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Platelet high affinity low density lipoprotein binding and import of lipoprotein derived phospholipids

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Abstract The binding of low density lipoprotein (LDL) to the platelet cell membrane could facilitate the transfer of phospholipids from LDL to the platelets. A polyclonal antibody against the platelet glycoproteins IIb/IIIa inhibited the high affinity binding of ^{125}I-LDL by up to 80%. The transfer of pyrene (py)-labeled sphingomyelin (SM), phosphatidylcholine and phosphatidylethanolamine from LDL to the platelets was unaffected by the antibody. The lectin wheat germ agglutinin (WGA) reduced the binding of ^{125}I-LDL to the platelets by approximately 80%. In contrast, the lectin stimulated the transfer of SM from LDL into the platelets by about three-fold. WGA also specifically augmented the transfer of py-SM between lipid vesicles and the platelets, the stimulation being abolished in the presence of *N*-acetylglucosamine. Dextran sulfate (DS) increased the specific binding of ^{125}I-LDL to the platelets by up to 2.8-fold. On the other hand, the import of LDL-derived py-phospholipids was unaffected by DS. Together, the results indicate that the phospholipid transfer from LDL to the platelets is independent of the high affinity LDL binding to the platelets and is specifically stimulated by WGA. Thus, the interactions of platelets with LDL phospholipids differ markedly from those with the apoprotein components of the lipoproteins.

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Key words: Phospholipid import; Low density lipoprotein binding; Wheat germ agglutinin; Anti-glycoprotein IIb/IIIa antibody; Dextran sulfate

1. Introduction

In recent years, defined functions in intracellular signaling events have been assigned to nearly all types of membrane phospholipids. Furthermore, distinct phospholipids were shown to interact with specific proteins thereby regulating cellular functions implicated in different physiological situations. For example, the exposure of phosphatidylserine at cellular surfaces was shown to play a prominent role in coagulation as well as in early apoptosis [1,2]. In view of these essential functions of membrane phospholipids it appears obvious that the composition and localization of phospholipids in cellular membranes has to be tightly controlled. This is accomplished by enzymes involved in the de novo synthesis of phospholipids [3], by remodelling of the phospholipids (e.g. due to the actions of acyltransferases and transacylases) at the level of the membranes [4] and by proteins mediating the transfer of phospholipids between the two halves of the bilayer [5,6].

We recently obtained evidence that an additional mechanism might be relevant for the biogenesis of the platelet phospholipid composition, namely the import of lipoprotein-derived phospholipids [7–9]. The major phospholipids of low and high density lipoproteins (LDL and HDL) (phosphatidylcholine (PC), sphingomyelin (SM) and phosphatidylethanolamine (PE)) were found to be rapidly transferred to the platelets in an endocytosis-independent manner [7]. The uptake of LDL-derived phospholipids was shown to contribute to supplying platelets with arachidonic acid. The newly imported phospholipid-bound arachidonic acid was shown to be metabolized subsequently to eicosanoids such as thromboxane A_2 [8]. When platelets were activated by the agonists thrombin and collagen, the incorporation of ethanolamine phospholipids was specifically stimulated [9]. This was apparently mediated by proteins secreted from the platelets. The acceleration of import of ethanolamine phospholipids enhanced the prothrombinase activity of the platelets [9].

Platelets possess saturable binding sites for LDL particles [10,11]. The platelet membrane glycoprotein (GP) IIb/IIIA complex (integrin $\alpha_{IIb}\beta_3$) was previously shown to be part of the high affinity binding sites for LDL particles [12]. Binding of LDL to their platelet receptors allows the lipoproteins to physically interact with the platelet surface. We hypothesized that this might facilitate the transfer of phospholipids from the lipoproteins to the platelets. In order to test this hypothesis, we evaluated whether agents that modulated specific LDL binding to platelets affected the transfer of phospholipids from LDL to the platelets. In further experiments we analyzed the potential involvement of LDL apoproteins in the phospholipid transfer process.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-pyrenedecanoyl-*sn*-3-glycerophosphorylcholine (py-PC), 1-palmitoyl-2-pyrenedecanoyl-*sn*-3-glycerophosphorylethanolamine (py-PE) and (*N*-pyrenedecanoyl)-sphingomyelin (py-SM) were from Sigma (Deisenhofen, Germany) or from Molecular Probes (Eugene, OR, USA). 1-Palmitoyl-2-[^{14}C]arachidonoyl-*sn*-glycero-3-phosphorylcholine (^{14}C-20:4-PC) and [*N-methyl*-^{14}C]sphingomyelin (^{14}C-SM) were obtained from NEN DuPont (Homburg). Polyclonal antibodies against GPIIb and GPIIIa were raised in rabbits by application of a mixture of both glycoproteins [13]. The IgG fraction was isolated by chromatography using protein A-Sepharose. Specificity was evaluated by Western blot techniques. Thereby, the antibody solution was found to bind to GPIIb and GPIIIa up to dilutions of 1:4000. Iloprost was kindly donated by Schering (Berlin, Germany). Pancreatic elastase (type IV), apyrase, α_1-antitrypsin, WGA and *N*-acetyl-D-glu-

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Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; py, pyrenedecanoic acid; GPIIb/IIIa, glycoprotein IIb/IIIa complex; WGA, wheat germ agglutinin; DS, dextran sulfate; apo B, apoprotein B

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cosamine were from Sigma (Deisenhofen, Germany). Dextran sulfate was from Pharmacia (Freiburg, Germany).

2.2. Preparation of platelets

Fresh venous blood was anticoagulated with acid-citrate-dextrose (15 mM citric acid, 90 mM trisodium citrate, 16 mM Na_2HPO_4, 160 mM glucose, pH 5.0; 1 part anticoagulant to 6 parts blood). The mixture was centrifuged for 10 min at $180\times g$ to obtain platelet-rich plasma. Iloprost (100 nM) and apyrase (50 mg/l) were added and the platelet-rich plasma centrifuged at $1000\times g$ for another 10 min. The pellet was resuspended in Tyrode solution without Ca^{2+} ('Tyrode buffer': 138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 2 mM $MgCl_2$, 5 mM glucose, PGI_2 (20 ng/ml) and apyrase (50 mg/l), pH 6.2) and washed twice with the same solution. After the last centrifugation, the platelets were resuspended in a modified Tyrode buffer containing Ca^{2+} ('Tyrode-Ca^{2+}': 138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM glucose, 5 mM HEPES, pH 7.35). The platelet suspension was kept at room temperature for 30 min before the start of the incubations with lipoproteins and/or substances.

2.3. Determination of ^{125}I-LDL binding to the platelets

LDL was isolated from plasma of healthy donors by sequential flotation at $100\,000\times g$ in the density range (d) = 1.019–1.063 and was washed by flotation through KBr solution at d = 1.063 g/ml [14]. Subsequently, the particles were filtered (0.45 μm). No contaminating plasma proteins were detected by immunoelectrophoresis. No signs of oxidation were detectable with the thiobarbituric acid reactive substance assay. Removal of KBr and rebuffering was performed by size-exclusion chromatography with Sephadex G-25 immediately prior to use. Radiolabeling was performed by means of *N*-chloro-benzenesulfonamide-conjugated polystyrene beads (Iodo Bead) following the manufacturer's instructions (Pierce, Rockford, IL, USA). The specific radioactivities of the ^{125}I-LDL ranged from 100 to 300 cpm/ng protein.

Usually, the washed platelets were incubated with ^{125}I-LDL at room temperature for 30 min. After centrifugation through 20% aqueous sucrose in an Eppendorf centrifuge, the supernatant was removed and the infranatant containing the platelets washed once with the Tyrode buffer. The radioactivities of both the bound and unbound fractions were determined. Unspecific binding was determined as platelet-associated radioactivity in the presence of a 50-fold excess of unlabeled LDL.

2.4. Enrichment of LDL with pyrene-labeled phospholipids

Pyrene (py)-labeled phospholipids were incorporated into lipoproteins essentially as described previously [7]. Briefly, 1 μmol of either py-SM, py-PC or py-PE were dissolved together with 3 μmol of egg PC in 20 μl of ethanol. The phospholipids were directly added to 3 ml of human plasma at 37°C under argon by very slow injection with gentle stirring. 0.74 mM diethyl-*p*-nitrophenylphosphate (DPNP) and 3 mM NaN_3 were added and the suspension incubated for 24 h at 37°C under argon. Pyrene-labeled lipoproteins were isolated by ultracentrifugation at 4°C [14]. Low density lipoproteins were recovered at a density range (g/ml) between 1.019 and 1.063.

Before starting the incubations with platelets, the lipoproteins were extensively dialyzed at 4°C under argon against a buffer containing 10 mM Tris-HCl, 150 mM NaCl, 0.3 mM EDTA (pH 7.4). The protein contents of the labeled lipoproteins were determined [15]. Pyrene-labeled lipoproteins thus obtained migrated at the same distance as native lipoproteins as evidenced by agarose gel electrophoresis. Lipids from LDL labeled with pyrene-containing phospholipids were extracted [16] and phospholipids separated by one-dimensional thin layer chromatography using the solvent chloroform/methanol/acetic acid/H_2O (90:40:12:2; v/v). The plates were viewed under UV light. Fluorescence was detected exclusively in the phospholipid fraction originally labeled in the vesicles.

2.5. Incorporation of ^{14}C-phospholipids into LDL

^{14}C-SM and ^{14}C-PC were incorporated into LDL particles by preparing egg PC vesicles with the ^{14}C-phospholipids and subsequent incubation of the vesicles with fresh plasma for 24 h at 37°C under argon [7]. ^{14}C-labeled LDL was isolated by ultracentrifugation at 4°C [14]. The specific activities thus obtained were $1.8–4.2\times10^3$ cpm/nmol of the respective LDL-associated phospholipids. Before the start of incubations with platelets, the lipoproteins were extensively dialyzed at 4°C under argon against a buffer containing 10 mM Tris-HCl, 150 mM NaCl, 0.3 mM EDTA (pH 7.4). ^{14}C-Labeled lipoproteins migrated at the same distance as the native lipoproteins as determined by agarose gel electrophoresis.

2.6. Incubation of platelets with labeled lipoproteins and vesicles

Washed platelets were suspended with either pyrene-labeled LDL, vesicle suspensions containing pyrene phospholipids, ^{125}I-apolipoprotein B (apo B)-LDL or ^{14}C-labeled LDL in Tyrode-Ca^{2+} buffer at either 37°C or room temperature. After the incubation, labeled lipoproteins were removed by centrifugation and one washing step. In the case of pyrene-labeled donors, the fluorescence was monitored directly in the suspensions every 60 s (on line conditions). In some cases, following incubation of platelets with pyrene-labeled donors, fluorescence intensities were also determined after separation of donors and acceptors by centrifugation (off-line experiments). Monomer and excimer fluorescence of the suspensions were determined at emission wavelengths of 380 nm and 480 nm, respectively, with excitation at 340 nm (excitation and emission slits of 5 and 10 nm). Incorporation of pyrene-labeled phospholipids into the platelets was followed by the increase in monomer intensity after addition of platelets to the donors. Fluorescence measurements were carried out using a Shimadzu RF-5001-PC spectrofluorometer (Shimadzu Germany, Duisburg). All mean values are given ± S.E.M.

3. Results and discussion

Binding of LDL to the platelet surface was estimated by incubating washed platelets (1.3×10^8) with two different concentrations of ^{125}I-LDL (2 or 8 μg/ml) at room temperature. Under these conditions, the majority of ^{125}I-LDL binding is specific as 81% of the platelet-associated ^{125}I could be removed by an excess of unlabeled LDL (Fig. 1). The effect of a polyclonal anti-GPIIb/IIIa antibody on platelet ^{125}I-LDL binding was evaluated. The lower concentration of the antibody (47 μg/ml) reduced ^{125}I-LDL binding by 56% (2 μg/ml) and 49% (8 μg/ml). Doubling the amount of the antibody diminished binding of ^{125}I-LDL to the platelets by 81% and 78% at the two concentrations of LDL (Fig. 1). Addition of a non-immune IgG barely influenced the interaction of ^{125}I-LDL with the platelets.

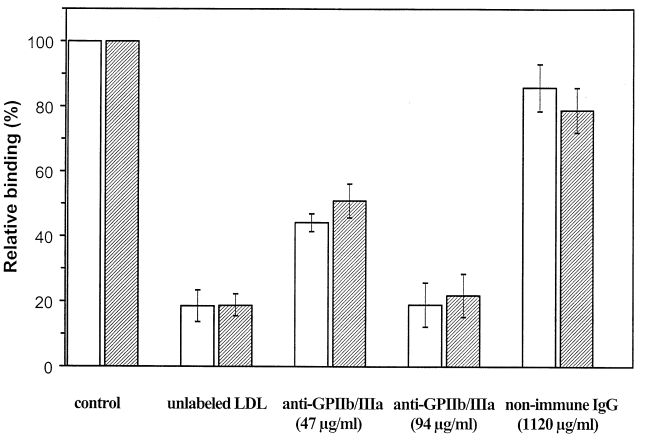

Fig. 1. Inhibition of ^{125}I-LDL binding to platelets by an antibody against GPIIb/GPIIIa. The indicated amounts of the anti-GPIIb/IIIa antibody were given to the platelet suspensions (1.3×10^8) and thereafter ^{125}I-LDL was added either at 2 μg protein/ml (empty columns) or 8 μg/ml (filled columns) for a total of 30 min (room temperature). Aliquots without addition of the antibody were run in parallel to determine the control binding. Non-specific binding was determined in the presence of a 50-fold excess of unlabeled LDL. Mean values obtained on platelets from three different donors.

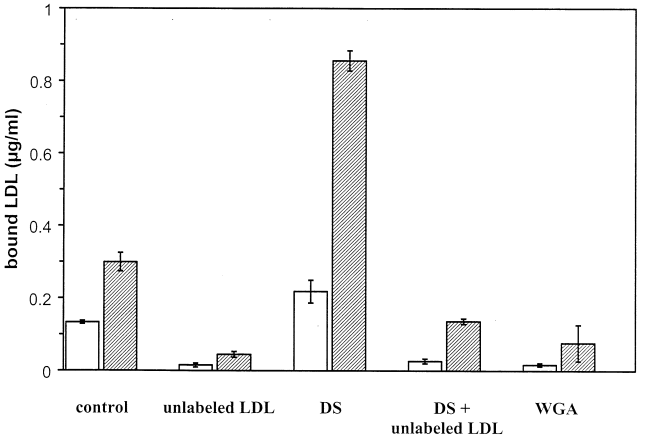

Fig. 2. Influences of WGA and DS on platelet binding of ^{125}I-LDL. Platelet suspensions (1.5×10^8) were incubated for 30 min at room temperature with either 2 μg/ml (empty columns) or 8 μg/ml ^{125}I-LDL (filled columns) in the absence or presence of WGA (20 μg/ml) or DS (27 μg/ml). Mean values on platelets of three different donors.

In our search for modulators of LDL binding to platelets, several substances were tested. Dextran sulfate (DS; 27 μg/ml) was found to enhance binding of ^{125}I-LDL to platelets by 1.6-fold (2 μg/ml) and 2.8-fold (8 μg/ml LDL protein) (Fig. 2). The increased binding of the lipoprotein to the platelets elicited by DS nearly entirely represented specific binding since it could be removed by an excess of unlabeled LDL (Fig. 2). The screening for modulators of LDL binding furthermore revealed that wheat germ agglutinin (WGA, 20 μg/ml) lowered total binding of ^{125}I-LDL to the platelets by 75–86% (Fig. 2). WGA decreased binding of ^{125}I-LDL to the platelets to a similar extent as the excess of unlabeled LDL. At the concentration of WGA applied, no platelet aggregation was found as analyzed using an aggregometer.

Table 1
Effect of WGA on platelet-associated ^{14}C following incubation of platelets with ^{14}C-phospholipid LDL

	Platelet-associated ^{14}C (cpm/10^8 platelets)	
	^{14}C-SM	^{14}C-PC
Control	405 ± 30	310 ± 40
+unlabeled LDL	185 ± 20	140 ± 25
WGA	1050 ± 70	360 ± 30
+unlabeled LDL	610 ± 80	155 ± 35

Platelets (1×10^8) were incubated for 5 min at 37°C with LDL (15 μg/ml, containing either ^{14}C-SM or ^{14}C-PC) in the absence or presence of WGA (20 μg/ml). ^{14}C-Lipoproteins were removed by centrifugation and one washing step. In some cases the suspensions were subsequently incubated for 40 min with a 40-fold excess of unlabeled LDL. Mean values from a total of four determinations on platelets from two donors.

On the basis of these results we evaluated whether the modulations of platelet ^{125}I-LDL binding exerted by the antibodies, WGA and DS, affected platelet phospholipid import. LDL particles were labeled with py-SM, py-PC or py-PE and incubated with the platelets at 37°C. The platelets rapidly acquired py-SM and py-PC from the donor lipoproteins within 5 min (Fig. 3A, upper and lower panels). Addition of the two concentrations of the anti-GPIIb/IIIa antibody neither altered import of py-SM nor affected uptake of py-PC. The incorporation of py-PE into the platelets as determined after a 3 min incubation of py-PE-labeled LDL (2 μg/ml) with the platelets at 37°C was not changed by the presence of the anti-GPIIb/IIIa antibody (94 μg/ml) (1.2 ± 0.4 (control) vs.

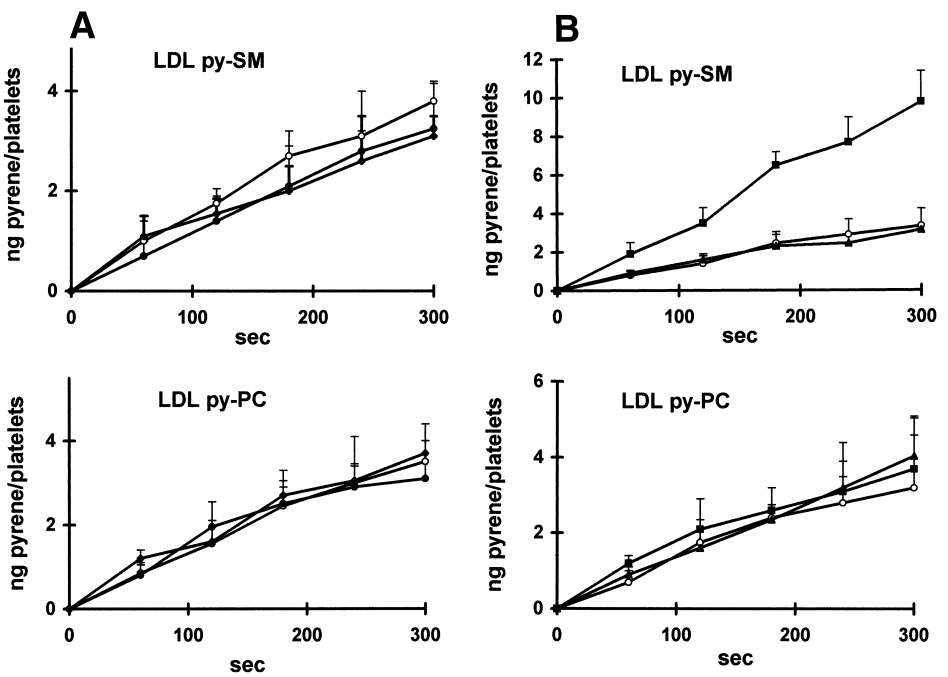

Fig. 3. Effects of the anti-GPIIb/IIIa antibody, WGA and DS on transfer of py-phospholipids from LDL to platelets. Platelet suspensions (1.3×10^8) were incubated with 2 μg/ml LDL (containing either py-SM or py-PC (30–36 ng pyrene)) in the absence or presence of the anti-GPIIb/IIIa antibody, WGA (20 μg/ml) or DS (27 μg/ml) at 37°C. A: ○, control; ◆, 47 μg/ml of antibody; ●, 94 μg/ml of antibody. B: ○, control; ■, WGA; ▲, DS. The platelet uptake of the py-phospholipids was estimated from the increase in monomer fluorescence under on-line conditions. Mean values on platelets from 4–6 different donors.

1.1 ± 0.3 ng pyrene/1.3 × 10^8 platelets (+antibody); means of four experiments). In addition, at the LDL concentration of 8 μg protein/ml, the transfer of py-SM, py-PC and py-PE was also unaffected by the anti-GPIIb/IIIa antibody (94 μg/ml) (data not shown). Following a 3 min incubation with py-SM-labeled LDL (2 μg/ml, containing 33 ng py-SM) at room temperature, the amount of labeled phospholipid incorporated int the platelets was not altered by the anti-GPIIb/IIIa antibody (94 μg/ml; 1.3 ± 0.3 (control) vs. 1.4 ± 0.3 ng py/1.2 × 10^8 platelets (+antibody); means of three experiments).

In further experiments, the influence of DS and WGA on the platelet uptake of py-labeled choline phospholipids was tested (Fig. 3B). The transfer of py-SM and py-PC from LDL to the platelets was not affected by DS (27 μg/ml). In contrast, WGA (20 μg/ml) time-dependently increased the import of py-SM into platelets. After 3 and 5 min of incubation, the incorporation of py-SM was stimulated by 2.7- and 2.9-fold at 37°C. The incorporation of py-PC (Fig. 3B) and of py-PE (not shown) was unaffected by WGA. The effect of WGA (20 μg/ml) on the transfer of py-SM from LDL (2 μg/ml; 33 ng py-SM) to the platelets was also determined at room temperature. Under these conditions, the lectin stimulated platelet py-SM uptake by 2.8-fold within 3 min (from 1.4 ± 0.2 (control) to 3.9 ± 0.5 ng py/1.3 × 10^8 platelets (+WGA); means of three experiments).

In order to determine whether WGA affected the transfer of ^{14}C-phospholipids from LDL to the platelets, platelets were incubated with LDL (15 μg/ml, containing either ^{14}C-SM or ^{14}C-PC) in the absence or presence of WGA (20 μg/ml) at 37°C. Thereafter, the amount of platelet-associated ^{14}C was determined. In platelets treated with WGA, the quantity of platelet ^{14}C-SM was 2.6-fold higher compared to the unstimulated platelets (Table 1). After removal of platelet bound ^{14}C-LDL by unlabeled LDL, the amount or platelet ^{14}C was 3.3-fold higher in the WGA-treated platelets. Following incubation of platelets with ^{14}C-PC-LDL in the presence of WGA, the quantity of platelet ^{14}C was similar to the amount determined after incubation of untreated platelets with ^{14}C-PC-LDL. This was also evident in platelets subsequently incubated with unlabeled LDL (Table 1). Taken together, the data indicate that ^{125}I-LDL binding and phospholipid transfer between lipoproteins and platelets are differentially affected by the anti-GPIIb/IIIa antibody, DS and WGA.

Also the concentration dependences of both processes argue against a decisive role of specific LDL binding for the phospholipid uptake process. Specific binding of ^{125}I-LDL to the platelets is half maximal at about 0.01 mg LDL protein/ml [13]. On the other hand, the half-maximal transfer of LDL derived ^{14}C-SM and ^{14}C-PC into the platelets is observed at about 0.5 mg LDL protein/ml [8]. We conclude from these data that phospholipid transfer from LDL to platelets is largely independent of the high affinity ^{125}I-LDL binding to the platelets. Therefore, the residual (low affinity) interaction of the particles with the platelets is expected to be involved in the phospholipid transfer. The extracellular surfaces of different cell types were previously reported to possess receptors for specific phospholipids, in particular for phosphatidylserine [17,18].

Specific binding of LDL to the platelets may involve protein-protein interactions between the LDL associated apo B-100 and the platelet GPIIb/IIIa complex [19]. In order to evaluate whether the apoprotein component of LDL was necessary for platelet phospholipid import, we investigated whether the platelets incorporated phospholipids from pure phospholipid vesicles. During incubation of platelets with lipid vesicles containing py-labeled phospholipids, a time-dependent increase in pyrene monomer fluorescence was observed for the three types of phospholipids analyzed (Fig. 4). This was accompanied by a decrease in excimer/monomer ratios. The amount of py-SM taken up within a 10 min incubation interval was similar to the quantity of py-PC incorporated by the platelets (Fig. 4). The transfer of py-PE was lower compared to the one of py-labeled choline phospholipids. These results indicate that lipid vesicles can serve as donors for phospholipid transfer into the platelets. The import of the lipoprotein-derived choline phospholipids PC and SM into the platelets is reduced after preincubation of platelets with elastase [7]. In platelets pretreated with the protease, the transfer of py-SM from vesicles to platelets was diminished by 44% (Fig. 4). The import of py-PC was lowered by 41%, while the incorporation of vesicle-derived py-PE was not altered by elastase pretreatment.

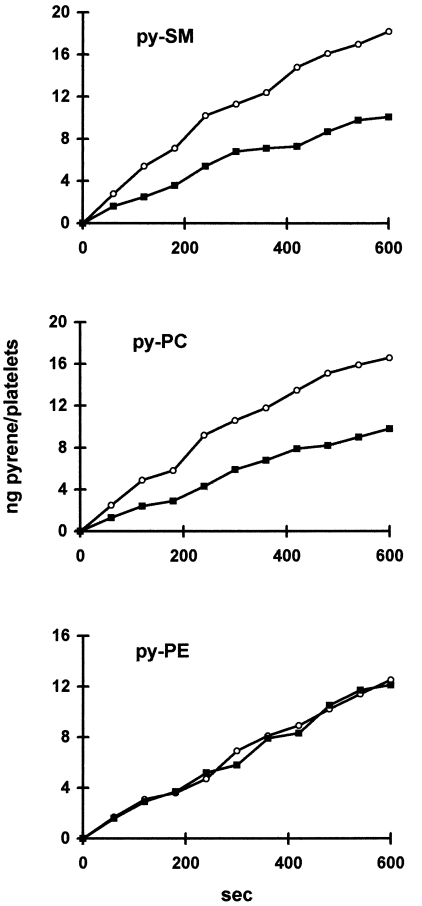

Fig. 4. Inhibition of py-phospholipid transfer from lipid vesicles to platelets by elastase. Platelets (1 × 10^8) were pretreated for 10 min with elastase (9 U/ml), proteolysis stopped by a 5-fold amount of α_1-antitrypsin and egg PC vesicles added for a further 10 min at 37°C. The vesicles contained 31 μg egg PC and 0.6 μg of py-phospholipids. The platelet uptake of the py-phospholipids was estimated from the increase in monomer fluorescence under on-line conditions. ○, control; ■, elastase-pretreated platelets. Data are shown from one representative of a total of three experiments.

Next, we evaluated whether WGA affected the transfer of py-phospholipids from lipid vesicles to platelets. Within a

Table 2
Effect of WGA on transfer of py-labeled phospholipids from lipid vesicles to the platelets

	Platelet import of py-phospholipids (ng pyrene/10^8 platelets)
py-SM	
no addition	10.6 ± 2.5
N-acetylglucosamine	9.4 ± 1.8
WGA	56.0 ± 11.1
WGA+*N*-acetylglucosamine	9.7 ± 3.2
py-PC	
no addition	9.8 ± 2.9
WGA	10.3 ± 2.5

Platelets (1×10^8) were incubated for 5 min at 37°C with egg PC vesicles containing either py-SM or py-PC under the same conditions as detailed in legend to Fig. 4. *N*-acetylglucosamine (5 mM) and/or WGA (20 μg/ml) were given to the platelet suspension immediately before addition of the vesicles. Mean values on platelets from 3–5 different donors.

5 min incubation interval, WGA (20 μg/ml) stimulated the import of py-SM by 5.3-fold (Table 2). The platelet uptake of py-PC was not altered in the presence of the lectin. WGA may exert its stimulatory effect on platelet phospholipid import by specifically binding to *N*-acetylglucosamine residues (present on platelet cell membrane proteins or lipids) or by unspecific interactions. When adding the monosaccharide alone to the platelet suspension, the transfer of py-SM from the vesicles to the platelets was not altered. On the other hand, *N*-acetylglucosamine completely prevented the stimulation of py-SM import induced by WGA (Table 2). This suggests that the enhanced incorporation of SM into WGA-treated platelets is mediated by a specific interaction of the lectin with *N*-acetylglucosamine residues on the platelet surface.

On the basis of these data it is evident that the transfer of phospholipids from either LDL or lipid vesicles into the platelets shares several common characteristics. Firstly, py-PC and py-SM were incorporated faster as compared to py-PE (Figs. 3 and 4). Secondly, protease pretreatment of the platelets reduced the import of py-labeled PC and SM but did not influence the uptake of py-PE (Fig. 4 and [7]). Thirdly, WGA stimulated uptake of SM into the platelets from LDL and the vesicles without affecting the incorporation of PC and PE (Fig. 3, Tables 1 and 2). Thus, the characteristics of phospholipid import into the platelets are independent of the apoprotein component of LDL.

WGA is known to induce platelet activation [20,21], the effect being in part due to the stimulation of tyrosine and other protein kinases [22,23]. The platelet stimulation elicited by WGA differs from that induced by thrombin and other platelet agonists [24]. In line with this observation, thrombin, collagen and ADP – while accelerating uptake of ethanolamine phospholipids – did not increase the incorporation of py-SM into the platelets [9]. Together, these data strongly suggest that different routes exist for the import of phospholipids into the platelets which are specifically activated according to the type of platelet agonist present.

LDL particles deliver phospholipid-bound arachidonic acid to the platelets which, in turn, can be further metabolized to autocrine and paracrine eicosanoids. This supply markedly depends on the LDL concentration, an increase being observed in the physiological and pathological range of lipoprotein concentrations [8]. Within a comparable concentration range, low affinity LDL binding sites on the platelet surface have been described earlier [25]. Several of the multiple influences of LDL on the functional responses of platelets (summarized in [26]) require LDL concentrations at or above the upper physiological range. It is conceivable, therefore, that some of these effects may be related to changes in phospholipid import. In future studies on platelet LDL interactions, the rapid import of phospholipids should be considered a potential mediator of LDL effects on platelet function.

In conclusion, selective phospholipid import pathways are stimulated by different types of platelet activation. The data suggest that apart from the previously described high affinity binding of LDL to the platelets there is a low affinity interaction between the two compartments which mediates the supply of phospholipids to the platelets.

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