Localization of Free Fatty Acids Taken Up by Human Platelets

By John C. Hoak, Arthur A. Spector, Glenna L. Fry, and Brian C. Barnes

Human platelets take up and metabolize long-chain free fatty acids (FFA). Experiments in which platelets were loaded with 14C-palmitate, homogenized, and then fractionated indicated large amounts of membrane-bound FFA. In other studies, platelet membrane fractions took up FFA from incubation media containing albumin-bound FFA. These findings suggested that a large portion of the platelet FFA uptake might be associated with the platelet surface. Radioautography with electron microscopy using 3H-palmitate-labeled platelets provided additional evidence that much of the newly incorporated FFA was associated with the platelet surface.

HUMAN PLATELETS take up and metabolize long-chain free fatty acids (FFA) from solutions containing albumin-bound fatty acids.1-5 The extent of uptake is dependent on the FFA albumin molar ration (v), the chain length of the fatty acid, and the degree of saturation of the fatty acid.4 Much of the FFA taken up by the platelet in short-term incubations remained in unesterified form, i.e., it was recovered as platelet FFA.

The present studies were performed to delineate the location of fatty acids taken up by the platelet after a short period of incubation. Cell fractionation experiments and radioautography with electron microscopy were used to localize the 3H-labeled palmitic acid that was incorporated into human platelet suspensions.

MATERIALS AND METHODS

Preparation of Platelets

Venous blood, from normal, nonfasting human donors, was added to siliconized tubes containing 0.77 M EDTA and centrifuged from 15 min at 300 g. The platelet-rich plasma
was then centrifuged at 750 g for 15 min in order to obtain the platelet pellet. The platelets were resuspended in a solution containing 0.123 M NaCl, 0.0045 M KCl, and 0.015 M Tris buffer adjusted to pH 7.4. The platelet count of each suspension was determined, and Tris-buffered salt solution was added so that 1 ml of the suspension contained 10^9 platelets.

Preparation of FFA-Albumin Solutions

Unlabeled palmitic acid was purchased from the Hormel Institute. Palmitic acid-1-14C was supplied by New England Nuclear. 3H-palmitic acid was supplied by Amersham-Searle. Crystalline human serum albumin was obtained from Miles Laboratories. Fatty acids were removed from the albumin by the method of Chen.6 The desired labeled and unlabeled FFA-albumin solutions were prepared by incubation with fatty acid-coated Celite as described in an earlier publication.4

Fractionation Studies

Two methods were used to separate subcellular fractions of the platelet homogenate prepared by the method of Marcus et al.7 The first method was identical to that described by others.7,8 The second method was designed to obtain the membrane fraction in larger yield. The total homogenate was diluted to 11.6 ml with 0.25 M sucrose and centrifuged at 3°C for 15 min at 9600 g. The sedimented material was removed, and the supernatant solution was then centrifuged at 3°C for 60 min at 100,000 g. The sedimented membranes were resuspended in 11 ml of 0.25 M sucrose. Protein concentration was determined by the method of Lowry et al.9 using bovine serum albumin as the standard. Contents of platelet fractions were identified using transmission electron microscopy.

Incubation of 1 ml of the platelet membrane suspension with 3 ml of the radioactive FFA-albumin solution was performed in a 37°C water bath with shaking. After incubation, the flasks were chilled, and their contents were transferred to centrifuge tubes containing 7.6 ml of 0.25 M sucrose. The membranes were sedimented at 100,000 g for 60 min at 3°C. They were resuspended in 11.6 ml of chloroform-methanol solution, and lipid separation and total lipid-soluble radioactivity were performed.4

In other studies, washed platelets that had been incubated with 1-14C palmitate were homogenized, and platelet fractions were separated on sucrose density gradients.7,8 Contents of each platelet fraction were identified using transmission electron microscopy. The FFA radioactivity was determined in each of the fractions. In platelet fractionation studies in which radioautography was performed, tritium-labeled palmitate-albumin was used.

Preparation of Platelets for Radioautography

Platelets were incubated with 3H-labeled palmitate, bound to albumin, as in the studies described above. The incubation period was 5 min. The final platelet pellet was fixed in 3% glutaraldehyde. In several experiments, the platelets were fixed in freshly prepared formalin instead of glutaraldehyde. The platelets were postfixed in osmium tetroxide, dehydrated in graded alcohol solutions and propylene oxide, and embedded in Epon-Araldite. After thin sections were cut on a Reichert ultramicrotome, they were stained with uranyl acetate and then with lead acetate. A Denton vacuum evaporator DV-502 was used to give the sections a thin coating of carbon. Then Ilford L4 emulsion was applied by the method of Budd and Pelc.10 After an exposure of 8 wk, the radioautographs were developed in Microdol-X and fixed in 20% sodium thiosulfate. The sections were examined in a Philips EM 300 electron microscope.

RESULTS

Platelet fractionation studies were used to localize the uptake of newly incorporated radioactive palmitate. Good separation of the subcellular fractions was obtained with platelets that had not been incubated with fatty acids.
However, when platelets that had been incubated with labeled palmiate were fractionated, the separation of the fractions was less discrete. One aliquot of each subcellular fraction was fixed and examined with electron microscopy to determine its purity. The radioactivity contained in another aliquot of the individual fraction was measured, and this was expressed as per cent of total cpm present in the intact platelets. In five experiments, those platelet fractions composed chiefly of membranes contained \(42 \pm 10\%\) of total cpm (± standard error of the mean). Fractions composed of membranes, granules and mitochondria had \(25 \pm 9\%\) of total cPM. Fractions composed of granules and mitochondria had \(26 \pm 4\%\) of the total counts.

Isolated platelet membranes took up labeled FFA when they were incubated with the FFA-albumin solutions. The time courses of FFA uptake by two different preparations of membranes incubated with 1-\(^{14}\)C-albumin solutions of molar ratio 2 are illustrated in Fig. 1. In both cases, most or all of the palmitate was taken up by 5 min, the earliest time point that was tested. Analysis by thin-layer chromatography of the lipid extracted from the membranes revealed that from 72 to 97\% of the radioactivity was present as FFA. Most of the remainder of the incorporated radioactivity was in phospholipids. Most of the radioactive FFA taken up by the platelet membranes was bound reversibly, i.e., it was available for release to fatty acid-poor albumin.

The rapidity of release of FFA from the platelets to albumin and the ability of isolated platelet membranes to take up FFA suggested that much of the FFA taken up by the intact platelet might be associated with the cell surface. The results of the radioautography studies with \(^{3}\)H-palmitate-loaded platelets using electron microscopy support this hypothesis. Grain counts of six preparations from three experiments using 5-min incubation periods revealed: \(61 \pm 2\%\) of total grain counts (± SEM) associated with the platelet membrane, \(17 \pm 2\%\) found in vacuoles and canaliculi, \(2 \pm 1\%\) in granules, less than \(1\%\)
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Fig. 2. Radioautograph of a section that demonstrates FFA radioactivity associated with platelet membrane. × 36,000.

in mitochondria, and 16 ± 2% in nonspecific areas of the cytoplasm. Background counts represented 4% ± 1 of the grain counts. Electron micrographs demonstrating the localization of radioactivity are shown in Fig. 2 and 3. Similar results were obtained when formalin was used to fix the platelets, instead of glutaraldehyde.

Radioautographic studies with electron microscopy were also performed on the subcellular fractions prepared from labeled platelets. These studies demonstrated that most of the grain counts were associated with membrane components. In fact, they indicated that much of the FFA radioactivity present in “nonmembrane” fractions actually was present on small amounts of membranous material contaminating these fractions. For example, Fig. 4 illustrates radioactivity with membranes in a platelet fraction that was essentially a “pure” granule fraction.

DISCUSSION

Exposure of platelets to high concentrations of long-chain fatty acids alters platelet function. Unbound long-chain saturated fatty acids are known to cause platelet aggregation associated with the release of adenine nucleotides and serotonin.11,12 Albumin-bound long-chain saturated fatty acids have been observed to enhance ADP-induced platelet aggregation13 and to increase
platelet adhesiveness. These changes occurred after only a short exposure of the platelets to the FFA. In earlier studies, i.v. injections of high concentrations of albumin-bound fatty acids caused the formation of thrombi containing platelet aggregates in the lungs of rabbits.

Since platelet adhesiveness and platelet aggregation are phenomena related to the platelet surface, we postulated that the effects of FFA on platelet function might be due to a FFA-induced change in the platelet membrane. There-
fore, the localization of the FFA taken up after a short incubation with platelets became an important consideration in attempting to elucidate the mechanism of the FFA-induced effects.

In the present study, platelet membrane fractions took up FFA rapidly. This phenomenon was reversible, and release of FFA occurred when the FFA-“loaded” membranes were exposed to FFA-poor albumin. Thus, the results obtained with platelet membranes were similar to those obtained with intact platelets. When the subcellular fractions were prepared from platelets that had been incubated with labeled FFA, most of the FFA radioactivity was in fractions composed chiefly of membranes. This technique actually underestimated the radioactivity associated with membranes (Fig. 4).

Finally, when radioautography studies with electron microscopy were used to determine the site of uptake of 3H-palmitate, over 75% of the radioactivity was localized to the platelet membrane and surface-connected structures. While information from any one of the investigative approaches that we employed can be considered as only suggestive, the cumulative data provide strong support for the platelet membrane as being the site of much of the newly incorporated FFA.

These studies support the hypothesis that during a short exposure of platelets to albumin-bound FFA, most of the FFA is associated with the platelet surface. The presence of increased amounts of membrane-bound FFA may affect platelet function, creating a transient state in which platelets become more adhesive and more prone to aggregate. This process could culminate in the formation of thrombi.

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REFERENCES


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