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Directed Shift of Fatty Acids from Phospholipids to Triacylglycerols in HL-60 Cells Induced by Nanomolar Concentrations of Triethyl Lead Chloride: Involvement of a Pertussis Toxin-Sensitive Pathway

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SUMMARY

Triethyl lead chloride (Et_3PbCl) was found to induce a shift of fatty acids from membrane phospholipids to triacylglycerols in the human promyelocytic leukemia cell line HL-60. High concentrations of Et_3PbCl (>10 μM) caused a substantial liberation of [^{14}C]arachidonic acid within 10 to 20 min in dimethyl sulfoxide-differentiated cells, comparable to the effect of the calcium ionophore A23187 (10 μM). Following liberation of arachidonic acid, its metabolites could be detected. Prolongation of the incubation time and reduction of Et_3PbCl concentration resulted in a shift of fatty acids from phospholipids to triacylglycerols. Deacylation of phospholipids and reacylation into phospholipids and triacylglycerols were in equilibrium when the cells were treated with Et_3PbCl at concentrations of $\leq$10 μM for 5 hr or $\leq$1 μM for 24 hr; no increase of free fatty acids could be observed, and the loss of fatty acids within the phospholipids was equivalent to the increase of fatty acid content within the triacylglycerols. Moreover, under these conditions, no loss of viability was seen after 24 hr, as compared with untreated differentiated cells. This concentration- and time-dependent effect of Et_3PbCl might be due to a stimulated liberation of fatty acids via phospholipase A_2, because this stimulation could be totally prevented by the phospholipase inhibitors quinacrine and *p*-bromophenacylbromide. Additionally, pretreatment of differentiated HL-60 cells with pertussis toxin resulted in a drastic reduction of [^{14}C]arachidonic acid liberation when cells were stimulated with Et_3PbCl. These results suggest the involvement of a pertussis toxin-sensitive GTP-binding protein and of a signal transduction mechanism during stimulated fatty acid release; release does not seem to be via a direct stimulation of phospholipase activity by the lead compound.

Heavy metal compounds are ubiquitously distributed toxicants, and lead is still first among world-wide emissions of all trace elements (1). Although organic lead antiknock motor fuel additives have been used for the last 60 years, most information about their toxic effects has appeared during the last 10 years. Et_3PbCl was found in human brains (2), in association with microtubules (3, 4) and intermediate filaments (5), and it affects membrane function (6, 7) and stimulates liberation of fatty acids in human blood platelets (8) and in the human promyelocytic leukemia cell line HL-60 (9). Moreover, toxic effects of lead were described at the micromolar level (10), the nanomolar level (11), and the picomolar level (12).

In this study, we investigated the fatty acid composition of phospholipids, triacylglycerols, and the free fatty acid fraction of HL-60 cells. We demonstrated a strong effect, even at low concentrations of Et_3PbCl, on the distribution of fatty acids within the lipid classes. Furthermore, these experiments gave a greater insight into the mechanism by which Et_3PbCl induces this rearrangement of fatty acids. The results shown here suggest a participation of GTP-binding protein-dependent activation of phospholipase A_2.

Materials and Methods

Chemicals. Quinacrine, thimerosal, and pBPB were obtained from Serva (Heidelberg). The calcium ionophore A23187, pertussis toxin, and fMLP were from Sigma (Munich), and the SIL G Polygram thin layer plates were from Macherey & Nagel (Düren). [1-^{14}C]Arachidonic acid (58.3 mCi/mmol) was purchased from Amersham (Braunschweig). All other chemicals were of analytical grade, and solvents for HPLC were obtained from Promochem (Wesel).

Cell culture and incubation. The HL-60 cells were grown in suspension culture in RPMI 1640 medium supplemented with 15% fetal calf serum. The suspension was split in order to maintain cell number between 2×10^5 and 2×10^6 cells/ml. Cells were induced to differentiate to mature granulocytes by the addition of 1.3% dimethyl sulfoxide for 6 days, because only the mature nonproliferating cells possess the activity of phospholipase A_2 (13). The percentage of differentiated cells was quantified using the nitroblue tetrazolium reduction method, as described (14), and if the extent of differentiation exceeded

ABBREVIATIONS: Et_3PbCl, triethyl lead chloride; pBPB, *p*-bromophenacylbromide; fMLP, fMet-Leu-Phe; PC, phosphatidylcholine; PE, phosphatidylethanolamine; HPLC, high pressure liquid chromatography; CoA, coenzyme A.

70% the cells were used for the experiments. The cells were harvested by centrifugation, washed once with RPMI without any additives, and finally resuspended in medium containing 1% dimethyl sulfoxide and 3.3% fetal calf serum, at a concentration of 1×10^7 cells/ml. Experiments were started after 30 min of rest. The cell suspensions (3 ml) were then incubated at 37° with 10 μM calcium ionophore A23187 or Et_3PbCl, as indicated. In the case of radioactive prelabeling, [^{14}C] arachidonic acid was dissolved in dimethyl sulfoxide and added at day 5 (2.5 μCi/50 ml) to the culture medium, and the cells were incubated overnight. The labeled cells were washed twice with RPMI and resuspended as described above.

Lipid extraction and separation of lipid classes. After incubation of the cell suspensions, they were extracted by the addition of 11.75 ml of chloroform/methanol (1:2), and phase separation was induced with an additional 3.75 ml of chloroform and 3.75 ml of 0.2% formic acid. The organic phase was dried under nitrogen, taken up in chloroform, applied to bonded phase aminopropyl columns (Waters), and separated into three fractions (15). After hydrolysis of the triacylglycerol and the phospholipid fractions, the fatty acids were esterified with pBPB (16). The phenacyl esters of the fatty acids were then analyzed using HPLC.

Radioactive lipids were extracted as described above. The dried lipids were taken up in $CHCl_3$, spotted onto SIL G polyester plates (20 cm × 20 cm), and separated by thin layer chromatography. A complete separation of phospholipid classes, as well as the arachidonic acid metabolites, free arachidonic acid, triacylglycerols, and cholesterol esters, on one plate was achieved by combining the methods of Billah *et al.* (17) and Lagarde *et al.* (18). Plates were developed in solvent system I (chloroform/methanol/acetic acid/water, 56:33:9:2) for the first 11 cm, dried, and developed in solvent system II (hexane/diethyl ether/acetic acid, 80:20:1) up to the end of the plate. The R_F values for the phospholipid classes, free arachidonic acid, and triacylglycerols were determined by comparison of their migration with that of commercial standards. This system gives good separation of the phospholipids sphingomyelin, PC, phosphatidylinositol, phosphatidylserine, and PE, as well as of the neutral lipids (arachidonic acid and its metabolites, triacylglycerols, and cholesterol esters).

HPLC analysis. Standards or cellular fatty acid esters were separated by use of a Waters chromatographic system, consisting of two HPLC pumps (model 510), an automated sample processor (WISP), and a programmable multiwavelength detector (model 490), and were evaluated with a Maxima 820 chromatography data station. The analysis was carried out by use of a Nucleosil C8 5-μm column (25 cm × 4.6 mm; Macherey & Nagel). A good resolution overall was achieved by application of a gradient after 1 min, from 60:40 (acetonitrile/water) to 73:27 within 3 min (gradient curve 2), at a flow rate of 1.5 ml/min. The fatty acid esters were detected at 254 nm, and the amounts were determined by the external standard method. The recovery from the whole procedure (extraction, separation, hydrolysis, and derivatization) was estimated, by the addition of the internal standard margaric acid, as 87 ± 7%.

Results

Et_3PbCl-induced liberation of arachidonic acid. HL-60 cells, differentiated with dimethyl sulfoxide to mature granulocytes, were incubated for 24 hr in the presence of [^{14}C] arachidonic acid. Although nearly 80% of the label was incorporated during the first 2 hr, an equilibrium in arachidonic acid distribution within the lipid classes was reached after 24 hr (results not shown). The time course of incorporation into PC and PE of HL-60 cells coincided almost exactly with that reported by Froissart *et al.* (19) for rat basophilic leukemia (RBL-2H3) cells. The distribution of the radioactivity within the cellular lipids was determined after 24 hr and compared with that of A23187- or Et_3PbCl-treated cells. The stimulation of arachidonic acid liberation was obvious both for the calcium ionophore and for Et_3PbCl (Fig. 1). The time course of the alterations within the different lipid classes during the first 30 min of incubation either with A 23187 or with Et_3PbCl showed that stimulation with the ionophore led to large amounts of free fatty acid during the first minutes of the incubation, whereas the early effect of Et_3PbCl was a shift of arachidonic acid from PC and PE to phosphatidylinositol and phosphatidylserine. An increase of free arachidonic acid could be observed after 10 min and it reached a maximum after 30 min; the level of free arachidonic acid then decreased slowly, just as could be seen between 10 and 30 min after ionophore stimulation (Fig. 1). The incorporation of arachidonic acid, especially into triacylglycerols, reached a higher level after ionophore stimulation than after Et_3PbCl treatment.

On the other hand, when HL-60 cells were treated with lower concentrations of the xenobiotic for longer periods of time, a loss of [^{14}C]arachidonic acid within the phospholipids was obvious as well, although no free arachidonic acid could be detected. The total amount of liberated arachidonic acid was reesterified continuously into both fractions, the triacylglycerols as well as the phospholipids. By use of the radiolabeling

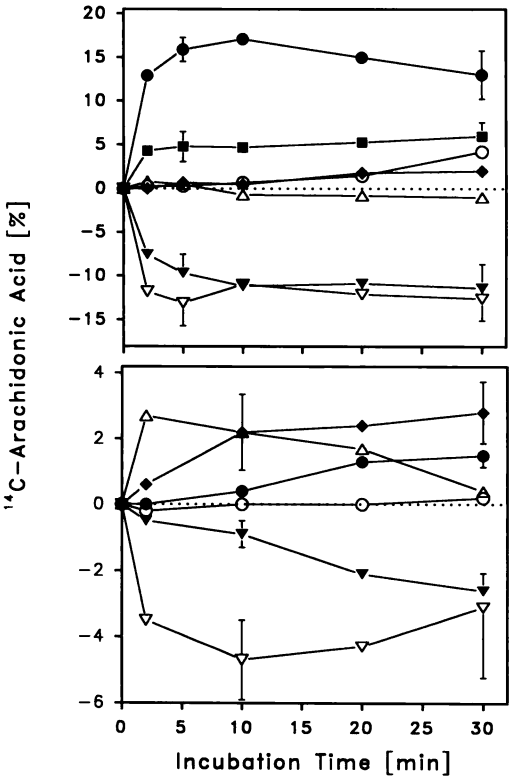

Fig. 1. Time courses of stimulated liberation and rearrangement of arachidonic acid induced by calcium ionophore A23187 or Et_3PbCl. [^{14}C] Arachidonic acid-prelabeled and differentiated HL-60 cells were incubated with 10 μM A 23187 (A) or 50 μM Et_3PbCl (B) for different periods of time (as indicated on the *abscissa*). Lipids were extracted, separated by thin layer chromatography, and counted for radioactivity in a liquid scintillation counter. *Ordinate*, decrease (negative) or increase (positive) of radioactivity (cpm × 10^{-3}) in the different lipid classes. Values at time zero for all lipid classes are given below. Results are expressed as the mean ± standard error of three to seven separate experiments. ●, Arachidonic acid (2,330 ± 1,140 cpm); ■, eicosanoids (1,019 ± 540 cpm); ○, triacylglycerols (32,032 ± 1,360 cpm); ♦, phosphatidylserine (39,330 ± 3,336 cpm); △, phosphatidylinositol (8,411 ± 846 cpm); ▽, PE (178,959 ± 8,502 cpm); ▼, PC (154,654 ± 2,323 cpm).

method, the reesterification back into the phospholipids is not detectable (unless the distribution of [^{14}C]arachidonic acid between the different phospholipids is altered, compared with the initial composition). Table 1 shows the results of the incubations with lower concentrations of Et_3PbCl. It demonstrates an increase of the label within the triacylglycerol fraction and a corresponding decrease within the phospholipids (PE and PC). Furthermore, concentrations of Et_3PbCl as low as 250 nM caused, within 24 hr, 40% of the effect caused by the 200-fold greater concentration within 30 min.

Alteration of fatty acid composition within cellular lipids induced by Et_3PbCl. In another series of experiments, without prelabeling of the cells, the fatty acid composition of the cellular lipid classes was measured by HPLC. These experiments revealed information on nearly all of the fatty acids present in the cells. The results shown in Fig. 2 corroborated those obtained by radioactive labeling and gave additional information about the other fatty acids. Stimulation of the cells with ionophore led to liberation of more than 20% of the total cellular content of arachidonic acid, whereas other fatty acids played only a minor role (Fig. 2A). The results obtained in experiments after incubation of differentiated HL-60 cells with 100 and 0.5 μM Et_3PbCl for 30 min and 24 hr, respectively, demonstrated that the liberation of fatty acids was quite different, compared with ionophore treatment; with Et_3PbCl treatment, the liberation of fatty acids other than arachidonic acid was increased (Fig. 2, B and C). Above all, the noncytotoxic concentration of Et_3PbCl (0.5 μM) induced a substantial shift from cellular phospholipids to triacylglycerols, not only of arachidonic acid but also of the other fatty acids measured.

Effect of phospholipase inhibitors and pertussis toxin on Et_3PbCl-stimulated liberation of arachidonic acid. Quinacrine is known to inhibit the liberation of arachidonic acid from human platelet phospholipids (8, 20, 21), as is pBPB, which binds irreversibly to phospholipase A_2 (21, 22). Preincubation of HL-60 cells with either quinacrine or pBPB almost completely prevented the appearance of arachidonic acid after stimulation with A23187 or Et_3PbCl (Table 2). In the case of quinacrine pretreatment, a small loss of label within the phospholipids and an equivalent increase within the triacylglycerols could be detected, whereas pBPB prevented all lead-induced effects (results not shown).

In another set of experiments, prelabeled HL-60 cells were incubated for 3 hr at 37° with 500 ng/ml pertussis toxin. During this period of time, no alteration of incorporation and distribution of [^{14}C]arachidonic acid within the lipid classes could be detected. The pertussis toxin-pretreated cells lost their ability to release arachidonic acid after stimulation with the chemotactic peptide fMLP (Fig. 3). This was not observed when the cells were stimulated with A23187. In that case, the effect was reduced to only a small extent, compared with incubations without pertussis toxin. Furthermore, Fig. 3 shows that Et_3PbCl stimulation is pertussis toxin sensitive by nearly the same order of magnitude as fMLP induction. The Et_3PbCl-induced functional response of arachidonic acid liberation was shown to be inhibited by pertussis toxin treatment even at high concentrations of the lead compound.

Comparison with the effects of thimerosal, a lysophospholipid acyltransferase inhibitor. The incubation of HL-60 cells with exogenous [^{14}C]arachidonic acid for 60 min resulted in nearly 75% uptake of the fatty acid, one third into the neutral lipids and two thirds into the phospholipids (the bulk was found in PC).

The organomercury compound thimerosal has been described by many investigators (e.g., Ref. 23) as an inhibitor of the reacylation of acyl-CoA into lysophospholipids. Additionally, other alkylated mercury compounds, such as methylmercury chloride, act in a similar manner (24). As shown in Table 3, thimerosal had no significant effect on the liberation of arachidonic acid within HL-60 cells, at a concentration of up to 50 μM. On the other hand, this concentration was sufficient to inhibit the incorporation of exogenous arachidonic acid into PC, PE, and triacylglycerols by 70, 66, and 95%, respectively (Table 4). As compared with these results, Et_3PbCl inhibited the incorporation of arachidonic acid into the three lipid classes equally. In contrast to this identical effect of the two different compounds, Et_3PbCl additionally stimulated the liberation of incorporated arachidonic acid at this concentration (Fig. 1). Moreover, 100 times lower concentrations (0.5 μM) had no effect on the incorporation at either 1 or 24 hr of preincubation with this alkylated lead compound but still induced the redistribution of fatty acids, as shown in Fig. 2.

Discussion

We have shown earlier (8) that human blood platelets were stimulated to liberate and metabolize arachidonic acid by high

TABLE 1

Liberation of arachidonic acid in HL-60 cells after stimulation with calcium ionophore A23187 or different concentrations of Et_3PbCl

Suspensions of differentiated HL-60 cells were prelabeled with [^{14}C]arachidonic acid and incubated at 37° for the indicated periods of time, in RPMI 1640 medium, with calcium ionophore A23187 or Et_3PbCl or with vehicle only (control). More than 90% of the label taken up (≈430,000 cpm) was esterified into phospholipids, with the remainder being incorporated into triacylglycerols. Free arachidonic acid could be detected in only trace amounts (<1%). After incubation with either A23187 or Et_3PbCl, cellular lipids were then extracted and separated by thin layer chromatography. Radioactive spots were localized by scanning, cut out, and counted for radioactivity in a liquid scintillation counter. Free acid, liberated arachidonic acid and its metabolites; TG, triacylglycerols. Results are given as differences from vehicle-treated control incubations (control cells). Values are the mean ± standard error of four to 13 experiments.

Treatment		Radioactivity, as difference from control cells		
Compound	Duration	PE + PC	TG	Free acid
			cpm	
10 μM A23187	30 min	−81,990 ± 5,605	+14,850 ± 3,350	+66,420 ± 5,513
50 μM Et_3PbCl	30 min	−20,250 ± 2,579	+1,130 ± 380[a]	+19,940 ± 3,216
10 μM Et_3PbCl	5 hr	−18,140 ± 970	+17,600 ± 728	
5 μM Et_3PbCl	5 hr	−18,720 ± 1,825	+19,400 ± 633	
1 μM Et_3PbCl	5 hr	−12,330 ± 1,909	+12,600 ± 2,877	
1 μM Et_3PbCl	24 hr	−13,950 ± 2,270	+14,220 ± 4,014	
500 nM Et_3PbCl	24 hr	−9,450 ± 2,038	+10,620 ± 3,951	
250 nM Et_3PbCl	24 hr	−7,920 ± 1,987	+7,380 ± 3,227	

[a] All values except this are different from those of control cells ($p < 0.05$; unpaired t test).

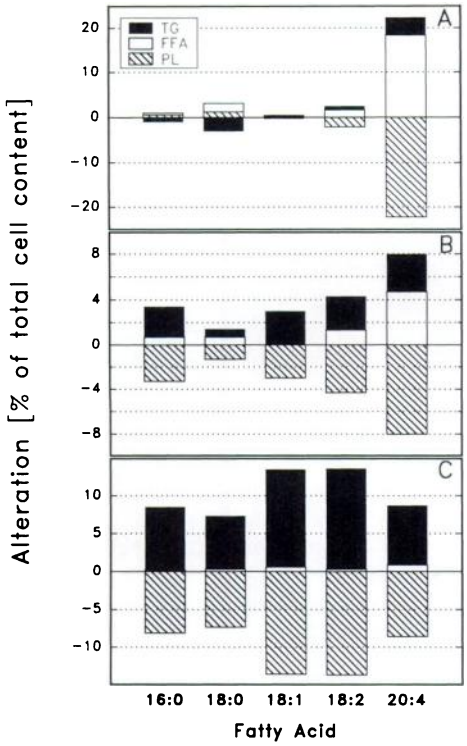

Fig. 2. Redistribution of the five major fatty acids within the lipid classes of HL-60 cells. Differentiated HL-60 cells were treated with 10 μM A23187 for 30 min (A), 100 μM Et_3PbCl for 30 min (B), or 0.5 μM Et_3PbCl for 24 hr (C). Lipids were extracted and separated using bonded phase columns, into phospholipid (*PL*), free fatty acid (*FFA*), and triacylglycerol (*TG*) fractions. After hydrolysis of the phospholipid and triacylglycerol fractions, the fatty acid phenacyl esters were prepared and analyzed using HPLC analysis. *Ordinate*, decrease (negative) and increase (positive) of fatty acid content within the lipid fractions (percentage of total cell content of the specific fatty acid). *16:0*, Palmitic acid; *18:0*, stearic acid; *18:1*, oleic acid; *18:2*, linoleic acid; *20:4*, arachidonic acid. Values are the mean of three or four experiments. Differences for free arachidonic acid (A and B) and for arachidonic, linoleic, oleic, and palmitic acids in the triacylglycerol fraction (C) are significant ($p < 0.025$), compared with vehicle-treated control cells (unpaired *t*-test).

TABLE 2

Effect of phospholipase inhibitors quinacrine and pBPB on A23187- or Et_3PbCl-stimulated liberation of arachidonic acid

Prelabeled HL-60 cells were preincubated with 1 mM quinacrine (5 min) or 50 μM pBPB (30 min) before calcium ionophore A23187 or Et_3PbCl was added and the incubation was continued for 30 min. Extraction and separation of cellular lipids were as described in Table 1. Values are the mean ± standard error of four experiments (except for the experiment with pBPB and A23187, only one experiment).

Preincubation addition	Incubation addition	[^{14}C]Arachidonic acid	Inhibition
		cpm	%
None	10 μM A23187	51,866 ± 1,906[a]	
1 mM Quinacrine	10 μM A23187	1,270 ± 995	98
50 μM pBPB	10 μM A23187	582	99
None	50 μM Et_3PbCl	12,239 ± 1,652[a]	
1 mM Quinacrine	50 μM Et_3PbCl	942 ± 577	92
50 μM pBPB	50 μM Et_3PbCl	2,300 ± 1,242[b]	81
None	100 μM Et_3PbCl	20,539 ± 3,703[a]	
1 mM Quinacrine	100 μM Et_3PbCl	1,024 ± 686[c]	95
50 μM pBPB	100 μM Et_3PbCl	1,719 ± 205[d]	92

[a] $p < 0.0005$, compared with control incubations (free arachidonic acid ≈1000–3000 cpm).
[b] $p < 0.01$; [c] $p < 0.005$; and [d] $p < 0.025$, as compared with corresponding control stimulations without preincubation (unpaired *t* test).

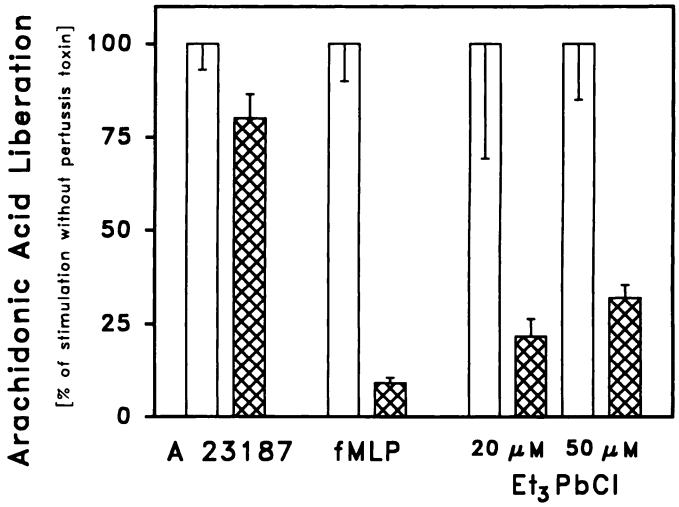

Fig. 3. Effect of pertussis toxin on arachidonic acid liberation in HL-60 cells. [^{14}C]Arachidonic acid-prelabeled and differentiated HL-60 cells were preincubated for 3 hr with 500 ng/ml pertussis toxin before the cells were stimulated with either 10 μM A23187, 1 μM fMLP (10 min), or 20 or 50 μM Et_3PbCl (20 min). Lipid extraction and separation were as in Fig. 1. *Ordinate*, arachidonic acid liberated, as percentage of corresponding stimulations without pertussis toxin. □, Stimulation without pertussis toxin; ■, stimulation after pretreatment with 500 ng/ml pertussis toxin. Each *column* represents the mean ± standard error of four experiments.

TABLE 3

Effect of thimerosal on liberation of [^{14}C]arachidonic acid

Prelabeled HL-60 cells were incubated with the indicated concentrations of thimerosal for 30 min. All other experimental details were as in Table 1. Results are given as differences from vehicle-treated control incubations. Values are the mean of three experiments, and no statistically significant liberation of arachidonic acid could be detected.

Treatment	Free arachidonic acid
	% of incorporated label
1 μM Thimerosal	0.0
10 μM Thimerosal	0.0
50 μM Thimerosal	0.7

TABLE 4

Inhibition of arachidonic acid incorporation into lipids of HL-60 cells after preincubation with thimerosal or Et_3PbCl

Suspensions of differentiated HL-60 cells were preincubated with thimerosal or Et_3PbCl for the indicated times before radioactively labeled arachidonic acid was added to the incubation mixtures. The cells were then incubated for an additional 60 min. Cellular lipids were extracted, and the amount of incorporated label in the various lipid classes was estimated (for details, see Table 1). PI, phosphatidylinositol; TG, triacylglycerols. Values are the mean of four experiments. The standard error for all values was less than ±2%.

Pretreatment		Incorporation of [^{14}C]arachidonic acid			
Compound	Time	PC	PE	PI	TG
		%			
	40 min	31.7	9.9	3.8	22.9
50 μM Thimerosal	40 min	8.9[a]	3.3[a]	6.4	1.1[a]
50 μM Et_3PbCl	10 min	6.7[a]	3.2[a]	4.0	1.5[a]
0.5 μM Et_3PbCl	1 hr	29.2	8.6	3.0	17.7
0.5 μM Et_3PbCl	24 hr	30.9	11.0	3.2	19.4

[a] Different from value for vehicle-treated cells ($p < 0.005$; unpaired *t* test).

concentrations of Et_3PbCl. The results presented here demonstrate a strong effect, even at low concentrations of Et_3PbCl, on the cellular fatty acid metabolism of differentiated HL-60 cells. The concentrations of this xenobiotic used in the experiments come close to those in normal human brains, as reported by Nielsen *et al.* (2). They found organic lead in quantities up

to 50 ng of Pb/g of wet weight, and the lowest amount used to stimulate HL-60 cells was 70 ng of Pb/ml.

The quantity of free unsaturated fatty acids within human blood platelets and HL-60 cells is very low (Fig. 2) (25). Both cell types respond to exogenous stimuli, e.g., thrombin, collagen, A23187, or fMLP, with a rapid increase, above all, of free arachidonic acid. Because this is one important metabolic pathway, these cells are provided with an efficient regulatory mechanism for controlling free fatty acid concentration. Involved in these processes are the fatty acid-liberating enzymes, phospholipase C and diacylglycerol lipase or phospholipase A_2, and the reacylating enzymes, arachidonoyl-CoA synthetase, lysophospholipid acyltransferase, and diacylglycerol acyltransferase (24, 26).

The inhibition of lysophospholipid acyltransferase by organomercury compounds (23, 24) can account for stimulation only in those cells that possess a high steady state activity of liberating and reacylating enzymes, creating an equilibrium at a very low level of free fatty acids in resting cells. This might be true within macrophages (23) and human blood platelets (24, 27), although some problems are not well explained in the case of lysophospholipid acyltransferase inhibition by thimerosal or methyl mercury, as follows: the accumulation of arachidonoyl-CoA does not lead *per se* to free arachidonic acid and subsequently eicosanoid production, and the stimulation of phospholipases by physiological agents could not account for such a dramatic increase in free fatty acids when the reacylating enzymes are still at this postulated high activity level.

A second possibility for reincorporation of free fatty acids is via diacylglycerol acyltransferase, giving triacylglycerol. This enzyme seems to be affected only by high concentrations ($\geq$50 μM) of the organometallic compounds used. As demonstrated (Table 1, Fig. 1), only a very small increase in the labeled arachidonic acid is found within the neutral lipids in such treated cells. On the other hand, at lower concentrations ($\leq$10 μM), the liberation is still inducible and the acylation of diacylglycerol still occurs (Tables 1 and 4, Fig. 2).

In HL-60 cells, the thiol-blocking agent thimerosal inhibits the reacylation of subsequently added [^{14}C]arachidonic acid, but in the reverse sequence, i.e., first labeling with [^{14}C]arachidonic acid and then addition of thimerosal up to cytotoxic concentrations, no accumulation of free arachidonic acid is detected (Tables 3 and 4). Therefore, one can assume that the liberation-reacylation cycle is passed through slowly in resting differentiated HL-60 cells. However, Et_3PbCl inhibits the incorporation of [^{14}C]arachidonic acid into cellular lipids as well but also enhances the level of free arachidonic acid, as shown here. Furthermore, at low concentrations (<1 μM), no inhibition of [^{14}C]arachidonic acid incorporation is observed, even after 24 hr of preincubation with Et_3PbCl (Table 4), but the liberation and subsequent redistribution of fatty acids still occurs at these low concentrations (Fig. 2).

Quinacrine and pBPB are known to inhibit phospholipases (20–22). Both of these compounds have an inhibitory effect on arachidonic acid liberation (Table 2). Although neither substance is specific in this respect, these findings could indicate that increased levels of unsaturated free fatty acids are caused by Et_3PbCl-induced phospholipase activation in HL-60 cells.

In the context of receptor-coupled activation of fatty acid liberation, however, more and more evidence points to GTP-binding protein regulation of phospholipase A_2 (26, 28). In order to determine whether a direct stimulation of phospholipase A_2 by Et_3PbCl occurs or whether the preceding components within the signal transduction mechanism are the targets of Et_3PbCl action, we tested the pertussis toxin sensitivity of this mechanism. The results of these experiments show clearly that about 70–80% of the Et_3PbCl-mediated effect is prevented by pertussis toxin. The thiol-blocking action of Et_3PbCl on lysophospholipid acyltransferase is not sufficient to explain this pertussis toxin effect. On the other hand, the slow steady state activity within the fatty acid liberating-reacylating cycle in HL-60 cells can account for the 20–30% pertussis toxin-insensitive fraction of liberated arachidonic acid, in comparison with nearly 100% inhibition when the receptor-stimulated liberation caused by fMLP is examined (Fig. 3).

Membrane phospholipids and free fatty acids play important roles in the regulation of, for instance, protein kinase C (29, 30), and hydrolysis of membrane phospholipids by phospholipase A_2 leads to structural alterations of membrane receptors (31). By examination of the results shown above, it is obvious that exactly these cellular parameters are affected by Et_3PbCl. This compound induces a redistribution of mainly unsaturated fatty acids, resulting in a decrease in phospholipids and an increase in triacylglycerols. The lower the concentration of Et_3PbCl, the greater is the degradation of PE, the primary phospholipid concerned (results not shown). In this case, not only the fatty acid composition within the phospholipids but also the phospholipid composition of the membranes is altered.

This action of Et_3PbCl not only leads to a simple shift of fatty acids within the cellular lipid classes; it also alters the cellular content of triacylglycerols and phospholipids and the composition of these fractions. In this way, Et_3PbCl may have subsequent effects on membrane functions and phospholipid-dependent enzymes (e.g., Ref. 7). Our earlier studies (8) point to phospholipase A_2 as the target enzyme, and the results presented here define the action of Et_3PbCl as a combination of pertussis toxin-sensitive stimulation of fatty acid liberation and, to a minor extent, inhibition of reacylation.

Very low concentrations of lead compounds, close to those reached within the environment, are able to induce enzyme activities, as shown here and reported elsewhere (12). Because of the large number of important cellular processes that are dependent on signal transduction mechanisms and that could be affected, such as protein kinase C activity, arachidonic acid metabolism, and membrane functions, it could be assumed that chronically toxic concentrations of heavy metals, especially lead compounds, within living organisms are already being reached.

Acknowledgments

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