impossible to know if they were intact or in part hydrolysed within 90 min of incubation. They were probably not hydrolysed at position 1 by phospholipase A_1 which is not active in stimulated (A 23187) macrophages.[40] Also probably not by etherases which split 1-alkyl (alk-1′-enyl)-2- acylglycerophospholipids, nor by phospholipase A_2 which cannot split alkyl-linked fatty chains at position 2. A non specific phospholipase C may have been active : it is known that 1-octadecyl 2-methyl-GPC is attacked by this enzyme in transformed cells;[41,42] it would be present in macrophages.[43] This enzyme may have produced 1,2-di-alkyl-glycerols which are analogs of diacylglycerol, the main activator of protein kinase C. Indirectly, they may have inhibited phospholipase A_2 assuming that they first inactivated protein kinase C. Dialkyl-GPC and dialkylglycerol may have subtoxic effects in macrophages after prolonged contact and at high concentrations. On mouse peritoneal macrophages, 30 min of incubation with a wide concentration range (0.1 to 100 μM) for dodecylglycerol or 1-octadecyl-2-methyl-GPC or-GPE (alkyllyso-GPC and -GPE) followed by 5 hours post-treatment, provoked subtoxic

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effects above 10 μM.[44] For these reasons, rat peritoneal macrophages in this study were only allowed 90 min contact at low concentrations (2, 10 or 20 μM) with dialkyl-GPC.

Uptakes of tritiated phospholipid by macrophages were lowered in the presence of dialkyl-GPC. This had been previously observed with 1,2-dihexadecyl-GPC at 10 μM (data not published but associated with experiments, in reference.[19] In Tables 1 and 2, the highest efficient alkyl-linked structures were with 8 and 10 carbons, at a concentration as low as 2 μM. All dialkyl-GPCs were in the medium at concentrations about 10^7 higher than tritiated phospholipid, so they may have lowered its uptake by a competitive effect. This may be also understood assuming that more 1,2-di-octyl-GPC or 1-decyl-2-octyl-GPC molecules than other dialkyl-GPCs had diffused across plasma membranes, in relation to fluidity at 37°C. The melting points of dialkyl-GPCs and diacyl-GPCs, at maximum hydration, differ only by 2 or 3°C, when they have the same chain lengths.[45–47] Melting points were lower than 0°C for 1,2 di-octyl-GPC or 1-decyl 2-octyl-GPC, and about 15°C for two others (while it is about 44°C for 1,2-di-hexadecyl-GPC). In addition, chain lengths of 8 or 10 carbons lowered lipophilicity compared to longer chains. However all were water insoluble while dialkyl-GPCs with chain lenghts of 6 or 7 carbons would probably be water soluble and would not be recognized by the enzyme.

Phospholipase A_2 was active since the most predominant lipid class to become labelled was non esterified fatty acids, in macrophages (Table 1 and 2) and in culture medium (Table 3). In addition, tritiated eicosanoids appeared in higher amounts in culture medium of activated cells than in non activated cells (Table 3). But tritiated DG + MG and TG were synthesized, in non activated cells and, to smaller amounts, in activated cells. They never reached the radioactivity associated with the fatty acids. Assuming that tritiated phospholipid was hydrolyzed only by phospholipase A_2, this would mean that non phosphorous glycerides were esterified by [^{3}H] arachidonic acid which had been split from tritiated phospholipid. However it may have been hydrolysed by other phospholipases.

After phospholipase A_1 activity, 1-lyso-2-[^{3}H] arachidonoyl-GPC could be hydrolysed by lysophospholipase D. But, according to Wyckle, *et al.*,[48] this enzyme hydrolyses only 1-alkyl-2-lyso-GPC and GPE. Furthermore, phospholipase A_1, in murine peritoneal macrophages, is not active.[40]

After phospholipase C activity, 1-stearoyl-2-[^{3}H]arachidonoyl-sn-glycerol may appear, but probably not to an important extent. Firstly, because macrophages which have been activated by A 23187 have a very active phospholipase A_2 which is Ca^{++}–dependent. Secondly, because ionophore A 23187 acts after membrane stimulations by an agonist, and it is this early event which is associated with phosphatidyl-inositol specific phospholipase C hydrolysis. However, non specific phospholipase C may have been activated by A 23187 and may have hydrolyzed the tritiated phospholipid; following this, a lipase may have released[^{3}H] arachidonic acid. Data (Tables 1 and 2) did not support such an hypothesis since fatty acids were always more radioactive than any of the non phosphorous glycerolipids. Furthermore, dialkyl-GPCs never modified this distribution. In conclusion, phospholipase A_2 was the main active enzyme on this radioactive phospholipid, in activated and non activated macrophages.

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It was found that phospholipase A_2 activity, in macrophages activated by A 23187, was more inhibited by 1,2-di-hexadecyl-GPC than by bromophenacy lbromide.[19] The IC_{50} (evaluations of radioactive fatty acids released from 1-stearoyl 2-[^{3}H]arachidonoyl-GPC) was 4.7 μM in the medium but 10 μM in cells (respectively 10 μM and 9 μM with bromophenacylbromide). These results showed that this dialkyl-GPC had a higher effect upon secreted phospholipase than upon the endocellular enzyme. It might be explained by poor diffusion of this dialkyl-GPC through plasma membranes. In the present study, 1-decyl 2-octyl-GPC was the only molecules as active in cells as in the medium. In activated cells, the IC_{50} (radioactive fatty acids) was 17 μM with 1-hexadecyl-2 octy-GPC, 6 μM with 1-decyl-2-octyl-GPC and 4 μM with the other two molecules. In the medium of activated cells, IC_{50} was higher than 100 μM with 1-hexadecyl-2 octyl-GPC, 9 μM with 1-dodecyl-2octyl-GPC or 1 di-octyl-GPC and 5 μM with 1-decyl-2octyl-GPC. Applied to radioactivities associated with TG + DG + MG, measures of IC_{50} would also give a best inhibitory effect with 1-decyl-2-octyl-GPC.

Inhibition of eicosanoid synthesis was observed (Table 3), but only in the medium of activated cells and at 20 μM for dialkyl-GPC with 8 or 10 carbons.

From these results, four dialkyl-GPCs with various chains lengths inhibited phospholipase A_2 activity in peritoneal macrophages labelled with 1-stearoyl-2-[^{3}H]arachidonoyl-GPC as source of [^{3}H] arachidonic acid. As judged by inhibition of arachidonic acid release, in cells which were activated by A 23187, 1-decyl-2-octyl-GPC was the most efficient inhibitor (IC_{50} at 4 μM). This was also found to be the case with this molecule in culture medium (IC_{50} at 5 μM). This molecule was also the more efficient inhibitor in lowering radioactivity associated with non phosphorous glycerides. Since phospholipase inhibition by dialky-GPCs, in intact cells, might occur after diffusion of the inhibitor across cell membranes, 1-decyl-2-octyl-GPC, but not other dialkyl-GPC, convincingly inhibited the hydrolysis of radioactive phospholipid.

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