Binding of apoE-rich high density lipoprotein particles by saturable sites on human blood platelets inhibits agonist-induced platelet aggregation

Kalpna Desai, K. Richard Bruckdorfer, Ronald A. Hutton, and James S. Owen[1]

Departments of Medicine, Biochemistry, and Haemophilia, Royal Free Hospital School of Medicine, University of London, Hampstead, London NW3 2PF, United Kingdom

Abstract High density lipoproteins (HDL, d 1.063–1.21 g/ml) are reported to stimulate, to have no effect on, or to inhibit agonist-induced platelet aggregation. We have hypothesized that these conflicting reports might be explained by opposing effects of individual HDL subclasses on platelet aggregability. Physiologic concentrations of HDL_3 had little effect on ADP-induced aggregation of washed platelet suspensions, although higher levels were stimulatory. Normal concentrations of HDL_2 (0.2–0.4 mg of protein/ml) inhibited aggregation; further fractionation by heparin–Sepharose chromatography identified the particles rich in apolipoprotein E, termed HDL-E, as the major anti-aggregatory subclass. Washed platelets bound radioiodinated HDL-E to a uniform class of saturable sites; they numbered 4,200 per platelet and the K_D was 7.9×10^{-7} M. Binding of HDL-E by platelets, and its anti-aggregatory action, showed a similar rapidity and both occurred within the physiologic concentration range. Moreover, the two processes were independent of the presence of divalent ions and were impaired by chemical modification of the apolipoprotein constituents of HDL-E. We conclude that occupation of cell-surface receptors by HDL-E particles impairs platelet responsiveness to exogenous agonists and that platelet aggregability in the presence of whole HDL may reflect the relative concentrations of the individual subclasses in the particular sample. —**Desai, K., K. R. Bruckdorfer, R. A. Hutton and J. S. Owen.** Binding of apoE-rich high density lipoprotein particles by saturable sites on human blood platelets inhibits agonist-induced platelet aggregation. *J. Lipid Res.* 1989. **30:** 831–840.

Supplementary key words apoE • high density lipoprotein subclasses • lipoprotein receptors

Several studies suggest that plasma lipoproteins can influence the reactivity of blood platelets, including their aggregatory response to a variety of agonists. Platelet-rich plasma containing elevated levels of low density lipoproteins (LDL) has an enhanced sensitivity to certain aggregating agents, including the weak agonists, epinephrine and ADP (1, 2). When platelets are freed from their plasma environment by gel filtration, they are rapidly sensitized by incubation with normal physiologic amounts of LDL (3–5). The initial step in this sensitization process is thought to involve binding of LDL particles by saturable sites, distinct from the classical LDL-receptors of nucleated cells (6), on the platelet surface (7–10); subsequent events are uncertain although normal agonist-receptor coupling may be affected (11–14).

By contrast, there have been few studies on interactions between platelets and high density lipoprotein (HDL) particles, and the effects of HDL on platelet aggregability have been conflicting. In a large normal population a weak negative correlation was found between the sensitivity of platelet-rich plasma to ADP and its HDL concentration (2) although addition of isolated HDL to platelet-rich plasma was reported not to affect ADP-induced aggregation (15). Addition of physiologic amounts of HDL to gel-filtered platelets either decreased (4) or had no effect of aggregability (5), whereas excess concentrations of HDL were considered to stimulate aggregation (5).

Plasma HDL (d 1.063–1.21 g/ml) comprises a heterogeneous group of particles (16), most commonly separated by sequential, isopycnic ultracentrifugation into two major classes, HDL_2 (d 1.063–1.125 g/ml) and HDL_3 (d 1.125–1.21 g/ml). A minor subclass, HDL_1, is also recognized; it floats largely within the HDL_2 density range by isopycnic ultracentrifugation but can be isolated either by

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43); LDL, low density lipoproteins.

[1]To whom correspondence should be addressed at the Academic Department of Medicine, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, U.K.

rate-zonal ultracentrifugation (17) or, because it is rich in apolipoprotein E (apoE), by heparin-Sepharose affinity column chromatography (18). In the present study we have investigated whether these individual HDL subclasses have different effects on agonist-induced aggregation of isolated platelets and have examined the ability of washed platelets to bind the HDL particles rich in apoE.

MATERIALS AND METHODS

Materials

Soluble collagen was purchased from Hormon-Chemie (Munich, West Germany) and Na ^{125}I was supplied by Amersham International (Bucks, U.K.). Dow Corning silicone oils (200/1 cs and 550) were purchased from Hopkins and Williams (Essex, U. K.) and mixed to give d 1.02 g/ml. Enzymobead radioiodination reagents were supplied by Bio-Rad Laboratories (Watford, U.K.). Prostacyclin was a kind gift from Dr. S. Moncada (Wellcome Research Laboratories, Beckenham, Kent, U. K.).

Plasma lipoprotein preparation

Sequential, isopycnic ultracentrifugation was used to isolate HDL (d 1.063–1.21 g/ml), or HDL_2 (d 1.063–1.125 g/ml) and HDL_3 (d 1.125–1.21 g/ml), as described previously (19). All fractions were washed by recentrifugation at the appropriate densities and extensively dialyzed against Tyrode's buffer.

In some cases HDL_2 or whole HDL were applied to a heparin-Sepharose affinity column to isolate the apoE-rich subclass, termed HDL-E.[2] Heparin (porcine intestinal mucosa, grade II; Sigma Chemical Co., Dorset, U.K.) was covalently coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions and the affinity chromatography was carried out by Method A of Weisgraber and Mahley exactly as described (18). Modification of apolipoprotein arginine, lysine, and tyrosine residues of HDL-E was accomplished by established procedures using cyclohexanedione (20), potassium cyanate (20), and tetranitromethane (21), respectively. Freshly isolated HDL-E was labeled with ^{125}I using immobilized lactoperoxidase and glucose oxidase as described elsewhere (9). The newly radioiodinated HDL-E was separated from the reaction products by chromatography on Sephadex G-10, followed by extensive dialysis to remove residual non bound iodide. Immediately before addition to platelet suspensions the ^{125}I-labeled HDL-E was passed through a 0.2-μm filter. Lipoprotein concentrations are expressed as μg of protein per ml, as measured with the Folin-Ciocalteau reagent (22) using bovine serum albumin as standard, and all values refer to final concentration in the incubation medium.

Platelet isolation

Blood was withdrawn with minimal stasis from the antecubital vein of normal volunteers who gave informed verbal consent and was mixed with one-sixth volume of acid citrate dextrose. Platelet-rich plasma was obtained by centrifugation at 750 *g* for 5 min and freed of any contaminating erythrocytes by recentrifugation at 120 *g* for 10 min. Prostacyclin (300 nM) was then added to temporarily inhibit platelet activation (23) and the platelets were sedimented at 750 *g* for 10 min. The platelet pellet was transferred to a clean tube with 5 mM HEPES buffer, pH 7.4, containing 150 mM NaCl, 2.7 mM KCl, 7.0 mM $NaHCO_3$, 0.5 mM $MgCl_2$, 5 mM glucose and the volume was adjusted to give a platelet count of 6×10^8 cells/ml; fibrinogen and human serum albumin were added at final concentrations of 0.4 mg/ml and 0.3 mg/ml, respectively. The platelet suspensions were left for up to 2 h at room temperature by which time full responsiveness to the weak agonists, ADP and epinephrine, was recovered (5, 23).

Platelet aggregation

Isolated platelet suspensions were incubated for 2 min at 20°C with an equal volume of Tyrode's buffer. Aggregation was initiated by addition of increasing concentrations of ADP, adrenaline, or collagen at 37°C in a Payton dual-channel aggregometer (Centronic Sales, Croydon, U.K.) fitted with 0.1-ml cuvettes. The minimum amount of each agonist required to induce secondary aggregation within 3 min was determined; this "threshold concentration" of agonist was used in the subsequent experiments in which the Tyrode's buffer was replaced by buffer containing lipoproteins at a series of concentrations. In some studies, twice-threshold and one-fifth threshold concentrations of ADP were also used. To avoid any variation in platelet responsiveness during a particular time needed to complete the experiment, the following protocol was adopted. The test concentrations of a particular lipoprotein class were arranged in increasing order: the even numbers were tested first in increasing concentration, followed in order by the odd numbers. The aggregation of platelet-buffer mixtures, interspaced between the lipoprotein test samples, was also measured to ensure that the threshold concentration of agonist remained unchanged during the course of the study; when the extent of aggregation with the threshold concentration did vary by more than 5%, the experiment was abandoned.

[2]HDL particles containing apoE have been termed HDL_1 when isolated by ultracentrifugation (17,45); their concentration is increased by cholesterol feeding in animals and in man when they are usually designated HDL_C (45). Although the HDL subclass bound by heparin-Sepharose has many properties in common with HDL_1 (18), we prefer to use the term HDL-E to distinguish between the two methods of fractionation.

Platelet binding studies

Isolated platelets (2×10^8/ml) were incubated with ^{125}I-labeled HDL-E at concentrations up to 800 μg of protein/ml in the presence or absence of other unlabeled lipoproteins or agents. Aliquots of the incubation mixures (usually 200 μl from a total volume of 250 μl) were layered onto 100 μl of silicone oil in 400-μl polyethylene microcentrifuge tubes and centrifuged at 14,000 *g* for 1 min to pellet the cells below the oil layer. The tubes were then sliced and ^{125}I radioactivity associated with the platelets was measured. The counts obtained from control incubations, containing radioiodinated HDL-E but no platelets, were subtracted from all test incubations; the correction was small, usually less than 3% of that bound. Any radioactive acid-soluble, noniodide degradation products of ^{125}I-labeled HDL-E catabolism by the platelets were measured as previously described (19). The binding constant and the number of binding sites for HDL-E per platelet were calculated using computer-assisted analysis (The KINETIC-EBDA/LIGAND program (24), Elsevier-BIOSOFT, Cambridge, U. K.). Nonspecific binding was not measured directly but instead was estimated from the analysis of total binding (25); this technique is considered to give more accurate information on the binding characteristics of saturable sites than the use of excess, unlabeled ligand to estimate nonspecific binding (26).

To assess the reversibility of binding, isolated platelets (4×10^8 in 2 ml) were pre-incubated for 20 min at 23°C with 20 μg of ^{125}I-labeled HDL-E. The cells were pelleted at 800 *g* for 2 min, resuspended in 2 ml of Tyrode's buffer, and washed twice more by centrifugation and resuspension. The platelet suspension was then diluted to 20 ml in Tyrode's buffer and incubated at 23°C for 30 min. Aliquots (2 ml) were removed at zero time and at appropriate intervals thereafter; the platelets were immediately sedimented by centrifugation and the ^{125}I radioactivity in the cell pellet was counted.

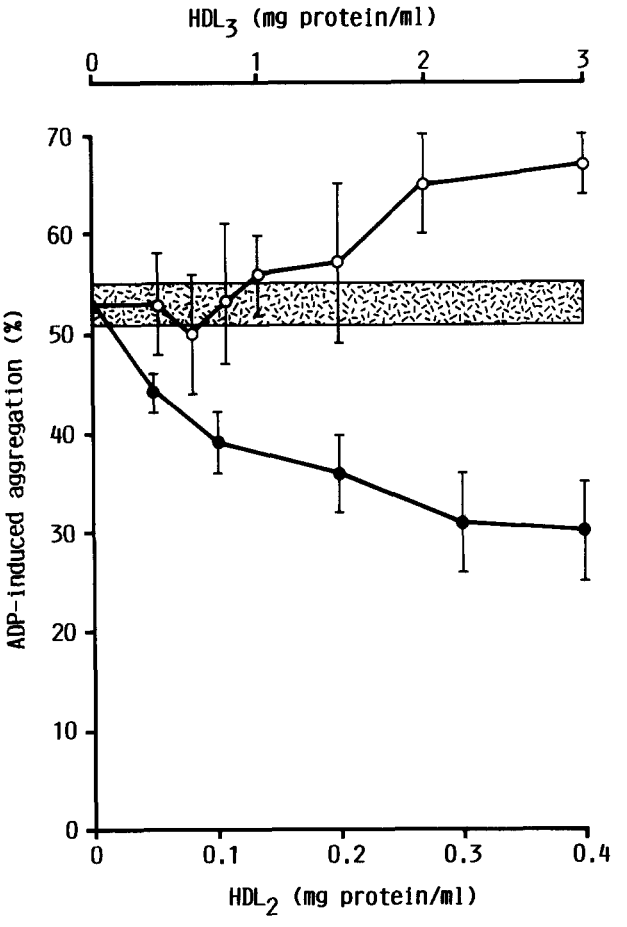

Fig. 1. Inhibition of ADP-induced aggregation of isolated platelets by HDL_2. Platelet suspensions (6×10^8/ml) were mixed with an equal volume of Tyrode's buffer containing either HDL_2 (●; d 1.063–1.125 g/ml; final concentration 0.05–0.4 mg of protein/ml) or HDL_3 (○; d 1.125–1.21 g/ml; 0.05–3.0 mg of protein/ml) and pre-incubated for 2 min at 20°C. Platelet aggregation was initiated by addition of the threshold concentration of ADP (see Materials and Methods) and was measured as the maximum percentage decrease in absorbance within 3 min at 37°C in a dual-channel aggregometer. Each point is the mean percentage aggregation (± SEM) for 14 different HDL_2 or 12 different HDL_3 samples tested on a total of six separate isolated platelet preparations. The shaded area gives the range of values obtained when HDL was replaced by buffer alone.

RESULTS

Platelet aggregation

Normal physiologic concentrations of HDL_3 (0.8–1.2 mg of protein/ml) had little effect on the percentage aggregation of isolated platelet suspensions induced by ADP (**Fig. 1**). At 2–3 times these concentrations the HDL_3 particles appeared to sensitize the platelets; aggregation with threshold concentrations of ADP increased from 52–57% to 60–67%. A similar sensitizing effect was also seen when one-fifth threshold levels of ADP were used; aggregation increased from 10 ± 1% with buffer to 25 ± 5% when 1.5 mg/ml of HDL_3 protein was present. By contrast, HDL_2 (d 1.063–1.125 g/ml) inhibited ADP-induced aggregation in a stepwise manner throughout the range tested (0.05– 0.4 mg of protein/ml) (Fig. 1). These opposing effects of HDL_2 and HDL_3 on platelet aggregability were not restricted to ADP as agonist; similar differences in their effects were seen when epinephrine and collagen were used at threshold concentrations (data not shown).

Fractionation of HDL_2 by heparin–Sepharose affinity chromatography yielded one class of particles deficient in apoE and another class that was apoE-rich (**Fig. 2**). The apoE-poor fraction had only a minor inhibitory effect on ADP-induced aggregation. The apoE-rich fraction, however, was a potent inhibitor; it reduced aggregation from 50% to 32% and 26% when present at 0.05 mg and 0.1

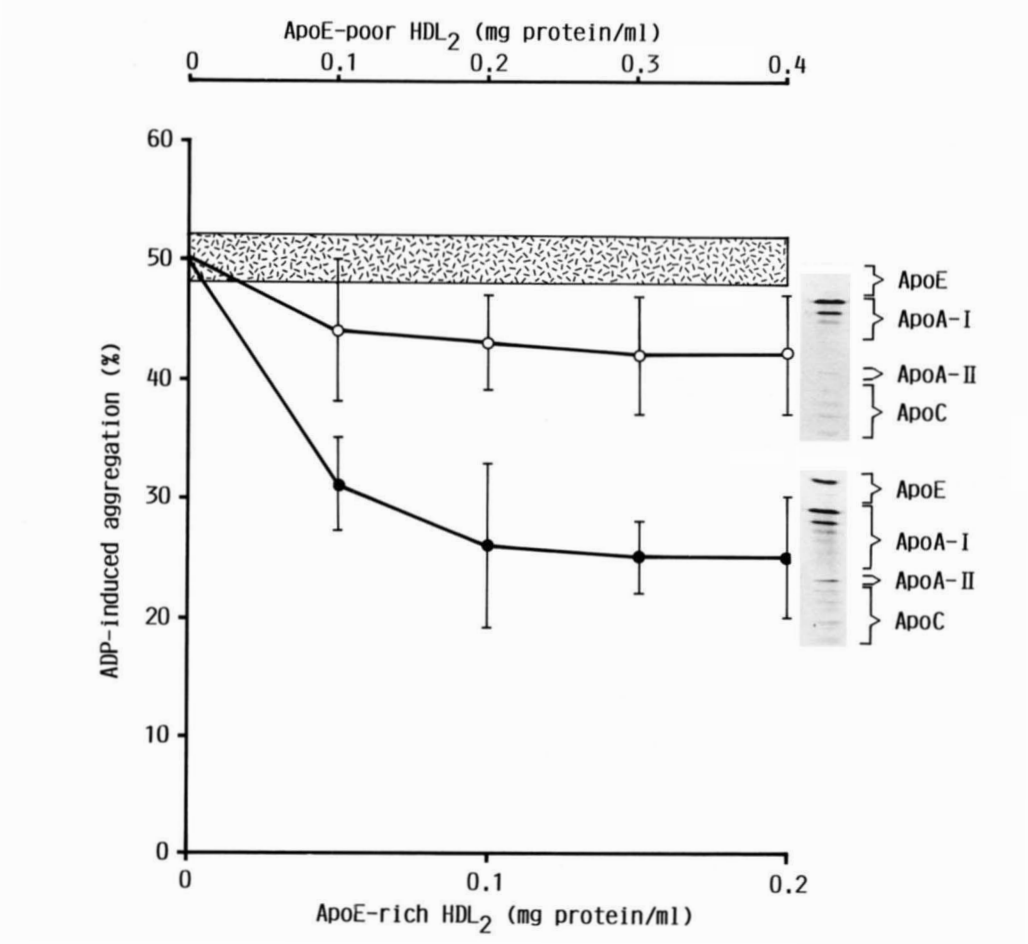

Fig. 2. Inhibition of ADP-induced aggregation of isolated platelets by apoE-rich HDL_2 particles. HDL_2 (d 1.063–1.125 g/ml) was subfractionated by heparin–Sepharose affinity column chromatography using Method A of Weisgraber and Mahley (18). Isoelectric focusing of the delipidated samples was carried out as before (19) and confirmed, as shown to the right, that the unbound column fraction was apoE-poor, whilst the second fraction eluted was enriched in apoE. The ability of both fractions to inhibit ADP-induced aggregation of isolated platelet suspensions was then examined as described in the legend to Fig. 1. Each point is the mean percentage aggregation (± SEM) of four different experiments and the shaded area delineates the range of values obtained when the HDL sample was replaced by buffer alone.

mg of protein/ml, respectively (Fig. 2), concentrations similar to the average plasma level of 7.4 ± 2.3 mg/dl reported by Weisgraber and Mahley (18) for six normal adults. The HDL-E particles were similarly anti-aggregatory at twice-threshold levels of ADP and were also able to suppress collagen- or epinephrine-induced aggregation (**Fig. 3**). As shown by the representative aggregation traces in Fig. 3, HDL-E inhibited the initial rate of aggregation by ADP and epinephrine and prolonged the lag phase before aggregation commenced with collagen. The progressive decrease in the thickness of the traces indicates that the size of the aggregates formed was also reduced by the presence of HDL-E. However, HDL-E particles chemically modified by carbamylation or by cyclohexanedione treatment had only a minor inhibitory effect on ADP-induced aggregation even at concentrations of 0.2 mg of protein/ml (Fig. 3A, traces d and e).

Our HDL-E preparations were characterized further. Following separation of delipidated HDL-E by isoelectric focusing and densitometric scanning as described previously (19), the amount of apoE relative to the other apolipoproteins was calculated to be 18.5% and 26.3% in two different preparations. The biological activity of the HDL-E was confirmed by measuring its ability to compete with LDL for binding at 4°C by apoB,E-receptors on the surface of cultured human skin fibroblasts (18, 19): two separate HDL-E preparations reduced binding of ^{125}I-labeled LDL (5 μg protein/ml) by 49% and 42% respectively, when tested at a concentration of 25 μg protein/ml. We conclude that our HDL-E preparations are chemically and metabolically similar to those originally reported (18).

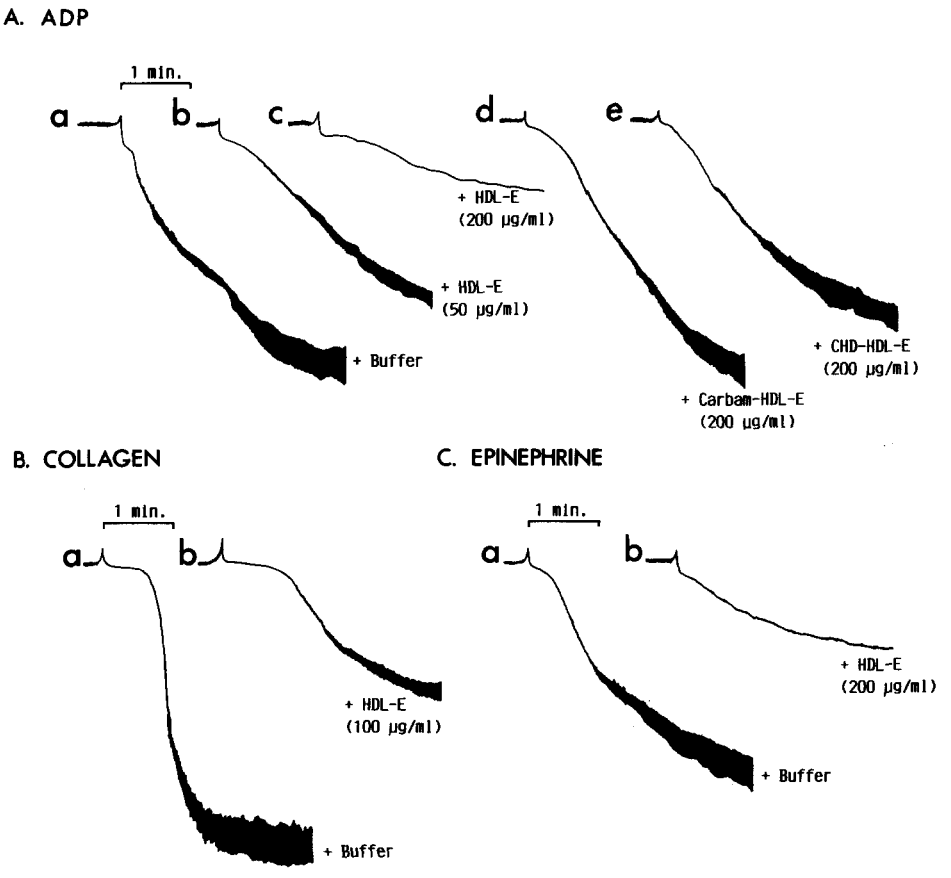

Fig. 3. Effects of native and chemically modified HDL-E on agonist-induced aggregation of isolated platelets. The experimental procedure was as described in the legend to Fig. 1; threshold concentrations of ADP (A), collagen (B), or epinephrine (C) were added to platelet suspensions pre-incubated with either Tyrode's buffer (trace a), HDL-E (traces b and c; final protein concentrations as indicated), or chemically modified HDL-E (traces d and e; 200 µg protein/ml of carbamylated and cyclohexanedione-treated HDL-E, respectively).

Binding studies

Binding of ^{125}I-labeled HDL-E by isolated platelets increased in a curvilinear manner as the concentration of HDL-E in the incubation medium was increased (**Fig. 4**). The total binding curve was subjected to analysis using the LIGAND nonlinear curve-fitting technique (24, 25); the data closely fitted a one-site model with the nonspecific binding subtracted, and consideration of an additional saturable site did not improve the fit. A Scatchard plot of the data gave a linear correlation coefficient of $r = -0.98, P < 0.001$ (Fig. 4, insert) and, in six independent experiments, the number of these identical and independent HDL-E binding sites was estimated to be 4193 ± 1337 (mean ± SEM) with a K_D of 7.9 ± 6.0 × 10^{-7} M, assuming a mean particle molecular weight of 6 × 10^5.

Binding of ^{125}I-labeled HDL-E by platelets was rapid, reaching a maximum within 15 min at room temperature (**Fig. 5**, inset), and was also readily reversible since resuspension of preloaded platelets in buffer led to 50% dissociation of the radiolabel within 20 min (data not shown). Such binding was also largely insensitive to temperature; the binding curves of ^{125}I-labeled HDL-E by platelets at 0°C, 23°C (room temperature), and 37°C were similar (Fig. 5). Moreover, binding of HDL-E at 37°C was not associated with degradation of the apolipoprotein residue; following incubation of ^{125}I-labeled HDL-E with platelets for up to 2 h, the noniodide, acid-soluble radioactivity in the incubation medium did not exceed 0.5% of that added and was similar to that in parallel cell-free incubations (about 0.4%). Addition of disodium EDTA (up to 10 mM) or Ca^{2+} (up to 2 mM) to the incubation medium had little effect on the ability of platelets to bind HDL-E, nor was binding impaired by the presence of heparin (1000 U/ml). Unlabeled HDL-E readily reduced the binding of 50 µg of protein/ml of ^{125}I-labeled HDL-E by platelets, with 50% displacement at about 400 µg protein/ml (**Fig. 6**). Modification of the arginyl, lysyl, or tyrosyl residues of apolipoprotein molecules in HDL-E by treatment with cyclohexanedione, potassium cyanate, and tetranitromethane, respectively,

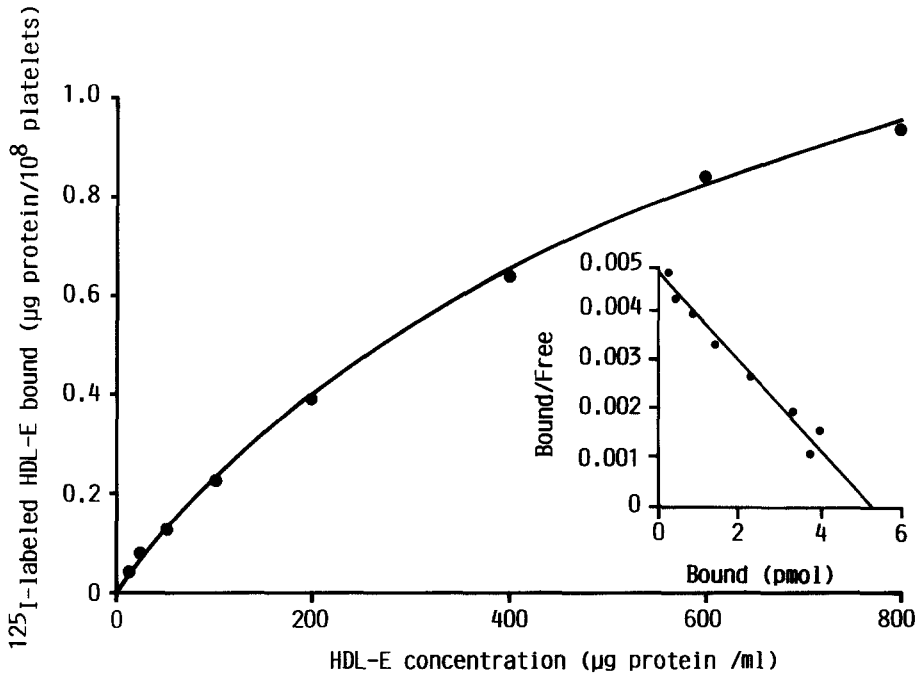

Fig. 4. Binding of ^{125}I-labeled HDL-E by isolated platelets as a function of concentration. Suspensions of isolated platelets (100 µl: 5 × 10^8/ml) were incubated with ^{125}I-labeled HDL-E (150 µl; up to 800 µg/ml) for 15 min at room temperature. Aliquots (200 µl) were layered onto silicone oil (100 µl, d 1.2 g/ml) in polyethylene microcentrifuge tubes and were rapidly centrifuged (14000 *g*, 1 min) to pellet the cells below the oil layer. The tubes were then sliced and the ^{125}I radioactivity associated with the platelets was measured. Control incubations, containing radioiodinated HDL-E but no platelets, were treated similarly and the values were subtracted from the test incubations; each point shown is the mean of three determinations. The inset shows the Scatchard plot of the data corrected for nonspecific binding by the LIGAND program.

markedly impaired its ability to compete with unmodified ^{125}I-labeled HDL-E for platelet binding (Fig. 6). When the blocked arginyl residues of HDL-E were regenerated by incubation with hydroxylamine (20), the resulting HDL-E was still competitive, suggesting that impaired binding of modified HDL-E by platelets was due to the loss of positively charged arginine groups and not to a secondary deterioration of the HDL-E during the chemical blocking reaction.

DISCUSSION

Our results establish that the two major subclasses of plasma HDL, as isolated by isopycnic ultracentrifugation, have opposite effects on agonist-induced aggregation of isolated platelet suspensions: HDL_3 are pro-aggregatory, albeit at concentrations near or above the upper limit of the normal range, whereas HDL_2 are anti-aggregatory at physiologic levels. Subfractionation of the particles floating within the HDL_2 density range, using heparin–Sepharose affinity chromatography, revealed that their inhibitory action resulted predominantly from the presence of HDL-E, large particles enriched in cholesteryl ester and apoE (17, 18); inhibition of aggregation by the apoE-deficient fraction was moderate. These findings suggest that the influence of whole HDL (d 1.063–1.21 g/ml) on platelet responsiveness may depend on the relative concentrations of the different subclasses present. Whether this can also explain the previous conflicting reports of HDL effects on agonist-induced platelet aggregation (2–5, 15) is uncertain. However, the relative proportion of HDL_3 to HDL_2 in plasma is known to vary widely and to be affected by a number of factors including sex, alcohol intake, exercise, smoking, obesity, and hypercholesterolemia (14, 27, 28). Unfortunately, there is little information on the variability of HDL-E per se in normal plasma, although data on HDL_1 levels by both analytical ultracentrifugation (28) and rate zonal ultracentrifugation (17) suggest that considerable variation may occur; nor is it clear whether HDL-E concentrations are influenced by factors which affect the HDL_3 to HDL_2 ratio.

Several experiments were carried out to help delineate the interactions between HDL-E particles and platelet surfaces. We established that isolated platelet suspensions bound ^{125}I-labeled HDL-E to a uniform class of saturable sites, numbering about 4,200 per cell. Various studies have concluded that binding of LDL particles by satur-

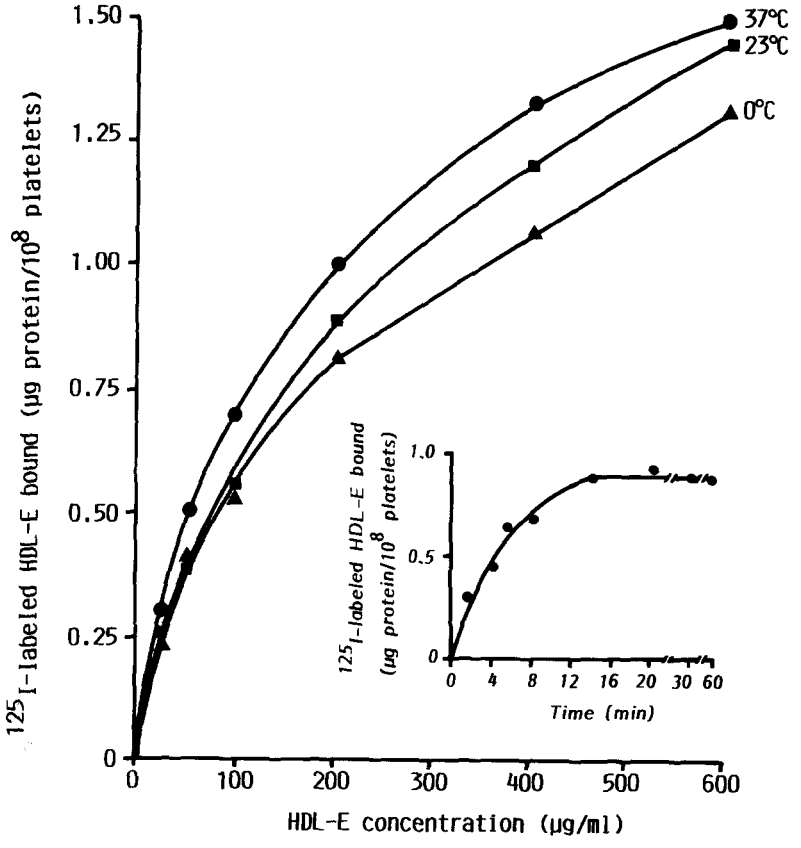

Fig. 5. Binding of ^{125}I-labeled HDL-E by isolated platelets as a function of temperature and time. The experimental procedures were as described in the legend to Fig. 4; a 15-min incubation period was used at three different temperatures (●; 37°C; ■, 23°C; ▲, 0°C), whilst the time course for binding of HDL-E at a concentration of 100 µg protein/ml was carried out at 23°C (insert).

able receptors on the platelet surface represents the initial step for sensitization of platelets by LDL (6, 7, 9). Although caution should always be used in assigning a biological significance to saturable binding (29), we believe it reasonable to conclude that occupation of cell-surface receptors by HDL-E particles impairs platelet responsiveness to exogenous stimuli. Several of our experiments supported this view; binding of HDL-E and its inhibitory action showed similar rapidity and occurred within the same concentration range, whilst chemical modification of apolipoproteins of HDL-E prevented not only binding but also the anti-aggregatory effect. Although other groups have not studied the interactions between HDL-E and platelets, there have been reports of platelets binding radioiodinated HDL. Koller, Koller, and Doleschel (7) showed that washed platelets bind about 3200 HDL_3 particles per cell and that the process, as we report herein for HDL-E, is rapid, independent of divalent ions and largely insensitive to temperature. Such binding of HDL_3 presumably occurs through apoA-I and/or apoA-II, since the particles contain only traces of apoE and apoC (18, 30), but it remains to be established whether the interaction is related to the pro-aggregatory action of HDL_3 (ref. 3 and this study). More recently, Curtiss and Plow (9) found that maximum binding of whole HDL by gel-filtered platelets required 2 h of incubation at 37°C and that divalent ions or lower temperatures were markedly inhibitory. As HDL_3 is the major subclass of whole HDL, it is unclear why the results of these authors differ from those of Koller et al. (7). Their relevance to our aggregation studies is also unclear since very little HDL was bound at 5 min (the total incubation period used in our aggregation measurements); this study of Curtiss and Plow (9) cannot explain, therefore, how HDL particles exert their effects on platelet aggregability.

Each of the major polypeptide constituents of HDL-E viz., apoA-I (approx. 55%; Fig. 2 and ref. 15), apoE (25%), and apoA-II (15%), has been designated as the signal within a particular lipoprotein particle recognized by certain cell-surface receptors. In the liver, receptors on hepatocyte plasma membranes bind apoE in chylomicron remnants or in HDL_c (the cholesterol-induced HDL con-

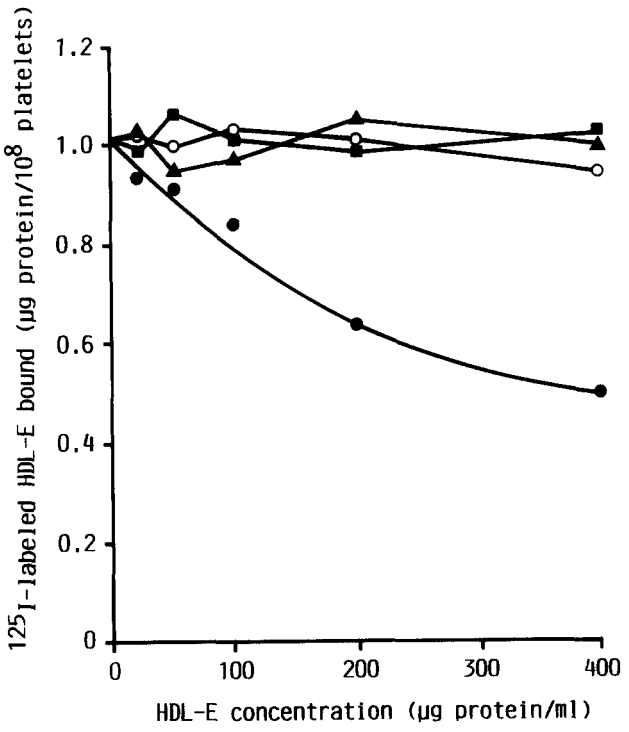

Fig. 6. Competitive inhibition of ^{125}I-labeled HDL-E binding to isolated platelets by chemically modified or untreated HDL-E particles. These experiments were carried out as described in the legend to Fig. 4, except that a fixed concentration of radioiodinated HDL-E (50 μg of protein/ml) was incubated in the presence of increasing concentrations (up to 400 μg protein/ml) of unlabeled lipoproteins. The unlabeled competitors were native HDL-E (●) or HDL-E particles treated with cyclohexanedione (▲), tetranitromethane (○), or potassium cyanate (■) as described in the Materials and Methods section. Each point is the mean of three determinations.

taining predominantly apoE) (31), whilst in adipocytes (32) and in macrophages (33), apoA-I is considered the ligand involved in specific binding of apoE-free HDL_3 particles. However, receptors binding HDL in other cell lines appear to have a more relaxed specificity; the amphipathic helical regions of both apoA-I and apoA-II are recognized (30, 32). We suspect that apoE is the anti-aggregatory component of HDL-E, not only because the other HDL subclasses contain low amounts of apoE, but also because chemically blocking arginine and lysine residues of HDL-E prevents its inhibitory effect; these treatments prevent recognition of apoE by its receptor on hepatic membranes (31) but leave binding of apoA to extrahepatic cells unimpaired (21, 30). The mechanism by which tetranitromethane treatment prevents HDL-E recognition by platelets is uncertain, but may result from cross-linkage of apoE molecules to each other or to apoA (21, 34), or possibly from nitration of apoE tyrosine residues [although these residues are not present in the region of apoE recognized by its hepatic receptor (35)]. Also relevant are our previous observation that abnormal, apoE-rich HDL (36) in familial lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) deficiency is anti-aggregatory (37) and our recent report of a strong correlation ($r = 0.70$, $P < 0.001$) between the apoE content of liver disease HDL and its ability to inhibit ADP-induced platelet aggregation (38). Clearly, these findings are consistent with apoE as the active constituent of HDL-E.

Our observation that a subpopulation of HDL particles has anti-aggregatory properties may have pathophysiologic significance, since many epidemiological studies show that HDL levels, particularly those of the HDL_2 class, are inversely correlated with atherogenesis (39–41). At the cellular level this protective effect of HDL may primarily reflect its ability to reduce cholesterol in extrahepatic cells by transporting it to the liver for excretion (42). However, other beneficial effects are also reported: HDL can not only stimulate endothelium production of prostacyclin (43), a potent vasodilator and an inhibitor of aggregation, but it can also act to stabilize it in plasma (44). The direct effects of HDL on platelet reactivity have hitherto been poorly defined, but our finding that certain HDL particles within the HDL_2 density range are anti-aggregatory is consistent with the epidemiological evidence and may represent, therefore, an additional mechanism by which HDL helps prevent coronary heart disease.

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