The Effects of Thrombin on Adenyl Cyclase Activity and a Membrane Protein from Human Platelets

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A B S T R A C T   Washed human platelets were incu-
bated with 0.1–1.0 U/ml human thrombin and the ef-
fects on adenyl cyclase activity and on a platelet mem-
brane protein (designated thrombin-sensitive protein)
were studied. Adenyl cyclase activity was decreased
70–90% when intact platelets were incubated with throm-
bin. The $T_{\frac{1}{2}}$ for loss of adenyl cyclase activity was less
than 15 sec at 1 U/ml thrombin. There was no decrease
of adenyl cyclase activity when sonicated platelets or
isolated membranes were incubated with these concentra-
tions of thrombin. Loss of adenyl cyclase activity was
relatively specific since the activities of other platelet
membrane enzymes were unaffected by thrombin. Prior
incubation of platelets with dibutyryl cyclic adenosine
monophosphate (AMP), prostaglandin E_1, or theophyl-
line protected adenyl cyclase from inhibition by
thrombin.

Incubation of intact but not disrupted platelets with
thrombin resulted in the release of thrombin-sensitive
protein from the platelet membrane. The rapid release
of this protein ($T_{\frac{1}{2}} < 15$ sec) at low concentrations of
thrombin suggested that removal of thrombin-sensitive
protein from the platelet membrane is an integral part
of the platelet release reaction. This hypothesis is sup-
ported by the parallel effects of thrombin on adenyl
cyclase activity and thrombin-sensitive protein release
in the presence of dibutyryl cyclic AMP, prostaglandin
E_1, and theophylline at varying concentrations of
thrombin.

INTRODUCTION

Previous studies from several laboratories have indi-
cated that human platelets contain adenyl cyclase and
cyclic nucleotide phosphodiesterase (cAMP phospho-
diesterase)[1] (1–6). Compounds which affect the activity
of these enzymes and, as a result, the concentrations of
adenosine 3',5'-monophosphate (cyclic AMP, cAMP)
in platelets also have marked effects on platelet ag-
gregation (2, 6–10) and on the platelet release reac-
tion (11). For example, incubation of platelets with
prostaglandin (PGE₁), N^6-2'-0-dibutyryl cyclic adeno-
sine 3',5'-monophosphate (DBcAMP), or theophylline
before addition of thrombin inhibits thrombin-induced
platelet aggregation (9, 10, 12) and release (11). These
observations have led to the hypothesis that agents
which inhibit platelet aggregation increase the concen-
tration of cAMP in platelets by stimulating adenyl
cyclase or by inhibiting cAMP phosphodiesterase, and
agents which stimulate aggregation and release, de-
crease platelet cAMP by the opposite mechanisms. One
difficulty in explaining the action of thrombin by this
hypothesis is that 0.1–1.0 U/ml of thrombin induces
aggregation and release in intact platelets while 10–200
U/ml of bovine thrombin are required to demonstrate
inhibition of adenyl cyclase activity in disrupted platelet
preparations (5, 9).

Recently we have described a thrombin-sensitive pro-
tein (TSP) which is located in the membrane fraction
of human platelets and which rapidly disappears from
the platelet membrane after addition of 0.1–1.0 U/ml
thrombin to intact but not to disrupted platelets (13).

[1] *Abbreviations used in this paper:* cAMP, cyclic adeno-
sine 3',5'-monophosphate; cAMP phosphodiesterase, cyclic
nucleotide phosphodiesterase; DBcAMP, N^6-2'-0-dibutyryl
cyclic adenosine 3',5'-monophosphate; PGE₁, prostaglandin
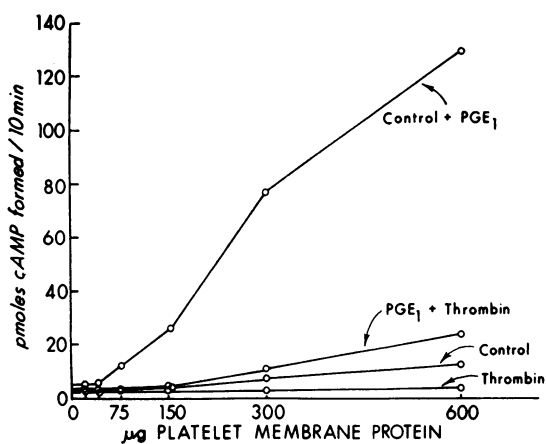E₁; SDS, sodium dodecylsulfate; TSP, thrombin-sensitive
protein.

FIGURE 1 Intact platelets were incubated for 20 min with thrombin or without (control) thrombin, 1 U/ml. After sonication, the platelet membranes were isolated and assayed for adenyl cyclase activity with and without 75 mμg PGE₁ in the reaction medium.

Since intact platelets are required to demonstrate this interaction, we postulated that intact platelets rather than preparations of platelet membranes might also be required to demonstrate an effect of low concentrations of thrombin on platelet adenyl cyclase activity.

In the current study we report that adenyl cyclase activity from human platelets is rapidly decreased by low concentrations of thrombin when thrombin is added to intact but not to disrupted platelets. Further, there is a striking parallel between the effect of thrombin on TSP and inhibition of adenyl cyclase. Both effects of thrombin are inhibited in parallel by prior incubation of platelets with PGE₁, theophylline, and DBcAMP. In more recent experiments we have shown that TSP is actually released from the platelet membrane into the medium after incubation of intact platelets with thrombin.[2]

METHODS

Preparation of platelets. Human platelets, collected and isolated as described previously (14), were washed twice in an isotonic buffer, pH 6.5, containing 0.113 M NaCl, 0.0043 M K₂HPO₄, 0.0043 M Na₂HPO₄, 0.0244 M NaH₂PO₄, and 1 mg/ml glucose, and were resuspended in 0.154 M NaCl-0.154 M Tris Cl pH 7.4; 9:1, with 1 mg/ml glucose. All experiments were carried out within 4 to 6 hr of initial blood collection. Suspensions of platelets (2–4 × 10⁹/2 ml) were incubated in a shaking incubator at 37°C with various additions as noted below. After incubation, the cells were disrupted by sonication for 15 sec at 70% intensity with a Biosonik[3] sonifier equipped with microprobe. The platelet membrane fraction was collected by centrifugation at 50,000 g for 20 min and the membrane pellets to be used for de-

[2] Baenziger, N. L., G. N. Brodie, and P. W. Majerus. Properties of the thrombin-sensitive protein of human platelet membranes. In preparation.

[3] Bronwill Scientific, Inc., Rochester, N. Y.

termination of adenyl cyclase activity were immediately frozen in liquid nitrogen and stored at −90°C. Adenyl cyclase activity was not affected by freezing under these conditions. Membrane pellets for sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis were stored at −20°C. The thrombin used in these experiments was highly purified human thrombin, supplied by Dr. David Aaronson, National Institutes of Health, Bethesda, Md. Thrombin was assayed as described previously (15). The membrane preparations used in these experiments contain the total membranous material of the platelet.

Adenyl cyclase assay. Adenyl cyclase was assayed by measuring the conversion of ATP-α³²P to cAMP-³²P. ATP-α-³²P was purchased from International Chemical & Nuclear Corporation, Burbank, Calif. (SA 2–5 Ci/mM). Membrane pellets were thawed at room temperature and resuspended in 0.05 M Tris-Cl pH 7.4 with two strokes in a Potter-Elvehjem homogenizer to give a concentration of 4–6 mg protein/ml. All reactions were carried out in a final volume of 70 μliters containing 3.5 μmoles Tris-Cl pH 7.4, MgCl₂ 0.7 μmole, bovine serum albumin 10 μg, MgNa₂ EDTA 0.35 μmole,[4] phosphoenol pyruvate 0.167 μmole, pyruvate kinase 5 μg, ATP-α-³²P 0.08 μmole (10 cpm/pmole) and platelet membrane homogenate (0.1–0.3 mg protein). Each membrane preparation was assayed with no additions, with PGE₁, 75 mμg, and with NaF 0.7 μmole. Reaction mixtures were incubated at 37°C for 10 min and stopped by adding 0.1 ml of a solution containing 4 μmoles ATP and 0.625 μmole ³H-cAMP (40,000 cpm/μmole) and boiling for 3 min. Isolation of cAMP and correction for recovery were carried out according to Krishna, Weiss, and Brodie (16). In initial assays of adenyl cyclase, MgNa₂ EDTA was not used and only 0.04 μmole of ATP-α-³²P was added to reaction mixtures. Lower values for adenyl cyclase activity were obtained using these less than optimal assay conditions but decrease of enzyme activity by thrombin was relatively the same. Adenyl cyclase activity is expressed as mμmoles cAMP formed per milligram of membrane protein/10 min. cAMP phosphodiesterase was assayed according to the method of Chase, Fedak, and Aurbach (17).

³H-cAMP (SA 1.4 Ci/mM) was purchased from Mann Research Labs, Inc., New York. PGE₁ was a gift from Dr. John Pike, Upjohn Co., Kalamazoo, Mich. Theophylline was purchased from Mann Research Labs, New York. Pyruvate kinase (400 U/mg) was purchased from Sigma Chemical Co., St. Louis, Mo.

SDS polyacrylamide gel electrophoresis for determination of TSP. SDS was purchased from Fisher Chemical Company, St. Louis, Mo., and recrystallized by the method of Burgess (18). Membrane solubilization and electrophoresis were carried out as described previously using 220 μg protein from each membrane sample per 0.5 × 20 cm gel (13). The gels were stained with Coomassie Brilliant Blue (19, 20) and destained electrophoretically (21).

Densitometry of the gels stained with Coomassie Brilliant Blue was carried out using a Gilford recording spectrophotometer[5] at 555 mμ with a Model 2410-S Gilford linear transport scanner. The recorder speed and sensitivity were adjusted so that an area of 1 cm² corresponded to approximately 1 μg protein (chart speed 2.5 cm/min, full scale 0–3.0 OD). The content of TSP in each gel was estimated by integrating the area under the densitometer peak. 1 U of TSP is defined as an area of 1 cm².

[4] Experiments where this compound was used in the reaction medium are indicated in legends for tables and figures.

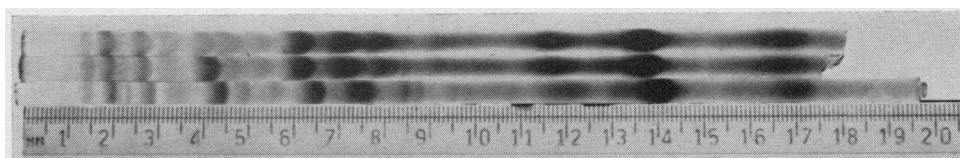[5] Gilford Instrument Labs, Inc., Oberlin, Ohio.

FIGURE 2 SDS-polyacrylamide electrophoresis of platelet membranes. The gels from lower to upper are as follows: (*a*) platelet membranes; (*b*) membranes incubated with 1 U/ml thrombin for 10 min after sonication of platelets; (*c*) membranes derived from intact platelets incubated with 1 U/ml thrombin for 1 min. Note diminished TSP at 42–44 mm migration from the origin in upper gel.

Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (22), or by a microbiuret assay (23).

RESULTS

Since platelet adenyl cyclase activity is markedly stimulated by PGE₁, we have reported the results of most experiments in terms of these higher values; however, in all experiments similar relative results were obtained using unstimulated or NaF-stimulated adenyl cyclase activity.

Initial experiments confirmed the observation that 1 U/ml of thrombin has no effect on adenyl cyclase activity in platelet membrane fractions. Adenyl cyclase activity was 1.09 and 1.04 mμmoles cAMP formed per milligram protein/10 min after incubation of platelet membranes with and without 1 U/ml thrombin for 20 min respectively. Thrombin at 100 U/ml did inhibit adenyl cyclase 50–75% as reported previously (5, 9). In contrast, when 1 U/ml of thrombin was added to intact platelets, marked inhibition of adenyl cyclase was detected (Fig. 1). The degree of inhibition was similar with or without the addition of PGE₁ or sodium fluoride. Adenyl cyclase activity was not inhibited when platelets were sonicated prior to the addition of thrombin. These results parallel our previous observations showing that

thrombin causes release of TSP from intact platelets but not from isolated platelet membranes (Fig. 2). When cells were disrupted by several other techniques (13), similar results were obtained.

Time course of disappearance of TSP and inhibition of adenyl cyclase activity. The finding that thrombin did not affect disrupted platelets allowed for studies of the time course of the effects of thrombin on TSP release and inhibition of adenyl cyclase activity, since each of these reactions could be stopped by sonication of the incubation mixture. Results of these studies are shown in Fig. 3. In this experiment adenyl cyclase was inhibited 42% by 15 sec and only 10% of initial activity remained after 2 min. TSP was released rapidly in this experiment with greater than 50% release by 15 sec and complete release by 2 min. We next attempted to quantitate TSP release using densitometry of SDS polyacrylamide gels.

Quantitative determination of protein content in polyacrylamide gels is usually not feasible with Coomassie
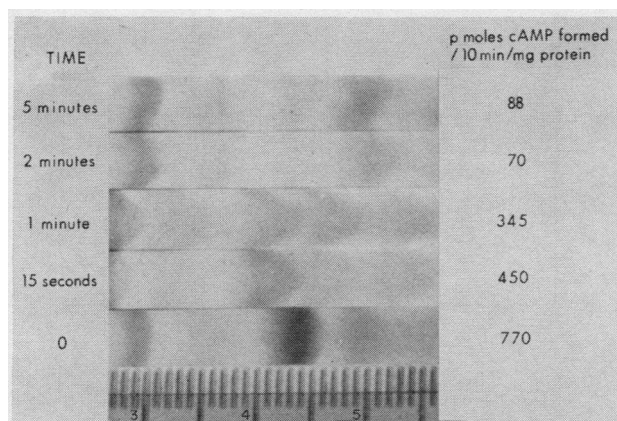

FIGURE 3 SDS polyacrylamide gel electrophoresis and adenyl cyclase activities of platelet membranes after thrombin treatment of intact platelets. Note progressive disappearance of TSP at 42 mm.
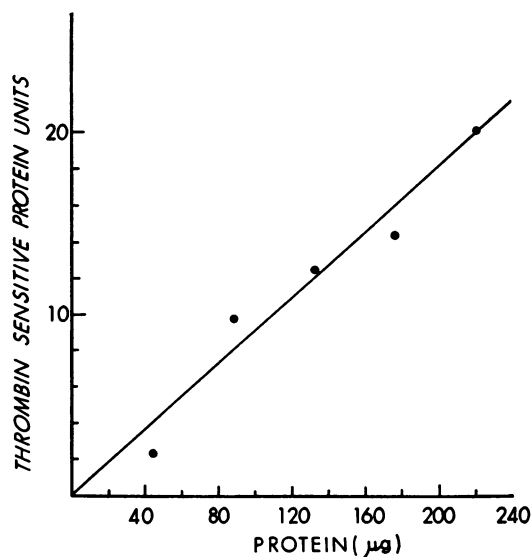

FIGURE 4 Densitometry of TSP in SDS polyacrylamide gels. Increasing amounts of platelet membrane protein were applied to the gels. Densitometry was carried out as described in Methods. (1 U of TSP is defined as an area of 1 cm² under the densitometer peak.)
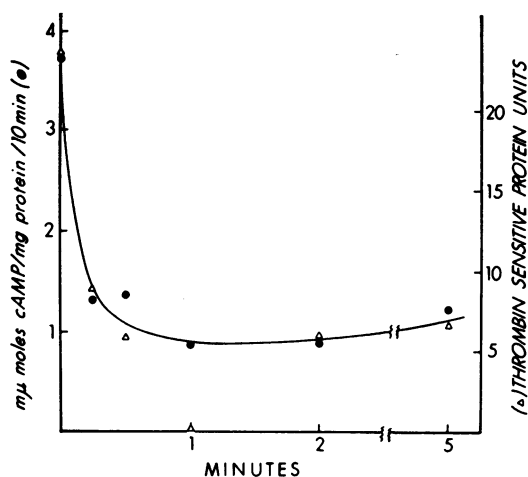
FIGURE 5 Effect of thrombin on adenyl cyclase activity and TSP from platelet membranes. Intact platelets were incubated with 1 U/ml thrombin and sonicated at the times indicated on the abscissa. Adenyl cyclase was assayed with 75 mμg PGE₁ and 0.35 μmole MgNa₂ EDTA in the reaction medium. △——△ TSP; ●——● adenyl cyclase activity.

Brilliant Blue stain, since all proteins do not stain equally in proportion to the actual amount of protein present (24). However, quantitation of a specific protein is possible using this technique. As shown in Fig. 4, the amount of TSP in platelet membranes is a linear function of the amount of membrane protein applied to each gel. The rate of TSP release from the platelet membrane after addition of thrombin was measured using densitometry and was compared with the rate of inhibition of adenyl cyclase activity. A close relationship was found between these two effects of thrombin (Fig. 5). The T½ for removal of TSP and inhibition of adenyl cyclase activity was less than 15 sec. In this experiment there was a better correlation between release of TSP and inhibition of adenyl cyclase activity than the experiment represented in Fig. 3. Part of this variation relates to the difficulty in obtaining early and frequent time points in this type of experiment. Complete disruption of platelets requires 15 sec under these conditions and it is not known whether sonication stops the thrombin reaction instantly or whether several seconds are required.

We also have examined the effect of varying concentrations of thrombin on adenyl cyclase activity and TSP content of platelet membranes (Fig. 6). The results of this experiment also demonstrate a close relationship between the amount of TSP released and the decrease of adenyl cyclase activity. In this experiment, 1 U/ml of thrombin caused 80% inhibition of adenyl cyclase activity and 90% release of TSP.

Inhibition of the effects of thrombin. Aggregation of platelets by a variety of agents, including thrombin,

can be prevented by preincubating the platelets with PGE₁, DBcAMP, or theophylline (2, 6–10, 12). We next determined whether prior incubation of platelets with these compounds would protect the platelet adenyl cyclase activity and the TSP from the effects of thrombin. Results of these experiments are tabulated in Tables I, II, and III. Prior incubation of platelets with 1 μg/ml PGE₁ caused almost 50% inhibition of release of TSP by 1 U/ml thrombin and completely prevented release by 0.2 U/ml thrombin (Table I). At this concentration PGE₁ did not prevent inhibition of adenyl cyclase activity by 1 U/ml thrombin, but decreased the inhibition of adenyl cyclase activity by 0.2 U/ml thrombin from 71 to 20%. A concentration of 0.1 μg/ml PGE₁ caused 23% inhibition of release of TSP by 1 U/ml thrombin and prevented release of TSP by 0.2 U/ml thrombin. At 0.1 μg/ml, PGE₁ did not prevent inhibition of adenyl cyclase activity by 1 U/ml thrombin, but caused a decrease in inhibition of adenyl cyclase activity by 0.2 U/ml thrombin from 71 to 49%. Thus, PGE₁ inhibited thrombin-induced release of TSP more effectively than it inhibited the effect of thrombin on platelet adenyl cyclase activity.

Prior incubation of platelets with 5.5 mM theophylline decreased the release of TSP by 1 U/ml thrombin from 86 to 50% and caused a parallel decrease in inhibition of adenyl cyclase activity (Table II). When 0.2 U/ml of thrombin was added, release of TSP was decreased from 66 to 2% by theophylline, and inhibition of adenyl cyclase activity was decreased from 61 to 22%. Since
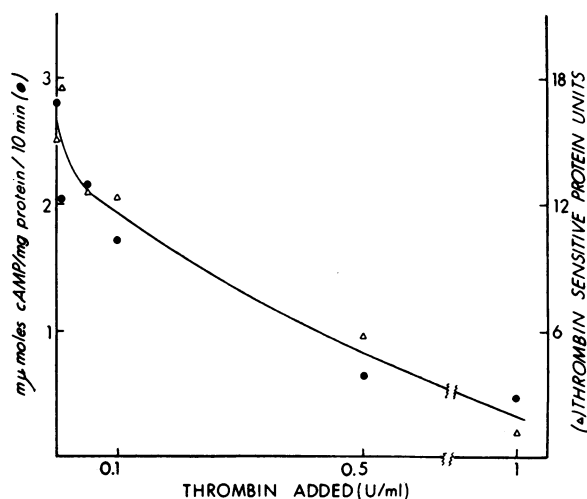

FIGURE 6 Effect of increasing concentrations of thrombin on adenyl cyclase activity and TSP from platelet membranes. Intact platelets were incubated with thrombin as indicated on the abscissa and sonicated after 20 min. Adenyl cyclase was assayed with 75 mμg PGE₁ and 0.35 μmole MgNa₂ EDTA in the reaction medium. △——△ TSP; ●——● adenyl cyclase activity.

TABLE I

Effect of Prostaglandin PGE₁ (PGE₁) on Thrombin-Sensitive Protein (TSP) and Adenyl Cyclase Activity from Platelets Incubated with and without Thrombin

| | Thrombin-sensitive protein | | Adenyl cyclase activity | |
| | | | Amount cAMP formed/mg protein/10 min | Inhibition |
Additions	U	Amount released		
		%	mμmoles	%
—	13.35	—	1.59	—
Thrombin 1 U/ml	0	100	0.50	68
Thrombin 0.2 U/ml	3.03	77	0.46	71
PGE₁ 1 μg/ml, thrombin 1 U/ml	6.13	54	0.43	73
PGE₁ 1 μg/ml, thrombin 0.2 U/ml	14.8	0	1.27	20
PGE₁ 0.1 μg/ml, thrombin 1 U/ml	3.09	77	0.49	69
PGE₁ 0.1 μg/ml, thrombin 0.2 U/ml	14.8	0	0.81	49

Platelet suspensions were incubated with PGE₁ for 20 min before adding thrombin for an additional 9 min. Platelets were then sonicated and the membranes isolated and assayed for adenyl cyclase activity as described in Methods. Adenyl cyclase was assayed with 75 mμg PGE₁ in the reaction medium. SDS polyacrylamide electrophoresis and densitometry of gels for quantitation of TSP were carried out as described in Methods.

theophylline presumably acts by inhibiting cAMP phosphodiesterase, we measured activity of this enzyme in sonicates of platelets which had been incubated with and without thrombin. Thrombin had no effect on the activity of cAMP phosphodiesterase.

When platelets were incubated with 1 mM DBcAMP for 20 min before the addition of 1 U/ml thrombin, the release of TSP was decreased from 68 to 57% while inhibition of platelet adenyl cyclase activity was decreased from 77 to 47% (Table III). At 0.2 U/ml thrombin, 1 mM DBcAMP decreased the release of TSP from 57 to 7% while inhibition of adenyl cyclase activity was decreased from 67 to 22%. When platelets were preincubated with 1 mM 5'-AMP or adenosine there was no inhibition of thrombin-induced TSP release or adenyl cyclase inhibition.

The possibility that PGE₁, DBcAMP, and theophylline might have a direct inhibitory effect on thrombin was excluded by experiments showing that these compounds did not affect thrombin activity in catalyzing the clotting of fibrinogen.

Effects of thrombin on platelet enzymes. In order to assess the specificity of the effect of thrombin on adenyl

TABLE II

Effect of Theophylline on Thrombin-Sensitive Protein (TSP) and Adenyl Cyclase Activity from Platelets Incubated with and without Thrombin

| | Thrombin-sensitive protein | | Adenyl cyclase activity | |
| | | | Amount cAMP formed/mg protein/10 min | Inhibition |
Additions	U	Amount released		
		%	mμmoles	%
—	19	—	0.88	—
Thrombin 1 U/ml	2.64	86	0.12	86
Thrombin 0.2 U/ml	6.45	66	0.34	61
Theophylline 5.5 mM, thrombin 1 U/ml	9.35	50	0.34	61
Theophylline 5.5 mM, thrombin 0.2 U/ml	18.6	2	0.69	22

Platelet suspensions were incubated with theophylline for 20 min before adding thrombin for an additional 3 min. Platelets were then sonicated, the membranes isolated and assayed for adenyl cyclase activity, and TSP content as described in Methods. Adenyl cyclase was assayed with 75 mμg PGE₁ in the reaction mixture.

cyclase, the activities of several enzymes from platelet membranes were examined after incubating intact platelets with thrombin. There was no significant effect of

TABLE III

Effect of Dibutyryl Cyclic AMP (DBcAMP) on Thrombin-Sensitive Protein (TSP) and Adenyl Cyclase Activity from Platelets Incubated with and without Thrombin

| | Thrombin-sensitive protein | | Adenyl cyclase activity | |
| | | | Amount cAMP formed/mg protein/10 min | Inhibition |
Additions	U	Amount released		
		%	mμmoles	%
—	26.9	—	6.42	—
Thrombin 1 U/ml	8.50	68	1.48	77
Thrombin 0.2 U/ml	11.6	57	2.09	67
DBcAMP 1 mM, thrombin 1 U/ml	11.6	57	3.39	47
DBcAMP 1 mM, thrombin 0.2 U/ml	25	7	4.98	22

Platelet suspensions were incubated with DBcAMP for 20 min before adding thrombin for an additional 20 min. Platelets were then sonicated, the membranes isolated and assayed for adenyl cyclase activity and TSP content as described in Methods. Adenyl cyclase was assayed with 75 mμg PGE₁ and 0.35 μmole MgNa₂ EDTA in the reaction medium.

TABLE IV
Effect of Thrombin on Platelet Enzyme Activities

Membrane enzymes	Enzyme activity, μmoles product formed/mg protein/hr	
	Control	Thrombin
Adenyl cyclase (16)*	0.0078	0.0018
Ca²⁺-ATPase (25)	0.25	0.41
5' Nucleotidase (26)	0.03	0.03
Mg²⁺-ATPase (25)	0.77	0.83
(Na⁺ + K⁺ + Mg²⁺)-ATPase (27)	0.30	0.24
Bis p-nitrophenol phosphatase (28)	70	77
Soluble enzymes		
Glucose-6-phosphate dehydrogenase (29)	58	63
Lactic dehydrogenase (30)	14.8	13.8

* References to the methods used are indicated in parentheses.

thrombin on the activity of $(Na^+ + K^+ + Mg^{2+})$-ATPase, Ca^{2+}-ATPase, 5′ nucleotidase, Mg^2-ATPase, or bis p-nitrophenol phosphatase (Table IV). There was also no effect of thrombin on the activity of the two soluble enzymes, glucose 6-phosphate dehydrogenase and lactic dehydrogenase. In contrast adenyl cyclase was inhibited 77% by thrombin indicating that the effect of thrombin on platelet membranes is relatively specific for adenyl cyclase.

DISCUSSION

Numerous previous experiments suggest that thrombin-induced platelet aggregation and release may be mediated by a fall in the concentration of platelet cAMP. However, Zieve and Greenough (5), and Saltzman and Levine (9), have shown that high concentrations of bovine thrombin (10–200 U/ml) are required to inhibit adenyl cyclase in isolated platelet membranes. We have confirmed these observations using human thrombin and have now shown that low concentrations of human thrombin (< 1 U/ml) will inhibit adenyl cyclase when it is incubated with intact platelets. This inhibition of adenyl cyclase occurs rapidly with a T½ of less than 15 sec, and is relatively specific since the activities of several other enzymes from platelet membranes are unaffected by thrombin (Table IV). Furthermore, preincubation of platelets with DBcAMP, PGE₁, or theophylline protects adenyl cyclase from inhibition by thrombin. This protection is competitive with thrombin since increasing concentrations of thrombin result in adenyl

cyclase inhibition even in the presence of these compounds. Wolfe and Shulman (11) have shown that PGE₁, DBcAMP, and theophylline competitively inhibit the thrombin-induced platelet release reaction. Release of Ca^{++} and nucleotides occurs rapidly and precedes visible platelet aggregation. Competitive inhibition of the effect of thrombin on the platelet release reaction parallels the competitive inhibition of the effect of thrombin on inhibition of adenyl cyclase.

In a previous study of the effects of thrombin on human platelets we demonstrated that incubation of intact but not disrupted platelets with thrombin resulted in the disappearance of a major protein (TSP) from the platelet membrane (13). The rapid release of TSP from platelet membranes at low concentrations of thrombin suggests that removal of TSP from the platelet membrane is an integral part of the platelet release reaction. This hypothesis is further supported by the parallel effects of thrombin on TSP release and adenyl cyclase inactivation. Thus TSP is released and adenyl cyclase is inhibited when thrombin is incubated with intact platelets, but neither effect is noted when disrupted platelets or isolated membranes are used. When TSP release and inhibition of adenyl cyclase activity are compared using different incubation times and concentrations of thrombin, there is a striking correlation between these two parameters (Figs. 5 and 6). Further correlation between TSP release and adenyl cyclase inhibition is noted in experiments using DBcAMP, PGE₁, and theophylline, although there was a tendency for the inhibition of TSP release to be greater than the protection of adenyl cyclase activity suggesting partial dissociation between these phenomena.

Neither the nature of the relationship between TSP and adenyl cyclase nor of the interaction of thrombin with the platelet has been defined. Thrombin may act initially on TSP, adenyl cyclase, or on some other component of the platelet membrane. The initial effect of thrombin might be to alter the conformation of the platelet membrane which in turn results in release of TSP, inactivation of adenyl cyclase, the platelet release reaction, and platelet aggregation. The fact that TSP release and adenyl cyclase inhibition by thrombin require an intact membrane suggests that these reactions may be complex and involve the interactions between multiple components of a structurally and functionally intact membrane system. There are several mechanisms by which TSP and adenyl cyclase may be related. Thrombin might act primarily to release TSP resulting in structural changes in the platelet membrane which in turn lead to inactivation of adenyl cyclase by any of several mechanisms including inhibition by throm-

bin or some other membrane component. The possibility that TSP is a part of adenyl cyclase has not been excluded although released TSP does not have any adenyl cyclase activity (i.e. there is no adenyl cyclase activity in incubation supernatants after thrombin treatment of intact platelets). Alternatively, the primary effect of thrombin might be to inactivate adenyl cyclase in the platelet membrane. The subsequent change in concentrations of cAMP in the membrane might secondarily lead to release of TSP. The parallel protection of adenyl cyclase activity and TSP release by agents which maintain cAMP levels (DBcAMP, PGE$_1$, and theophylline) would support this mechanism. This hypothesis can also be tested further by using other agents known to cause a fall in platelet cAMP levels such as epinephrine or collagen. Finally it is possible that TSP release accompanies platelet aggregation from any cause. Thus, while platelet aggregation may follow adenyl cyclase inactivation under physiological conditions, other nonphysiologic aggregating agents may also release TSP, since we have recently shown that erythrophytohemagglutinin from kidney beans causes platelet aggregation and releases TSP. In these experiments there was marked platelet aggregation but inconsistent release of TSP and decrease in adenyl cyclase activity ranging from 0–50%.

Recently we have isolated TSP both from platelet membranes and from platelet incubation medium after TSP release by thrombin.[3] The protein has been partially characterized and its relation to platelet function in coagulation is under study.

ACKNOWLEDGMENTS

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