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CATABOLISM OF PLATELET-ACTIVATING FACTOR BY HUMAN COLONIC MUCOSA

CALCIUM DEPENDENCE OF THE CATABOLIZING ENZYMES

CAROLINE B. APPLEYARD and KEITH HILLIER*
Clinical Pharmacology Group, Medical Faculty, University of Southampton, Southampton SO9 3TU, U.K.

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Abstract—The catabolism of platelet-activating factor (PAF) and lyso PAF by a supernatant fraction of human colon mucosa homogenates has been studied *in vitro*. PAF is initially catabolized to lyso PAF by mucosal enzymes via removal of its acetyl group. Incubates in Ca^{2+}-free Tris with EDTA showed that the acetyl hydrolase was Ca^{2+} independent. Addition of the hydrolase inhibitor, phenyl methyl sulphonyl fluoride, significantly reduced the catabolism of PAF. Lyso PAF was further catabolized in at least two ways. An acyl group was incorporated into the *sn*-2 position of lyso PAF to give 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkyl acyl GPC); this step was Ca^{2+} independent as shown by omitting Ca^{2+} and adding EDTA to the incubate. Formation of alkyl acyl GPC was confirmed by HPLC. Alternatively, choline was removed from the head group of lyso PAF by a calcium-dependent lyso phospholipase D. Under the experimental conditions utilized a neutral lipid product was formed but significant amounts of the intermediate lysophosphatidic acid could not be detected. A substance with a chromatographic mobility of $R_f = 0.8$ on TLC plates having an intact phosphorylcholine head group was also formed but has not yet been identified. It is concluded that the human colon mucosa contains enzymes that actively catabolize pro-inflammatory PAF and lyso PAF.

Platelet-activating factor (PAF) is 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkyl acetyl GPC). It can be synthesized by many cells including platelets, mast cells, macrophages, basophils, eosinophils and neutrophils. It has a range of biological activities some of which may be important in the pathology of the gastrointestinal tract. In the intestine, PAF has been shown to cause hyperaemia and haemorrhagic damage both by a direct action and also by sensitizing the mucosa to the effects of other irritants [1]. It also probably mediates bowel necrosis induced by bacterial endotoxin [2]. Colon mucosa biopsies taken from patients with inflammatory bowel disease have been shown to synthesize greater amounts of PAF than mucosa from normal patients [3, 4].

The amount of PAF present in biological fluids can be regulated by a balance of the synthetic and catabolic mechanisms. Firstly, there are two pathways of synthesis (reviewed in Ref. 5); secondly, catabolism of PAF is initially effected by an acetyl hydrolase that hydrolyses the acetate to form lyso PAF. Lyso PAF is 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (alkyl lyso GPC). Alkyl lyso GPC can then be reacylated with long chain fatty acids to yield 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine [6] or can be hydrolysed by phospholipases [7, 8].

Hydrolysis of alkyl lyso GPC by phospholipase C forming the respective neutral lipid, alkyl lyso glycerol, and phosphocholine has been shown to occur although the rates of hydrolysis appear to be low [7]. Phospholipase D can metabolize alkyl lyso GPC yielding choline and phosphatidic acid; the latter can then be converted to a neutral lipid by a phosphatidate phosphohydrolase [8]. This paper describes for the first time the catabolism of PAF by the mucosa of human colon and the dependence of the metabolic steps upon calcium.

MATERIALS AND METHODS

Materials. Amersham International supplied 1-*O*-[^{3}H]alkyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]alkyl acetyl GPC, sp. act. 80 curies/mmol, a mixture of C16 and C18 alkyl ethers); 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phospho[*N*-*methyl*-[^{14}C]choline] (alkyl acetyl[^{14}C]GPC, sp. act. 55 mCi/mmol); 1-*O*-[^{3}H]octadecyl-*sn*-glycero-3-phosphocholine ([^{3}H]alkyl lyso GPC, sp. act. 109–163 Ci/mmol). Acetonitrile was UV grade and methanol HPLC grade from May & Baker. Scintillation counting fluid was Optiphase Hisafe 3 from LKB. Other chemicals were supplied by Sigma U.K. Sodium vanadate and EDTA were prepared as solutions in Tris buffer and phenyl methyl sulphonyl fluoride (PMSF, 40 mM) was prepared in dimethyl sulphoxide.

Methods. Macroscopically normal samples of

* Corresponding author: Dr Keith Hillier, Clinical Pharmacology Group, Biomedical Sciences Building, Bassett Crescent East, Southampton SO9 3TU, U.K. Tel. (0703) 594264; FAX (0703) 594262.

† Abbreviations: PAF, platelet-activating factor; BSA, bovine serum albumin; GPC, glycero-3-phosphocholine; PMSF, phenyl methyl sulphonyl fluorides.

human colon were obtained from patients undergoing resection for carcinoma from the ascending or sigmoid colon; samples were taken at least 10–15 cm from the tumour and away from the cut edge. Tissue was placed immediately in ice-cold 0.05 M Tris–HCl buffer pH 8 containing 2.5 mg/mL bovine serum albumin (BSA) and all further preparative procedures were carried out at 4°. Specimens were washed in fresh buffer, the mucosa and submucosa were blunt-dissected away from the muscle and the mucosa was weighed and cut into small pieces with scissors, buffer was added (40 mL/g tissue) followed by homogenization in a motor-driven glass-teflon homogenizer for 1 min. A 1000-*g* supernatant was prepared by centrifuging at 4° for 15 min. The protein concentration in the supernatant was measured by the method of Lowry *et al.* [9] and was generally about 2 mg/mL buffer. The supernatant was pipetted into aliquots of 1 mL and stored at −20°. The activity of the supernatant was stable for at least 3 months. An aliquot of [^{3}H]alkyl acetyl GPC (0.2 μCi), alkyl acetyl [^{14}C]GPC (0.2 μCi), [^{3}H]alkyl lyso GPC (0.5 μCi) or alkyl lyso [^{14}C]GPC (0.02 μCi) was pipetted into a 1.5 mL capacity plastic microfuge tube and organic solvent evaporated under nitrogen at room temperature. Incubations were carried out for different times up to a maximum of 90 min in a final volume of 250 μL Tris buffer containing 5 mM Ca^{2+}, 2.5 mg/mL BSA and an amount of prepared colon supernatant containing 200 μg protein. PMSF was added in dimethyl sulphoxide (initial concentration 40 mM) and other drugs were prepared in Tris buffer. The incubation was stopped by adding 1 mL of chloroform:methanol 2:1; in some experiments one drop of 1 M citric acid was also added. The tubes were vortexed, stood for 10 min and were then centrifuged for 1 min in an MSE microcentrifuge at 8700 *g*. The volumes of organic and aqueous phases were measured separately and a 20-μL aliquot of each counted for radioactivity in 5 mL LKB scintillation fluid in a Packard Tricarb 3255 counter with subsequent corrections to convert to dpm.

The organic phases and aqueous phases were evaporated to dryness at room temperature and 35°, respectively, under a stream of nitrogen and reconstituted in 20 μL of 2:1 chloroform:methanol and 50 μL water, respectively. Twenty microlitres were applied as 1-cm narrow bands to Merck TLC plates coated with silica gel G. Tubes were rinsed with a further 15 μL of solvent and this was also applied. Plates were developed to a distance of 15 cm in saturated filter paper-lined tanks. The organic extract was developed in chloroform:methanol:acetic acid:water in proportions 50:25:8:2.5, v/v and the aqueous extract in methanol:0.9% NaCl:conc. NH_4OH 50:50:5, v/v. In some experiments aqueous extracts were applied in a volume of 50 μL to the concentration zone of Whatman LK5 plates. After development and drying overnight the plates were scanned for radioactivity using a Berthold linear analyser. The radioactive peaks corresponding to organic phase soluble catabolic degradation products were identified by co-chromatography with phospholipid and neutral lipid standards which were run in parallel and visualized in iodine vapour. The standards routinely utilized were L-α-phosphatidylcholine β-acetyl-γ-*O*-octadec-9-*cis*-enyl (PAF); DL-α-lyso phosphatidylcholine γ-*O*-hexadecyl (lyso PAF); phosphatidylcholine dilinoleoyl (which had been shown previously to co-chromatograph with phosphatidylcholine β-palmitoyl-γ-*O*-hexadecyl); DL-α-phosphatidyl-L-serine dipalmitoyl; L-α-phosphatidylethanolamine dioleoyl; 1-hexadecyl glycerol. The water soluble metabolites were compared by co-chromatography with choline ($R_f = 0.12$) and phosphocholine ($R_f = 0.58$). The radioactivity in the regions of interest on the plate was expressed as a percentage of the total radioactivity on the TLC plate. Where additions of PMSF or EDTA were made parallel control experiments without additions were always performed. In some experiments, areas of the plate co-chromatographing with choline were scraped into scintillation counting vials and 0.5 mL methanol was added with 5 mL scintillation fluid. Disintegrations per minute were calculated and expressed as a percentage of the radioactivity added to the incubate. To confirm the identity of the major peaks of radioactivity that co-chromatographed with the phospholipid standards, phosphatidylcholine and phosphatidylethanolamine, areas of TLC plates corresponding to the radioactive peaks were scraped into separate 10-mL glass tubes. Four millilitres of chloroform:methanol:2 M HCl (1:2:0.8, v/v) was added and vortexed, and then stood for 10 min. After centrifugation the silica gel was re-extracted as before. Each supernatant was extracted by the addition of 4 mL of water and rendered neutral by the dropwise addition of 1 M NaOH. Standard phosphatidylcholine and phosphatidylethanolamine (10 μg) were then added. The combined extracts were then evaporated to dryness and reconstituted in 100 μL mobile phase and 10 μL subjected to HPLC (Waters UK 6 injector and 510 pump) using a Hypersil APS 5 μm NH_2 column (Shandon, Runcorn, U.K.) and a CO:PELLPAC amino-cyano guard column (HPLC Technology, Macclesfield, U.K.). The column was developed isocratically using acetonitrile: methanol:phosphate buffer (15 mM, pH 5.64) in proportions 2.4:1:0.17 by volume. The flow rate was 1.5 ml/min and phospholipids were detected at a wavelength of 205 nm using a Waters 481 detector. Aliquots (1.5 mL) from the column were collected, added to scintillation fluid and radioactivity was assessed in terms of dpm.

RESULTS

Comparison of the products of [^{3}H]*alkyl acetyl GPC and* [^{3}H]*alkyl lyso GPC metabolism*

Radiolabelled products resulting from metabolism at the *sn*-2 and *sn*-3 position of PAF or lyso-PAF were followed using substrates labelled at the 1-*O*-alkyl moiety. Figure 1 shows that when [^{3}H]alkyl lyso GPC (peak 1) was incubated with 1000-*g* supernatant preparations of mucosal homogenates prepared from human colon mucosa and containing 5 mM Ca^{2+} and 2.5 mg/mL BSA, for 90 min, three major catabolites were identified co-chromatographing with alkyl acyl GPC (peak 3), neutral

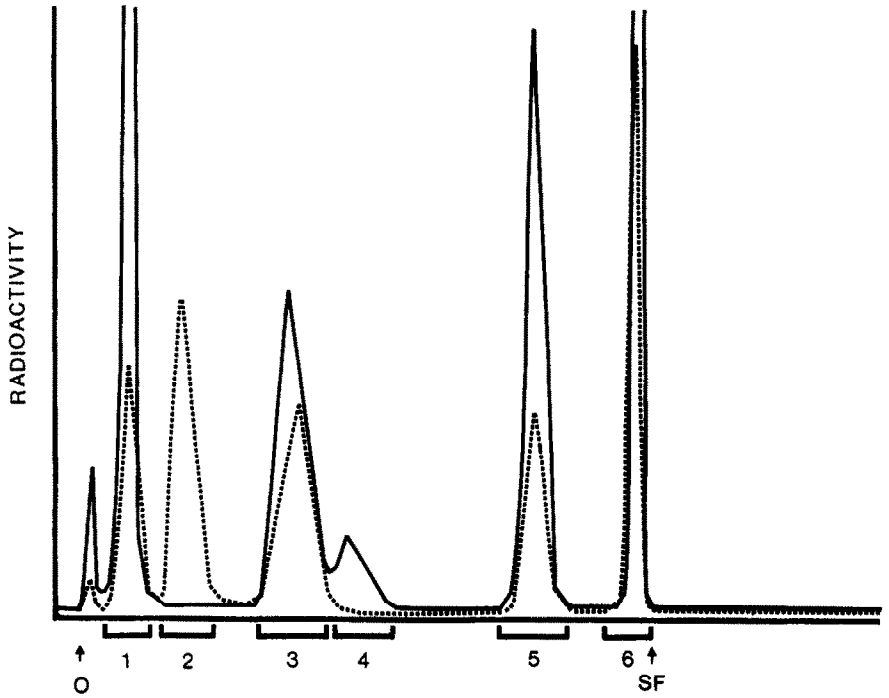

Fig. 1. Metabolism of [^{3}H]alkyl acetyl GPC and [^{3}H]alkyl lyso GPC by human colon mucosa. Representative profile scans of products formed by incubating supernatants of human colon mucosal homogenates (≡ 200 μg protein) with substrates 1-*O*-[^{3}H]alkyl-2-acetyl GPC (PAF) (----) or 1-*O*-[^{3}H]alkyl-2-lyso-GPC (lyso PAF) (——) for 90 min under conditions described in Materials and Methods. The results with the two separate substances have been superimposed for comparison. When alkyl acetyl [^{14}C]GPC was used as the substrate, products were similar to (----) except no labelled neutral lipid at R_f 0.98 was present. The peak numbers identify the following lipids: alkyl lyso GPC (peak 1 $R_f = 0.12$), PAF (peak 2 $R_f = 0.21$), alkyl acyl glycerophosphocholine (peak 3 $R_f = 0.4$), two unknown peaks (peak 4 $R_f = 0.5$, peak 5 $R_f = 0.8$) and neutral lipid (peak 6 $R_f = 0.98$).

lipid (peak 6) and a major unknown product (peak 5 $R_f = 0.8$). Occasionally a minor secondary peak with an R_f value slightly greater than that of alkyl acyl GPC was seen (peak 4). Evidence that alkyl acetyl GPC (PAF) was metabolized initially to alkyl lyso GPC (lyso PAF) was obtained in incubates of supernatants with the substrate [^{3}H]alkyl acetyl GPC (peak 2). A radioactive product co-chromatographing with [^{3}H]alkyl lyso GPC (peak 1) was identified; together with this peak other radioactive products had mobilities identical with those of the catabolites of [^{3}H]alkyl lyso GPC.

We were unable to demonstrate significant metabolism of 0.2 μCi 1-L-[^{3}H]alkyl-2-arachidonyl-GPC (NEN) by colon mucosal enzymes in incubations of up to 90 min under the same conditions utilized for [^{3}H]alkyl acetyl GPC metabolism.

Time-course of 1-*O*-[^{3}H]*alkyl-2-lyso GPC catabolism*

Figure 2 shows the time-course of the metabolism of [^{3}H]alkyl lyso GPC by colon mucosal supernatant. Within 5 min, 17% of added substrate had been metabolized during which time 8% had been converted to [^{3}H]alkyl acyl GPC and 3.5% to neutral lipid. After 30 min, 52% of added substrate had been metabolized of which 25% was present as [^{3}H]-alkyl acyl GPC and 9% was present as neutral lipid.

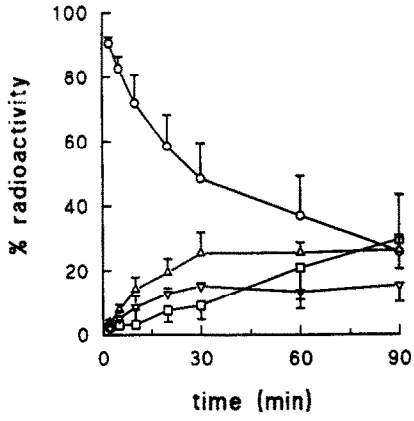

Fig. 2. Time-course of [^{3}H]alkyl lyso GPC metabolism. Aliquots of colon mucosal supernatants (200 μg protein) were incubated with 1-*O*-[^{3}H]alkyl-2-lyso GPC for periods of up to 90 min. Incubations were stopped at time-points shown, and extraction and separation by TLC performed as described in Materials and Methods. Each point is the mean ± SE of supernatant from three different patients. Incubates of each supernatant were replicated. Alkyl lyso GPC (lyso PAF) (○); alkyl acyl GPC (△); neutral lipid (□); unknown substance R_f 0.8 (▽).

Between 30 and 90 min incubation metabolism of the added substrate was still appreciable. At 90 min, 74% had been metabolized; moreover, the formation of the neutral lipid continued to increase and was 29% of the total radioactivity present at this time; however, [^{3}H]alkyl acyl GPC formation did not increase beyond that seen at 30 min. The unknown metabolite (R_f 0.8) increased during the first 30 min of incubation to 15% of the total radioactivity present but it did not alter thereafter.

A comparison of the percentages of products formed after 90 min incubation of [^{3}H]alkyl acetyl GPC and [^{3}H]alkyl lyso GPC can be made from the control experiments carried out in the presence of 5 mM Ca^{2+} shown in Table 1. The neutral lipid formed from the [^{3}H]alkyl acetyl GPC was relatively greater than the [^{3}H]alkyl acyl GPC or the unknown peak formed whereas with [^{3}H]alkyl lyso GPC as substrate, degrees of [^{3}H]alkyl GPC and neutral lipid formation were similar. In another series of experiments (N = 4–5) this difference in percentage product formation between [^{3}H]alkyl acetyl GPC and [^{3}H]alkyl lyso GPC was confirmed (data not shown).

Comparison of products obtained from alkyl acetyl [^{14}C]GPC and alkyl lyso [^{14}C]GPC

To investigate phospholipases C and D, which are known to act at the head group, supernatants were incubated with alkyl acetyl [^{14}C]GPC and alkyl lyso [^{14}C]GPC and extracted by partitioning with chloroform/methanol and water as described in methods. TLC of the water-soluble products from alkyl acetyl [^{14}C]GPC metabolism resulted in only one product which co-chromatographed on Whatman LK5 plates with authentic choline (R_f = 0.12). Phosphocholine had an R_f value of 0.58. Figure 3 shows that the formation of [^{14}C]choline increased with time. In 90 min, 27.1 ± 4% of added alkyl acetyl [^{14}C]GPC was metabolized. TLC of the organic phase gave products having identical R_f values to alkyl lyso GPC (peak 1), alkyl acyl GPC (peak 3) and the unknown peak (R_f = 0.8) (Fig. 1). No radiolabelled neutral lipid was present because the ^{14}C-labelled choline was removed. These data provide evidence that the unknown peak has an intact glycero-*N*-methyl[^{14}C]-containing head group, and that the substance running at R_f 0.98 is a neutral lipid. Utilizing alkyl lyso[^{14}C]GPC as substrate, only [^{14}C]choline was identified in the aqueous phase but due to shortage of this substrate only one experiment was performed.

Influence of calcium ions

Chelation of Ca^{2+} with 0.5 mM EDTA showed that the acetyl hydrolase and acyl transferase responsible for the metabolism of [^{3}H]alkyl acetyl GPC to [^{3}H]alkyl lyso GPC and acylation of [^{3}H]-alkyl lyso GPC to [^{3}H]alkyl acyl GPC, respectively, do not require Ca^{2+}. Analysis of the results from the utilization of [^{3}H]alkyl acetyl GPC as substrate showed that, when Ca^{2+} was chelated, [^{3}H]alkyl lyso GPC and [^{3}H]alkyl acyl GPC significantly increased but the formation of neutral lipid and unknown substance at R_f 0.8 was decreased (Table 1). The total amount of [^{3}H]alkyl acetyl GPC metabolized

Table 1. The influence of Ca^{2+} chelation on catabolism of 1-*O*-[^{3}H]alkyl-2-acetyl GPC and 1-*O*-[^{3}H]alkyl-2-lyso GPC

	N	Alkyl acetyl GPC	Alkyl lyso GPC	Alkyl acyl GPC	Neutral lipid	Unknown peak R_f 0.8
5 mM Ca^{2+} Tris	4	73.7 ± 1.58*	5.36 ± 1.58	2.62 ± 1.39	15.8 ± 5.10	3.34 ± 2.81
Ca^{2+}-free Tris plus 0.5 mM EDTA	4	68.5 ± 6.92*	14.9 ± 3.47§	5.80 ± 2.78‡	8.34 ± 2.25‡	ND
5 mM Ca^{2+} Tris	3	ND	26.1 ± 5.30†	26.5 ± 3.86	28.9 ± 13.7	15.4 ± 5.20
Ca^{2+}-free Tris plus 0.5 mM EDTA	3	ND	41.4 ± 8.07†‡	53.5 ± 8.21‡	3.10 ± 0.57	1.38 ± 0.99

Aliquots of 1000-*g* supernatants containing 200 μg protein were incubated for 90 min with [^{3}H]alkyl acetyl GPC* or [^{3}H]alkyl lyso GPC†. Incubates containing Ca^{2+} or Ca^{2+}-free Tris plus EDTA were processed in parallel. Data show each radioactively labelled product as a percentage of total radioactivity on the TLC plate (mean ± SEM). Supernatants from different patients were each replicated 2–4 times. Tissues studied with [^{3}H]alkyl acetyl GPC as substrate were different to those studied with [^{3}H]alkyl lyso GPC as substrate.

‡ $P < 0.05$, § $P < 0.001$ compared with values carried out in the presence of Ca^{2+} (paired *t*-test).

ND, not detectable.

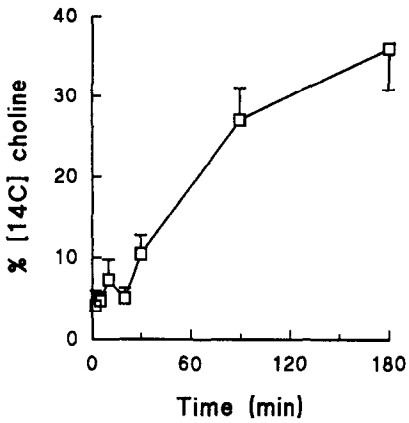

Fig. 3. Time-course of formation of [^{14}C]choline from alkyl acetyl [^{14}C]GPC. Supernatants (1000 *g*) of colon mucosa homogenates (200 μg protein) were incubated with alkyl acetyl [^{14}C]GPC. At time intervals specified extractions were performed as described in Materials and Methods and radioactivity in the aqueous extract was chromatographed on TLC. Radioactivity co-chromatographing with [^{14}C]choline was counted and expressed as a percentage of radioactivity added to the incubate. Data are means ± SEM (N = 3).

was not altered significantly; this indicates that the [^{3}H]alkyl lyso GPC may be the immediate precursor of the neutral lipid and the unknown substance at R_f 0.8. Further evidence that [^{3}H]alkyl lyso GPC is an intermediate in the formation of neutral lipid and unknown peak from [^{3}H]alkyl acetyl GPC was also obtained as Ca^{2+} chelation reduced their formation when [^{3}H]alkyl lyso GPC was used as substrate. Moreover, the total amount of [^{3}H]alkyl lyso GPC catabolized was significantly reduced and the formation of alkyl acyl GPC significantly increased when Ca^{2+} was chelated.

The effect of Ca^{2+} chelation on the phospholipases acting at the head group was examined using alkyl acetyl [^{14}C]GPC as substrate. The substrate and supernatant enzyme were incubated with 5 mM Ca^{2+}, or Ca^{2+} was omitted and 0.5 mM EDTA added. After TLC and scintillation counting the water soluble [^{14}C]choline which was formed was found to be reduced by 91%. Because metabolism of alkyl acetyl [^{14}C]GPC to alkyl lyso GPC is not reduced by Ca^{2+} chelation (Table 1) it is likely that the majority of [^{14}C]choline liberated is derived from the alkyl lyso [^{14}C]GPC intermediate.

Effect of PMSF on [^{3}H]*alkyl acetyl GPC and* [^{3}H]-*alkyl lyso GPC metabolism*

PMSF, an inhibitor of acetyl hydrolase, was used to examine whether the first step in the metabolism of alkyl acetyl GPC by colonic mucosal enzymes was to alkyl lyso GPC utilizing acetyl hydrolase. PMSF (10 mM) significantly reduced all the identified products from [^{3}H]alkyl acetyl GPC, yet when [^{3}H]-alkyl lyso GPC was the substrate PMSF caused no significant alteration in overall catabolism although there was considerable variability in the formation of individual catabolites (Table 2).

Effect of sodium vanadate

Sodium vanadate is a phosphatidate phosphohydrolase inhibitor. It was investigated to determine if the metabolism of alkyl lyso GPC was initially to alkyl lyso phosphatidic acid via lysophospholipase D and then to alkyl lyso glycerol utilizing a phosphatidate phosphohydrolase. If this route was used then sodium vanadate would be expected to increase the amount of alkyl lysophosphatidic acid while decreasing neutral lipid formation. Sodium vanadate at 2 mM had no significant effect on product formation from alkyl lyso GPC while 10 mM suppressed metabolism to all identified products by almost 100% (data not shown).

DISCUSSION

This study provides preliminary evidence for four metabolites when PAF is incubated with human colon mucosa: these lipids include alkyl lyso GPC, alkyl acyl GPC, neutral lipid (which is probably alkyl-*sn*-glycerol) and an unknown lipid running with an R_f value of 0.8 in a solvent system of chloroform/methanol/acetic acid/water (50:25:8:2.5, v/v).

We have shown that the first step in PAF metabolism by human colon mucosa is initially by an acetylhydrolase to alkyl lyso GPC and that alkyl lyso GPC is then acylated to alkyl acyl GPC or further metabolized by phospholipases to neutral lipids. Evidence for a mandatory first metabolic step to alkyl lyso GPC is provided by data showing that the products of PAF metabolism include alkyl lyso

Table 2. The effect of PMSF on catabolism of 1-*O*-[^{3}H]alkyl-2-acetyl GPC and 1-*O*-[^{3}H]alkyl-2-lyso GPC

	N	Alkyl acetyl GPC	Alkyl lyso GPC	Alkyl acyl GPC	Neutral lipid	Unknown peak R_f 0.8
Control	4	50.2 ± 7.07†	12.2 ± 0.51	11.1 ± 4.47	16.8 ± 2.58	8.36 ± 2.72
10 mM PMSF	4	87.1 ± 4.69*†	2.40 ± 2.41*	1.02 ± 0.72*	7.99 ± 1.84*	0.70 ± 0.42*
Control	3	ND	37.64 ± 8.2†	26.9 ± 7.86	14.3 ± 2.47	17.6 ± 1.9
10 mM PMSF	3	ND	47.1 ± 14.35†	12.6 ± 7.22	29.8 ± 16.0	7.92 ± 6.89

* Significantly different from control; paired *t*-test $P < 0.05$.
† Substrate added.
N is the number of different tissues studied; each was studied in duplicate. Other details are as in Table 1.
ND, not detected.

GPC; moreover, when alkyl lyso GPC was utilized as the substrate, products formed were identical to those formed using PAF as substrate; furthermore, the inclusion of PMSF which is known to inhibit acetyl hydrolase [10] reduced the formation of all products including alkyl lyso GPC, but when alkyl lyso GPC was used as substrate PMSF did not significantly inhibit metabolism. When Ca^{2+} was chelated in incubates containing [^{3}H]alkyl acetyl GPC (PAF) as the substrate there was a significant reduction in neutral lipid formation and an increase in the percentage of alkyl lyso GPC but not PAF. This suggests that the neutral lipid is formed directly from alkyl lyso GPC and not PAF.

The human mucosal enzymes, therefore, metabolize PAF by the same initial obligatory acetyl hydrolase-mediated step that exists in all cell types examined so far. The amounts of alkyl lyso GPC that accumulate, however, do vary markedly among different cell types, between species and are dependent on whether cells have been stimulated. Unstimulated platelets [11] and neutrophils [6] have a high acyl transferase activity and thus when PAF is metabolized the ratio of alkyl lyso GPC: alkyl acyl GPC is low; however, in human endothelial cells [12] and alveolar macrophages [13] with low acyl transferase activity PAF is converted to alkyl lyso GPC and a substantial amount is not reacylated. In this study, acylation of alkyl lyso GPC was readily apparent: within a 5-min incubation period 17% of alkyl lyso GPC had been catabolized and almost 50% of this had already been acylated to alkyl acyl GPC.

We also found evidence for significant metabolism of alkyl lyso GPC by a phospholipase that has the properties of a lysophospholipase D; lysophospholipase D hydrolyses the choline moiety from alkyl lyso GPC to form 1-*O*-alkyl-2-lyso-*sn*-glycero-P (lysophosphatidic acid) which can then be dephosphorylated by phosphatidate phosphohydrolases to 1-*O*-alkyl-*sn*-glycerol. Our evidence for the presence of lysophospholipase D was that using alkyl acetyl [^{14}C]GPC or alkyl lyso [^{14}C]GPC as substrate the water-soluble product of catabolism was [^{14}C]choline. No [^{14}C]phosphocholine (indicative of phospholipase C activity) was found in incubations of from 2 min to 3 hr.

The lyso phospholipase D enzyme identified by Kawasaki and Snyder [8] in rabbit kidney medulla bears similarities to the enzyme we have identified. It is Ca^{2+} dependent and has little hydrolytic activity towards alkyl acyl GPC (unpublished data). However, we cannot as yet exclude unequivocally the possibility that phospholipase C is active in our system and that [^{14}C]phosphocholine is formed and hydrolysed to [^{14}C]choline by phosphatases [14]. Moreover, using [^{3}H]alkyl acetyl GPC or [^{3}H]alkyl lyso GPC as substrate we were able to detect only small amounts of radiolabelled material with the mobility of lysophosphatidic acid ($R_f = 0.05$). Additionally, inclusion of sodium vanadate, a phosphohydrolase inhibitor, did not increase the amounts of lysophosphatidic acid present as has been shown in incubates of alkyl lyso GPC with rabbit kidney medulla microsomes [8]. It is possible that the colon mucosa contains large amounts of phosphohydrolases that readily convert lysophosphatidic acid to neutral lipids; in the absence of sodium vanadate the amounts of lysophosphatidic acid detected by Kawasaki and Snyder [8] were small. The lack of significant metabolism in preliminary experiments utilizing 1-*O*-[^{3}H]alkyl-2-arachidonyl GPC provides further evidence that the neutral lipid formed is derived from alkyl lyso GPC.

The role of Ca^{2+} in regulating the catabolism of PAF has given rise to some contradictions. In intact platelets, extracellular Ca^{2+} removal had no effect on PAF catabolism by acetylhydrolase [10, 15] whereas if intracellular Ca^{2+} was elevated in intact platelets by the addition of the calcium ionophore A23187, acetyl hydrolase was inhibited and this could be reversed by addition of EGTA [16]. In platelet membrane preparations acylation of alkyl lyso GPC was inhibited by Ca^{2+} [16, 17]. In the current investigation we showed that the activity of the acetyl hydrolase associated with the 1000-*g* supernatant of human colon mucosa homogenate was not altered by chelation of Ca^{2+} in the incubates and, therefore, is calcium independent. The activity of PAF acetyl hydrolase from human plasma is similarly independent of calcium concentrations [18]. It is, therefore, clear that the properties of acetyl hydrolases differ between species and tissues.

Significant amounts of alkyl acyl GPC were formed from alkyl lyso GPC by human mucosal acyl transferase in the presence of 5 mM Ca^{2+}. Chelation of Ca^{2+} resulted in a significant increase in the amount of alkyl acyl GPC formed which might suggest that 5 mM Ca^{2+} is, in fact, exerting an inhibitory effect on the acyl transferase enzymes. There is, however, an alternative explanation: in the presence of EDTA less neutral lipid and unknown substance (R_f 0.8) were formed, perhaps providing less competition for the substrate and allowing greater conversion to alkyl acyl GPC. This experiment also provides evidence that acyl CoA is not rate limiting in the formation of alkyl acyl GPC.

Our studies have shown that the lysophospholipase D is calcium dependent. This dependence has also been shown previously in human neutrophils and erythroleukaemia cells [19–21] and in rabbit kidney medulla [8] but not in rat tissues [22] or bovine endothelial cells [23]. Overall it must be said that the exact role of Ca^{2+} in activating lysophospholipase D is unclear and further studies are required [24]. Our HPLC studies were able to verify the TLC peak co-chromatographing with alkyl acyl GPC but preliminary experiments failed to detect a peak on HPLC for the unknown substance $R_f = 0.8$ which had a mobility similar to the phospholipid, phosphatidylethanolamine. Moreover, the unknown peak must have an intact choline head group as it is present as an organic phase soluble metabolite ($R_f = 0.8$) when 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-methyl[^{14}C]choline is used as substrate.

In summary, our data show for the first time that human colon mucosal enzymes can actively catabolize PAF via the intermediate lyso PAF to alkyl acyl GPC and to neutral lipid products. The conversion of PAF to lyso PAF then to alkyl acyl GPC is a process that does not require the presence of Ca^{2+},

but the production of neutral lipid from lyso PAF is Ca^{2+} dependent.

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