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Arachidonate mobilization in diacyl, alkylacyl and alkenylacyl phospholipids on stimulation of rat platelets by thrombin and the Ca^{2+} ionophore A23187

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Platelet stimulation by thrombin or Ca^{2+} ionophore induces mobilization of arachidonate from lipid stores. We have previously shown that, in [^{14}C]arachidonic acid-prelabelled resting platelets, [^{14}C]arachidonate was transferred from diacyl-*sn*-glycerophosphocholine to ethanolamine and choline-containing ether phospholipids. This transfer reached an equilibrium after 5 h incubation [Colard, Breton & Bereziat (1984*a*) Biochem. J. **222**, 657–662]. [^{14}C]Arachidonate-prelabelled platelets having reached this transfer equilibrium were used to study the mobilization of arachidonate in etheracyl and diacyl phospholipids. Upon thrombin stimulation, arachidonate decreased in diacyl-*sn*-glycero-3-phosphoinositol, in alkylacyl- and diacyl-*sn*-glycero-3-phosphocholine and increased in alkenylacyl- and diacyl-*sn*-glycero-3-phosphoethanolamine. Upon challenge with Ca^{2+} ionophore A23187, arachidonate decreased in diacyl-*sn*-glycero-3-phosphoethanolamine, in diacyl- and alkylacyl-*sn*-glycero-3-phosphocholine and increased in alkenylacyl-*sn*-glycero-3-phosphoethanolamine. We also compared arachidonate mobilization in platelets stimulated immediately after [^{14}C]arachidonic acid chase with platelets stimulated after 5 h reincubation. We observed that the arachidonate newly incorporated into diacyl-*sn*-glycero-3-phosphocholine and triacylglycerols was rapidly released upon stimulation. This suggests the presence in these two lipids of a rapidly-turning-over arachidonate pool.

INTRODUCTION

We recently demonstrated that, when platelets were incubated in the presence of labelled arachidonic acid, the radioactivity was first incorporated into diacyl-GPE and diacyl-GPC, then transferred from diacyl-GPC to alkylacyl-GPE and -GPC and to alkenylacyl-GPE by a long-term exchange (Colard *et al.*, 1984*a*). A similar transfer was also observed in rat alveolar macrophages (Sugiura *et al.*, 1984). By the use of rat (Colard *et al.*, 1984*b*) and human (Kramer & Deykin, 1983; Kramer *et al.*, 1984*a*, 1984*b*) platelet homogenates, CoA-dependent and CoA-independent pathways were shown to be involved in this arachidonyl transfer.

Platelet stimulation induces the release of arachidonate from phospholipid stores for the synthesis of biologically active metabolites. Two pathways have been involved in this release process, i.e. the phosphatidylinositol-specific phospholipase C pathway, and the direct hydrolysis of phospholipids by phospholipase A_2 [reviewed by Rittenhouse-Simmons & Deykin (1981) and Irvine (1982)]. The experiments using arachidonate-prelabelled platelets to elucidate which phospholipids are involved in the supply of arachidonate have disregarded the ether phospholipids for two reasons: first, alkylacyl phospholipids were never separated from the corresponding diacyl phospholipids, and secondly, when short-term arachidonate incorporations were performed, the ether phospholipids were very poorly labelled. However, a role for the alkylacyl-GPC in cell activation has recently been demonstrated. In addition to being the precursor of the phospholipid mediator PAF-acether (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in a number of stimulated cells (Albert & Snyder, 1983; Chilton *et al.*, 1984; Touqui *et al.*, 1985), alkylacyl-GPC was shown to be a significant source of metabolizable arachidonic acid in neutrophils (Chilton *et al.*, 1984), alveolar macrophages (Albert & Snyder, 1984) and rabbit platelets (Chignard *et al.*, 1984).

After 5 h incubation of [^{14}C]arachidonate-prelabelled rat platelets, the transfer of [^{14}C]arachidonate from diacyl-GPC to the various ether phospholipids reached an equilibrium. In the present study we used platelets having reached this [^{14}C]arachidonate equilibrium state to investigate the role of diacyl and ether phospholipids in the mobilization of arachidonic acid when platelets were stimulated by thrombin and the Ca^{2+} ionophore A23187. We also compared the changes observed upon stimulation when platelets were stimulated just after the [^{14}C]arachidonate has been removed or 5 h later.

MATERIALS AND METHODS

Materials

[1-^{14}C]Arachidonic acid (58 Ci/mol) and [1-^{14}C]palmitoyl lysophosphatidylcholine (57 Ci/mol) were obtained from Amersham International. Silica-gel-G-coated plates were purchased from Schleicher and Scheull. Thrombin from bovine plasma, acetylsalicylic acid and

Abbreviations used: GPC, sn-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GPI, *sn*-glycero-3-phosphoinositol; GPS, *sn*-glycero-3-phosphoserine.

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fatty-acid-free human albumin were purchased from Sigma Chemical Co. Ionophore A23187 was obtained from Boehringer Mannheim. DEAE-Sepharose CL-6B was from Pharmacia and gelatin was from Merck. Phospholipase A_1 was prepared from guinea-pig pancreas as described by Fauvel *et al.* (1981), with some modifications. The pancreatic gland was sliced and homogenized in 5 vol. of 0.1 M-Tris/HCl buffer, pH 8.0, by using an Ultra-Turrax homogenizer (two 10 s bursts). The homogenate was centrifuged at 900 *g* for 20 min. The pellet was resuspended in Tris/HCl buffer and centrifuged again. Both supernatants were pooled and mixed with DEAE-Sepharose suspended in 0.05 M-Tris/HCl, pH 9.0, containing 20% (v/v) glycerol. This mixture was gently shaken overnight, then the supernatant from the gel was recovered. The gel was resuspended in buffer, pH 9.0, and this mixture shaken for 4 h. These supernatants, which contained the cationic lipase I non-adsorbed on DEAE-Sepharose, were concentrated by ultrafiltration (UM-10 membrane; Amicon Corporation), adjusted to 50% (v/v) glycerol and stored frozen at −20 °C. Phospholipase A_1 activity was assayed by using 400 μM-[1-^{14}C]palmitoyl lysophosphatidylcholine in 0.2 M-Tris/HCl, pH 8.0, containing 2.4 mM-sodium deoxycholate.

Preparation and labelling of platelets

Platelet-rich plasma was prepared from citrated blood collected from about 2-month-old Wistar rats by centrifugation at 375 *g* for 7 min. Platelets were then isolated by centrifugation at 1400 *g* for 15 min at 30 °C and gently resuspended in the same volume of Tyrode buffer, pH 6.5, containing 2.6 mM-KCl, 1 mM-$MgCl_2$, 137 mM-NaCl, 12 mM-$NaHCO_3$, 0.2 mM-EGTA, 5.5 mM-glucose and 0.25% gelatin (Ardlie *et al.*, 1970). Acetylsalicylic acid (0.1 mM) was added for 15 min, then platelets were pelleted and resuspended at a concentration of 1×10^9 platelets/ml in the same buffer (pH 6.5). For labelling of platelets, the platelet suspension was incubated with [^{14}C]arachidonic acid (0.8 μCi/ml) and 0.25% fatty-acid-free albumin for 30 min at 37 °C. The platelets were then washed twice. Platelet samples taken from the last resuspension were considered as 'short-term labelled' and after 5 h reincubation at 37 °C were considered as 'platelets at equilibrium labelling'.

Platelet challenge

Before addition of thrombin or ionophore A23187, platelets were centrifuged and resuspended in Tyrode buffer, pH 7.4, containing 2.6 mM-KCl, 1 mM-$MgCl_2$, 137 mM-NaCl, 10 mM-Tris/HCl, 0.86 mM-$CaCl_2$, 5.5 mM-glucose and 0.25% gelatin. They were left in this buffer for 20 min at 37 °C. Platelets were then challenged with thrombin (1 unit/ml) or 1 μM-ionophore A23187 and vigorously shaken. All incubations were terminated by the addition of chloroform/methanol (1:2, v/v) to the platelet suspension. Measurement of aggregation responses were performed at 37 °C on a dual-channel aggregometer.

Analysis of lipids

Platelet lipids were extracted by the method of Bligh & Dyer (1959). Solvents were removed by a stream of N_2 and the lipids were resuspended in chloroform/methanol (1:1, v/v). Phospholipids and neutral lipids were separated by double migration in a single direction on layers of silica-gel G, first in chloroform/methanol/acetic acid/water (75/45/12/6, by vol.) and after drying, in light petroleum (b.p. 40–65 °C)/diethyl ether/acetic acid (90:30:1, by vol.). This system well separated individual phospholipids and triacylglycerols from 'other lipids'. Alkyl, alkenyl and diacyl species of phospholipids were separated by a method based on the selective hydrolysis of diacyl phospholipids by guinea-pig phospholipase A_1 and of alkenylacyl phospholipids by acidolysis (El Tamer *et al.*, 1984) as previously described (Colard *et al.*, 1984*a*). The reaction was stopped by adding chloroform/methanol (1:2, v/v) and lipids extracted by the method of Bligh & Dyer (1959). The lipid extract was spotted on silica-gel-G plates and developed for 50 min in chloroform/methanol/acetic acid/water (45:20:6:1, by vol.). After drying, the plate was subjected to HCl fumes for 10 min and left to evaporate for at least 1 h before developing in a second direction with chloroform/methanol/14 M-NH_3/water (180:108:11:11, by vol.). Spots were revealed by autoradiography. Radioactivity was assayed with an Intertechnique liquid-scintillation counter.

RESULTS

After the transfer of [^{14}C]arachidonate from diacyl-GPC to diacyl-GPE, alkylacyl-GPE, alkenylacyl-GPE and to alkylacyl-GPC, platelet phospholipid labelling after 5 h exchange (named 'platelets at equilibrium labelling') was markedly different from a 30 min labelling period (named 'short-term labelled'). Table 1 gives the levels of [^{14}C]arachidonate recovered in the various lipid fractions at short term and at equilibrium labelling. After treatment of platelet phospholipids with guinea-pig phospholipase A_1, [^{14}C]arachidonyl-GPI and -GPS had

Table 1. [^{14}C]Arachidonate labelling of platelet lipids at short-term and at equilibrium labelling

Platelets were incubated for 30 min with [^{14}C]arachidonic acid (0.8 μCi of platelet suspension/ml). Platelets were then washed twice and reincubated for 5 h more. Platelets after the two washings are designated 'short-term labelling' and platelets after 5 h reincubation are designated 'equilibrium labelling'. The lipids were extracted and the radioactivity was determined as described in the Materials and methods section. These data are given as percentages of the total radioactivity incorporated (50000 ± 10000 d.p.m./ml of platelet suspension) and are the means ± S.E.M. for five experiments. The fraction called 'other lipids' included mono- and di-acylglycerols as well as arachidonate derivatives and free arachidonic acid.

	[^{14}C]Arachidonate labelling (%)	
Lipid class	Short term labelling	Equilibrium labelling
Alkylacyl-GPC	4.2 ± 1.4	6.6 ± 0.8
Diacyl-GPC	43.2 ± 3.2	26.2 ± 2.1
Diacyl-GPI	19.1 ± 1.1	20.0 ± 1.4
Diacyl-GPS	6.4 ± 0.9	7.2 ± 1.1
Alkylacyl-GPE	3.6 ± 1.0	9.0 ± 0.6
Alkenylacyl-GPE	3.6 ± 0.9	16.2 ± 1.5
Diacyl-GPE	9.6 ± 2.2	10.7 ± 2.4
'Other lipids'	5.2 ± 1.9	4.7 ± 1.5
Triacylglycerols	4.2 ± 1.2	0

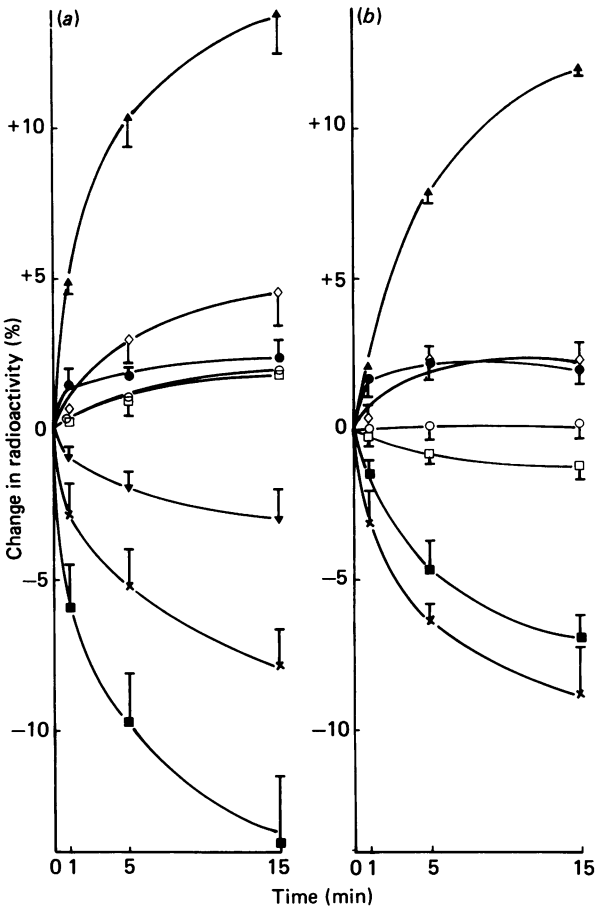

Fig. 1. Time course of [^{14}C]arachidonate mobilization (*a*) at short-term and (*b*) at equilibration labelling upon platelet stimulation with thrombin

Platelets suspended in Tyrode buffer, pH 6.5, without Ca^{2+} were prepared and labelled as described in the Materials and methods section, then centrifuged and resuspended in Tyrode buffer, pH 7.5, containing 0.9 mM-Ca^{2+} and 1 mM-Mg^{2+}. They were then challenged with thrombin (1 unit/ml) and chloroform/methanol (1:2, v/v) was added to the platelet suspension after the indicated time. Lipid extraction and analysis were as described in the Materials and methods section. Data are expressed as percentage changes in the various compounds and are the means from three experiments with S.E.M. values indicated by the vertical bars. ▼, Triacylglycerols; ▲, 'other lipids'; ◇, alkenylacyl-GPE; ○, alkylacyl-GPE; ●, diacyl-GPE; ×, diacyl-GPI; □, alkylacyl-GPC; ■, diacyl-GPC.

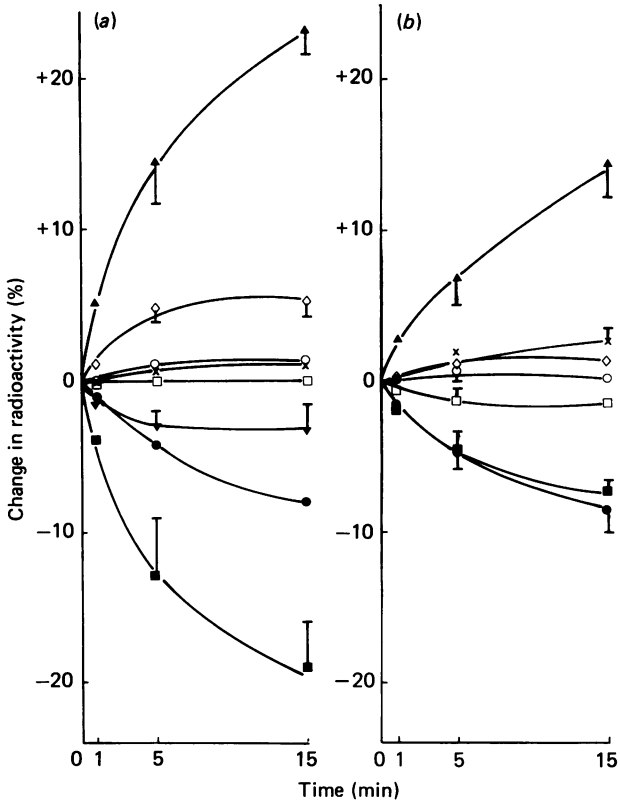

Fig. 2. Time course of [^{14}C]arachidonate mobilization at (*a*) short-term and (*b*) at equilibrium labelling upon platelet stimulation ionophore with A23187

Platelets resuspended in Tyrode buffer, pH 7.5, as indicated in Fig. 1 were challenged with the Ca^{2+} ionophore A23187 (1 μM). Results are expressed as the percentage change in the various compounds and are the means ± S.E.M. from three different determinations. Symbols are as in Fig. 1.

disappeared. Thus these compounds destroyed by phospholipase A_1 did not contain ether arachidonyl species. Upon platelet reincubation, radioactivity in diacyl-GPS and in diacyl-GPI did not change, whereas etheracyl-GPE and -GPC labelling was increased concomitantly with a decrease in diacyl-GPC, and radioactivity in triacylglycerols disappeared. Fig. 1 and Fig. 2 compare [^{14}C]arachidonate mobilization in the platelet lipids upon challenge of short-term or equilibrium labelled platelets by thrombin or Ca^{2+} ionophore A23187.

Thrombin (Fig. 1), as expected, induced a large, rapid (half maximum after 2–3 min) loss of [^{14}C]arachidonate from diacyl-GPI. If, after prolonged incubation, this decrease in acyl[^{14}C]arachidonyl-GPI, whose labelling did not vary upon reincubation, remained unchanged, the decrease in acyl[^{14}C]arachidonyl-GPC was considerably reduced. When platelets were short-term labelled, the radioactivity loss from acyl[^{14}C]arachidonyl-GPC was almost twice as high as that from acyl[^{14}C]arachidonyl-GPI. Approximately half of the [^{14}C]arachidonate loss from diacyl-GPI, diacyl-GPC and also triacylglycerols was recovered in alkenylacyl-GPE, diacyl-GPE, alkylacyl-GPE and alkylacyl-GPC, and the other half in the 'other lipids'. This fraction (so-called 'other lipids') could contain free arachidonate as well as arachidonate oxygenated derivatives, mono- and di-acylglycerols and phosphatidic acid. When equilibrium-labelled platelets were stimulated with thrombin, [^{14}C]arachidonyl transfer from diacyl-GPC to diacyl-GPE remained unchanged, and arachidonyl transfer to ether phospholipids was reduced as compared with short-term-labelled platelets. In fact, alkylacyl-GPC was decreased. The appearance of the 'other lipids' was slower under conditions of equilibrium than under those of short-term labelling. Thus, 1 min after thrombin stimulation, 2% as against 5% of label was recovered in this fraction.

When rat platelets were stimulated with ionophore A23187 (Fig. 2), there was no breakdown of acyl[^{14}C]arachidonyl-GPI. Conversely, we observed a small increase

in diacyl-GPI labelling. Arachidonate release from acyl-[^{14}C]arachidonyl-GPE was identical at short term and at equilibrium labelling (9%). Acyl[^{14}C]arachidonyl-GPC disappearance was the same as acyl[^{14}C]arachidonyl-GPE disappearance at equilibrium labelling. By contrast, under conditions of short-term labelling, twice as much acyl[^{14}C]arachidonyl-GPC as acyl[^{14}C]arachidonyl-GPE disappeared. The labelling lost by diacyl-GPE and diacyl-GPC was mostly recovered in the 'other lipids'. Labelling in the ethanolamine-containing ethers was strongly increased when platelets were short-term-labelled and slightly increased under conditions of equilibrium labelling. A decrease in alkyl[^{14}C]arachidonyl-GPC was observed at equilbrium labelling. A considerably lower fraction of 'other lipids' was released at equilbrium as compared with short-term labelling.

As prolonged incubations could have modified platelet reactivity and phospholipid metabolism, the short-term-labelled platelets were incubated in buffer for 5 h before [^{14}C]arachidonate labelling and then stimulated. The changes in labelling did not differ at either time from those obtained with platelets labelled without this preincubation. We also measured aggregation responses. After 5 h reincubation at 37 °C in pH 6.5 buffer without Ca^{2+}, platelets showed slightly reduced aggregation responses to thrombin (1 unit/ml) and to 1 μM-Ca^{2+} ionophore A23187 (15% and 20% respectively when compared with platelets just after the arachidonic acid chase). Prolonged incubation also induced a slower response rate to thrombin. Specifically, 40% rather than 60% of the maximum response was reached 1 min after addition of thrombin. There was no difference in the response rate upon stimulation by ionophore A23187.

DISCUSSION

At 5 h after free [^{14}C]arachidonic acid had been washed from platelets, [^{14}C]arachidonate had reached the equilibrium between the various classes of phospholipids (Colard *et al.*, 1984*a*). We hypothesize from these results that the changes in [^{14}C]arachidonate then observed represent the real movements of arachidonate.

When platelets having reached this equilibrium were stimulated by thrombin, [^{14}C]arachidonate decreased in diacyl-GPC, diacyl-GPI and in alkylacyl-GPC and increased in diacyl-GPE, alkenylacyl-GPE and in the 'other lipids' fraction. It is not possible to differentiate between [^{14}C]arachidonate coming from diacyl-GPC, alkylacyl-GPC and diacyl-GPI. However, in resting platelets, [^{14}C]arachidonate was exchanged from diacyl-GPC to diacyl-GPE and to the ether phospholipids (Colard *et al.*, 1984*a*). Moreover, rat platelet homogenates could not transfer [^{14}C]arachidonate from diacyl-GPI to the various ethanolamine-containing phospholipids (Colard *et al.*, 1984*b*). So it is likely that, when platelets are stimulated by thrombin, the transfer occurring in resting platelets is activated and the increase in diacyl-GPE and alkenylacyl-GPE (2% each after 5 min) observed at [^{14}C]arachidonate equilibrium labelling can be accounted for by the decrease in diacyl-GPC (4.5%). The appearance of ^{14}C-labelled 'other lipids' would be due to the breakdown of diacyl-GPI and alkylacyl-GPC. It thus appears that ethanolamine-containing phospholipids do not contribute to the arachidonate release, even when the uptake capacity of diacyl-GPE and alkenylacyl-GPE was reached 5 min after thrombin addition. After this point, diacyl-GPC contributed to the appearance of the ^{14}C-labelled 'other lipids'.

When platelets at [^{14}C]arachidonate equilibrium were challenged by ionophore A23187, the ^{14}C decrease in diacyl-GPC was about the same as when platelets were challenged by thrombin. By contrast, there was no [^{14}C]arachidonate decrease in diacyl-GPI. Diacyl-GPE labelling decreased in a similar manner to diacyl-GPC. There was only a slight increase in alkenylacyl-GPE. Diacyl-GPI also increased. These slight increases cannot account for the decrease in diacyl-GPC. Thus most of [^{14}C]arachidonate lost by diacyl-GPC and diacyl-GPE was recovered in the 'other lipids' fraction.

The results presented here allowed us to calculate the percentage change in each phospholipid class (Table 2). As is well documented in the literature, the addition of thrombin resulted in a significant breakdown of acylarachidonyl-GPI. In contrast with what has been observed in horse and human platelets (Rittenhouse-Simmons, 1981; Billah & Lapetina, 1982*a*,*b*), under our incubation conditions ionophore A23187 did not induce degradation of acylarachidonyl-GPI. Whereas no significant change in alkylarachidonyl-GPE was observed with either stimulus, they both induced a slight decrease in alkylarachidonyl-GPC. The decrease upon thrombin stimulation (12% in 5 min after thrombin addition) is in accordance with hydrolysis of [^{3}H]alkylacyl-GPC observed by Chignard *et al.* (1984) (10% in 5 min) and by

Table 2. Percentage increase or decrease in each acyl[^{14}C]arachidonyl or ether[^{14}C]arachidonyl phospholipid at exchange equilibrium upon challenge with thrombin or with ionophore A23187

Values were calculated from Fig. 1 or Fig. 2 and Table 1. Abbreviation used: n.s., not significant.

		Percentage change			
	Challenger...	Thrombin		Ionophore A23187	
Lipid class	Time (min)...	5	15	5	15
Diacyl-GPE		↗20	↗19	↘43	↘78
Alkenylacyl-GPE		↗12	↗12	↗8	↗8
Alkylacyl-GPE		n.s.	n.s.	n.s.	n.s.
Diacyl-GPI		↘30	↘41	↗5	↗13
Diacyl-GPC		↘19	↘28	↘17	↘28
Alkylacyl-GPC		↘12	↘18	↘16	↘21

Touqui *et al.* (1985) (15% in 10 min) in rabbit platelets. However, the amount of arachidonate released from alkylacyl-GPC remained low relative to the amount released from diacyl-GPI upon thrombin stimulation or from diacyl-GPE and diacyl-GPC upon challenge with ionophore A23187.

Thrombin and ionophore A23187 both induced the same decrease in arachidonate content of diacyl-GPC. But, as we have previously seen, arachidonate lost from diacyl-GPC appeared to be transferred to ethanolamine-containing phospholipids upon thrombin stimulation, whereas it appeared to be primarily released upon challenge with ionophore A23187. Concomitantly, ionophore A23187 induced a rapid and almost total (80% after 15 min) breakdown of acylarachidonyl-GPE and these two hydrolyses (acylarachidonyl-GPE and -GPC hydrolyses) occurred in parallel as a function of time (Fig. 2*b*).

The changes in [^{14}C]arachidonate observed at exchange equilibrium do not take into account the possible involvement of rapidly-turning-over pools. Now, when platelets were challenged at [^{14}C]arachidonate equilibrium labelling, the radiolabelled 'other lipids' appeared at a slower rate than when platelets were challenged just after free [^{14}C]arachidonic acid had been removed. At short-term labelling, the 'other lipids' represented 5% 1 min after the addition of thrombin or ionophore A23187 as against 2 and 3% at equilibrium labelling. Acylarachidonyl-GPI breakdown in the case of thrombin stimulation and acylarachidonyl-GPE breakdown in the case of ionophore A23187 stimulation were identical after a short-term labelling and after reincubation, so this difference in the released 'other lipids' can be only attributed to acylarachidonyl-GPC and triacylglycerols, whose labelling was completely different at the two times.

Thus if free [^{14}C]arachidonic acid is rapidly incorporated into diacyl-GPC and triacylglycerols before entering the ether phospholipids, it appears that this newly incorporated [^{14}C]arachidonate can be rapidly released after platelet stimulation. This suggests the existence of different diacyl-GPC and triacylglycerol pools and the possible involvement in early aggregation responses of the rapidly-turn-over pool.

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REFERENCES

Albert, D. H. & Snyder, F. (1983) J. Biol. Chem. **258**, 97–102
Albert, D. H. & Snyder, F. (1984) Biochim. Biophys. Acta **796**, 92–101
Ardlie, M. G., Packman, M. A. & Mustard, J. F. (1970) Br. J. Haematol. **19**, 7–17
Billah, M. M. & Lapetina, E. G. (1982*a*) J. Biol. Chem. **257**, 5196–5200
Billah, M. M. & Lapetina, E. G. (1982*b*) J. Biol. Chem. **257**, 11856–11859
Bligh, E. G. & Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
Chignard, M., Le Couedic, J. P., Coeffier, E. & Benveniste, J. (1984) Biochem. Biophys. Res. Commun. **124**, 637–643
Chilton, F. H., Ellis, J. M., Olson, S. C. & Wykle, R. L. (1984) J. Biol. Chem. **259**, 12014–12019
Colard, O., Breton, M. & Bereziat, G. (1984*a*) Biochem. J. **222**, 657–662
Colard, O., Breton, M. & Bereziat, G. (1984*b*) Biochim. Biophys. Acta **793**, 42–48
El Tamer, A., Record, M., Fauvel, J., Chap, H. & Douste-Blazy, L. (1984) Biochim. Biophys. Acta **793**, 213–220
Fauvel, J., Bonnefis, M. J., Sarda, L., Chap, H., Thouvenot, J. P. & Douste-Blazy, L. (1981) Biochim. Biophys. Acta **663**, 446–456
Irvine, R. F. (1982) Biochem. J. **204**, 3–16
Kramer, R. M. & Deykin, D. (1983) J. Biol. Chem. **258**, 13806–13811
Kramer, R. M., Pritzker, C. R. & Deykin, D. (1984*a*) J. Biol. Chem. **259**, 2403–2406
Kramer, R. M., Patton, G. M., Pritzer, C. R. & Deykin, D. (1984*b*) J. Biol. Chem. **259**, 13316–13320
Rittenhouse-Simmons, S. (1981) J. Biol. Chem. **256**, 4153–4155
Rittenhouse-Simmons, S. & Deykin, D. (1981) in Platelets in Biology and Pathology 2 (Gordon, J. L., ed.), pp. 349–371, Elsevier/North-Holland, Amsterdam
Sugiura, T., Katayama, O., Fujui, J., Nakagawa, Y. & Waku, K. (1984) FEBS Lett. **165**, 273–276
Touqui, L., Jacquemin, C., Dumarey, C. & Vargaftig, B. B. (1985) Biochim. Biophys. Acta **833**, 111–118