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Metabolism of platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) and lyso-PAF (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) by cultured rat Kupffer cells

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The metabolism of platelet-activating factor (PAF; identified as AGEPC: 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) and lyso-PAF (lyso-GEPC: 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) was investigated in cultured rat Kupffer cells. The rat Kupffer cells accumulated [^{3}H]AGEPC and deacetylated this compound to the corresponding [^{3}H]lyso-GEPC, which was the major metabolic product of [^{3}H]AGEPC. [^{3}H]Lyso-GEPC was distributed primarily in the supernatant fraction of incubated cells throughout the experimental interval. Only a very small portion of the [^{3}H]lyso-GEPC was further converted to 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC), indicating that this acylation process was not particularly active in these cells. When [^{3}H]lyso-GEPC was incubated with Kupffer cells, the conversion of lyso-GEPC to AGEPC via the acetyltransferase reaction increased up to 30 min and declined thereafter. Bovine serum albumin (BSA) had a substantial influence on both the cellular uptake and the metabolism of [^{3}H]AGEPC. An increase in the BSA concentration in the incubation media reduced the cellular uptake of [^{3}H]AGEPC and the subsequent formation of lyso-GEPC. The results of this study suggest that the hepatic Kupffer cells play an important role in the metabolism of PAF. Moreover, these results infer that the regulation of the PAF level in certain hepatic pathophysiological situations may be a consequence of the production and subsequent metabolism of this potent lipid autacoid in the Kupffer cells of the liver.

INTRODUCTION

Platelet-activating factor [PAF, identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC)] is a biologically active phospholipid which is involved in several physiological and pathophysiological activities, such as allergic reactions [1,2], activation of platelets and polymorphonuclear leukocytes [2–4], negative inotropic effects in guinea pig heart [5], hypotension [6] and hepatic glycogenolysis [7,8]. PAF is produced following appropriate stimuli in several tissues and cells including blood cells [2,9], peritoneal and alveolar macrophages [10,11], isolated rat kidney cells [12], human endothelial cells [13,14], perfused guinea pig heart [5], perfused rat liver [15] and rabbit lung [16].

The metabolism of AGEPC has been studied previously in HL-60 cells [17], Madin–Darby canine kidney cells [17], intact rat [18], transformed and nontransformed mouse embryonic cells [17], human and rabbit neutrophils [19,20], rabbit and human platelets [21–23], rat alveolar macrophages [24], interstitial and epithelial cells from rat lungs [25], rat capillary endothelial cells [26], hepatocytes [27], cultured human endothelial cells [28] and hepatic nonparenchymal cells in the present study. Although most reports have shown that AGEPC is first converted to 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso-GEPC) which is then reacylated to 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC) [17,19–26], it has been suggested that AGEPC is probably transacylated to alkylacyl-GPC directly [21] and that lyso-GEPC is converted to 1-alkyl-2-lyso-glycerol via phospholipase C. 1-Alkyl-2-lyso-glycerol then may be converted to fatty aldehyde via alkyl-monooxygenase [27].

There are a limited number of reports on the metabolism of lyso-GEPC. Alam *et al.* [29] monitored the metabolism of [^{3}H]lyso-GEPC, a substrate of acetyltransferase, in human iliac vein endothelial cells and found that these cells converted [^{3}H]lyso-GEPC to [^{3}H]AGEPC during the first 30 min of incubation. In addition, Prescott *et al.* [13] observed the stimulatory effect of thrombin on the metabolism of [^{3}H]lyso-GEPC in cultured human endothelial cells.

The Kupffer cell is a major cell type of the reticulo-endothelial system in the liver. Kupffer cells are in intimate contact with circulating blood, and the microvilli of the surface of these cells intermingle with microvilli of the hepatic parenchymal cells [31]. Hence Kupffer cells may play an important role in receiving the stimulatory signals of PAF from the hepatic circulation, whereupon the stimulated Kupffer cell may transmit the signal in some fashion to the parenchyma thus mediating previously observed biological responses of PAF in the perfused rat liver [7,8], as this potent autacoid is cleared from the circulation. In fact, previous studies in our laboratory have shown that various particulate sub-

Abbreviations used: PAF, platelet-activating factor; AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; lyso-PAF (lyso-GEPC), 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine; lyso-PC, lyso-phosphatidylcholine; alkylacyl-GPC, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; BSA, bovine serum albumin; TNS, toluidinyl-2-naphthalenesulphonate.

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stances, e.g. heat-aggregated IgG [15], zymosan, latex-coated beads [37] or bacterial lipopolysaccharide (G. Hanson & M. S. Olson, unpublished work), which are expected to stimulate reticulo-endothelial cells in the liver, cause the hepatic responses similar to those seen with AGEPC, and these particulates stimulate the formation of this potent agonist in the perfused rat liver but not in suspensions of isolated hepatic parenchymal cells. Recently, our laboratory has shown that the cultured rat Kupffer cells synthesize PAF when given certain stimuli [30]. Thus, because of the apparent central role of the Kupffer cell in the hepatic actions of AGEPC, it seemed essential to elucidate the mechanism by which a biologically active phosphoglyceride, such as AGEPC, is metabolized by this cell type.

MATERIALS AND METHODS

Materials

1-*O*-[1′,2′-^{3}H]Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]AGEPC; 59.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). 1-*O*-[^{3}H]Octadecyl-2-lyso-*sn*-glycero-3-phosphocholine ([^{3}H]lyso-GEPC, 80 Ci/mmol) was obtained from Amersham (Arlington Heights, IL, U.S.A.). The purities of [^{3}H]AGEPC and [^{3}H]lyso-GEPC were greater than 93%, as determined by t.l.c. Polar lipid standards were purchased from Avanti Polar Lipids Inc. (Birmingham, AL, U.S.A.) and unlabelled PAF was from BACHEM BioScience, Inc. (Bubendorf, Switzerland). Collagenase type IV and protease type XIV were purchased from Sigma. Metrizamide [(2,3-acetamido-5-*N*-methyl-acetamido-2,4,6-tri-iodobenzamido)-2-deoxy-D-glucose] was obtained from NYCOMED Co. (Oslo, Norway).

Isolation and primary culture of rat Kupffer cells

Rat Kupffer cell suspensions were purified by centrifugal elutriation and cultured as described previously [30]. The viability of Kupffer cells was greater than 95% as determined by Trypan Blue exclusion.

Incubation conditions and lipid extraction

Before cultured cells were used in an incubation, the maintaining media were carefully removed from the cultured cells and the cells were rinsed twice with 2 ml of 0.1% bovine serum albumin (BSA) containing serum-free RPMI-1640 media. Kupffer cells (2×10^6) were incubated in 1 ml of the serum-free medium for 10 min at 37 °C, and then 0.5 pmol of [^{3}H]AGEPC (0.03 μCi; 0.5 nM final concentration) was added to each plate. Each incubation was conducted in duplicate or triplicate for the desired time and the metabolic reaction was stopped by quickly removing the media from each plate for subsequent lipid analysis (set A). Then 1 ml of methanol was added to each dish. The cells were scraped with a rubber policeman and each culture dish was rinsed with 1.25 ml of methanol, again for analysis of the component lipids (set B). Lipids in the medium (set A) and the cellular fraction (set B) were extracted separately according to the procedure of Bligh & Dyer [32]. The experiment to characterize the metabolism of [^{3}H]lyso-GEPC in each sample was performed following the same procedure except that 12.5 pmol of lyso-GEPC (1.0 μCi) was added to each dish instead of [^{3}H]AGEPC.

Thin layer chromatography

The lipid-rich chloroform phase of each sample was collected and evaporated under N_2. The residue was immediately dissolved in 100 μl of chloroform/methanol (2:1, v/v) and subjected to t.l.c. on a 250 μm silica gel H plate (Uniplate, Analtech, Newark, NJ, U.S.A.), with a separate lane containing unlabelled standard polar lipids, i.e. AGEPC, lysophosphatidylcholine (lyso-PC) and phosphatidylcholine (PC). Chromatography was performed using a solvent system of chloroform/methanol/water (65:35:7, by vol.) as described previously [33,34]. The standard phospholipids were detected under ultraviolet light after spraying with toluidinyl-2-naphthalenesulphonate (TNS). Each spot of phospholipid was scraped and the amount of radioactivity in each lipid class was determined by lipid scintillation spectrometry.

Data presentation

The quantification of the results of the phospholipid analysis performed in this study is based on the specific radioactivity of the labelled compounds added to the various incubations, except in the recovery study of [^{3}H]AGEPC where the percentage of total radioactivity was used. Unless otherwise stated, all data are presented as the means ± S.D. of triplicate determinations and are representative of at least two separate experiments.

RESULTS

Experimental conditions: the effect of BSA on the recovery and the metabolism of [^{3}H]AGEPC by rat Kupffer cells

The strong adsorption of AGEPC to glassware or plastic cultureware has been observed previously [35]. Therefore, it was important to establish suitable incubation conditions to improve the recovery of AGEPC and its metabolites. We used different concentrations of BSA and observed its effect on the recovery of [^{3}H]AGEPC and on the uptake and the metabolism of [^{3}H]AGEPC by rat Kupffer cells. Culture medium was incubated with 0.5 nM-[^{3}H]AGEPC (0.03 μCi) at 37 °C in the plastic culture dishes for 30 min in the presence of Kupffer cells. About 84% of the total added radioactivity was recovered when the culture medium contained 0.0025% BSA in the presence of Kupffer cells, and the recovery rate rose to 99 and 98% when the concentration of BSA was increased to 0.1% and 0.25% respectively (results not shown). Hence, all subsequent metabolic experiments were performed in the presence of 0.1% BSA. Furthermore, BSA was shown to have an obvious inhibitory effect on the cellular uptake of [^{3}H]AGEPC by rat Kupffer cells. If the concentration of BSA was increased from 0.0025% to 0.1% or 0.25%, the cellular uptake of [^{3}H]AGEPC by Kupffer cells decreased from 127.9 fmol/2×10^6 cells to 38.8 and 29.0 fmol respectively (results not shown). When the BSA content of the incubation system was increased and the amounts of different metabolites of [^{3}H]AGEPC were analysed at 30 min after addition of [^{3}H]AGEPC to the cells, we found that lyso-GEPC decreased rapidly, both in the cellular fraction (from 88.7 fmol at 0.0025% BSA to 5.9 fmol at 0.25% BSA) and in the medium fraction (from 62.8 fmol at 0.0025% BSA to 46.8 fmol at 0.25% BSA), suggesting an inhibitory effect on the formation of

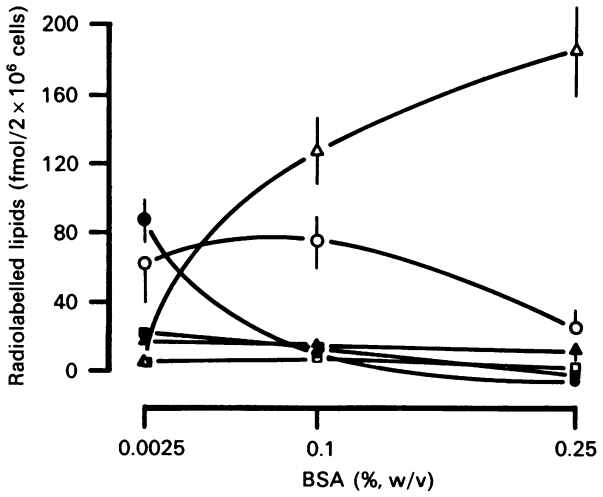

Fig. 1. The effect of BSA on the metabolism of [^{3}H]AGEPC by rat Kupffer cells

This experiment was done as described in the Materials and methods section. The radiolabelled lipids from medium (open symbols) and cellular (solid symbols) fractions were extracted and analysed separately. AGEPC (△, ▲), lyso-GEPC (○, ●) and alkylacyl-GPC (□, ■) are shown.

lyso-GEPC (Fig. 1). Also, alkylacyl-GPC, a reacylated metabolite of lyso-GEPC, decreased in the cellular fraction from 21.3 fmol to 8.7 fmol when the concentration of BSA was increased from 0.0025% to 0.25%, though this change was not as pronounced as with lyso-GEPC. In contrast, there was an increased retention of [^{3}H]AGEPC in the medium with the increase in the concentration of BSA.

Cellular uptake and metabolism of [^{3}H]AGEPC

Kupffer cells (2×10^6) in 1 ml of medium containing 0.1% BSA were incubated with 0.5 pmol of [^{3}H]AGEPC (0.03 μCi) for the desired time, and the phospholipids were separated and analysed by t.l.c. as described in the Materials and methods section. Three areas of labelled phospholipids were identified and removed by scraping: AGEPC, lyso-GEPC and alkylacyl-GPC. The metabolic products of [^{3}H]AGEPC found in the complete incubation mixture and in the cellular and medium subfractions were analysed and are shown in Figs. 2(*a*), 2(*b*), and 2(*c*) respectively. Fig. 2(*a*) demonstrates that the amount of [^{3}H]AGEPC in the total fraction (cells plus medium) declined, with a concomitant and subsequent increase of [^{3}H]lyso-GEPC from 13.4 fmol at 0 min to 73.5 fmol at 60 min. The major metabolic product of [^{3}H]AGEPC was [^{3}H]lyso-GEPC, whereas the amount of alkylacyl-GPC appeared not to change significantly during the entire incubation period (60 min). When the cellular and the medium fractions were analysed separately, it was observed that the peak uptake of [^{3}H]AGEPC, approx. 30.1 fmol, occurred about 10 min after the addition of [^{3}H]AGEPC, and this was maintained at that level thereafter, as shown in Fig. 2(*b*). The amount of [^{3}H]AGEPC found intracellularly increased rapidly to 17.9 fmol at 5 min and 21.9 fmol at 10 min, then decreased slowly thereafter. At the same time, the amounts of lyso-GEPC and alkylacyl-GPC in the cellular fraction increased at a relatively constant rate

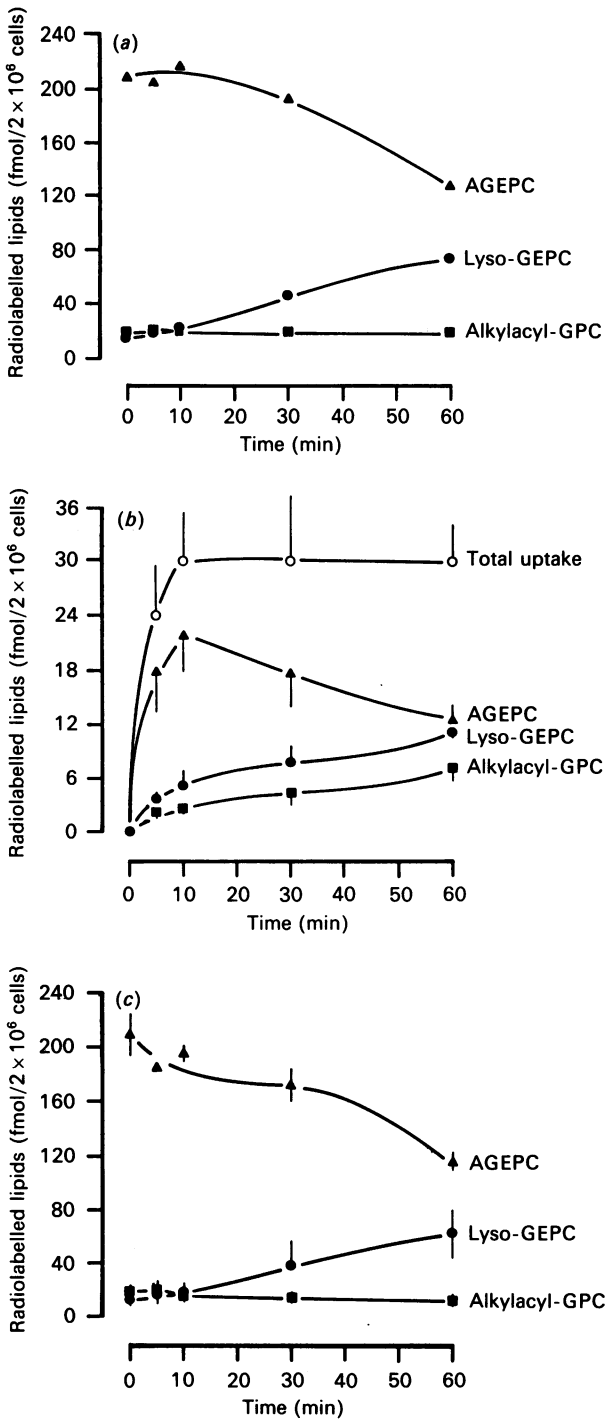

Fig. 2. Time-course of the metabolism of [^{3}H]AGEPC incubated with rat Kupffer cells

[^{3}H]AGEPC (0.5 nM; 0.03 μCi) was incubated with 2×10^6 cells in 1 ml of 0.1% BSA-containing medium for the indicated time at 37 °C, and then the ^{3}H-labelled lipids in total (*a*), cellular (*b*) and medium (*c*) fractions were extracted and analysed separately on t.l.c. plates. Total cellular lipids (○), lyso-GEPC (●), AGEPC (▲), and alkylacyl-GPC (■) are shown.

(Fig. 2*b*). In the medium fraction (Fig. 2*c*), the amount of alkylacyl-GPC showed no significant change, whereas the amount of lyso-GEPC rose constantly within 10 min of the addition of [^{3}H]AGEPC. Lyso-GEPC was the

major metabolic product found in both the cellular and the medium fractions, with primary distribution in the medium. In order to verify that the lyso-GEPC found in the medium was formed in and released by the Kupffer cells instead of being formed in the medium resulting from the release of acetylhydrolase, [^{3}H]AGEPC was incubated for 60 min in medium in which the Kupffer cells had been preincubated for 10 min then the cells removed; no lyso-GEPC was produced (results not shown).

Cellular uptake and metabolism of [^{3}H]lyso-GEPC by rat Kupffer cells

The analysis of the total incubation mixture (Fig. 3*a*) indicated that the amount of AGEPC rapidly increased from 0.3 pmol at 0 min to 0.4 pmol at 30 min and decreased thereafter. There occurred a 30 min delay in the formation of alkylacyl-GPC compared to AGEPC and the increase in the amount of alkylacyl-GPC was not as much as for AGEPC. When the cellular and the medium fractions were analysed separately, it was found that the cultured Kupffer cells took up [^{3}H]lyso-GEPC in a typical time-dependent manner (Fig. 3*b*). Within 10 min of the addition of [^{3}H]lyso-GEPC to the cultured cells, 59.1 fmol of ^{3}H-labelled lipids were found in the cellular fraction, and this increased to 91.5 fmol at 30 min and 110.7 fmol at 60 min. The amount of [^{3}H]lyso-GEPC in the cellular fraction increased rapidly during the first 10 min of incubation (from 1.4 fmol at 0 min to 46.1 fmol at 10 min), reached a maximum level (68.4 fmol) at 30 min, remaining approximately constant for 60 min thereafter. Concomitantly, alkylacyl-GPC associated with the cell fraction increased at a slower but constant rate (Fig. 3*b*). Analysis of the medium fraction revealed that during the first 30 min, the amount of [^{3}H]lyso-GEPC decreased rapidly, with a corresponding increase in [^{3}H]AGEPC which then decreased rapidly thereafter; whereas the amount of alkylacyl-GPC in the medium remained almost at the same level (Fig. 3*c*).

DISCUSSION

The results of the present study demonstrate that cultured rat Kupffer cells can take up and hydrolyse [^{3}H]AGEPC to [^{3}H]lyso-GEPC, and then reacylate the lyso-GEPC to alkylacyl-GPC. When [^{3}H]AGEPC was incubated with the cells for different periods of time at 37 °C, lyso-GEPC was the major radiolabelled metabolite detected throughout the incubation period. This observation differs from the reports of others using HL-60 cells [17], rabbit and human platelets [21–23], rat lung epithelial cells [25] and rabbit and human neutrophils [19,20], where the major metabolite of AGEPC was alkylacyl-GPC, with only a small amount of lyso-GEPC formed. Results similar to the present study have been reported previously in rat capilliary endothelial cells [26], human endothelial cells [28] and rat alveolar macrophages [24]. The experiment in which [^{3}H]AGEPC was incubated with the cell-free medium in which the Kupffer cells had been preincubated confirmed that the lyso-GEPC found in the medium was, indeed, the major metabolite of [^{3}H]AGEPC, and was formed intracellularly and released into medium, suggesting an intracellular location of acetylhydrolase. However, the biological significance of the production of a large amount of lyso-GEPC and its extracellular distribution is presently unclear.

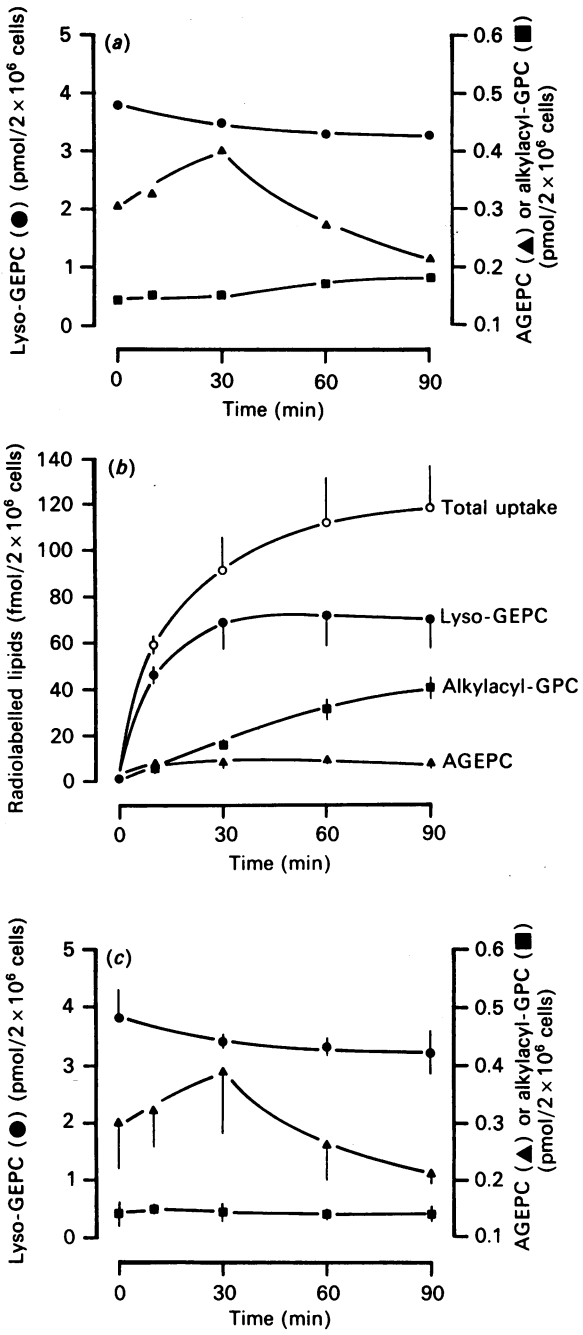

Fig. 3. Time-course of the metabolism of [^{3}H]lyso-GEPC incubated with rat Kupffer cells

[^{3}H]Lyso-GEPC (12.5 nM; 1.0 μCi) was incubated with 2×10^6 cells in 1 ml of 0.1 % BSA-containing medium for the indicated time at 37 °C and then the ^{3}H-labelled lipids in total (*a*), cellular (*b*) and medium (*c*) fractions were extracted and analysed separately on t.l.c. plates. Total cellular lipds (○), lyso-GEPC (●), AGEPC (▲) and alkylacyl-GPC (■) are shown.

When [^{3}H]lyso-GEPC was added to cultured Kupffer cells and incubated for various time intervals, it was observed that during the first 30 min [^{3}H]lyso-GEPC decreased with a corresponding rapid increase in

[^{3}H]AGEPC, which then itself decreased thereafter. Similar results have been reported by Alam *et al.* [29] in adult human iliac vein endothelial cells. Interestingly, there was a 30 min delay before any [^{3}H]alkylacyl-GPC was detected, comparable with [^{3}H]AGEPC, i.e. at 30 min after addition of [^{3}H]lyso-GEPC to the cultured cells when AGEPC begun to decline, the amount of alkylacyl-GPC increased at a slower and constant rate (Fig. 3*a*). These results suggest that exogenous lyso-GEPC stimulated either the acetyltransferase to convert lyso-GEPC to AGEPC, or the acyltransferase to transform lyso-GEPC to alkylacyl-GPC. Also, it appears that the acetyltransferase may be more sensitive than the acyltransferase to the stimulatory action of this exogenous substrate.

Finally, it has been reported that BSA has an influence on the recovery and the cellular uptake of [^{3}H]AGEPC by various cell types [17,25,36]. Also, BSA has effects on PAF synthesis and PAF-induced platelet and neutrophil activation [38]. In agreement with these reports, it has been shown in the present study that BSA can improve the recovery of [^{3}H]AGEPC from the plastic culture plates and can inhibit the cellular uptake of this compound by cultured rat Kupffer cells. Furthermore, BSA appeared to have a significant influence on the metabolism of [^{3}H]AGEPC, mainly decreasing the formation of lyso-GEPC, which may be the result of the inhibition of the cellular uptake of [^{3}H]AGEPC by BSA. This observation stands in contrast to a previous report in which BSA had no effect on the metabolism of [^{3}H]AGEPC in MDCK cells [17], but is in agreement with the report of Yamashita *et al.* [36].

Even though certain phenomena cannot be explained definitively in this study, it is clear that Kupffer cells play an important role in the metabolism, including both catabolism and synthesis, of this potent phospholipid autacoid, and hence in the regulation of the PAF levels in certain pathophysiological situations to which the liver may be exposed.

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