High-density lipoproteins induce a rapid and transient release of Ca^{2+} in cultured fibroblasts

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Several different cell types showed increased rates of proliferation and cholesterol mobilization in response to treatment with high-density lipoprotein (HDL). This would suggest that one main function of HDL is the activation of signal pathways in cells. In the current study we have used the fluorescent indicator fura-2 to monitor the level of cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) in human skin fibroblasts. Exposure of subconfluent as well as confluent fibroblasts to HDL₃ (20–60 $\mu\text{g}/\text{ml}$) resulted in a rapid and transient increase in $[\text{Ca}^{2+}]_i$. Sequential additions of HDL₃ resulted in diminished rises in $[\text{Ca}^{2+}]_i$. The transient rise in $[\text{Ca}^{2+}]_i$ was observed with HDL prepared from plasma either by conventional ultracentrifugation or by precipitation with dextran sulphate. Chelation of the extracellular Ca^{2+} with EGTA prior to the addition of HDL₃ did not prevent the HDL₃-induced rise in $[\text{Ca}^{2+}]_i$, suggesting that the mobilized Ca^{2+} was derived mainly from intracellular stores. Covalent modification of the apoproteins of HDL₃ with dimethyl suberimide or tetranitromethane did not inhibit the HDL₃-induced rise in $[\text{Ca}^{2+}]_i$. This indicates that the binding of HDL₃ to cell surface receptors may not be necessary for the mobilization of intracellular Ca^{2+} . Moreover, the Ca^{2+} -releasing effect of HDL₃ was not inhibited by the presence of albumin (1%, w/v) in the extracellular medium, suggesting that non-esterified fatty acids were not the cause of the increased $[\text{Ca}^{2+}]_i$. The exposure of fibroblasts to lysophosphatidic acid, a potent mitogen and Ca^{2+} -releasing agent, before addition of HDL₃ completely inhibited the HDL₃-induced rise in $[\text{Ca}^{2+}]_i$. Furthermore, phorbol 12-myristate 13-acetate blocked the HDL₃-induced rise in $[\text{Ca}^{2+}]_i$. The results of this study imply that exposure of cells to HDL generates an intracellular signal which is induced by a component of the lipid fraction.

INTRODUCTION

High-density lipoproteins (HDLs) induce several different metabolic responses in cultured cells. First, they deplete peripheral cells of cholesterol by two different mechanisms: (a) an unspecific exchange of cholesterol molecules between HDL particles and the plasma membrane of cells [1], and (b) binding of apoproteins to specific binding proteins on the cell surface, which induces cholesterol transport from intracellular stores to the plasma membrane, but does not directly promote efflux to the HDL particle [2–5]. Secondly, HDLs have been reported to be mitogenic for several cell types [6,7].

With the exception of macrophages, the binding of HDL to specific receptors on the surface of peripheral cells apparently does not result in endocytosis of the receptor–HDL complex [8,9]. Thus there may be other ways for the HDL particle to induce its intracellular effects. HDLs may act through cell surface receptors to activate intracellular second messenger pathways, the best characterized of which are the cyclic AMP pathway [10] and the phosphoinositide pathway [11,12]. The mechanisms by which HDLs mediate their mitogenic effects, including the necessity of receptor binding for this process, are also unclear.

An important event in the activation of different metabolic pathways, as well as in mitogenesis, is a rise in intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$). This occurs either as Ca^{2+} influx through the plasma membrane or release of Ca^{2+} from intracellular stores such as the endoplasmic reticulum and putative ‘calcosomes’ [13–15]. In the current study a possible role of Ca^{2+} in the intracellular effects of HDL was investigated by monitoring $[\text{Ca}^{2+}]_i$ upon exposure of cultured fibroblasts to HDL. The

results show that a lipid component of HDL induces release of Ca^{2+} from intracellular stores in cultured fibroblasts without the binding of HDL to high-affinity binding sites on the cell surface.

EXPERIMENTAL

Materials

Fura-2 AM was purchased from Molecular Probes, and fetal calf serum was obtained from Gibco. Cell culture dishes were purchased from Costar. Tetranitromethane (TNM) was from Aldrich. Dimethyl suberimide (DMS), Ham F-12 nutrient mixture, phosphatidylinositol (PI; soybean), L- α -lysophosphatidic acid (LPA) (1-oleyl), phorbol 12-myristate 13-acetate (PMA) 5,5'-dithio-bis(2-nitrobenzoic acid) and bradykinin were from Sigma. Compound 58-035 {3-(decyldimethylsilyl)-N-[2-(4-methylphenyl)-1-phenylethyl]propanamide} was provided by Sandoz. [^3H]Thymidine (27 Ci/mmol) and [^{35}S]methionine (800 Ci/mmol) were obtained from Amersham. Dextran sulphate was from Pharmacia LKB and ionomycin was from Calbiochem.

Cell culture

Human skin fibroblasts (GM 8333) were obtained from the NIGMS Human Genetic Mutant Cell Repository, Camden, NJ, U.S.A. Cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 12% fetal calf serum, 2 mM-L-glutamine, 100 units of penicillin/ml, 100 μg of streptomycin/ml and 1 mM non-essential amino acid. Cells were cultured in 75 cm^2 culture flasks at 37 °C in a humidified atmosphere (5% CO_2). Prior to experiments, cells were seeded in culture dishes and treated as described.

Abbreviations used: HDL(s), high-density lipoprotein(s); LDL, low-density lipoprotein; DMEM, Dulbecco's modified Eagle medium; $[\text{Ca}^{2+}]_i$, cytosolic Ca^{2+} concentration; ACAT, acyl-CoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; BSS, balanced salt solution; DMS, dimethyl suberimide; TNM, tetranitromethane; PA, phosphatidic acid; LPA, lysophosphatidic acid; PC, phosphatidylcholine; PKC, protein kinase C; PI, phosphatidylinositol; PIP₂, phosphatidylinositol 4,5-bisphosphate; IP₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PMA, phorbol 12-myristate 13-acetate.

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Isolation and modification of lipoproteins

Low-density lipoproteins (LDL; d 1.019–1.063) and high density lipoproteins (HDL₃; d 1.12–1.21) were prepared from fresh human plasma (EDTA 4 mM) by sequential ultracentrifugation [16,17]. The dialysed lipoproteins were stored in sterile conditions in the dark at 4 °C and were used within 4 weeks of preparation. Modification of HDL₃ with TNM was performed as described by Brinton *et al.* [18]. Briefly, 20 μ l of a freshly prepared solution of 0.6 M-TNM in absolute ethanol was added to 2 ml of HDL₃ (2 mg/ml in 0.9% NaCl; final TNM concentration 6 mM) and the reaction was allowed to proceed in the dark at room temperature for 60 min. The sample was chilled on ice and subsequently desalted on a Sephadex PD-10 column (Pharmacia). The resulting TNM-HDL₃ was stored at 4 °C in the dark and used within 3 days of preparation. The treatment of HDL₃ with DMS was performed according to the method of Chacko *et al.* [19]. Precipitation of HDL₂ from EDTA/plasma with dextran sulphate (M_r 500 000) and MgCl₂ was performed as described by Bachorik & Albers [20].

Measurement of $[Ca^{2+}]_i$

GM 8333 cells on coverslips in 60 mm culture dishes were either used as such or incubated for 18 h in serum-free DMEM supplemented with 100 μ g of LDL/ml and 5 μ g of Compound 58-035/ml, an inhibitor of acyl-CoA:cholesterol acyltransferase (ACAT). Cells were exposed for 20 min at 37 °C to fura-2 AM dissolved in dimethyl sulphoxide (2 μ g/ml final concentration in serum-free DMEM). Cells were then rinsed with a balanced salt solution (BSS) containing 137 mM-NaCl, 5 mM-KCl, 0.44 mM-KH₂PO₄, 4.2 mM-NaHCO₃, 1.2 mM-MgCl₂ and 20 mM-Tes, adjusted to pH 7.4. The coverslips were mounted diagonally into a quartz cuvette containing BSS with 1 mM-CaCl₂ and 10 mM-glucose at 37 °C with magnetic stirring. Fluorescence measurements were carried out with a Hitachi F-2000 fluorescence spectrophotometer at 340/380 nm (excitation) and 505 nm (emission). For calibration, maximum levels were obtained by addition of ionomycin and minimum levels by a subsequent addition of EGTA. A value of 220 nM was used for the K_d of the fura-2-Ca²⁺ complex [21]. Calculation of $[Ca^{2+}]_i$ was as described by Tsien *et al.* [22].

Determination of the mitogenic effects of HDL₃

The mitogenic effects of HDL₃ were determined by measuring the incorporation of [³H]thymidine into the DNA of GM 8333 cells. The procedure was briefly as follows. Cells in 60 mm culture dishes were labelled with [³⁵S]methionine (0.5 μ Ci/ml of medium) shortly after plating. The [³⁵S]methionine was present in the dishes during the entire experiment, to correct for variations in protein mass. The culture medium used during these experiments was composed of serum-free DMEM and Ham F-12 nutrient mixture (1:1, v/v). At 24 h after plating different concentrations of HDL₃ were added to the incubation medium. Finally, 48 h after plating, [³H]thymidine (1.0 μ Ci/ml) was added to the medium and dishes were incubated at 37 °C for an additional 2 h. Cells were then harvested by treatment with trypsin, and the cell suspension was pipetted on to a Whatman filter. The filter was rinsed with 0.9% NaCl (3 $\times$ 1.5 ml) and dried at 60 °C for 30 min. The dry filter was placed in a scintillation vial together with 3 ml of scintillation cocktail, and radioactivity was counted. The ³H/³⁵S ratio was calculated and used as a function of mitogenic effect.

Lipoprotein lipid analysis

Extraction of lipoprotein lipids was performed with water-saturated butanol. Phospholipids and lysophospholipids were

then separated two-dimensionally on Silica Gel 60 t.l.c. plates (Merck), with chloroform/methanol/acetic acid/water (50:30:8:4, by vol. [23]) as the first solvent system, and chloroform/methanol/7 M-ammonia (65:30:4, by vol.) as the second solvent system. Lipid spots were developed by I₂ staining, and phospholipids containing free amino groups were visualized with the aid of ninhydrin spray. The lipids were identified from standards.

RESULTS

Effects of native HDLs on $[Ca^{2+}]_i$

Exposure of subconfluent as well as confluent human skin fibroblasts on coverslips to HDL₃ (isolated by ultracentrifugation) resulted in a rapid and transient increase in fura-2 fluorescence, indicating a rise in $[Ca^{2+}]_i$ (Fig. 1a). The increase was observed in 75% of the measurements performed ($n = 80$ in 20 independent experiments) upon addition of 20–60 μ g of HDL₃/ml. In three independent experiments (different batches of cells), the increase in $[Ca^{2+}]_i$ upon addition of HDL₃ was $54 \pm 16\%$ ($n = 3$), $120 \pm 79\%$ ($n = 5$) and $104 \pm 50\%$ ($n = 4$). There seemed to be no difference in the Ca²⁺-mobilizing properties of different batches of HDL₃. HDL₃ isolated in the presence of 5,5'-dithio-bis(2-nitrobenzoic acid), an inhibitor of lecithin:cholesterol acyltransferase (LCAT), caused a similar rise in $[Ca^{2+}]_i$ (results not shown). HDL₂ isolated by precipitation from plasma with dextran sulphate, a procedure which takes only about 3 h, also showed a Ca²⁺-releasing effect (Fig. 1b). The exposure of cells to dextran sulphate alone did not cause any change in $[Ca^{2+}]_i$ (results not shown). Chelation of extracellular Ca²⁺ with EGTA (final concn. 2 mM) before exposure to HDL₃ did not diminish the increase in $[Ca^{2+}]_i$ (Fig. 1c). Hence it appears that the HDL-induced elevation in $[Ca^{2+}]_i$ was mostly or entirely derived from intracellular stores. The magnitude of the HDL-induced rise in $[Ca^{2+}]_i$ varied, as shown in Figs. 1 and 2. The magnitude of the increase was, however, independent of the amount of HDL₃ added,

Fig. 1. Effects of native HDL on $[Ca^{2+}]_i$.

GM 8333 fibroblasts loaded with fura-2 were exposed to HDL₃ isolated by ultracentrifugation (a and c) or HDL₂ precipitated with dextran sulphate (b). Fluorescence recordings were performed as described in the Experimental section. Additions of HDL are indicated with arrows. The $[Ca^{2+}]_i$ is expressed in nM, except for in (c), where the initial addition of EGTA made a normal calibration impossible, and $[Ca^{2+}]_i$ is expressed as the ratio between fluorescence recordings at 340 nm and 380 nm (excitation). Each trace is a representative of at least five independent measurements, except for (a), which is a typical signal for 20 independent experiments ($n = 80$).

Fig. 2. Features of the HDL-induced rise in $[\text{Ca}^{2+}]_i$

Fibroblasts loaded with fura-2 were exposed to indicated amounts of HDL₃ isolated by ultracentrifugation. In (c), arrows indicate the addition of HDL₃ (45 µg/ml). The $[\text{Ca}^{2+}]_i$ is expressed in nM. Each trace is a representative of at least three independent measurements.

Fig. 3. Effect of HDL₃ with modified apoproteins on $[\text{Ca}^{2+}]_i$

Fibroblasts loaded with fura-2 were exposed to indicated amounts of HDL₃ (isolated by ultracentrifugation) which had been covalently modified with (a) DMS or (b) TNM, or (c) treated with trypsin, as described in the Experimental section. Calculated values of $[\text{Ca}^{2+}]_i$ are expressed in nM. The signals are representative of at least three similar independent measurements.

in the range 20–60 µg/ml (Figs. 2a and 2b). Subsequent additions of HDL₃ to the same coverslip resulted in diminishing Ca^{2+} peaks (Fig. 2c). The exposure of fibroblasts to LDL (isolated by ultracentrifugation, 20–40 µg/ml) caused an increase in the fluorescence at both 340 and 380 nm (excitation; results not shown). Therefore this lipoprotein was not used any further in our study. Sachinidis *et al.* [24] reported that LDL caused a transient increase in $[\text{Ca}^{2+}]_i$ in several cultured cell types, in particular smooth muscle cells. The differences in response to LDL between their study and the present can perhaps be explained by the use of different cell lines.

Effects of HDL₃ particles with modified apoproteins on $[\text{Ca}^{2+}]_i$

To investigate whether the binding of HDL₃ to binding sites at the cell surface was necessary for the induction of Ca^{2+} release from intracellular stores, we exposed GM 8333 fibroblasts to LDL (300 µg/ml) and Compound 58-035 (5 µg/ml) for 24 h at 37 °C in order to induce expression of HDL₃-binding sites [25]. The $[\text{Ca}^{2+}]_i$ of cells was then measured during exposure to covalently modified HDL₃. The modification of HDL₃ with DMS or TNM did not prevent the Ca^{2+} -releasing effect of HDL₃

Fig. 4. Effects of albumin, LPA and PMA on the HDL₃-induced rise in $[\text{Ca}^{2+}]_i$

Fura-2-loaded fibroblasts were exposed to indicated amounts of LPA, PMA, bradykinin and HDL₃ (isolated by ultracentrifugation). In (a), albumin (1%, w/v) was present in the experimental medium during fluorescence recordings. Values of $[\text{Ca}^{2+}]_i$ are expressed in nM. Each trace is a representative of at least three similar measurements.

(Fig. 3). Moreover, after treatment with trypsin (1.25%, 1 h at 37 °C) the HDL₃ still possessed Ca^{2+} -releasing properties (Fig. 3c).

Characterization of the Ca^{2+} -releasing component in HDL

The results obtained from Ca^{2+} measurements with covalently modified HDL₃ did not indicate a role for the apoproteins in the signal transduction. Thus it appears that some component(s) of the lipid fraction of HDL₃ is responsible for the transient rise in $[\text{Ca}^{2+}]_i$. It is known that polyunsaturated non-esterified fatty acids mobilize intracellular Ca^{2+} [26,27], and since the preparation of HDL₃ by ultracentrifugation takes 120 h, an accumulation of unphysiological metabolites, such as non-esterified fatty acids, is possible. Chow & Jondal [27] reported that Ca^{2+} mobilization induced by polyunsaturated non-esterified fatty acids was inhibited by 1% albumin in the experimental medium. We therefore performed Ca^{2+} measurements with albumin present in the cuvette (Fig. 4a). The HDL₃-induced increase in $[\text{Ca}^{2+}]_i$ was unaffected by albumin, which indicates that non-esterified fatty acids were not the cause of the Ca^{2+} release.

A recent report by Jalink *et al.* [28] showed that LPA, but not phosphatidic acid (PA), mobilizes intracellular Ca^{2+} . We tested the effects of commercial LPA on GM 8333 fibroblasts and found it to be a potent Ca^{2+} -releasing agent in the present system (Fig. 4b). Addition of LPA (10 µg/ml) prior to the addition of HDL₃ completely inhibited the Ca^{2+} -releasing effect of HDL₃ (Fig. 4b). The phorbol ester PMA has been shown to cause activation of protein kinase C (PKC) [29], and thereby has an indirect effect on the cellular Ca^{2+} level. As shown in Fig. 4(c), the HDL₃-induced Ca^{2+} release in GM 8333 cells was inhibited when PMA was added to the cuvette before HDL₃, but subsequent addition of bradykinin still resulted in an elevated $[\text{Ca}^{2+}]_i$.

The similarity between the rises in $[\text{Ca}^{2+}]_i$ caused by HDL and LPA led us to investigate whether HDLs contain any LPA. The two-dimensional t.l.c. analysis of HDL₃ lipids did not result in any visible spot of LPA when compared with standards. In addition to phosphatidylcholine (PC) and sphingomyelin, which are the major constituents of HDLs, the analysis revealed spots of lyso-PC, PI and PA (Fig. 5). No phospholipids containing

Fig. 5. HDL lipids separated by two-dimensional t.l.c.

The lipids of HDL₃ (450 µg of protein) were extracted and separated on t.l.c. as described. Lipid spots were stained with cupric acetate (3%, w/v) in phosphoric acid (8%, v/v) and developed by heating the plate for 30 min at 150 °C. The application spot is marked with a circle. Abbreviations: LPC, lyso-PC; SPM, sphingomyelin.

Table 1. Mitogenic effect of HDL₃ on GM 8333 fibroblasts

GM 8333 cells cultured in the presence of [³⁵S]methionine were incubated with the indicated amounts of HDL₃ (isolated by ultracentrifugation) for 24 h and were then pulsed for 2 h with [³H]thymidine. The results presented are mean values of five dishes ± S.D. from two separate experiments.

HDL ₃ (µg/ml)	Proliferation (%)
0	100
5	103 ± 4
20	120 ± 7
100	158 ± 3

free amino groups (e.g. phosphatidylserine and phosphatidylethanolamine) were found in HDL₃, as determined by spraying with ninhydrin.

Mitogenic effects of HDL₃

The proliferation rate has been shown to increase linearly with increasing amounts of HDL present in the culture medium [6,7], but the mechanism underlying the mitogenic effect of HDL is unknown. To verify that HDL₃ had a mitogenic effect on the cultured cells in the present system, we measured the incorporation of [³H]thymidine in the presence of different concentrations of HDL₃, which was isolated by ultracentrifugation. As shown in Table 1, cell proliferation increased with increasing amounts of HDL₃ present in the culture medium.

DISCUSSION

The rate of cholesterol efflux as well as the proliferation rate of cultured cells have been shown to be linearly dependent on the extracellular concentration of HDL ([6,7,30]; in the present study). The precise mechanism of HDL action has remained unknown, however. An M_r -110000 binding site for HDL₃ at the cell surface has been fairly well characterized [25,31], and binding of HDLs to this putative receptor seems to promote transport of cholesterol from intracellular stores to the plasma membrane [2,5]. On the other hand, the efflux of cholesterol from the plasma membranes of fibroblasts to HDL particles does not necessarily involve the binding of HDL to any receptor [2,3]. The mechanism

by which HDLs produce their mitogenic effect is also not known. In the current study we have attempted to elucidate a cellular signalling pathway used by HDL, and we chose to study the cytosolic Ca²⁺ balance, since this ion plays an important role in different signalling pathways [32,33].

The results in this study show that HDLs induce a transiently increased [Ca²⁺]_i in cultured fibroblasts. The HDLs were isolated by two different methods: (a) the conventional ultracentrifugation, which is a time-consuming procedure, and may lead to the production of unphysiological metabolites in the HDL particle, and (b) a more rapid precipitation with dextran sulphate, which should result in HDL₂ free from degradation products. Both methods of isolation produced HDLs which readily induced a transient increase in [Ca²⁺]_i. In the case of several subsequent additions of HDL₃, Ca²⁺ release was diminished after each addition, suggesting a desensitization, a saturation or emptying of intracellular Ca²⁺ stores. Since the HDL₃-induced increase in [Ca²⁺]_i was also seen in the presence of extracellular EGTA, the bulk of the Ca²⁺ flow appeared to be derived from intracellular stores of Ca²⁺.

The results obtained from Ca²⁺ measurements with DMS-HDL₃, TNM-HDL₃ and trypsin-treated HDL₃ suggested that the binding of HDL₃ to the M_r -110000 binding site at the cell surface would not be necessary for induction of a Ca²⁺ release into the cytosol. The HDL-induced rise in [Ca²⁺]_i is thus probably not a signal for intracellular cholesterol mobilization, since this event seems to be mediated by binding of HDLs to their high-affinity receptors. The cholesterol-efflux-promoting effect of HDLs is not likely to involve an intracellular signalling system, since the efflux of cellular cholesterol is thought to occur by aqueous diffusion of cholesterol from the plasma membrane to HDL particles [1]. The HDL-induced Ca²⁺ release may be a signal for mitogenesis though, since several of the known fibroblast mitogens cause a rise in [Ca²⁺]_i.

The identity of the Ca²⁺-mobilizing component in HDLs is uncertain at present. Since the Ca²⁺-releasing effect of HDLs was not inhibited by albumin, we were able to rule out the polyunsaturated non-esterified fatty acids. Also, the increased [Ca²⁺]_i induced by polyunsaturated fatty acids returned to baseline only after 10–15 min [27], whereas in our study the Ca²⁺ level returned to baseline 30 s after exposure to HDL. The phospholipid components found upon lipid analysis of HDL₃ was lyso-PC, sphingomyelin, PI, PC and PA.

GM 8333 fibroblasts exposed to commercial LPA showed an increase in [Ca²⁺]_i which was of the same type and duration as the HDL-induced increase. Addition of LPA to cells prior to the addition of HDL₃ inhibited the HDL₃-induced increase in [Ca²⁺]_i, suggesting that HDLs mobilize Ca²⁺ by the same pathway as LPA. The roles of PA and LPA in cellular signal transduction have been extensively investigated in recent years. It has been reported that PA and LPA could function as agonists when added to cells, by stimulating DNA synthesis and cell division, activating phospholipase C, inhibiting adenylate cyclase and mobilizing intracellular Ca²⁺ [34–37]. A recent report by Jalink *et al.* [28] shows that many of the agonist-like effects which have earlier been attributed to PA are in fact effects of LPA contamination. The same report showed that lyso-PC did not possess any Ca²⁺-mobilizing properties. Thus it seems unlikely that the lyso-PC or PA found in both HDL₃ and LDL would be responsible for the Ca²⁺ mobilization observed in cells exposed to HDLs.

The interesting fact that HDL₃ contains PI could perhaps indicate an introduction of exogenous PI into the cellular phosphoinositide cycle, which plays a central role in the mediation of various agonist-induced intracellular effects. The hydrolysis of phosphatidylinositol bisphosphate (PIP₂) by

phospholipase C in the plasma membrane is induced upon ligand binding to specific receptors [36]. The products of this hydrolysis, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) respectively, mobilize intracellular Ca^{2+} and activate PKC [29]. The observed rapid mobilization of Ca^{2+} would, however, require that the PI in HDL_3 be transferred to the plasma membrane and there phosphorylated to PIP_2 , all within a few seconds. This seems improbable, since the exchange of phospholipids between lipid bilayers is a relatively slow process, with reported half-times of several hours [38]. Plasma membrane PI could also be directly utilized by phospholipase C to yield DAG and inositol phosphate [39]; however, neither mobilizes Ca^{2+} .

The HDL_3 -induced Ca^{2+} mobilization was completely inhibited by pretreatment with the phorbol ester PMA. Treatment of cells with PMA causes activation of PKC, which is thought to have multiple effects on the cellular Ca^{2+} balance. PKC may block the agonist-induced production of IP_3 [40] or directly inhibit the intracellular release of Ca^{2+} [41]. The results of the present study indicate that a lipid component of HDLs induces a Ca^{2+} mobilization in cultured cells, perhaps mediated by IP_3 .

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