Defective Platelet-Fibrinogen Interaction in Hereditary Canine Thrombopathia

By James L. Catalfamo, Sharon L. Raymond, James G. White, and W. Jean Dodds

A unique, intrinsic, hereditary canine platelet disorder attributable to abnormal fibrinogen receptor availability is described. Thrombopathic platelets from 13 severely affected basset hounds failed to aggregate in response to all agonists tested except thrombin. Normal platelet interaction with the various stimuli was inferred on the basis of their ability to elicit unimpaired shape change in thrombopathic platelets. No quantitative differences in major platelet membrane glycoproteins, intraplatelet fibrinogen, adenosine nucleotides, or serotonin uptake were detected. Dense granule secretion was impaired. The ultrastructural appearance of thrombopathic platelets was normal. Fibrinogen-platelet interaction was evaluated by reacting platelet-rich plasma (PRP) with fibrinogen coupled to polymeric acrylonitrile beads and scoring the extent of stimulus-induced agglutination. The aggregatory responses of normal and thrombopathic platelets were closely correlated with fibrinogen receptor availability. In contrast to human platelets, epinephrine-stimulated canine platelets did not interact with immobilized fibrinogen, and arachidonate generally induced only weak agglutination. Thrombopathic platelets agglutinated fibrinogen beads at reduced rates when stimulated with physiologic doses of thrombin and high-dose calcium ionophore, A23187. Our data suggest that thrombin-mediated induction of canine platelet fibrinogen receptors may proceed by pathway(s) alternate to those shared by other platelet agonists, and/or that secreted granule constituents may act synergistically with thrombin to overcome inhibition of signal-response-coupled reactions mediating the interaction of fibrinogen with its receptor. This congenital platelet defect provides further evidence, in a species other than human, for the pivotal role of fibrinogen receptor induction in platelet aggregation.

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solved in 0.15 mol/L of NaCl; SA was dissolved in 0.15 mol/L Tris-O.15 mol/L NaCl, pH 7.4, and diluted prior to use. SA was used to represent 100% aggregation. In a typical experiment, 0.45 mL of PRP (300,000/mL) was pipetted into each 1-mL siliconized glass cuvette and warmed to 37 °C for one minute with stirring (850 rpm); then 0.05 mL of the appropriate agonist was added. When MeSO was the agonist solvent, not more than 5 μL was added to the platelet suspension. The extent of platelet aggregation was measured after 3 minutes. When dual stimuli were used, they were applied one minute apart.

Formation of microaggregates in response to extremely high doses of ADP (>150 μmol/mL) was confirmed by phase-contrast microscopy. Shape change events were monitored photometrically and verified by electron microscopy. [14C]-Serotonin was used as a marker for release of dense granule constituents, and release was measured essentially as described by Jerushalmi and Zucker, with inclusion of a formalin fixation step prior to centrifugation to minimize reported secretion artifacts associated with rapid centrifugation of A23187-treated platelets. Imipramine (3-10 μmol/mL) was also included to inhibit serotonin reuptake.

Retention of canine platelets on glass bead surfaces was measured by using whole blood as previously described. The average number of platelets eluted in the fourth and fifth milliliter of blood was used to calculate percentage of retention. Collagen-platelet interaction was evaluated by using noncovalently immobilized collagen. The ability of thrombopathic platelets to support normal clot retraction was determined according to the method of Taylor and Muller-Eberhard.

Electron microscopy. For ultrastructural studies, citrated PRP (300,000/μL) was warmed with stirring (850 rpm) for 1 minute in an aggregometer; then 50 μL of the appropriate agonist or vehicle was introduced. The samples were fixed by rapidly adding one part 0.1% glutaraldehyde to the cuvette when the point of maximal optical density (platelet shape change) was reached. After ten minutes, the platelets were pelleted (800 g for five minutes); the supernatant was decanted; 1 mL of 3% glutaraldehyde was added; and the samples were sent to Dr James G. White, University of Minnesota, for processing.

Adenine nucleotides. ADP and ATP were quantitated in etha-nolic extracts of EDTA PRP by the firefly luciferase method of Holmsen et al using an Amino microphotometer (American Instruments Co, Silver Springs, Md) interfaced with a digital integrator.

Platelet fibrinogen. The amount of fibrinogen in normal and thrombopathic platelets was quantitated by rocket immunoelectrophoresis. The procedure of Nurden was adapted to measure canine platelet fibrinogen. In brief, washed normal and thrombopathic platelets (2 x 10^5 cells) were resuspended in 200 μL 0.038 mol/L of Tris and 0.1 mol/L of glycine, pH 8.6, containing 1% (vol/vol) Triton X-100 and extracted for 30 minutes at 4 °C. Triton insoluble material was removed by centrifugation. The extracts were then electrophoresed as detailed in Fig 1. The assay was standardized using canine fibrinogen. Peak heights for fibrinogen standards were linear with respect to fibrinogen concentration from 50 to 500 ng per well. Assays were performed in duplicate. A standard curve was constructed from which platelet fibrinogen levels were determined. Maximal receptor sharpness using canine antigen with rabbit anti-human fibrinogen immunoglobulins could be achieved only when the plate temperature was maintained at 28 °C during electrophoresis. Paired normal and thrombopathic platelet extracts were run on the same plates.

Fibrinogen receptor interaction. Canine fibrinogen was isolated from ACD plasma as described by Regoecci and Stannard and was further purified to remove possible traces of plasminogen, fibronectin, and factors VIII and XIII. Highly purified canine fibrinogen, judged to be free of trace contaminants by sensitive and specific methods described elsewhere, was 95% thrombin-clottable. When
In preliminary studies, crude (Kabi) or purified human fibrinogen was used. Beads to which BSA was similarly coupled served as controls. The extent of agglutination after platelet exposure to a stimulus was scored at three minutes, and with some agonists, at 15 seconds on a scale of 0 to 4+. A slight grainy reaction was considered 1+; a 4+ value was assigned to samples in which the beads were in relatively few but large clumps. Canine platelet interaction with bead-immobilized human fibrinogen was similar to that observed for canine fibrinogen.

Platelet glycoproteins. Washed normal or thrombopathic platelets were resuspended, solubilized, and reduced (5% vol/vol 2-mercaptoethanol) as described for human platelets.\(^{48}\) Nonreduced and reduced samples containing 100 to 400 \(\mu\)g of protein were analyzed on 6% or 7% polyacrylamide slab gels, overlaid with a 3.5% stacking gel and electrophoresed according to Laemmli,\(^{57}\) and fixed, washed, and stained with periodic-acid Schiff (PAS) reagent\(^{21}\) as detailed by Clemetson.\(^{53}\) Orosomucoid, 5 \(\mu\)g per sample, was used as an internal glycoprotein standard. Gels were scanned at 550 nanometers using a model 2400 Gilford spectrophotometer. Some gels were counterstained with Coomassie brilliant blue R 250.

Washed normal human, canine and thrombopathic platelets were also solubilized and phase extracted with Triton X-114 to obtain detergent phase fractions selectively enriched for integral membrane proteins (predominantly \(\alpha\)IIb and \(\alpha\)IIa). Reduced and nonreduced SDS-solubilized detergent phase proteins, 25 to 100 \(\mu\)g per lane, were electrophoresed, stained (PAS), and scanned under identical conditions and often on the same slab gel as SDS-solubilized platelets.

RESULTS

Aggregation. Aggregation profiles typical for severe hereditary canine thrombopathia are presented in Fig 2. Stimulation of thrombopathic platelets with physiologic concentrations of ADP, collagen, or SA failed to induce aggregation. Unlike human platelets, canine platelet sensitivity to exogenous arachidonate was variable, as others have pre-

![Fig 2. Typical aggregation profiles obtained for normal (control) and thrombopathic canine platelets in response to (A) ADP, (B) sodium arachidonate, (C) collagen, or (D) \(\gamma\)-thrombin. Aggregation is expressed as a function of increasing light transmission. Platelet activation was initiated by adding 50 \(\mu\)L of agonist (I) to citrated platelet-rich plasma (300,000/\(\mu\)L). Platelet response to \(\alpha\)-thrombin was evaluated in gel-filtered platelets (300,000/\(\mu\)L) without added fibrinogen (inset). The tracings shown are from a single experiment and are representative of results obtained for each severely affected dog (\(N = 13\)) and an equal number of controls. Occasional microaggregate formation in response to high-dose ADP (\(>50 \mu\)mol/L) was observed.](image-url)
DEFECTIVE PLATELET-FIBRINOGEN INTERACTION

Previously reported.55-57 Normal basset hound platelets (N = 15) responded to SA with consistent weak reversible aggregation. Heparinized (10 U per milliliter) PRP was used in a few experiments; the aggregation profiles obtained for all agonists were similar to those shown for the citrated system.

In marked contrast to the other physiologic stimuli, thrombin induced aggregation of thrombopathic platelets. When quantitated with GFP, thrombopathic platelets aggregated to the same maximal extent as their normal counterparts over a wide range of thrombin concentrations (Fig 3A). However, the rate of the response was impaired (Fig 3C). In heparinized-GFP, no significant differences in the rate of α-thrombin-induced aggregation were observed. This may be due to the reported proaggregatory effects of heparin.58-59 Bell and co-workers and Patterson and co-workers60,61 have reported that thrombopathic platelets fail to aggregate in response to thrombin; however, they used a single dose of thrombin (0.2 U per milliliter PRP).

In PRP, stimulation of thrombopathic platelets with high-dose calcium ionophore A23187 failed to induce aggregation (Fig 4). In GFP made 1 mmol/L with respect to CaCl₂ (Fig 4; Inset) aggregation occurred but only in response to ionophore doses eight- to tenfold higher than required by normal platelets (Fig 3B). Likewise, the rate of aggregation was markedly impaired (Fig 3D).

In other experiments, increasing the concentration of extraplatelet ionic calcium had no demonstrable effect on the reactivity of thrombopathic platelets, even in response to agonist concentrations 20- to 50-fold higher than those shown. Likewise, the severe platelet dysfunction could not be corrected by resuspending Stratcan-washed platelets for up to two hours in normal canine plasma. When equal volumes of normal and thrombopathic PRP (300,000 platelets per microliter) were mixed and incubated for up to 10 minutes at 37 °C, the presence of thrombopathic platelets had no effect.
on the aggregatory response of normal platelets stimulated with ADP, collagen or γ-thrombin.

**Shape change and morphology.** Thrombopathic platelets underwent dose-dependent shape change in response to all stimuli. For all agonists examined, the onset and degree of shape change (quantitated photometrically on the basis of slope and extent of increased light scatter) paralleled those observed for normal canine platelets.

Agregrometric evidence for shape change events was confirmed by ultrastructural analysis of platelets fixed at the point of maximal light scatter (Fig 2, open arrow). Morphological changes consistent with shape change were evident upon platelet stimulation. Platelet ultrastructural reorganization in response to ADP, collagen, or thrombin was similar. The general features of thrombopathic platelet morphology appeared normal. In comparison to normal canine platelets, thrombopathic platelets appeared to be the same size and to contain the same number of dense bodies and other organelles, whose size and shape were also within normal limits.

**Potentiation by epinephrine.** The sensitivity of thrombopathic platelets to ADP appeared to be enhanced immediately after the animals were exposed to the mild stress of a change in kennel environment. Stress-induced activation of the sympathoadrenal system is often associated with an increase in epinephrine secretion. Because epinephrine has been proposed as a "gain controller" in canine platelet function, due to its ability to increase markedly without apparent activation, platelet sensitivity to various agonists, we examined its possible potentiating effect on thrombopathic platelets. Epinephrine (5.4 μmol/L) did not activate thrombopathic platelets (Fig 5) or normal controls (not shown). It has no apparent potentiating effect on the response of thrombopathic platelets to collagen. However, preexposure to epinephrine enhanced the sensitivity of thrombopathic platelets to physiologic concentrations of ADP. Such platelets reacted with irreversible aggregation; although at rates four- to fivefold lower than those of non-epinephrine-exposed, ADP-stimulated, normal canine platelets. For thrombopathic platelets, aggregation rates approaching normal could not be achieved even when epinephrine and ADP concentrations were increased to 20 and 150 μmol/L, respectively.

**Serotonin release.** When the release reaction of thrombopathic platelets was evaluated in PRP (Table 1), <6% of the total platelet 14C-serotonin was secreted in response to collagen (100 μg per milliliter of PRP) or calcium ionophore A23187 (40 μmol/L). Thrombin at 5 U per milliliter of PRP induced secretion exceeding 70%. ADP (8 to 10 μmol/L)-induced secretion was variable for individual thrombopathic dogs, ranging from 2% to 20%. Release was maximal at 30 seconds in contrast to platelets from normal dogs which maximally released (<6%) 14C-serotonin between one to three minutes. At high ADP concentrations (68 to 100 μmol/L), secretion by thrombopathic platelets was impaired. For 12 dogs examined, the early 14C-serotonin release induced by ADP represents, at its highest, less than one-third of the total secretable pool. This atypical-release response was also observed in thrombopathic platelets by Patterson and colleagues. They suggested that thrombopathic platelets secrete the bulk of their dense granule constituents in response to 10 μmol/L of ADP and that the early release of large amounts of platelet ATP may explain why thrombopathic platelets fail to aggregate. The fact that several of the bassets (N = 5) in this study released <4% of their 14C-serotonin in response to 10 μmol/L of ADP and failed to aggregate does not support this hypothesis.

Dose–response curves typical for 14C-serotonin release by normal and thrombopathic GFP in response to α-thrombin and calcium ionophore A23187 are shown in Fig 3E and F. No significant differences in release were observed over a wide range of thrombin concentrations. In contrast, A23187 induced maximal secretion by thrombopathic platelets only.

### Table 1. Serotonin Release in Citrated PRP Obtained From Normal and Thrombopathic Dogs

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Final Concentration</th>
<th>Normal</th>
<th>Thrombopathic</th>
</tr>
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<tbody>
<tr>
<td>ADP</td>
<td>8–10 μmol/L</td>
<td>4 ± 2</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>Collagen</td>
<td>68–100 μmol/L</td>
<td>51 ± 9</td>
<td>23 ± 11</td>
</tr>
<tr>
<td>α-Thrombin</td>
<td>100 μg</td>
<td>66 ± 9</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>γ-Thrombin</td>
<td>5 U</td>
<td>75 ± 3</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>A23187</td>
<td>0.12 μmol/L</td>
<td>81 ± 9</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>A23187</td>
<td>40 μmol/L</td>
<td>50 ± 16</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

PRP, platelet-rich plasma.

*14C-Serotonin uptake, after 30 minutes, 21 °C, was 92% ± 2% and 91% ± 1% for normal and thrombopathic platelets, respectively; release at three minutes.

†Canine platelets exhibit irreversible aggregation in response to doses of ADP ranging from 8 to 12 μmol/L.
at ionophore concentrations three- to fivefold higher than required by normal canine platelets.

Adenine nucleotides. The abnormal release reaction of thrombopathic platelets could not be attributed to low concentrations of platelet adenine nucleotides. No significant differences in either ADP, 1.44 ± 0.34 and 1.42 ± 0.37 μmol/10^11 platelets or ATP, 7.07 ± 0.91 and 6.75 ± 45 μmol/10^11 platelets were detected for thrombopathic (N = 8) and normal (N = 5) platelets, respectively.

Platelet fibrinogen. Total platelet protein and platelet fibrinogen levels were also determined. Intraplatelet fibrinogen, as illustrated for a typical experiment (Fig 1), was quantitated by rocket immunoelectrohoresis. No significant differences were found in total platelet protein, 1.73 ± 0.21 and 1.62 ± 0.27 mg/10^9 platelets, or platelet fibrinogen, 60 ± 12 and 56 ± 13 μg/10^9 platelets, for normal (N = 5) and thrombopathic (N = 12) platelets, respectively.

Retention, adhesion, and clot retraction. Functional parameters of the basic platelet reaction other than aggregation were also evaluated. No significant differences were observed between normal and thrombopathic dogs (N = 6) in percentage of platelet adhesion to fibrillar type I collagen, 51% ± 6% and 52% ± 5%, or their ability to support normal clot retraction, 90% ± 6% and 94% ± 3% retraction, respectively. However, platelets from thrombopathic dogs exhibited a marked impairment in their ability to react with and be retained by glass bead columns, 10% ± 10% 94% ± 3% retention for normal platelets; despite normal levels (90% to 120% control) of factor VIII-related antigen.

Fibrinogen receptor interaction. The abnormal retention of thrombopathic platelets on glass-bead surfaces prompted us to assess the interaction of these platelets with canine fibrinogen. The bead agglutination technique of Coller et al. was used to evaluate receptor availability. Results obtained for platelets from arachidonate-insensitive normal or thrombopathic dogs are presented in Table 2.

Unstimulated normal platelets failed to interact with fibrinogen. Stimulation of normal platelets with 10 μmol/L of ADP, 0.1 U per milliliter of PRP α-thrombin, 32 nmol/L of γ-thrombin, 40 μmol/L of A23187, but not 5.4 μmol/L of epinephrine, induced maximal fibrinogen–platelet interaction. In contrast, thrombin and high-dose ionophore were the only agonists capable of initiating fibrinogen receptor availability in thrombopathic platelets. The rate of response (agglutination after 15 seconds) for both agonists was impaired. Stimulation with a combination of epinephrine and ADP induced receptor availability; however, the extent of agglutination was consistently less than that for normal platelets exposed to ADP alone.

Platelet glycoproteins. Normal platelet interaction with fibrinogen requires the presence of GPIIb and GPIIIa in normal amounts. Thrombopathic platelet glycoproteins were therefore analyzed by SDS-PAGE (Fig 6). No quantitative differences in major platelet glycoproteins were detected. Under identical extraction conditions, no differences were observed in the protein content of detergent phases from human, normal canine, or thrombopathic platelets. Mobilities of PAS-staining bands in this fraction (predominantly IIB and IIIa) were identical to glycoprotein reactive bands from whole platelets. Canine PAS reactive bands designated as IIB and IIIa (Fig 6, arrows) comigrated with human IIB and IIIa and had relative mol wts (reduced and nonreduced) similar to those of their human counterparts.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Normal Agglutination</th>
<th>Thrombopathic Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>ADP [10 μmol/L]</td>
<td>4+</td>
<td>±</td>
</tr>
<tr>
<td>+ EDTA (10 mmol/L)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ Fibrinogen (5 mg/mL)</td>
<td>2+</td>
<td>±</td>
</tr>
<tr>
<td>+ Apyrase (2.0 mg/mL)</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>+ Epinephrine (5.4 μmol/L)</td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td>Epinephrine (5–20 μmol/L)</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>α-Thrombin (0.1 U/mL)</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>+ Apyrase (0.5 mg/mL)</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Arachidonate (0.125 mmol/L)</td>
<td>2+</td>
<td>±</td>
</tr>
<tr>
<td>+ Epinephrine (5.4 μmol/L)</td>
<td>4+</td>
<td>±</td>
</tr>
<tr>
<td>γ-Thrombin (32 nmol/L)</td>
<td>(4+)</td>
<td>(3+)</td>
</tr>
<tr>
<td>(16 nmol/L)</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>(8 nmol/L)</td>
<td>(1+)</td>
<td>(2+)</td>
</tr>
<tr>
<td>A23187 (120 μmol/L)</td>
<td>(4+)</td>
<td>(1+)</td>
</tr>
<tr>
<td>(80 μmol/L)</td>
<td>(4+)</td>
<td>(1+)</td>
</tr>
<tr>
<td>(40 μmol/L)</td>
<td>(4+)</td>
<td>(1+)</td>
</tr>
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</table>

Normal dogs were arachidonate insensitive.
*Scoring after three minutes at room temperature. Scores in parenthesis are after 16 seconds. Maximal agglutination shown.
†GP IIb was reacted with immobilized fibrinogen after the thrombin was neutralized with excess hirudin. Hirudin alone did not inhibit ADP-stimulated platelet reactivity with immobilized fibrinogen.
‡Required warming reaction mixture to 37 °C.

Table 2. Agglutination of Canine Fibrinogen Beads by Normal and Thrombopathic PRP

DISCUSSION

Canine thrombopathia appears to be a unique, autosomal inherited platelet disorder without a recognized human counterpart. Unlike platelets from human type I Glanzmann’s thrombasthenia, thrombopathic platelets aggregated in response to thrombin; exhibited impaired dense granule secretion; supported normal clot retraction; and had normal amounts of PAS-reactive GPIIb-GPIIIa and intraplatelet fibrinogen. No support for the proposal that thrombopathy may be a model for the even rarer type II form of Glanzmann’s thrombasthenia was obtained.

The failure of a wide array of platelet stimuli to induce aggregation, dense granule secretion, and fibrinogen receptor exposure suggests that the defect occurs at a locus or loci subsequent to agonist receptor occupancy. Indirect support for this is provided by the unaltered shape-change response of thrombopathic platelets. That thrombopathic membranes have normal collagen receptor density was demonstrated by our studies of platelet adhesion to immobilized collagen.
and retention—were abnormal, we examined the ability of these platelets to interact with fibrinogen.

Fibrinogen–platelet interactions were evaluated using the bead agglutination technique of Coller. This assay is sensitive and specific for fibrinogen receptor availability (fibrinogen binding sites). Although it does not provide the detailed kinetic data concerning fibrinogen–platelet interactions obtainable using 125I-radiolabeled fibrinogen, it had the advantage of allowing us to evaluate platelet-fibrinogen interaction in a large number of normal and thrombopathic dogs using an array of agonists under various experimental conditions. The essential features of normal canine platelet interaction with bead-immobilized fibrinogen were similar to those reported for human platelets. Platelet stimulation was required for receptor availability. Interaction required extra-platelet divalent cations, presumably for the maintenance of GPIIb–GPIIIa complexes. In contrast to human platelets, epinephrine-stimulated canine platelets did not interact with immobilized fibrinogen, and arachidonate induced weak agglutination. In the presence of apyrase, ADP-induced receptor expression was reduced whereas thrombin-induced exposure was unaffected. Fibrinogen binding sites were exposed on thrombopathic platelets only in response to thrombin and high-dose calcium ionophore. The possibility that the thrombin response was an artifact related to fibrin formation was considered; however, it seemed unlikely since γ-thrombin-stimulated thrombopathic platelets agglutinated bead-immobilized fibrinogen whereas γ-thrombin in the absence of platelets had no effect. For both thrombin and calcium ionophore, the rate of receptor induction was impaired, which correlated with the impaired aggregation rates seen in GFP for both agonists.

Electrophoretic analysis of thrombopathic platelets failed to demonstrate significant quantitative differences in the major platelet glycoproteins, in particular GPIIb–GPIIIa. Patterson and co-workers reported similar results. They also demonstrated normal levels of the GPIIb–GPIIIa complex, thus distinguishing canine thrombopathia from a recently described variant of human Glanzmann’s disease with reduced levels of the complex.

Stimulation of platelets with thrombin or ADP causes an increase in intracellular ionized calcium, which is a prerequisite for the secretory and aggregatory responses of platelets. The increase appears to result from a release of Ca2+ from intracellular sites and influx of extracellular Ca2+. In addition, 1,2-diacylglycerol and inositoltriphosphate may serve as intracellular messengers. Thrombin and collagen induce phosphoinositol breakdown and lead to a transient increase in cellular 1,2-diacylglycerol. It has been proposed that Ca2+ and 1,2-diacylglycerol act as coordinate second messengers in platelet activation by greatly increasing the affinity of protein kinase C for Ca2+. Thrombin stimulation results in an increase in both internal ionized calcium and 1,2-diacylglycerol, whereas collagen increases only 1,2-diacylglycerol and has no apparent effect on the concentration of intracellular ionized calcium. Although Ca2+ acts as an activator second messenger, cAMP can serve as an inhibitory second-messenger that antagonizes the
mobilization of Ca$^{2+}$. It is also known that cAMP inhibits the hydrolysis of inositol lipids. The impaired responsiveness of thrombopathic platelets to Ca$^{2+}$ ionophore A23187 implies that Ca$^{2+}$ mobilization and/or utilization may be abnormal. This is consistent with our recent observation of elevated cAMP levels in thrombopathic platelets. It appears probably that mobilized Ca$^{2+}$ is rapidly sequestered into intracellular storage sites. The potentiation by epinephrine of ADP-induced receptor exposure and aggregation is likely related to inhibition of adenylate cyclase in thrombopathic platelets.

The ability of thrombin and high-dose ionophore to initiate thrombopathic platelet fibrinogen receptor availability, although at impaired rates, may be due to their ability to initiate generation of coordinate second messenger(s) which serves to lower requirements for ionic calcium in reactions leading to platelet activation. The failure of thrombopathic platelets to secrete dense granule constituents and express fibrinogen binding sites appears to be related to abnormal second-messenger signal generation. Because the details of reactions leading to expression of fibrinogen binding sites on GPIIb-GPIIa complexes have yet to be established, thrombopathic platelets may serve as a model system for the assignment of functional significance to biochemical events implicated in receptor induction.

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