Albumin redirects platelet eicosanoid metabolism toward 12(*S*)-hydroxyeicosatetraenoic acid[1]

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Abstract Albumin is a major determinant of eicosanoid formation, affecting autacoids important in cell-cell interactions. We delineated three mechanisms by which albumin controlled platelet eicosanoid formation: *1*) Albumin diverted free arachidonate toward 12-lipoxygenation. *2*)Albumin enhanced release of arachidonate from phospholipids. *3*) Albumin inhibited incorporation of arachidonate from the medium into platelet phospholipids. 12(*S*)-Hydroxyheptadecatrienoic acid (12-HHTrE) formation was reduced 70% by albumin as compared to that formed in albumin-free medium. In sharp contrast, formation of 12(*S*)-hydroxyeicosatetraenoic acid (12-HETE), the platelet lipoxygenase product, was much less influenced by albumin. Moreover, 12-HETE production in the presence of albumin was markedly increased and prolonged after aspirin treatment. These data suggested that albumin redirected released endogenous arachidonate from cyclooxygenase to lipoxygenase. Therefore, the metabolic fate of arachidonate present in the medium of stimulated platelets was studied by adding tracer [^{3}H]arachidonate 30 sec before thrombin. Albumin increased arachidonate metabolism by lipoxygenase 7-fold as compared to albumin-free controls, while cyclooxygenation increased 2.7-fold. Redirection of eicosanoid metabolism by albumin toward lipoxygenase products constitutes a heretofore undescribed and potentially important physiological role for albumin. In vitro utilization of albumin may reflect in vivo events in thrombosis and hemostasis more accurately than previous studies without albumin could appreciate.—**Broekman, M. J., A. M. Eiroa, and A. J. Marcus.** Albumin redirects platelet eicosanoid metabolism toward 12(*S*)-hydroxyeicosatetraenoic acid. *J. Lipid Res.* 1989. **30:** 1925-1932.

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Activated platelets release arachidonate and enzymatically generate cyclooxygenase and lipoxygenase metabolites therefrom. The profile of eicosanoids formed by platelet suspensions in vitro consists of 12-HHTrE, TXA_2, and 12-HETE. Negligible quantities (less than 1.6%) of PGE_2, PGD_2, and $PGF_{2\alpha}$ are detectable (1). As studied in vitro this spectrum can be qualitatively and quantitatively extended by interactions with other cell types (2-6). Such cell-cell interactions involve transfer of arachidonate as well as eicosanoids and eicosanoid intermediates (7). Therefore, free arachidonate derived from stimulated cells constitutes a critical substrate for reactions involving formation of eicosanoids during cell-cell contact.

We recently found that interactions with albumin are of great importance in studying further metabolism of arachidonate following its release from cells under investigation. Albumin is an abundant plasma protein, of which 40% circulates but 60% is located extravascularly (8). Albumin binds fatty acids at two primary sites with affinity constants of 2.6×10^8/M and 1×10^8/M (oleate); the affinities of three or four secondary sites are at least 10-fold lower (9, 10). For this reason alone it is imperative to evaluate fatty acid binding by albumin when considering how eicosanoid metabolism transpires in vivo. Fatty acid binding sites on albumin are distinct from those for other ligands and do not involve lysine 199, which is acetylated by aspirin. The K_A for arachidonate of albumin is $\sim 3 \times 10^7$/M, 10-fold less than for oleate (11, 12).

For reasons of practicality, in vitro studies of eicosanoid formation have avoided albumin and utilized protein-free or gelatin-containing media in order to avoid binding of free fatty acids or eicosanoids extracellularly. In some instances, fatty acid binding by albumin was utilized to retain released fatty acids in the extracellular milieu (13-15). While the utility of this approach has been very important, it had not

Abbreviations: HPLC, high performance liquid chromatography; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; PA, phosphatidic acid; PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids; PI, phosphatidylinositol; PS, phosphatidylserine; TLC, thin-layer chromatography; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2. Abbreviations of eicosanoids follow recent recommendations (27): 5,12-diHETE, 5(*S*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid; 12,20-diHETE, 12(*S*),20-dihydroxy-5,8,10,14-*cis*-eicosatetraenoic acid; 12-HETE, 12(*S*)-hydroxy-5,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid; 15-HETE, 15(*S*)-hydroxy-5,8,11-*cis*,13-*trans*-eicosatetraenoic acid; 12-HHTrE, 12(*S*)-hydroxy-heptadecatrienoic acid.

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heretofore served to unmask in vivo realities concerning the physiological significance of eisosanoid metabolism.

Of particular interest is the continued formation of the lipoxygenase product, 12-hydroxyeicosatetraenoic acid (12-HETE), under conditions of aspirin ingestion or addition in vitro. Cyclooxygenation of arachidonate occurs over a much shorter time period following stimulation than does lipoxygenation. Therefore, binding of released arachidonate by albumin in the external milieu might affect cyclooxygenation more than lipoxygenation. This would result in redirection of arachidonate oxygenation by albumin from cyclooxygenase to lipoxygenase processing. The experimental data to be presented herewith support a new diversion hypothesis concerning the lipoxygenase pathway, with important in vivo implications. Albumin redirects eicosanoid formation from both endogenous and exogenous platelet arachidonate to the lipoxygenase product, 12-HETE. This altered profile of eicosanoids generated after platelet activation in a medium of greater physiological relevance, may thereby affect cellular interactions of critical importance to hemostasis, thrombosis, and the inflammatory response. In this communication, details of these newly characterized biochemical reactions will be delineated.

EXPERIMENTAL PROCEDURES

Platelet collection and processing

Washed human platelet suspensions were prepared from platelet-rich plasma (13–16). Platelets were washed twice (4°C) in Tris-citrate buffer (63 mM Tris, 95 mM NaCl, 5 mM KCl, 12 mM citric acid, pH 6.5), resuspended in 154 mM NaCl, and adjusted to 10^{10} platelets/ml.

Experimental design

Incubations were carried out at 37°C in open tubes, with or without stirring (13–16): 0.5 ml ($1.7–5 \times 10^9$) platelets were incubated in buffer (75 mM Tris, 95 mM NaCl, 7 mM glucose, 3 mM KH_2PO_4, pH 7.4) in a total volume of 1 or 2 ml. Thrombin was added in 50 or 100 μl 154 mM NaCl after 4 min preincubation. Incubations were terminated by rapid addition of 3.5 volumes chloroform–methanol 2:5 (v/v) for analyses of fatty acids (13–16), or 1.5 volumes acetone for measurements of eicosanoids (4, 17). [^{3}H]Arachidonate (1 μCi, 5 pmol, in 5 μl Na_2CO_3) was added 30 sec prior to thrombin.

In preliminary experiments different sources of albumin were evaluated. Effects of human essentially fatty acid-free albumin derived from fraction V (Sigma, St. Louis, MO, catalog #A-1887) on eicosanoid formation were indistinguishable from bovine albumin (Sigma #A-6003). Furthermore, no significant differences were noted between human essentially fatty acid-free albumin (Sigma), and non-defatted albumins such as human albumin containing less than 2.4% dimer (Calbiochem, San Diego, CA, #126654), or bovine albumin (ICN ImmunoBiologicals, Lisle, IL, #81-006). This ruled out effects due to lipid and fatty acid content of the albumins and effects of dimeric albumin. In addition, contamination with serine proteases was ruled out as follows: Sigma essentially fatty acid-free human albumin was pretreated with 2.5 mM diisopropylfluorophosphate. Calbiochem albumin was pretreated with glycine, followed by neutralization and incubation with 0.1 mM phenylmethylsulfonyl fluoride. Excess reagents were removed by dialysis; no changes in free arachidonate or its metabolites were observed when compared with untreated albumin.

Fatty acid analyses

Incubation mixtures were extracted by a modified Bligh and Dyer method and lipids were separated by two-dimensional TLC using chloroform–methanol–conc. ammonia–water 65:35:5:1.5 (v/v) (solvent 1), and chloroform–acetone–methanol–acetic acid–water 60:80:20:20:10 (v/v) (solvent 2) (13–16). Alternatively, one-dimensional TLC utilizing silica gel 60 and chloroform–methanol–acetic acid–water 60:50:1:4 (v/v) was performed for separation of major phospholipid classes (18). Lipid extracts (in chloroform–methanol 2:1) were spotted on the plates under N_2. Spotting tubes were rinsed twice with chloroform–methanol 2:1, which was also spotted to ensure quantitative transfer of lipid extract. Following development, plates were dried under N_2 and stained with purified 2′,7′-dichlorofluorescein. Visualized lipids were marked, plates were dried under N_2, and marked lipids were scraped into tubes containing heneicosanoic acid (21:0) as internal standard, prior to addition of 2 ml 3 N HCl in methanol for preparation of fatty acid methyl esters (14–16). Fatty acid methyl esters were quantitated utilizing a Hewlett-Packard 5880A gas chromatograph, equipped with two columns (1800 × 2 mm) packed with 10% SP2340 on 100/120 mesh Chromosorb WAW. Fatty acid content was measured relative to the known quantities of 21:0 added as internal standard. From these data fatty acids were calculated and expressed as nmol per 5×10^9 platelets (14–16). Free fatty acids in eicosanoid extracts were also quantitated. After completion of eicosanoid analyses (see below), solvent (ethanol) was evaporated, and lipids were redissolved in chloroform–methanol 2:1 prior to TLC and fatty acid analyses as above. In these instances, 21:0 internal standard was added at the same time as 15-HETE, the eicosanoid internal standard.

Eicosanoid analyses

Internal standard (15-HETE, an eicosanoid not formed by pure human platelet suspensions) was added immediately after incubations were terminated with 1.5 volumes of acetone. Platelet suspensions were extracted (4, 17), and the extracts were dissolved in 50–100 μl ethanol. Eicosanoids were separated by HPLC on a NovaPak C18 column (150 × 4 mm; Waters/Millipore, Milford, MA) using mixtures of solvent A (27% acetonitrile in water, pH 2.5 with phos-

phoric acid) and solvent B (100% acetonitrile) at a flow rate of 1 ml/min. After 5 min at 100% A, solvent composition was varied linearly: over 2 min to 78% A (43.1% acetonitrile); then in 25 min to 60% A (56.2% acetonitrile); finally in 1 min to 100% B (100% acetonitrile) for a "washoff" of 15 min. Solvent composition was returned in 5 min to 100% A (27% acetonitrile) for re-equilibration. Eicosanoids were detected by absorbance at 237 nm (5 nm bandwidth) utilizing a Kratos 783 spectrophotometer (ABI Analytical, Ramsey, NJ), governing two Waters model 590 pumps (Millipore, Bedford, MA), and quantitated (Shimadzu C-R4A, Shimadzu Scientific Instruments, Columbia, MD). At 237 nm, peak area per ng 12-HHTrE and 12-HETE related to peak area per ng 15-HETE as 0.86 and 1.12, respectively. This was determined from standard solutions whose purity and concentration were ascertained spectrophotometrically (Cary 118, Varian Associates, Palo Alto, CA), utilizing ϵ_{237} = 27,500 for 12- and 15-HETE, and ϵ_{231} = 33,400 for 12-HHTrE. Peak area varied linearly with quantity over the absorbance range encountered. Sensitivity was 1–5 pmol eicosanoid. Peak identity was confirmed by cochromatography with authentic standards, and by on-line spectral analyses (LC-235 diode-array detector, Perkin-Elmer, Norwalk, CT).

Radio-TLC and -HPLC

Radioactive eicosanoids were analyzed by radio-TLC utilizing the Nugteren TLC system (2, 19), and by radio-HPLC. The effluent from the HPLC column was mixed with Flo-Scint III (5 ml/ml HPLC effluent) and counted (Flo-One Beta, Radiomatic Instruments and Chemical Co., Tampa, FL).

Materials

Solvents for HPLC were obtained from Burdick and Jackson (Scientific Products, Edison, NJ) or Waters (Waters/Millipore, Milford, MA); eicosanoid standards were from Biomol (Plymouth Meeting, PA); [^{3}H]arachidonic acid (NET-298Z, 7030 GBq or 190 Ci/mmol) from DuPont (Boston, MA) was converted to the sodium salt immediately prior to use (2); silica gel 60 TLC plates were from Merck (EM Science, Cherry Hill, NJ). Other reagents were obtained as previously described (13–16).

RESULTS

Time course of thrombin-induced eicosanoid formation in the presence and absence of albumin

There was a pronounced effect of albumin on generation of platelet eicosanoids (**Fig. 1**). Albumin, at a concentration of 37 μM, blocked formation of 12-HHTrE 15 sec following thrombin addition, when 15 nmol 12-HHTrE had formed (Fig. 1A). When albumin was absent from the medium, rapid formation of 12-HHTrE persisted for more than 1 min (rather than 15 sec) after thrombin. Albumin reduced formation of 12-HHTrE by two thirds after 5 min stimulation (45 to 15 nmol, Fig. 1A). However, the initial rate of 12-HHTrE formation was albumin-insensitive. These data suggested that arachidonate released from stimulated platelets was retained extracellularly. This resulted in reduced availability of released arachidonate for oxygenation intracellularly by membrane-bound cyclooxygenase. Since platelet arachidonate cyclooxygenation results in parallel formation of TXA_2 and 12-HHTrE, our data imply that arachidonate released from stimulated platelets is diverted from all cyclooxygenase-mediated reactions by albumin in the medium.

In contrast, results with the lipoxygenase product, 12-HETE, differed from those of the cyclooxygenase metabolite, 12-HHTrE. The rate at which 12-HETE formed was

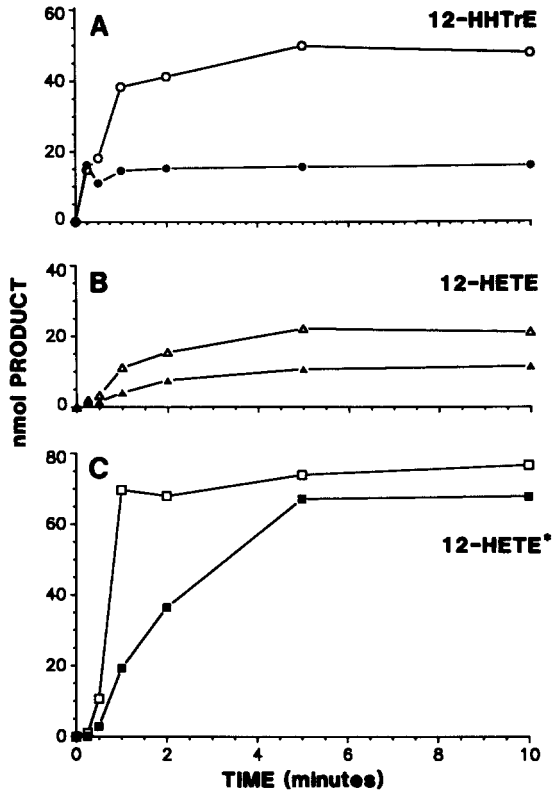

Fig. 1. Effect of albumin on eicosanoid formation by thrombin-stimulated platelets. A: Albumin (37 μM, closed symbols) limited formation of 12-HHTrE to less than one-third of its formation in albumin-free incubations (open symbols). B: 12-HETE formation in the presence of albumin persisted albeit at a lower initial rate than 12-HHTrE production. Greater than 50% of 12-HETE formation was retained in the presence of albumin. C: 12-HETE formation was much more extensive in the presence of 0.5 mM aspirin (asterisk). Albumin slowed 12-HETE formation without affecting its final level. Data are from one of three experiments that yielded similar results, and are expressed as nmol eicosanoid/5 × 10^9 platelets; 4.8 × 10^9 platelets were incubated in a total volume of 2 ml and stimulated with 15 U α-thrombin (0.31 U per 10^8 platelets). See Experimental Procedures for details of extraction and HPLC analyses.

slower, more continuous, and of longer duration than 12-HHTrE. Albumin reduced overall production of 12-HETE by one third to one half (Fig. 1B). However, this was considerably less than the two thirds reduction observed in 12-HHTrE production reported above (Fig. 1A). The data depicted in Fig. 1A and B lead to two conclusions. *1*) Albumin strongly reduced total eicosanoid formation in platelets by retention of arachidonate in the extracellular medium; this reduced availability of released arachidonate for oxygenation by intracellular oxygenases. *2*) Albumin affected cyclooxygenation of endogenous arachidonate to a much greater extent than it did lipoxygenation. These results differed significantly from those obtained in protein-free media.

Effects of albumin on 12-HETE formation in aspirin-treated platelets

Platelets were pretreated with 0.5 mM aspirin to block cyclooxygenation of arachidonate. When these platelets were stimulated with thrombin (5–10 min), 12-HETE formation increased threefold in the absence of albumin. However, when albumin was included in the medium, 12-HETE production increased sixfold (compare Figs. 1C and 1B). There was always a slight lag prior to 12-HETE synthesis (Fig. 1B) as compared to generation of 12-HHTrE. This lag was even more pronounced following aspirin treatment (20), especially when albumin was included in the incubation (Fig. 1C).

Whether aspirin was present or not, the initial rate of 12-HETE formation (following the lag phase) was reduced three- to fourfold by albumin (compare Figs. 1C and 1B). Formation of 12-HETE persisted in a continuous manner in the presence of albumin, and therefore total 12-HETE formation at late time points (5–10 min) in the presence of aspirin was minimally affected by albumin (Fig. 1C), in contrast to the effects of albumin in the absence of aspirin (Figs. 1A and 1B). These results stress the importance of time course studies of eicosanoid formation. This is because each metabolite is maximally produced at different times. Moreover, the results suggest that albumin may play a significant role in eicosanoid metabolism in vivo.

The abrupt cessation of 12-HHTrE formation (Fig. 1A) was in sharp contrast with the continuous formation of 12-HETE in the presence of albumin (Fig. 1C). Thus, sequestration of the eicosanoid precursor arachidonate by albumin was only temporary, and albumin was responsible for a metabolic diversion of arachidonate from cyclooxygenase to lipoxygenase.

The "reservoir" effect of albumin was studied in further detail. The approach was to compare levels of 12-HETE and free arachidonate after thrombin activation of aspirin-treated platelets. This was done in the presence of three different albumin concentrations. At the 1-min time point, the greatest differences in 12-HETE formation in albumin-free and in albumin-containing media occurred. However, these differences were greatly diminished at 5 min (Fig. 1C). At 1 min following thrombin addition, 12-HETE production in the presence of albumin (37 or 112 μM) was markedly reduced to 6–12% of that observed in the absence of albumin (**Fig. 2A**). The converse occurred with free arachidonate at 1 min. In the presence of albumin, approximately 75 nmol free arachidonate accumulated, while in the absence of albumin only 10 nmol arachidonate was identified in free form.

Thrombin stimulation (5 min) in the presence of albumin (37 μM) actually promoted formation of platelet 12-HETE in comparison with 12-HETE formation in its absence (Fig. 2B). With regard to free arachidonate, 65 nmol was measurable in the presence of albumin (37 μM), but only 6 nmol was free in its absence. Moreover, with 112 μM albumin, 105 nmol arachidonate remained free, but 12-HETE formation was still suppressed at the 5-min time point. In separate experiments using a higher thrombin-platelet ratio, the enhancing effect of albumin on 12-HETE formation was evident at the highest concentration of albumin practicable (150 μM). These observations constitute further evidence that albumin not only inhibited immediate oxygenation of arachidonate, but simultaneously promoted its availability for subsequenet metabolism.

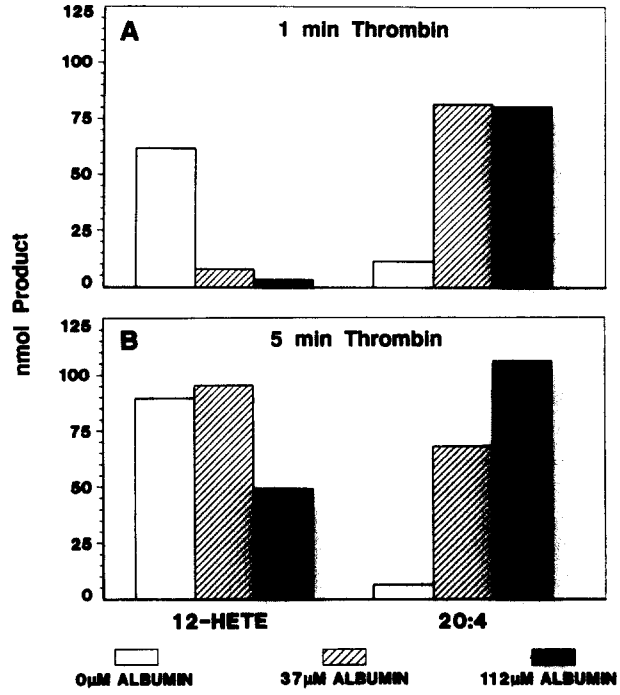

Fig. 2. Albumin promotes 12-HETE formation and arachidonate (20:4) accumulation by thrombin-stimulated platelets in the presence of 0.5 mM aspirin. A: Increasing albumin concentrations diminished 12-HETE formation and resulted in free arachidonate retention at 1 min thrombin. B: After 5 min, albumin promoted 12-HETE formation at 37 μM compared to zero albumin, but still retained free arachidonate. Increasing albumin to 112 μM diminished 12-HETE formation, even at 5 min, while free available arachidonate remained present in excess. In the absence of albumin, little if any arachidonate accumulated. Data are from one of three experiments yielding similar results, expressed as nmol per 5×10^9 platelets.

Effects of albumin on metabolism of arachidonate in the incubation medium

The data obtained above suggested that albumin promoted selective metabolism of extracellular arachidonate by lipoxygenase. To test this hypothesis further, platelets were preincubated in the presence or absence of 37 μM albumin, and tracer [^{3}H]arachidonate was added 30 sec prior to stimulation with thrombin (0.75 U/10^8 platelets). Patterns of radioactivity of lipid extracts from these incubations, as separated by TLC are depicted in **Fig. 3**. In the absence of albumin, 30 sec of [^{3}H]arachidonate incubation and 5 min thrombin stimulation resulted in association of 76% (3.8 pmol) of radioactivity with phospholipids. The remainder was distributed between 12-HETE (10%, 0.5 pmol), 12-HHTrE (9%, 0.4 pmol), TXB_2 (2%, 0.1 pmol), and free arachidonate (3%, 0.2 pmol). In contrast, in the presence of 37 μM albumin, only 42% (2.1 pmol) of the radioactivity added as [^{3}H]arachidonate was associated with phospholipids, while increased quantities of radioactivity were present as 12-HETE (23%, 1.2 pmol), 12-HHTrE (19%, 0.9 pmol), TXB_2 (5%, 0.3 pmol), and free arachidonate (11%, 0.5 pmol). As expected, no "classical" prostaglandins formed (1).

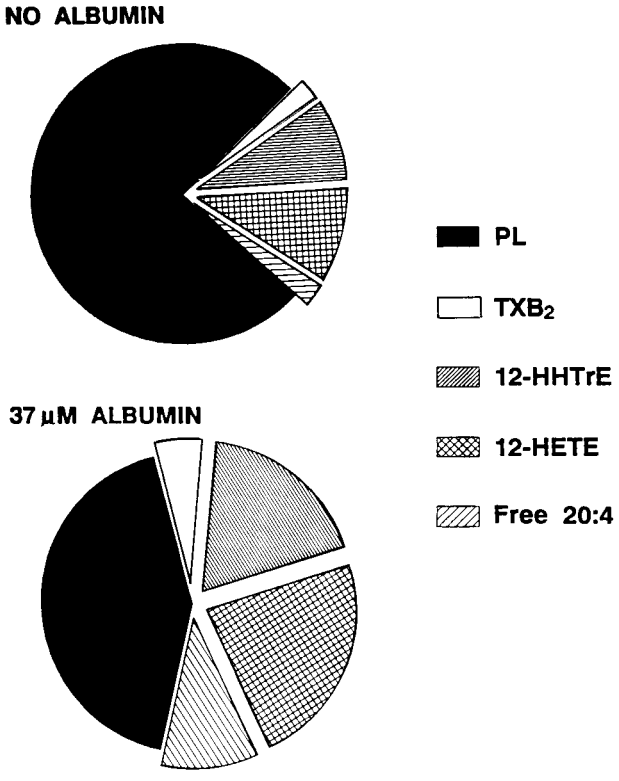

Fig. 3. Albumin promotes oxygenation of medium arachidonate (20:4) and decreases arachidonate incorporation into phospholipids (PL). [^{3}H]Arachidonate, when added 30 sec prior to 5 min of thrombin stimulation, was oxygenated to TXB_2, 12-HHTrE, and 12-HETE, and was retained as free fatty acid to a far greater extent in the presence of 37 μM albumin than in its absence. Conversely, albumin strongly suppressed incorporation of [^{3}H]arachidonate into platelet phospholipid. Data represent averages from two separate experiments.

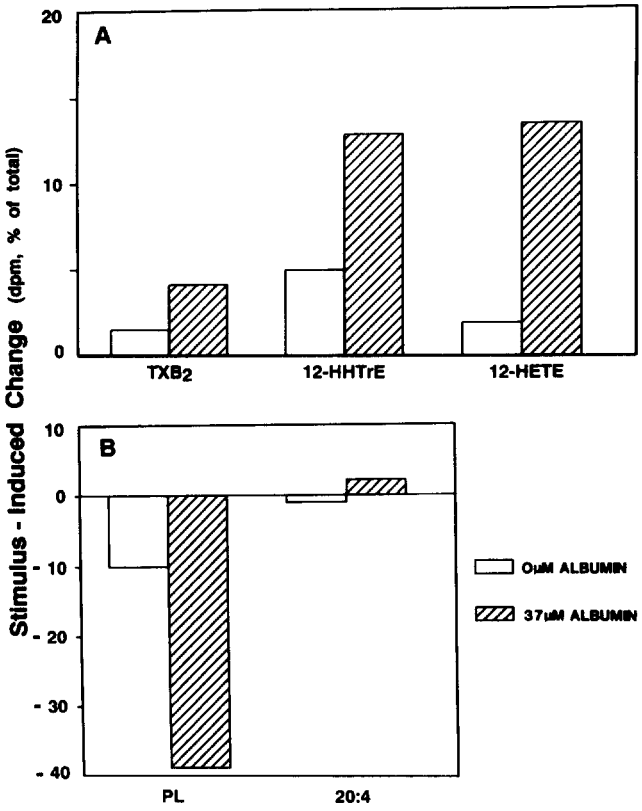

Fig. 4. Redirection of extracellular arachidonate metabolism by albumin in thrombin-stimulated platelets. A: Albumin redirected arachidonate metabolism to lipoxygenase resulting in a 7-fold increase in 12-HETE radioactivity as compared to a 2.7-fold increase in TXB_2 and 12-HHTrE. B: The thrombin-induced decrease in phospholipid [^{3}H]arachidonate was amplified 4-fold by albumin. Data are averages from two separate experiments, expressed as % of radioactivity added to incubations (see text for details).

The net effects of thrombin stimulation on metabolism of extracellular arachidonate were determined separately in order to exclude basal incorporation of exogenously provided arachidonate. Metabolism of [^{3}H]arachidonate, added 30 sec prior to thrombin, was compared in thrombin-stimulated and unstimulated platelet suspensions, both in the presence and absence of albumin. The net effect of stimulation was determined by subtracting radioactivity in each TLC zone of unstimulated platelets from radioactivity in corresponding zones of stimulated platelets. Thrombin activation induced albumin to preferentially enhance stimulus-dependent incorporation of radioactivity into 12-HETE (**Fig. 4A**): 12-HETE was increased 7-fold, but TXB_2 and 12-HHTrE only 2.7-fold (Fig. 4A). This indicated that albumin acted as "conduit" to divert free arachidonate away from platelet cyclooxygenase to lipoxygenase.

In addition to enhancing lipoxygenation of arachidonate, albumin also controlled the quantity of arachidonate-derived radioactivity in both free fatty acid and phospholipid fractions of platelets. In albumin-free media, radioactivity in free fatty acids decreased by 1.0% after stimulation (% of

total radiolabeled arachidonate added; Fig. 4B). With albumin present in the medium, free fatty acids increased by 2.2% of total radioactivity (Fig. 4B). The influence of albumin upon incorporation of radioactivity into phospholipid was even more pronounced, and in the opposite direction from that of free fatty acids. Thus, in albumin-free medium, thrombin stimulation induced a decrease of radioactivity in the phospholipid fraction of 10%. When cells were stimulated in the presence of albumin, a fourfold greater decrease in phospholipid radioactivity occurred (39% of total radioactivity added prior to thrombin; Fig. 4B).

Alterations in phospholipids as described above were examined in further detail. We utilized total separation of all phospholipid components (13–16, 18). We could thus quantitate the net effect of thrombin stimulation on [^{3}H]arachidonate content and correlate the changes in each phospholipid class (**Fig. 5**). The data demonstrated that after thrombin addition the [^{3}H]arachidonate content of PC, PS, and PI decreased, but radioactivity in PA and PE increased. When thrombin was added to platelets in the presence of albumin, there was a decrease in [^{3}H]arachidonate content of all phospholipids, especially PC, but no change in radioactivity of PI (Fig. 5A). When platelets were treated with aspirin to block arachidonate oxygenation by cyclooxygenase, results were similar, and again albumin induced a pronounced decrease in PC (Fig. 5B). These data indicate that albumin exerts a controlling influence on platelet eicosanoid metabolism by selectively modulating an increase in net phospholipase A_2 activity, and/or by blocking reincorporation of released free arachidonate. There is no evidence that the albumin effect is operative via phospholipase C activity on inositides.

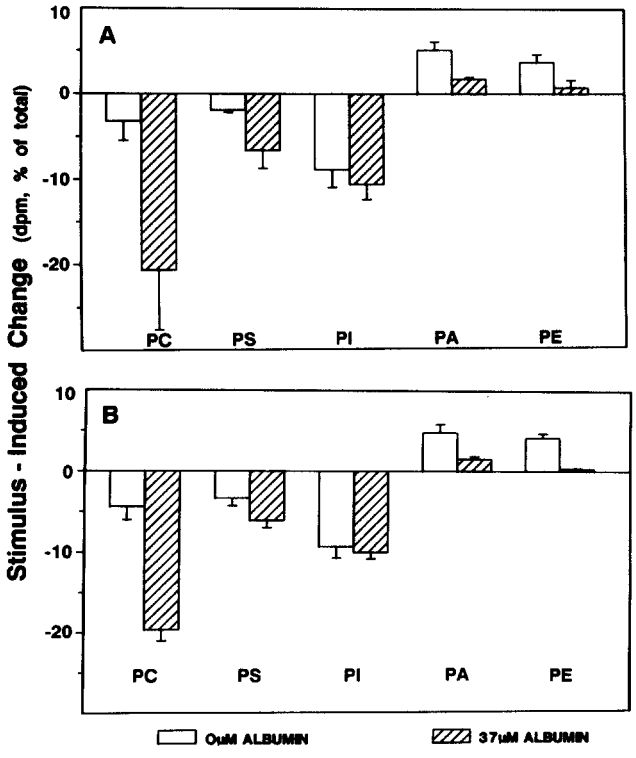

Fig. 5. Albumin selectively affects arachidonate incorporation into platelet phospholipids upon thrombin stimulation. Albumin (37 μM) increased the loss of [^{3}H]arachidonate from PC and PS, and reduced the gain in [^{3}H]arachidonate in PA and PE. PI was not affected by the absence or presence of albumin. A: No aspirin was present during preincubation or stimulation; B: treated with 0.5 mM aspirin. Data represent averages and standard errors of three separate experiments and were significant (two-tailed Student's *t*-test) with respect to the effect of albumin ($P < 0.05$) for PC, PA, PE, and PS, but not significant ($P > 0.42$) for PI (see Fig. 4 and Experimental Procedures for details).

DISCUSSION

The data presented demonstrate that a considerable fraction of arachidonate, liberated upon platelet stimulation with thrombin, is released extracellularly prior to further metabolism. Once extracellular, this arachidonate can be metabolized by other cells or bound by albumin. Subsequently, arachidonate can be released from the albumin for metabolism by cells in the microenvironment. In the case of platelets, our data demonstrate that such metabolism occurs preferentially via lipoxygenase, rather than cyclooxygenase.

The results of our experiments also suggest that previous estimates of eicosanoid formation as carried out in protein-free media in vitro require re-evaluation. This has implications for in vivo situations. The presence of albumin during platelet activation and subsequent biological responses represents a more physiological setting than studies conducted in the absence of this plasma protein. Redirection by albumin of arachidonate metabolism from cyclooxygenase to lipoxygenase constitutes a heretofore undescribed "conduit" function for albumin in platelet eicosanoid metabolism.

In the setting of decreased platelet cyclooxygenation of arachidonate, albumin caused a considerable persistence of lipoxygenation (Figs. 1A, 1B). Of potential clinical significance is our observation that in aspirin-treated platelets lipoxygenation of arachidonate was only delayed by albumin (Figs. 1C, 2B).

During platelet stimulation, albumin increased oxygenation of extracellular arachidonate, especially by the lipoxygenase enzyme (Fig. 4A). In marked contrast to the effect of albumin on most other phospholipids, incorporation of exogenous arachidonate by stimulated platelets into the PI fraction was not affected by albumin (Fig. 5). These observations demonstrate that albumin modulation of eicosanoid formation results in increased availability of free arachidonate for oxygenation. Thus, under in vivo conditions with albumin present virtually ubiquitously (8, 10), arachidonate oxygenation and further processing is likely to be a more active process than previously appreciated. Importantly, eicosanoid participation in cellular responses to stimulation, perturbation, or injury, especially by lipoxygenase products, may be far more prominent than estimated in the past.

The prolonged production of 12-HETE in albumin-containing medium demonstrated that arachidonate in albumin-bound form is not irreversibly "sequestered." Rather, it is readily available for utilization by stimulated platelets, suggesting that albumin served as a "reservoir" of substrate for eicosanoid formation, rather than as a "sink," as previously concluded (21–23). Our experiments also re-emphasize the importance of time course studies in eicosanoid metabolism to determine for each condition the time point at which maximal product formation occurs (Fig. 1). Our data demonstrate that in the presence of albumin 12-HHTrE formation was strongly inhibited at an early time point, while 12-HETE generation continued (Figs. 1 and 2). This observation was further emphasized by our data indicating a two-fold greater availability of albumin-bound exogenous arachidonate for lipoxygenase in comparison to cyclooxygenase (Fig. 4), demonstrating a dichotomy in processing of extracellular arachidonate bound to albumin. These data suggest that, in contrast to lipoxygenation, cyclooxygenation occurs in close physical association with the cell which, upon activation, originally released the arachidonate.

There are several consequences of albumin-induced redirection of arachidonate metabolism toward the lipoxygenase pathway. In the in vivo setting, albumin may serve to confine cyclooxygenase-dependent reactions to the immediate milieu of the cell. Simultaneously, albumin modulates formation of an alternate spectrum of eicosanoids, predominantly lipoxygenase products. Most notably, in our in vitro systems containing albumin, 12-HETE accumulated rapidly to final concentrations of 10–100 μM. At these levels 12-HETE has been demonstrated to have physiologically relevant effects (24). Thus, albumin could serve as an "endogenous aspirin-like substance" in that it promotes lipoxygenation to the detriment of cyclooxygenation.

Albumin-induced redirection of arachidonate metabolism may have clinical consequences not appreciated previously. In the in vivo situation, albumin is ubiquitously present (8, 10), and, as we have shown, platelet stimulation leads to prolonged formation of increasing quantities of 12-HETE, a trend that is enhanced during aspirin ingestion (Figs. 1C, 2, and 4). Whereas cellular interactions involving cyclooxygenase metabolites have been demonstrated in vitro (2, 3) and in vivo (25), increasing attention is now focusing on metabolism of lipoxygenase products. We previously demonstrated that cyclooxygenase-dependent interactions (prostacyclin formation) were reduced in the presence of albumin when platelets and endothelial cells interacted in vitro (2). These interactions do occur in vivo and may be of clinical importance (25). In addition, it was recently suggested that products of the 12-lipoxygenase pathway exerted an inhibiting influence upon thromboxane-mediated responses in platelets and vascular smooth muscle cells (26). Thus a new emphasis is emerging on a modulating role for 12-lipoxygenase metabolites in thrombosis and inflammation. Our data indicate that albumin exerts an important control mechanism in these cell–cell interactions.

In conclusion, we demonstrate novel and potentially significant clinical roles for albumin in the control of eicosanoid formation. Albumin redirected eicosanoid formation to the lipoxygenase pathway by stimulating utilization of arachidonate by cytosolic lipoxygenase rather than particulate cyclooxygenase, by promoting release of arachidonate, and by inhibiting its reincorporation into phospholipid. This albumin shift in eicosanoid metabolism, which is increased by aspirin, further emphasizes the importance of lipoxygenase products in vitro as well as in vivo, especially since previous ex vivo measurements of cyclooxygenase metabolite levels were not sufficient for a full understanding of in vivo eicosanoid physiology.

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