Biochemical Pharmacology, Vol. 30, No. 15, pp. 2089–2095, 1981.
Printed in Great Britain.

0006-2952/81/152089-07 $02.00/0
Pergamon Press Ltd.

PHOSPHOLIPID METHYLATION AND HUMAN PLATELET FUNCTION

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(*Received* 27 *October* 1980; *accepted* 9 *January* 1981)

Abstract—Lysed human platelet preparations were shown to methylate phosphatidylethanolamine to the mono-, di- and trimethyl products. At a low concentration of *S*-adenosylmethionine (SAM), 2 μM, the major product formed was phosphatidylmonomethylethanolamine (PNE). There was a broad pH optimum from 9 to 11 for the formation of PNE. The combined formation of phosphatidyldimethylethanolamine (PNNE) and phosphatidylcholine (PC) showed a peak of activity at pH 8.2 followed by a broad shoulder up to pH 12. At a higher concentration of SAM (200 μM), the predominant product formed was PC; the pH dependence for formation of the combination of PNNE and PC under these conditions showed a broad peak from 9 to 11. Inhibitors of SAM-dependent methylations, *S*-adenosylhomocysteine (SAH) and 3-deazaadenosine (3-DZA), blocked phospholipid methylation in lysed platelets and whole cells respectively. 3-DZA, did not inhibit platelet responses to thrombin, ADP, epinephrine, collagen, arachidonic acid and the calcium ionophore A23187 as measured by serotonin release, platelet aggregation, malondialdehyde formation, clot retraction, or arachidonic acid release at high agonist concentrations, and appeared to potentiate responses at low concentrations. Prostaglandin-induced inhibition of platelet function was unaffected by 3-DZA. Thrombin stimulation of platelets failed to produce the changes in methylation of phospholipids that have been associated with receptor activation of other cell types. Phospholipid methylation is not required for intial activation of platelets but the inhibition of this methylation may activate platelet function at low levels of agonists.

Phospholipid methylation has been shown to play an important role in receptor-mediated events in several systems such as β-receptor linked increases in cyclic AMP in rat reticulocytes [1], chemotaxis of neutrophils [2], histamine secretion in rat mast cells [3, 4], and mitogenesis in lymphocytes [5]. In these systems, inhibition of methylation blocked the ability of the cell to respond to the external signal; other processes associated with this signal transduction, such as calcium influx and phospholipase-induced arachidonic acid release, were also prevented by inhibition of phospholipid methylation.

Platelets are secretory cells that release adenine nucleotides, serotonin and calcium from granular stores in response to a variety of stimuli including thrombin, ADP, collagen, epinephrine and A23187. Initial stages of the platelet release reaction involve activation of phospholipases to produce free arachidonic acid. This fatty acid is further metabolized to prostaglandins and thromboxanes which can amplify platelet aggregation and secretion and which are rapidly converted to relatively stable metabolites such as malandialdehyde. Hydroxyeicosatetraenoic acids are also produced from arachidonic acid but do not appear to exert direct effects upon the platelet functions studied here [6]. The platelet was used to test the role of phospholipid methylation in receptor-mediated cell functions. We report the presence of phospholipid-methylating enzyme(s) in platelet membrane traction and the lack of effect of inhibition of these enzyme(s) on platelet function.

MATERIALS AND METHODS

A highly purified, lyophilized, preparation of human thrombin containing 2300 units/mg was obtained from Dr. David Aronson (Bureau of Biologics, NIH, Bethesda, MD). Adenosine diphosphate (ADP) (Sigma Chemical Co., St. Louis, MO), epinephrine hydrochloride (Parke, Davis & Co., Detroit, MI), and soluble skin collagen (Millipore Corp., Beford, MA) were diluted in 0.15 M NaCl to the appropriate concentration for use. A23187 (CalBiochem, San Diego, CA), 1 mg/ml, was stored in dimethylsulfoxide at −20°; initial dilutions were made in 95% ethanol followed by Tris-buffered saline assuring that the concentration of dimethylsulfoxide or ethanol never exceeded 1.1% in reaction mixtures. [^{3}H]Serotonin binoxalate (New England Nuclear Corp., Boston, MA) had a specific activity of 25.2 Ci/mmole. Human serum albumin (HSA) was obtained from the Nutritional Biochemical Corp., Cleveland, OH. *S*-Adenosyl-L-[^{3}Hmethyl]methionine, 14 Ci/mmole, and L-[^{3}H-methyl]methionine, 12 Ci/mmole, were purchased

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from the New England Nuclear Corp. Arachidonic acid[5,6,8,9,11,13,14,15-^{3}H], 90 Ci/mmole, was purchased from Amersham/Searle (Arlington Heights, IL). Protaglandins were supplied by Dr. John Pike (Upjohn Laboratories), 3-deazaadenosine was purchased from Southern Research Institute and L-homocysteine thiolactone was purchased from the Sigma Chemical Co.

Platelet and lysed platelet preparation. For platelet function studies, blood was drawn, using a two-syringe technique, from a panel of normal individuals who had taken no drugs for at least 2 weeks. Depending on the type of study, either 0.17 ml of 0.27 M ethylenediamine tetra acetate (EDTA) or 0.1 ml of 1.36 M sodium citrate dihydrate was added per 10 ml of whole blood for anticoagulation. Platelet-rich plasma (PRP) was obtained by centrifugation of anticoagulated whole blood at 1300 *g* for 3.5 min. Platelets were sedimented from PRP by centrifugation at 1400 *g* for 12 min. Platelet suspensions were prepared in modified Rossi's buffer [7] containing 5 mM 2-(*n*-morpholino) ethanesulfonic acid (MES), pH 6.0, without citrate, or were washed in this buffer before suspension in calcium-free Gey's solution with 0.1% human serum albumin added. Saline suspensions were centrifuged at 700 *g* for 8 min. Preparative procedures were carried out at room temperature. Platelet counts were performed in duplicate by the method of Brecher and Cronkite [8], and concentrations were adjusted as indicated below for testing. Other specific conditions are included in sections below on various platelet assays.

Lysed platelet preparations were made from random NIH blood blank donations which were collected in ACD anticoagulant. Washed platelet suspensions were prepared as described above, using Gey's balanced salt solution [9] with 25 mM HEPES* buffer (pH 7.4) without calcium, and then were frozen at −20° for cell disruption and storage. Upon thawing, the membranes were separated by centrifugation at 27,000 *g* for 30 min and suspended in the appropriate hypotonic buffer (see below).

Phospholipid methylation. Lysed platelets were suspended in 50 mM Tris-glycylglycine buffer, pH 8.0, except for pH studies. A 100-μl aliquot containing 0.1 to 1.0 mg protein, 5 mM $MgCl_2$, 3 μCi [^{3}H]SAM, and from 2 to 200 μM SAM was incubated at 37° for up to 30 min. The reaction products were precipitated with 0.5 ml of 10% trichloroacetic acid (TCA) followed by centrifugation at 27,000 *g* for 10 min. The pellet was extracted into 3.0 ml of chloroform/methanol (2:1, v/v). This extract was washed twice with 2 ml of 0.1 M KCl methanol (1:1). Total methylation was measured by drying 1.0 ml of the chloroform phase at 80°, adding scintillation mixture, and counting.

Methylated phospholipids were identified by thin-layer chromatography as follows. The washed chloroform phase was dried under nitrogen or vacuum and taken up in a small volume of chloroform/methanol (2:1) containing 10 μg each of the methylated phospholipids as carriers. The phospholipid extract was chromatographed on silica gel with chloroform/propionic acid/propanol/water (3:2:2:1) and visualized with iodine vapor after drying.

Methylation of phospholipids in intact platelets was determined by incubating the platelets, 5×10^8 per ml in Gey's buffer, with [methyl-^{3}H]methionine (10 μM, 100 μCi/ml). The [^{3}H]methionine was dried with nitrogen and dissolved in Gey's buffer just before the incubation. The reaction was stopped at various times by addition of 0.5 ml of 10% TCA containing 10 mM methionine. The extraction procedure for phospholipids was similar to that described for methylation in lysed platelets.

Arachidonic acid release. [^{3}H]Arachidonic acid released from thrombin-stimulated platelets was measured by counting the radioactivity in the supernatant fraction from prelabeled cells. [^{3}H]Arachidonic acid (2 μM, 2 μCi/ml) was incorporated into platelets by preincubating at 37° for 30 min in calcium-free Gey's buffer containing 0.1% HSA. EDTA was then added to a final concentration of 2 mM, and the cells were centrifuged at 1000 *g* for 10 min at room temperature, washed twice with calcium-free Gey's buffer, with EDTA and 0.1% HSA, and resuspended at a concentration of 5×10^9 ml in calcium-free Gey's buffer with 0.1% HSA. This suspension was diluted 1:9 with Gey's buffer, and 300-μl aliquots were incubated with or without thrombin, 1.0 unit/ml, for various times. The reaction was stopped by addition of 1.0 ml of cold buffer followed by centrifugation at 27,000 *g* for 10 min. The radioactivity in 1.0 ml of the supernatant fraction was counted.

Platelet aggregation. Aggregation was measured with a Payton aggregometer using 0.45 ml of platelet-rich plasma anticoagulated with citrate, containing 3 to 4.8×10^5 platelets/μl in different experiments. Cuvettes were siliconized, the temperature was 37°, and the stirring rate was 900 rpm. Aggregating agents in 0.05 ml were added, and changes in light transmission were recorded. Both the maximum degree of light transmission (maximum aggregation) obtained with a given concentration of agonist and the minimum concentration of agonist that increased light transmission were used as measures of comparative sensitivity of the response of different platelet preparations.

Serotonin release. To 10 ml of platelet-rich plasma anticoagulated with EDTA was added 0.03 ml (40 μM, 30 μCi) of [^{3}H]serotonin, and the mixture was incubated for 45 min at 18°. Platelets were sedimented, resuspended in modified Rossi's solution, then resedimented, and suspended at a final concentration of 10^6/μl in 0.3 M sucrose containing 1 mg HSA/ml and 0.02 M HEPES, pH 7.4. Reaction mixtures consisted of 0.04 ml of platelet suspension and 0.06 ml of buffered sucrose containing various agonists. The reaction proceeded for 5 min at 37° and was terminated by addition of 0.1 ml of 0.5% glutaraldehyde. Mixtures were centrifuged for 2 min in a Beckman microfuge, and tritium in 0.1 ml of supernatant fluid was measured. Release of [^{3}H]serotonin was calculated as the percentage of maximum release produced by 34 units of thrombin/ml.

Malondialdehyde (MDA) formation. Platelets

* HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

obtained from PRP anticoagulated with EDTA were washed in modified Rossi's solution and suspended in Ca^{2+}-free Gey's solution at a concentration of 4 $\times$ $10^5/\mu l$. The reaction mixture consisted of 1.5 ml of platelet suspensions plus 0.015 ml containing one of various stimulants; incubation time was 5 min at 37°. Reactions were stopped by adding 2 ml of 2.3% perchloric acid containing 0.53% of 2-thiobarbituric acid. The mixture was heated at 100° for 15 min, then centrifuged at 1700 *g* for 12 min and the O.D. read at 532 nm [10]. When collagen was used as a stimulus, platelet-rich plasma anticoagulated with citrate was used instead of platelets in saline, and MDA was measured by the method of Smith *et al.* [11].

Clot retraction. A modification of the quantitative clot retraction technique of Lucia *et al.* [12] was used. Platelet concentrations in citrated platelet-rich plasma were adjusted to 10^5, 5×10^4, 2.5×10^4, and $1.25 \times 10^4/\mu l$ with platelet-poor plasma and to 2 ml of each platelet suspension, and were added to 0.05 ml of 1 M $CaCl_2$ and 5 units of thrombin in 0.05 ml of 0.15 M NaCl. Platelet-poor plasma was the supernatant fraction from platelet-rich plasma centrifuged at 1000 *g* for 30 min. Tubes were quickly mixed with a stirring rod which was left in the tube. After 5 min, the clot that formed was gently rimmed with a wire; after 60 min at 37° the rod was withdrawn with the adherent clot, and the volume of residual serum in the tube was measured. Percent clot retraction was expressed as (the residual serum volume ÷ 2.1) $\times$ 100.

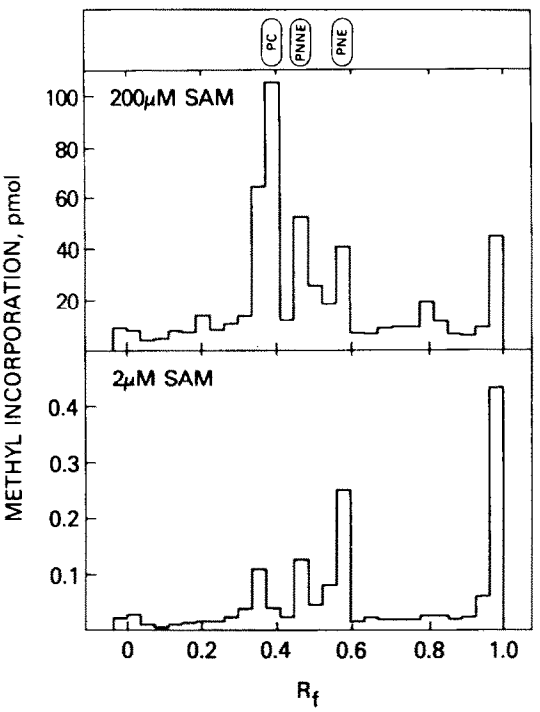

Fig. 1. Chromatographic pattern of ^{3}H-methylated phospholipids from platelet membrane fraction after incubation with [^{3}H-methyl] SAM. Chromatography was on silica gel G; 0.5 cm zones were scraped. Abbreviations: PNE, phosphatidylmonomethylethanolamine; PNNE, phosphatidyldimethylethanolamine; and PC, phosphatidylcholine.

RESULTS

Phospholipid methylation in platelets. Methylation of phospholipids in platelet membrane fragments was determined by incubation with 2.0 and 200 μM SAM. Incorporation of the ^{3}H-methyl group was linear over a 30 min period at both concentrations of SAM. The identity and relative abundance of the methylated phospholipids were determined by thin-layer chromatography. The distribution of products was dependent upon the concentration of SAM (Fig. 1). At 2μM SAM, the methyl group was incorporated into phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine and phosphatidylcholine, with the monomethyl product predominating. The same phospholipids were also found at 200 μM SAM; however, phosphatidylcholine was the major product. The unidentified peak running at the solvent front contributed about 50 per cent of the total activity at 2.0 μM SAM, 11 per cent at 200 μM SAM, and was about 17 per cent in whole platelets labeled with [^{3}H]-methionine (see below). The formation of all labeled products was completely inhibited in the presence of *S*-adenosylhomocysteine (SAH) at ten times the SAM concentration.

For the purpose of characterizing the effect of pH on phospholipid methylation, the products were divided into two groups: (1) phosphatidylmonomethylethanolamine, and (2) phosphatidyldimethylethanolamine and phosphatidylcholine. The pH optima at high and low concentrations of SAM for incorporation of the methyl group into the two groups of phospholipids were different. At 2 μM SAM, there was a broad pH optimum from 9 to 11 for formation of the monomethyl product. Phosphatidyldimethylethanolamine and phosphatidylcholine formation showed a peak at pH 8.2, followed by a shoulder extending to pH 12. At 200 μM SAM, there was a broad pH optimum from 9 to 11 for these two products, while the monomethyl product showed no peak.

Phospholipid methyltransferase activity in beef adrenal microsomes has been shown to have a strong Mg^{2+} dependence, specifically in the formation of phosphatidylmonomethylethanolamine [13]. However, Mg^{2+} dependence in platelet membrane fragments could not be demonstrated after overnight dialysis against Mg^{2+} free buffer and in the presence of 2 mM EDTA.

Phospholipid methylation in whole platelets was measured by labeling the intracellular SAM pool with [^{3}H]-methionine. The ^{3}H-methyl group was incorporated into the phospholipids linearly for up to 2 hr. The methylated lipids were separated and identified after a 30 min incubation at 37°. Under these conditions the relative proportions of the methylated products were 23% for the monomethyl product, 30% for the dimethyl product, and 47% for phosphatidylcholine. Total methylated phospholipid formed was 128 fmoles·(mg protein)$^{-1}$·hr^{-1}.

The effect of the intracellular methyltransferase inhibitors 3-deazaadenosine and homocysteine thio-

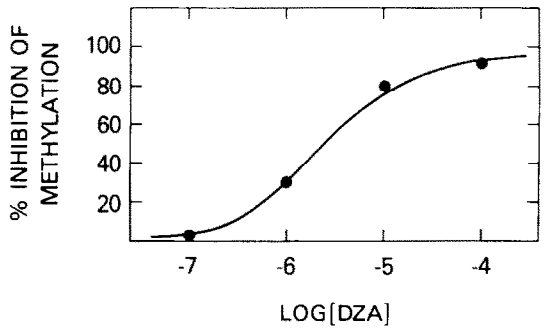

Fig. 2. Dose–response curve for inhibition of phospholipid methylation in intact platelets by 3-deazaadenosine. Homocysteine thiolactone (HCTL), 100 μM, was present at all concentrations of 3-DZA; the unit of concentration of 3-DZA is molar. Platelets were preincubated with [^{3}H-methyl]methionine for 40 min, at which time 3-DZA and HCTL were added. The reaction was stopped 50 min after addition of the inhibitor, and the ^{3}H-methylated phospholipids were extracted as described in Materials and Methods.

lactone on phospholipid methylation in intact platelets was measured. The combination of 3-deazaadenosine and homocysteine thiolactone was shown to inhibit the synthesis of methylated phospholipids in a dose-dependent manner (Fig. 2). The EC_{50} for 3-deazaadrenosine was 3 μM in the presence of 100 μM homocysteine thiolactone.

Lack of effect of receptor stimulation on platelet phospholipid methylation. In several other systems receptor occupation on the cell surface leads to transient changes in the formation of methylated phospholipids. Thrombin stimulation of platelets was studied to determine if similar changes in phospholipid synthesis could be induced. When platelets were prelabeled with [^{3}H]-methionine for 30 min and then stimulated with thrombin (0.2 units/ml), no changes in methylated phospholipid synthesis were observed over a 4 min period, samples having been taken at 10 sec intervals. Since other receptor systems linked to cyclic AMP formation show strong interaction with phospholipid methylation [1], the effect of inhibition of platelets by prostaglandins known to raise cyclic AMP levels was tested. Prostaglandin E_1 (5 μM), and PGI_2 (10^{-8} to 10^{-5} M), caused no net change in phospholipid methylation in methionine-labeled platelets. Other prostaglandins that failed to cause a net change in phospholipid methylation were: PGE_2 (10^{-8} to 10^{-5} M), $PGF_{2\alpha}$ (10^{-6} to 10^{-8} M), and thromboxane B_2 (10^{-6} to 10^{-8} M).

Phospholipid methylation and platelet function. To determine whether phospholipid methylation is involved in the physiological response of platelets after receptor-mediated activation, a variety of stimuli were used in the presence and absence of 3-deazaadenosine and homocysteine thiolactone at concentrations known to inhibit phospholipid methylation. Several agonists, ADP, thrombin, epinephrine, arachidonic acid, A23187 and collagen, were used to stimulate platelet functions such as serotonin release, aggregation, malondialdehyde formation, and clot retraction. None of these functions were inhibited significantly in platelets pretreated with 3-deazaadenosine and homocysteine thiolactone (Table 1); under these conditions platelets were actually sensitised to the lower concentrations of thrombin, A23187, ADP and epinephrine in all of the function tests.

The PGE_1- or PGI_2-induced increase in cyclic AMP in the platelet is known to inhibit platelet function. Since previous work [1] showed that coupling of the β-receptor to adenylate cyclase was influenced by phospholipid methylation, methyltransferase inhibitors were used to test whether the prostaglandin-induced inhibition of platelet function was dependent upon phospholipid methylation. When platelets were preincubated with and without 3-DZA and homocysteine thiolactone, 100 μM and 1.0 mM respectively, followed by 5×10^{-7} M PGE_1, the aggregation response of both preparations to 10^{-5} M ADP and 5.5×10^{-5} M epinephrine was completely abolished, and the response to A23187, 4×10^{-5} M, was decreased to the same degree.

Extracellular SAH was also tested for its ability to inhibit platelet aggregation induced by ADP (10^{-5} to 2×10^{-6} M) and collagen (0.125 mg/ml). Four conditions were used: platelet-rich plasma (PRP) without addition, PRP with 20 μM SAM, PRP with 300 μM SAH, and PRP with both 20 μM SAM and 300 μM SAH. All four conditions showed normal platelet aggregation in response to ADP and collagen.

Phospholipid methylation and arachidonic acid release. Previous work with rat leukemic basophils [14, 15] and fibroblasts* has shown that phospholipase-induced release of arachidonic acid from phospholipids is closely coupled to phospholipid methylation. Inhibition of phospholipid methyltransferase activity blocked the receptor-mediated release of arachidonic acid in these systems. A similar experiment was carried out on platelets. Thrombin caused a marked release of [^{3}H]arachidonic acid previously incorporated into platelet phospholipids (Fig. 3). The time course and extent of release, however, were identical in thrombin-stimulated platelets, with or without 3-deazaadenosine.

DISCUSSION

We have shown here that human platelet membrane fragments and whole platelets possess the capacity to incorporate the methyl group from SAM into phosphatidylethanolamine to form the phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine, and ultimately phosphatidylcholine. The distribution of these products is dependent on the SAM concentration, with the monomethyl product predominating at low concentrations of SAM and phosphatidylcholine predominating at the higher SAM concentrations. This result, in addition to differences in pH optima at low and high SAM concentrations, suggests that there may be two enzymes involved in the three successive methylations of phosphatidylethanolamine to phosphatidylcholine as has been shown in other systems

* F. Hirata, D. L. Bareis and V. Manganiello, unpublished results.

Table 1. Summary of platelet function studies*

	(A) Serotonin release†	
	Control	3-DZA + HCTL
Thrombin (units/ml)		
0.0425	14	35
0.17	84	86
0.68	83	95
34.00	100	100
A23187 (μM)		
0.5	7	6
1	9	34
2	89	65
	(B) Malondialdehyde production‡	
	Control	3-DZA + HCTL
Thrombin (units/ml)		
0.67	1.0	1.6
3.3	2.7	2.4
Arachidonic acid (μM)		
52	1.2	1.1
A23187 (μM)		
4	2.7	2.6
Collagen (mg/ml)		
0.36	3.6	3.6
	(C) Aggregation§	
	Control	3-DZA + HCTL
ADP (μM)		
0.8	8	11
1	28	62
2	39	56
4	62	56
8	66	76
10	76	73
Epinephrine (μM)		
5.5	34	85
55	83	82
550	71	71
A23187 (μM)		
40	85	79
	(D) Clot retraction‖ (%)	
Platelets	Control	3-DZA + HCTL
12,000	25	38
25,000	44	37
50,000	62	67
100,000	76	67

* Results of all platelet function studies represent an average of two experiments. Abbreviations: 3-DZA, 3-deazaadenosine; and HCTL, homocysteine thiolactone.

† Platelets were suspended in 0.3 M buffered sucrose with albumin preincubated for 35 min with or without 3-DZA (830 μM) and HCTL (830 μM). Values are percentages of the maximum release; see Materials and Methods.

‡ Platelets were suspended in Gey's buffer without Ca^{2+} and preincubated for 35 min with or without 3-DZA (83 μM) and HCTL (830 μM). Values are nmoles of MDA/10^9 platelets.

§ Platelets were suspended in citrate plasma and preincubated for 35 min with or without 3-DZA (100 μM) and HCTL (1.0 mM). Values are percentages of maximum transmission.

‖ Platelets were suspended in citrate plasma and preincubated with or without 3-DZA (830 μM) and HCTL (830 μM) for 35 min.

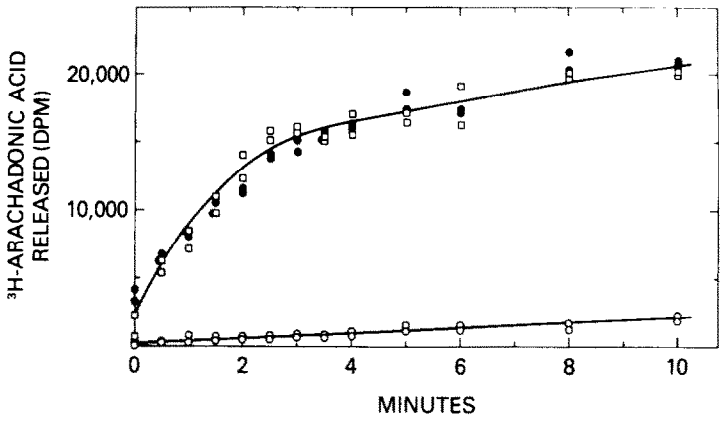

Fig. 3. [^{3}H]Arachidonic acid and metabolites released by thrombin stimulation. Platelets were labeled with [^{3}H]arachidonic acid as described in the text. Thrombin (1.0 units/ml) was added at time zero. Platelets were preincubated with 3-DZA (30 μM) and homocysteine thiolactone (HCTL) (100 μM) for 15 min after labeling with [^{3}H]arachidonic acid. Key: (○——○) no thrombin added, (□——□) thrombin, and (●——●) thrombin, 3-DZA and HCTL.

such as human red blood cell ghosts [16], beef adrenal microsomes [13], and rat synaptosomes [17].

Biochemical studies on whole platelets following stimulation of thrombin or prostglandin receptors consistently showed no changes in the rate of synthesis or degradation of the methylated phospholipids. Thus, the metabolism of phosphatidylcholine in platelets does not appear to be linked to these receptor-mediated events. These results are in contrast to other systems such as the β-receptor on rat reticulocyte [1] and C_6 astrocytoma [18] as well as other receptor classes such as benzodiazepine [19], concanavalin A [3], IgE [4], chemotactic peptide [2], and bradykinin* found on various cell types; in these systems, receptor-mediated events do interact with phospholipid methylation.

The release of arachidonic acid following receptor stimulation has also been shown to be linked to phospholipid methylation in leukemic basophils [15], neutrophils†, and fibroblasts.* In these cells it appears that a major source of this arachidonic acid is the phosphatidylcholine that arises from the methylation of phosphatidylethanolamine. In the platelet, however, arachidonic acid release is not impaired when methylation is suppressed. This suggests that phosphatidylcholine from the methylation pathway is not the phospholipid substrate which is cleaved by phospholipases to liberate arachidonic acid. Another type of phospholipid may be a major source of arachidonic acid in the platelet; others have reported that phosphatidylinositol is an important source of this fatty acid [20–23]. If phosphatidylcholine is a source of arachidonic acid in the platelet, this phospholipid may be synthesized by the base exchange pathway. Another possibility is that the phospholipases are topographically distant from the phospholipid-methylating enzymes, effectively isolating phosphatidylcholine arising from the methylation pathway from the phospholipases during short-term labeling studies. These suggestions are consistent with our observations reported here that the suppression of methylation does not affect arachidonic acid release and that there is little decrease in total ^{3}H-methylated phosphatidylcholine up to 4 min after thrombin stimulation.

Other workers have also reported that there is little change in [^{3}H]methionine-labeled phosphatidylcholine in thrombin-stimulated platelets, even when the thrombin to platelet ratio is fifty times higher than was used in the experiments reported here [24]. These workers also reported a decrease in [^{3}H]arachidonic acid-labeled phosphatidylcholine 10 min after thrombin stimulation. These results support our conclusion that the supply of phosphatidylcholine from the methylation pathway in the human platelet is not tightly linked to the phospholipases which are activated followed thrombin stimulation.

Platelet function studies also failed to demonstrate an association between methylation and receptor-mediated events. In the presence of methyltransferase inhibitors functions such as platelet aggregation, serotonin release and MDA formation induced by thrombin, collagen, ADP, epinephrine and A23187 were all normal at high doses of agonist and increased at low doses of agonist in most tests. The inhibitory effect of PGE_1 was unaltered by the suppression of phospholipid methylation.

The explanation of the differences between the platelet and cell types which have been reported to have functional and biochemical links with phospholipid methylation may lie in the structure of the membrane itself. Whatever the role of the phospholipid-methylating enzymes in platelet function, it appears that these enzymes are not biochemically associated with the proteolipid domains which include functional complexes of receptors, ion channels and associated proteins as has been found with other systems [1, 4, 15, *, †]. The methylated products of phosphatidylethanolamine appear to be

* F. Hirata, D. L. Bareis and V. Manganiello, unpublished results.

† D. L. Bareis, V. Manganiello, F. Hirata and J. Axelrod, unpublished observations.

inserted into domains of the membrane which are chemically, metabolically, and functionally different from the domain including platelet receptors and other proteins such as phospholipases. In cell types which show receptor-mediated events correlated with changes in phospholipid methylation and metabolism, receptors are probably closely coupled to phospholipid-methylating enzymes as interacting functional units.

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