The Inhibitory Effects of Exogenous Arachidonic Acid on Rabbit Platelet Aggregation and the Release Reaction

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Although arachidonic acid causes rabbit platelet aggregation and the release of granule contents in suspensions of washed platelets when used in concentrations of approximately 50–300 μM, higher concentrations (500 μM) cause neither aggregation nor release. Suspensions of platelets from rabbits were exposed to arachidonic acid (250 μM) for 15 min, allowed to recover in the presence of PGE₂ for 30 min, washed, and resuspended; in some experiments, the platelets were treated with aspirin before being exposed to arachidonic acid. Aggregation of platelets pretreated with arachidonic acid was inhibited in response to ADP; this effect was greater with the non-aspirin-treated platelets and persisted for at least 4 hr after resuspension. The association of 125I-fibrinogen with the platelets as a result of ADP stimulation was also inhibited. Aggregation and release of granule contents in response to collagen and low concentrations of thrombin was inhibited, but the inhibition could be overcome by higher concentrations. Thrombin induced further release of granule contents from platelets exposed to arachidonic acid without pretreatment with aspirin. Platelets that had been exposed to arachidonic acid, either with or without pretreatment with aspirin, did not aggregate or undergo further release upon stimulation with arachidonic acid after they were washed and resuspended. Inhibition of the lipoxygenase pathway with eicosatetraynoic acid (ETYA) or nordihydroguaiaretic acid (NDGA) did not affect the inhibition caused by arachidonic acid, so it is unlikely that a product of this pathway is responsible for the inhibition. Mixing experiments indicated that the pretreated platelets did not form a thromboxane-A₂-like activity, and that they were unresponsive to aggregation and release induced by products formed from arachidonic acid. Experiments with ³H-arachidonic acid showed that after 45 min of incubation with platelets, only 1.1% of the ³H-arachidonic acid remained as free arachidonic acid in the platelets. Although cyclic-AMP was slightly increased 1 min after the addition of arachidonic acid, the cyclic-AMP concentration was the same as that of control platelets after the platelets were washed and resuspended, indicating that increased cyclic-AMP is not likely to be responsible for the persistent inhibitory effect. Thus, the inhibitory effect of pretreatment with arachidonic acid is a general effect on responses to a variety of aggregating agents that act through different mechanisms, and the inhibition is not related to thromboxane-A₂ formation. The possibility of membrane perturbation resulting in the unavailability of receptors may explain the persistent inhibitory effect, but the responsible reactions have not been identified.

When platelets are exposed to exogenous arachidonic acid they aggregate and release the contents of their amine storage granules. The extent to which this occurs depends largely on the concentration of arachidonic acid to which the platelets are exposed: with low concentrations of arachidonic acid, little aggregation and release take place because the stimulus is too weak; with somewhat higher concentrations, human platelets aggregate and release their granule contents, whereas with still higher concentrations, little aggregation and release occurs. With optimal concentrations of arachidonic acid, the extent of release of amine storage granule contents is seldom greater than 50%. In addition to the inhibitory effects that high concentrations of arachidonic acid seem to have on arachidonic-acid-induced aggregation and release, these concentrations have also been found to inhibit platelet aggregation and release in response to thrombin and collagen with rabbit and human platelets. Other cis-unsaturated fatty acids have also been shown to inhibit human platelet aggregation induced by adenosine diphosphate (ADP), thrombin, or collagen. There are several possible reasons for an inhibitory effect of arachidonic acid or other cis-unsaturated fatty acids: (A) the cyclooxygenase responsible for converting arachidonic acid to its aggregating and release-inducing product, thromboxane-A₂, may become refractory; (B) arachidonic acid may be metabolized by the lipoxygenase pathway to form inhibitory products; several investigators have observed that HPETE (12-hydroperoxy-5,8,10,14-eicosatetraenoic acid) inhibits thromboxane formation, attributing this to inhibition of thromboxane synthetase or cyclooxygenase; (C) arachidonic acid may be converted to inhibitory end-products such as PGD₂, which would stimulate adenylate cyclase and raise the concentration of cyclic adenosine monophosphate (AMP) in the platelets. Goodman has sug-

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gested that arachidonic has a direct effect on platelet cyclic-AMP levels; (D) arachidonic acid may be incorporated into the platelet membrane and alter the ability of the membrane to respond to further stimulation; perturbation of the membrane by free fatty acids has been suggested as the mechanism of alteration of function in platelets and other cells.

The purpose of the experiments described in this article was to investigate the inhibitory effects of arachidonic acid on platelet aggregation and the release reaction.

MATERIALS AND METHODS

Arachidonic acid, nordihydropreguiaeric acid (NDGA),(4,4'-[2,3-dimethyl-1,4-butanediyl]-but-1,2-benzenediol), CDP, bovine tendon collagen, bovine thrombin, imipramine, and acetyl salicylic acid were from Sigma Chemical Company, St. Louis, Mo. Prostaglandin-E1 (PGE1) and eicosatetraynoic acid (ETYA) were generous gifts of the Upjohn Company, Kalamazoo, Mich. Bovine albumin (Percent, fraction V) was from Miles Laboratories, Inc., Elkhart, Ind.

14C-serotonin (as 5-hydroxytryptamine-3',4'-creatinine sulfate) (58 mCi/mmol) and 3H-arachidonic acid (120 Ci/mmol) were purchased from New England Nuclear, Dorval, Quebec. Silica gel H60 (Meck) was from Brinkmann Instruments (Canada) Ltd., Rexdale, Ontario. Solvents for thin-layer chromatography were distilled before use. Phospholipid standards were from Serdary Research Laboratories, London, Ontario.

Apyrase, prepared from potatoes by the method of Molnar and Lorand, was dissolved in 0.15 M sodium chloride and stored at –20°C.

All other chemicals were analytical grade.

Preparation of Washed Platelets from Rabbits

Suspension of washed platelets from rabbits were prepared according to the method of Ardile et al.14,15 In most experiments, the platelets were labeled in the first washing solution by incubation with the stimulus (at least 3 min after the addition of the aggregating agent). The extent of release was expressed as a percentage of the total 14C-serotonin in the platelet suspension.

Platelet Lysis

Platelet aggregation was recorded in an aggregation module (Payton Associates, Scarborough, Ontario) during a 3-min period after the addition of the aggregating agent. By adjusting the sensitivity of the aggregometer, changes in the oscillations of light transmission through the stirred platelet suspension were recorded as an indication of a change of platelet size in response to an aggregating agent.14 Suspensions of finely divided collagen were prepared as previously described. ADP and bovine thrombin were dissolved in modified Tyrode solution (no calcium or magnesium) and adjusted to pH 7.35. All concentrations given in the text are the final concentrations after all additions to the platelet suspensions.

Measurement of the Platelet Release Reaction and Platelet Lysis

The release of platelet constituents was measured by counting the amount of 14C-serotonin present in the platelet-suspending medium following incubation with the stimulus (at the end of the incubation period during which the platelets were treated with arachidonic acid, or 3 min after addition of other aggregating stimuli). The extent of release was expressed as a percentage of the total 14C-serotonin in the platelet suspension.

Platelet lysis was determined by measuring the amount of 51Cr present in the suspending medium following stimulation.14

In experiments in which the platelets were labeled with both 14C-serotonin and 51Cr, radioactivity was determined using the method of Sheppard and Marlow.22

Labeling Platelet Lipids With 3H-Arachidonic Acid

Platelets were incubated with 3H-arachidonic acid (250 μM) followed by PGE1 (10 μM) for 15 min, followed by PGE1 (10 μM) for 30 min. At the end of the incubation period, 1-ml portions of the platelet suspension were centrifuged (1,500 g for 10 min) and washed twice with calcium-free Tyrode solution (containing apyrase). The platelets were finally resuspended in 1-ml portions of Tyrode-albumin solution containing apyrase and mixed with 3.75 ml of chloroform/methanol (1:2 v/v) in a 50-ml conical glass centrifuge tube. The chloroform–methanol–platelet mixture was agitated continuously for 15 sec immediately following transfer and for a further 10 sec every 5 min for 1 hr.

The mixture was partitioned into two phases by consecutive additions of 1.25 ml of chloroform followed by water; the samples were mixed for 15 sec after each addition. Separation of phases was completed by centrifuging the samples for 10 min at 200 g. The lower, chloroform phase was removed using a Pasteur pipette (neutral extract).

A mixture (9.5 ml) of chloroform/methanol/10 N HCl (3:4:0.8 v/v/v) containing butylated hydroxytoluene (0.1 mg/100 ml) was added to the upper phase and mixed. After 5 min, the extract was partitioned into two phases by the consecutive addition of 3.75 ml of chloroform and 3.75 ml of water.

The contents were mixed during and for 15 sec after each addition. The samples were centrifuged at 200 g for 10 min and the lower, chloroform phase (acidic extract) was removed and pooled with the corresponding neutral extract. The combined extracts were evaporated to dryness under a N2 stream in a water bath at approximately 50°C. The dried material was redissolved in 100 μl of chloroform and spotted onto silica gel H plates. The plates were developed in petroleum ether/diethylether/acetic acid (90:10:1). After drying, the plates were sprayed with 0.005% Rhodamine GG in ethanol and the lipids visualized under ultraviolet light. Spots were scraped into scintillation vials and radioactivity counted in ACS counting solution (Amersham Company, Oakville, Ontario).
Assay of Cyclic-AMP (cAMP)

Suspensions of washed platelets (platelet count 10⁶/μl) were incubated for 1 min at 37°C with arachidonic acid (250 μM). Samples (1 ml) were mixed with 1 ml of cold 6% perchloric acid and neutralized with 2 N KOH. The precipitate was removed by centrifugation, and the amount of cAMP in the supernatant was determined by a cAMP-binding assay using the Amersham kit. Samples of platelets that had been incubated with arachidonic acid, deaggregated, washed, and resuspended in fresh medium were also assayed for cAMP.

RESULTS

Effects of Arachidonic Acid on Platelet Aggregation

The effects of arachidonic acid, added to suspensions of washed platelets from rabbits, depended on its concentration; with low concentrations of arachidonic acid (1–2.5 μM), the platelets changed shape but did not aggregate or release the contents of their amine storage granules (Fig. 1). Higher concentrations of arachidonic acid (250 μM) caused extensive aggregation and the release of approximately 40% of the contents of the amine storage granules. Very high concentrations of arachidonic acid (500 μM) did not cause extensive aggregation, and the release of granule contents was significantly less than observed with the lower concentrations of arachidonic acid (Fig. 1). At 500 μM, 3%–5% loss of ⁵¹Cr was observed, but only 1%–3% loss was observed with lower concentrations of arachidonic acid.

Effects of Preincubating Platelets With Arachidonic Acid

We have examined platelets treated in two ways: (1) platelets treated with arachidonic acid so that they aggregated and released some of their granule contents, and (2) platelets pretreated with aspirin before exposure to arachidonic acid so that aggregation and release of granule contents did not occur. The concentration of aspirin used (0.5 mM) completely blocked aggregation and release in response to 250 μM arachidonic acid.

ADP-Induced Platelet Aggregation

When a high concentration of arachidonic acid (500 μM) was added to aspirin-treated platelets before or with ADP (10 μM), ADP-induced aggregation was inhibited (Fig. 2A). With a lower concentration of arachidonic acid (250 μM), its inhibitory effect increased with the time of incubation before the addition of ADP (Fig. 2B).

When platelets were incubated with arachidonic acid, washed, and resuspended in fresh medium, their
aggregation response to ADP was diminished although platelet shape change was not inhibited. The effect of pretreatment with arachidonic acid was more pronounced when the platelets had not been treated with aspirin before exposure to arachidonic acid (Fig. 3).

In some experiments, washed rabbit platelets were treated with lower concentrations of arachidonic acid (50, 100, 150, or 200 μM) in the presence or absence of aspirin. When the non-aspirin-treated platelets were washed and resuspended in fresh medium, the platelets did not aggregate in response to ADP. With aspirin-treated platelets, the inhibitory effect was only evident when concentrations of arachidonic acid of 100 μM or greater had been used.

If the platelets that had been exposed to arachidonic acid were washed, resuspended in fresh medium, and incubated at 37°C for several hours, most of the inhibitory effect on ADP-induced aggregation persisted (Fig. 4). This was also observed with platelets that had been pretreated with aspirin before exposure to arachidonic acid.

The inhibitory effect of arachidonic acid on ADP-induced aggregation was less apparent when higher (40–50 μM) concentrations of ADP were used.

Association of 125I-Fibrinogen With the Platelets When They Were Stimulated With ADP

When arachidonic-acid-treated rabbit platelets were exposed to ADP, platelet aggregation was inhibited, and the uptake of 125I-fibrinogen was reduced to approximately one-half that observed with control platelets (Table 1).

**Table 1. 125I-Fibrinogen Associated With Control and Arachidonic-Acid-Pretreated Platelets Exposed to ADP**

<table>
<thead>
<tr>
<th>Time After Stimulation (sec)</th>
<th>−ADP</th>
<th>+ADP (9 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control platelets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.15 ± 0.03</td>
<td>2.92 ± 0.14</td>
</tr>
<tr>
<td>60</td>
<td>0.22 ± 0.04</td>
<td>2.14 ± 0.15</td>
</tr>
<tr>
<td>Arachidonic-acid-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.17 ± 0.01</td>
<td>1.62 ± 0.18†</td>
</tr>
<tr>
<td>60</td>
<td>0.14 ± 0.04</td>
<td>0.85 ± 0.14†</td>
</tr>
</tbody>
</table>

The platelets had been pretreated with arachidonic acid (250 μM) or its solvent, washed, and resuspended in fresh medium. The specific radioactivity of the fibrinogen was 45,400 cpm/μg; final concentration 66 μg/ml.

*Mean ± SEM of 5 samples from 2 different experiments.
†p < 0.001, compared with control platelets.
Aggregation and Release Induced by Arachidonic Acid

Platelets preincubated with arachidonic acid in the absence of aspirin, washed, and resuspended changed shape in response to a further addition of arachidonic acid, but did not aggregate or undergo further release; control platelets aggregated and released about 40% of their amine storage granule contents (Fig. 5D). Platelets treated with arachidonic acid in the presence of aspirin also changed shape in response to further stimulation with arachidonic acid, but the shape change was less extensive as judged by the change in the oscillations of light transmission through the stirred platelet suspension; these platelets did not release serotonin. Control platelets treated with aspirin behaved similarly (Fig. 5D). Platelets pretreated with 250 μM arachidonic acid without exposure to aspirin did not undergo extensive shape change in response to a further addition of 500 μM arachidonic acid. With control platelets, 500 μM arachidonic acid caused the platelets to change shape, but they did not aggregate (Fig. 1).

Effect of Inhibition of the Lipoxygenase Pathway

The arachidonic-acid-induced inhibition of ADP-induced aggregation of aspirin-treated platelets was not prevented by incubating the platelets with eicosatetraynoic acid (30 μM added 2 min before arachidonic acid). Similarly, NDGA (5–10 μM), which also inhibits the lipoxygenase pathway, did not prevent the inhibitory effect of preincubation with arachidonic acid on ADP-induced platelet aggregation.

Mixing Experiments

To determine whether the inhibitory effect caused by preincubating platelets with arachidonic acid was a result of inhibition of their ability to produce thromboxane-A2 or a result of inhibition of the effects of thromboxane-A2 after it had formed, two types of mixing experiments were done. In the first of these, 1 part of platelets pretreated with arachidonic acid (250
μM) was mixed with 9 parts of aspirin-treated platelets that had been labeled with 14C-serotonin; the mixture was then stimulated with 250 μM arachidonic acid. The mixture prepared with 1 part of platelets pretreated with arachidonic acid did not aggregate nor was 14C-serotonin released (Table 2). It is evident that the ability of the platelets (present as 1 part of the mixture) to form thromboxane-A₂ was impaired by pretreatment with arachidonic acid, since any thromboxane-A₂ formed would have stimulated the aspirin-treated platelets to aggregate and release 14C-serotonin; this occurred when the 1 part of platelets consisted of control platelets that had not been exposed to arachidonic acid. In this case, extensive aggregation occurred, and 12.2% ± 3.6% of the 14C-serotonin was released (Table 2).

In the second type of mixing experiment, 1 part of untreated normal platelets was mixed with 9 parts of platelets that had been labeled with 14C-serotonin and pretreated with arachidonic acid at a concentration of 250 μM in the presence of aspirin. When this mixture was stimulated with 250 μM arachidonic acid, the platelets did not aggregate upon the addition of arachidonic acid, nor release 14C-serotonin. Thus, platelets that have been incubated with arachidonic acid are unresponsive to aggregation and release induced by thromboxane-A₂, since the 1 part of untreated platelets would have formed thromboxane-A₂ upon stimulation with arachidonic acid.

Incorporation of 3H-Arachidonic Acid Into Platelet Phospholipids

To determine the proportion of the arachidonic acid incorporated into platelets in a 45-min period that became associated with membrane phospholipids and the proportion that remained free in the platelets, platelets exposed to 3H-arachidonic acid were subjected to lipid extraction. The majority of the radioactivity was present in the phospholipids (91%); smaller amounts became associated with the diacylglycerol, triacylglycerol, and cholesterol ester fractions, and only 1.1% of the 3H-arachidonic acid in the platelets remained as free arachidonic acid (Table 3).

Effect of Arachidonic Acid on Platelet cAMP

Platelet cyclic-AMP was slightly increased (2-3 times the basal concentration) 1 min after the addition of 250 μM arachidonic acid. When the platelets that

| Table 2. Arachidonic-Acid-Induced Aggregation and Release of 14C-Serotonin From Mixtures of Unlabeled Platelets and 14C-Serotonin-Labeled Platelets Pretreated With Aspirin and Preincubated With or Without Arachidonic Acid |
|---|---|---|---|
| Platelet Mixtures | One part unlabeled platelets | Nine parts ASA-treated, 14C-serotonin-labeled platelets | Change in light transmission† | Release of 14C-serotonin (%) of total |
| Preincubation solution* | Preincubation solution* | | |
| Arachidonic acid (250 μM) | Control | | 0 | 0.4 ± 0.3 |
| Control | Control | 96.1 ± 0.9 | 12.2 ± 3.6 |
| Control | Arachidonic acid (250 μM) | 0 | 0.3 ± 0.1 |

*The platelets were washed and resuspended after the preincubations.
†The maximum change in light transmission of an untreated platelet suspension aggregated with 250 μM arachidonic acid was arbitrarily assigned a value of 100%, and the maximum change in light transmission of each 1:9 part mixture, upon stimulation with 250 μM arachidonic acid, was expressed as a percentage of the value for the untreated suspension.

Means ± SEM of values from 3 experiments.
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had been incubated with arachidonic acid were washed and resuspended, the cAMP concentration (0.42 μmole/10⁸ platelets) was no greater than that of the control platelets.

DISCUSSION

The results of these studies are in agreement with the observations of other investigators that high concentrations of arachidonic acid inhibit platelet aggregation. Other cis-polysaturated fatty acids have also been shown to inhibit the responses of cells (including those of platelets) to stimuli. In the experiments described in the present study, we demonstrated that the inhibitory effects of arachidonic acid persisted when the platelets were washed, resuspended in fresh medium, and incubated for several hours. Vargaftig obtained similar results with platelets preincubated with arachidonic acid. These observations indicate that the inhibitory effect involves a relatively permanent change in the platelets, and that it is unlikely to be attributable to the formation of inhibitory products such as PGD₂ or intermediates of the lipoxygenase pathway, since they would be removed by washing and resuspending the platelets. The inhibitory effect of cis-unaturated fatty acids has been attributed to a “perturbation” of cell membranes that interferes with membrane-associated processes. The observation that arachidonic acid treatment of rabbit platelets does not inhibit ADP-induced shape change but does inhibit the interaction of fibrinogen with its receptor during ADP-induced aggregation is compatible with this concept, because the fibrinogen receptor becomes available only when platelets are stimulated and its availability may depend on rearrangement of the membrane glycoproteins IIb and IIIa. Whether other receptors are affected is not known, although the ADP receptor is apparently able to respond since the platelets can change shape in response to ADP.

The inhibitory effect of preincubating the platelets with arachidonic acid was a general effect. Not only was aggregation and release inhibited in response to a further stimulation with arachidonic acid, but ADP-induced aggregation, and aggregation and release in response to low concentrations of collagen and thrombin were inhibited. ADP-induced aggregation of rabbit platelets is not associated with activation of phospholipases and the generation of thromboxane-A₂, nor does it involve the release of granule contents. The inhibition of thrombin-induced aggregation was evident with aspirin-treated platelets, which would not be able to form thromboxane-A₂. Thus, several different mechanisms of aggregation are inhibited, not just the arachidonic acid pathway, and the inhibitory effect cannot be attributed solely to refractoriness of the cyclo-oxygenase to further stimulation nor to HPETE, which has only been reported to inhibit thromboxane formation.

The results from the mixing experiments indicate that arachidonic-acid-treated platelets do not form a thromboxane-A₂-like activity that can stimulate aspirin-treated platelets. Furthermore, the arachidonic-acid-treated platelets were refractory to stimulation by thromboxane-A₂ formed by normal untreated platelets.

One group of agents that has a general inhibitory effect on platelet aggregation and the release of granule contents is the group that increases platelet cyclic AMP. Two sets of observations, however, indicate that this is unlikely to be the mechanism of inhibition. First, the inhibitory effect was evident with platelets that had been treated with aspirin in which PGD₂ or other prostaglandins that stimulate adenylate cyclase could not form, and second, the cyclic AMP concentration in the platelets was not above basal levels when it was measured at the time when aggregation responses were examined, that is, after the platelets had been washed, resuspended, and incubated in fresh medium. (The slight increase in cyclic-AMP observed 1 min after the addition of arachidonic acid agrees with the increase reported by Linder and Goodman, but it did not persist, although the platelets remained refractory.) Rao et al. have reported that arachidonate did not influence the basal level of cyclic-AMP in their experiments.

Although a product formed from arachidonic acid via the lipoxygenase pathway, HPETE, has been reported to inhibit arachidonic-acid-induced platelet aggregation, it is unlikely that this compound was responsible for the inhibitory effects because inhibitors of the lipoxygenase pathway, ETYA and NDGA, did not alter the inhibitory effect of arachidonic acid when they were present during the preincubation of aspirin-treated platelets with arachidonic acid.

It was observed that although further stimulation of arachidonic-acid-treated platelets with arachidonic acid did not induce further release of ¹⁴C-serotonin, stimulation with thrombin did cause further release of ¹⁴C-serotonin from these platelets. Vargaftig also observed that thrombin caused aggregation of platelets that were refractory to arachidonic acid because of previous treatment with arachidonic acid. It is well established that thrombin can cause extensive release from platelets in which the arachidonate pathway is blocked. Thus, preincubation with arachidonic acid appears to have little effect on the main mechanism through which thrombin acts, although the contributions of ADP and thromboxane-A₂ to thrombin-induced aggregation and release are probably inhibited by the preincubation with arachidonic acid.
The findings with arachidonic acid provide another indication that although arachidonic acid can initiate the release reaction through the formation of thromboxane-A\(_2\), arachidonic acid also makes the platelets refractory to stimulation by thromboxane-A\(_2\) (or first stimulates and then prevents the formation of thromboxane-A\(_2\)). This may be one of the reasons why arachidonic acid has not been observed to cause the release of more than approximately half of the contents of the amine storage granules. Linder et al.\(^2\) have suggested that the endogenous arachidonic acid released from platelet phospholipids could conceivably produce local concentrations of arachidonic acid sufficient to have the inhibitory effect.

In these experiments, the inhibitory effect of pretreatment with arachidonic acid was greater with platelets that had been pretreated without aspirin in the medium than it was with platelets that were pretreated with arachidonic acid in the presence of aspirin. Although these observations could be interpreted as indicating that products of the arachidonic acid pathway contribute to the inhibitory effect of arachidonic acid, this explanation would require that such products would not be removed when the platelets were washed and resuspended in fresh medium. In addition, one of the known inhibitory products, PGD\(_2\), stimulates adenylate cyclase, but we could not detect an increase in cyclic AMP after the platelets were washed and resuspended. However, other inhibitory products may form and persist. Another possible explanation is that the non-aspirin-treated platelets underwent the release reaction during their pretreatment with arachidonic acid and that this altered responsiveness to further stimulation, possibly because of membrane changes resulting from the fusion of granule and plasma membranes.

It is not possible to draw firm conclusions concerning the mechanism by which the inhibitory effect of arachidonic acid is produced. In the experiments of MacIntyre and his associates,\(^1\) the cis-unsaturated fatty acids were present in the suspending medium when the inhibitory effects were observed. These investigators speculated that the free fatty acids perturbed the membrane and thus interfered with membrane receptors.\(^7\) In the present experiments, the inhibitory effect of arachidonic acid persisted after the platelets had been washed free of the fatty acids and incubated for several hours. Furthermore, during the incubation with arachidonic acid, most of it was incorporated into phospholipids and very little was free in the platelets at the time when inhibition was still apparent. The inhibition involved several different mechanisms of platelet aggregation, and therefore a general inhibitory action must have occurred. Since increased cyclic AMP was not responsible, one might speculate concerning changes in the mechanisms responsible for the availability of membrane receptors, perhaps for fibrinogen, or changes in the ability of the platelets to mobilize internal calcium in response to stimulation.

NOTE ADDED IN PROOF

Since this manuscript was submitted, a paper has appeared in the August issue of this journal in which the inhibitory effect of arachidonate is attributed to the increase in cyclic AMP. We noted a similar increase in cyclic AMP when platelets were exposed to arachidonate. However, we found that the inhibitory effect persisted when the platelets had been washed and resuspended and the cyclic AMP concentration had returned to basal levels. Also in agreement with Linder and Goodman (Blood 60:436-445, 1982), we found that prior exposure to arachidonate inhibited platelet responses to collagen and thrombin and, in our experiments, also to ADP.

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