Inhibition of Collagen-Induced Platelet Activation by 5'-p-Fluorosulfonylbenzoyl Adenosine: Evidence for an Adenosine Diphosphate Requirement and Synergistic Influence of Prostaglandin Endoperoxides


The relative roles of platelet autacoids such as adenosine diphosphate (ADP), prostaglandin endoperoxides, and thromboxane A2 (TXA2) in collagen-induced platelet activation are not fully understood. We reexamined this relationship using the ADP affinity analogue, 5'-p-fluorosulfonylbenzoyl adenosine (FSBA), which covalently modifies a receptor for ADP on the platelet surface, thereby inhibiting ADP-induced platelet activation. Collagen-induced shape change, aggregation, and fibrinogen binding were each fully inhibited under conditions in which FSBA is covalently incorporated and could not be overcome by raising the collagen used to supramaximal concentrations. In contrast, TXA2 synthesis stimulated by collagen under conditions that produced maximum aggregation was only minimally inhibited by FSBA. Since covalent incorporation of FSBA has been previously shown to specifically inhibit ADP-induced activation of platelets, the present study supports the contention that ADP is required for collagen-induced platelet activation. Under similar conditions, indomethacin, an inhibitor of cyclooxygenase, inhibited collagen-induced shape change, indicating that endoperoxides and/or TXA2 also play a role in this response. Shape change induced by low concentrations (10 nmol/L) of the stable prostaglandin endoperoxide, azo-PGH2, was also inhibited by FSBA. These observations indicate a role for ADP in responses elicited by low concentrations of endoperoxides. However, at higher concentrations of azo-PGH2 (100 nmol/L), inhibition by FSBA could be overcome. Thus, the effect of collagen apparently has an absolute requirement for ADP for aggregation and fibrinogen binding and for both ADP and prostaglandins for shape change. Aggregation and fibrinogen binding induced by prostaglandin endoperoxides also require ADP as a mediator, but ADP is not absolutely required at high endoperoxide concentration to induce shape change.

FOLLOWING INJURY to endothelial cells and exposure of subendothelium, blood platelets adhere to the newly exposed collagen and undergo a phenomenon called shape change.1 Shape change consists of a disk-to-sphere transformation and the extrusion of long, thin pseudopodia. Adenosine diphosphate (ADP) and thromboxane A2 (TXA2) released by the adherent platelets recruit additional platelets to form aggregates.2 The aggregated platelets then secrete part of the contents of their granules and together with fibrin form the tightly fused mass known as the hemostatic plug.

Since the identification of collagen as the active component of the connective tissue involved in hemostasis was made by Zucker and Borrelli,3 numerous investigators have attempted to decipher the nature of the collagen-platelet interaction. Many studies have focused on the structural requirements of the collagen molecule to stimulate platelet aggregation and the release reaction,7 and at least one study has focused on shape change,4 the most proximate activation event. Most investigators have concluded that the quaternary structure of collagen is critical for the platelet-collagen interaction. This study focuses on another important aspect of collagen-induced activation, the nature of the intercellular and intracellular mediators such as released ADP, newly synthesized prostanoid derivatives. These mediators have been implicated in the cellular responses of activation, including shape change8-13 and the exposure of fibrinogen binding sites.14 The recent demonstration in our laboratories that ADP is required for aggregation15,16 but not shape change15 mediated by cyclic endoperoxides/TXA2 resulted in a reexamination of the mechanism by which collagen may activate platelets.

5'-p-Fluorosulfonylbenzoyl adenosine (FSBA) has previously been used to explore many ADP-dependent interactions.17 FSBA does not inhibit all ADP-dependent reactions; for instance, it has no effect on ADP-induced inhibition of adenylyl cyclase in platelets prestimulated by prostaglandin E1 (PGE1).18 However, its suitability for the study of platelet activation mechanisms rests on the following lines of evidence. FSBA covalently modifies a single polypeptide (molecular weight [mol wt] = 100,000) on the membrane surface of intact platelets.19 Preceded by an electrophilic substitution and resulting in sulfonylbenzoyl adenosine (SBA)-labeled protein, this covalent incorporation parallels the inhibition of ADP-induced platelet shape change.19,20 platelet aggregation,20 and fibrinogen binding site exposure.20 A related affinity analogue with the purine base guanosine substituted for adenosine, 5'-p-fluorosulfonylbenzoyl guanosine (FSBG), has no effect on ADP-induced shape change.18 Further, FSBA inhibition of prostaglandin endoperoxide-induced aggregation parallels the activity of the ADP scavenger system—apyrase and creatinine phosphate/creatinine phosphokinase.13 Thus, the affinity analogue FSBA is an appropriate probe to further investigate the role of ADP in collagen-induced platelet activation.

MATERIALS AND METHODS

Materials. FSBA was prepared by the method of Colman et al.17 The prostaglandin endoperoxide analogue, 9,11-azo-PGH2, was

From the Thrombosis Research Center and the Hematology-Oncology Section of the Department of Medicine, Temple University School of Medicine, Philadelphia. Supported by research Grants HL14217, HL36359, and HL33337 from the National Institute of Heart, Lung and Blood. Presented in part at the 74th Annual Meeting of the American Society of Biological Chemists, San Francisco, June 5 to 9, 1983, and was published in abstract form in 1983. Federation Proceedings 42:1840.

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Address reprint requests to Dr Robert Colman, Thrombosis Research Center, Temple University School of Medicine, 3400 North Broad St, Philadelphia, Pa 19140.

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obtained from Dr K.C. Nicolau (Department of Chemistry, University of Pennsylvania, Philadelphia). Equine collagen fibrils were purchased from Hormon-Chemie, Munich. Human fibrinogen was purchased from Kabivitrum, Stockholm. 125I was purchased from New England Nuclear, Boston. Bovine serum albumin, indomethacin, and calf intestinal adenosine deaminase were purchased from Sigma Chemical Co, St Louis. All other reagents were of reagent grade or better.

Platelet isolation. Platelet-rich plasma (PRP) 4 to 6 10^9 platelets/mL was prepared by differential centrifugation of fresh whole human blood (120 g, 20 minutes, 23 °C). Volunteers claimed to have abstained from taking aspirin for 2 weeks prior to donation. Informed consent was obtained. The blood was drawn into a 3.8% sodium citrate solution at a ratio of 5 mL anticoagulant per 45 mL blood. Suspensions of washed platelets were prepared by the method of Mustard et al. After the final wash, the platelets were resuspended in a medium containing Na+ (130 mmol/L), K+ (2.7 mmol/L), Mg2+ (2 mmol/L), Cl− (130 mmol/L), HCO3− (12 mmol/L), PO43− (1 mmol/L), HEPES (10 mmol/L), glucose (560 mmol/L), and bovine serum albumin (3.5 mg/mL), at pH 7.4 and a platelet count of 5 to 8 10^9/mL. For TXA2 synthesis experiments, platelets were washed by the method of Mills et al. Calcium (2 mmol/L) was added for the fibrinogen-binding experiments.

Platelet aggregation and shape change. Aggregation was measured in an aggregometer (Chronolog, Havertown, Pa). The change in optical density was recorded on a chart recorder (LKB, Stockholm); the rate of aggregation was expressed in centimeters per minute. The instrument was calibrated at 100 mV for differential light transmission between the platelet suspension and the platelet suspension medium. The platelet suspension medium was platelet-poor plasma (PPP) for experiments conducted in PRP and Tyrode’s buffer for those conducted in gel-filtered platelets (GFP). The platelet suspensions in these experiments were all stirred at 37 °C.

Shape change was measured on gel-filtered platelets diluted with an equal volume of 5 mmol/L EDTA to prevent aggregation, using a Chronolog lumia aggregometer at 37 °C. The rate of change in optical density was recorded on either an LKB recorder or a Fisher recorder and expressed as distance of pen deflection in millimeters per second after addition of an agonist. The platelet suspensions were 0.5 to 1.0 10^9 cells/mL in the cuvette.

Since FSBA potentially could form adenosine on incubation (<0.05 mol adenosine per mole FSBA), all aggregation and shape change experiments were performed in the presence of adenosine deaminase at a concentration of 2 U/mL. Inclusion of this enzyme blocked the immediate inhibitory effects by FSBA (100 µmol/L) or adenosine (400 µmol/L).

Platelet modification by FSBA. FSBA was dissolved in dimethyl formamide (DMF) to a high enough concentration so that the final concentration of DMF added to the platelets was less than 2.5%. Adenosine deaminase (final concentration, 2 U/mL) was first added to the platelet suspensions of 2.4 10^9 platelets/mL prior to the addition of the FSBA-DMF solution. The mixture was incubated for 20 minutes at 37 °C. DMF in equivalent amounts was added to all control samples. At the conclusion of the incubation, the platelets were sedimented (11,100 g, 20 minutes, 23 °C) and resuspended in fresh Tyrode’s buffer. To ensure that the platelets were refractory to ADP, we challenged an aliquot of the platelets with 10 µmol/L ADP and measured aggregation and shape change. Only platelets that failed to aggregate or undergo shape change were used. Concentrations of FSBA required to produce complete refractoriness in different donor platelets varied from 40 to 100 µmol/L, and the time at which complete inhibition of shape change occurred varied from 15 to 60 minutes. Each experiment represents trials performed in duplicate from the platelets of a single donor and is representative of at least three separate donors.

Preparation of 125I-fibrinogen. Human fibrinogen radiolabeled with 125I was prepared as described by Figures et al.

Fibrinogen binding to platelets. Specific binding of 125I-fibrinogen to platelets was carried out using a separation technique of centrifugation through silicone oil. The range of 125I-fibrinogen concentrations used (5 to 80 µg/mL) was chosen to saturate high-affinity binding sites on the platelet surface. Incubation mixtures containing 125I-fibrinogen, EDTA, collagen, and FSBA were layered on silicone oil in microcentrifuge tubes, and the platelets were sedimented through the oil layer at 12,000 g for three minutes. The tips of the centrifuge tubes containing the platelet pellet were severed from the rest of the tubes and were counted in a gamma counter (Intertechnique, Baltimore) to yield the total amount of fibrinogen bound. Nonspecific binding of fibrinogen to the platelet surface was determined by adding 2 mmol/L EDTA to the incubation mixture to inhibit collagen-induced exposure by fibrinogen binding sites. Counts associated with the platelet pellet under these conditions were considered to be the result of nonspecific fibrinogen binding. Specific binding was calculated as the difference of total binding and nonspecific binding. Results presented here represent specific fibrinogen binding. All experiments were carried out at 37 °C.

Measurement of TXA2. TXA2 is an unstable compound and is rapidly converted into the more stable TXB2. In the present study, TXA2 was determined by radioimmunoassay in its TXB2 form. The assay was performed using the antiserum against TXB2 as described by Lewy et al. and provided by Dr J.B. Smith (Thrombosis Research Center). Two experiments were performed; the samples were assayed in duplicate.

RESULTS

Effect of FSBA on the rate of platelet aggregation induced by collagen. Washed platelets were treated with either FSBA or an equivalent volume of DMF, the carrier solvent for FSBA, for various times prior to the addition of collagen, 4 µg/mL (Fig 1). DMF had no significant effect on the rate of aggregation by collagen. Platelets incubated with FSBA, however, exhibited an aggregation rate that progressively diminished over a 20-minute period.

![Fig 1](https://via.placeholder.com/150)

Fig 1. Time-dependent inhibition of collagen-mediated platelet aggregation by FSBA. Washed platelets were incubated with 40 µmol/L FSBA at 37 °C in the presence of adenosine deaminase (2 U/mL) after centrifugation and resuspensions (see Methods). At indicated times, a 0.5-mL aliquot was withdrawn and placed in an aggregometer. Collagen (2 µg) was then added, and the aggregation, as indicated by the deflection of the chart recorder pen, was measured. Inhibition of aggregation was complete by 13 minutes.
To ascertain whether the inhibition could be overcome by increasing the concentration of collagen, we incubated washed platelets with FSBA (Table 1) and demonstrated that no response occurred to 10 µmol/L ADP. Collagen produced a maximum response in platelets incubated with DMF at 4 µg/ml, and little further increase was noted with 40 mg/mL. FSBA totally inhibited collagen-induced aggregation at both 4 and 40 µg/mL.

**Effect of FSBA on collagen-induced exposure of fibrinogen binding sites.** Platelet aggregation was induced by 4 µg/mL collagen in the presence of concentrations of 125I-fibrinogen consistent with binding of the protein to high-affinity sites (Fig 2). The platelets were incubated with FSBA (40 µmol/L) or DMF for 20 minutes. Fibrinogen bound to platelets incubated with DMF increased proportionally to the concentration of added fibrinogen. The fibrinogen bound to platelets incubated with FSBA was markedly diminished and was similar to that observed with EDTA, which inhibits specific binding of fibrinogen.

**Effect of FSBA on TXA2 formation stimulated by collagen in aggregating platelets.** The possibility that FSBA could block any of the reactions leading to TXA2 synthesis and thus inhibit platelet aggregation and exposure of fibrinogen binding sites by collagen was then investigated. In the absence of collagen but in the presence of the amount of calcium in HEPES-buffered Tyrode’s solution (~50 µmol/L), stirred washed platelets preincubated with DMF contained TXA2 at a concentration of 38 nmol/L and did not aggregate. Following incubation with collagen (4 µg/mL), the TXA2 level rose to 260 nmol/L, coincident with maximum aggregation at two minutes. When platelets were preincubated with FSBA (100 µmol/L) for 20 minutes and stimulated with collagen (4 µg/mL), the concentration TXA2 at two minutes was 170 nmol/L.

**Effect of FSBA on the rate of platelet shape change induced by collagen.** Washed platelets were incubated with FSBA (40 µmol/L) for various times prior to the addition of 4 µg/mL collagen (Fig 3). The velocity of shape change was measured in the presence of EDTA (2 mmol/L) to inhibit aggregation. A progressive inhibition of shape change was observed with a similar time course to that of aggregation (Fig 1). Platelets incubated with DMF, the solvent from FSBA, for the same time showed no inhibition of the velocity of shape change (not shown). The platelets were then incubated with various concentrations of FSBA for 20 minutes prior to measuring the velocity of shape change induced by collagen (Fig 4). A concentration-dependent inhibition was observed that reached a maximum of greater than 88% inhibition at concentrations between 25 and 40 µmol/L. The IC50 (concentration to produce 50% inhibition of maximum rate of shape change) was approxi-

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**Table 1. Effect of Increasing Collagen Concentrations on Inhibition of Platelet Aggregation by FSBA**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration</th>
<th>Maximum Rate of Aggregation (cm/min)</th>
<th>DMF</th>
<th>FSBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>10 µmol/L</td>
<td>23.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collagen</td>
<td>4 µg/mL</td>
<td>24.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collagen</td>
<td>8 µg/mL</td>
<td>24.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collagen</td>
<td>16 µg/mL</td>
<td>29.0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Collagen</td>
<td>20 µg/mL</td>
<td>29.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collagen</td>
<td>40 µg/mL</td>
<td>28.1</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Azo-PGH2</td>
<td>1 µg/mL</td>
<td>20.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Azo-PGH2</td>
<td>2 µg/mL</td>
<td>20.8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Washed platelets were incubated with 100 µl FSBA in DMF or DMF alone for 30 minutes at 37 °C. ADP, collagen, or Azo-PGH2 at various concentrations were added and the maximum rate of aggregation recorded. Each point is the mean of duplicate determinations in three different donors.

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**Fig 2.** Effect of FSBA on collagen-mediated fibrinogen binding. Washed platelets were preincubated with FSBA (40 µmol/L) as indicated in Fig 1. These platelets were then incubated with collagen (4 µg/mL) and various concentrations of 125I-fibrinogen for 20 minutes at 37 °C. Platelets incubated with DMF were also incubated separately with EDTA (2 mmol/L) for one minute prior to adding 125I-fibrinogen. FSBA was able to reduce the fibrinogen binding to background levels as indicated by the labeling in the presence of EDTA.

**Fig 3.** Time-dependent effect of FSBA on collagen-induced shape change. Washed platelets were incubated with 40 µmol/L FSBA as in Fig 1. At the indicated times, a 0.5-mL aliquot was placed in an aggregometer, and 4 µg collagen was added. Shape change was gradually inhibited over time, with complete inhibition observed at 16.5 minutes in this experiment.
mately 14 μmol/L FSBA, similar to that reported for inhibiting ADP-induced shape change.13 The inhibition could not be reversed by concentrations of collagen as high as 40 μg/mL (Table 2).

**Effect of indomethacin on the rate of collagen-induced shape change.** At concentrations of indomethacin that inhibit cyclooxygenase (10 μmol/L), shape change was inhibited at collagen concentration from 4 to 40 μg/mL (Table 2), whereas ADP-induced shape change was not inhibited.

**Effect of FSBA on Azo-PGH2-induced platelet shape change and aggregation.** Since collagen is known to cause both release of ADP and synthesis of prostaglandin endoperoxides, the effect of FSBA on shape change stimulated by endoperoxide was assessed. Washed platelets were incubated with FSBA or DMF for various times before the addition of azo-PGH2 (10 nmol/L). In the presence of DMF, the cells changed shape at all incubation times. On incubation with FSBA, the platelet shape change with azo-PGH2 was progressively inhibited until no shape change was observed at 60 minutes (Fig 5). However, increasing concentrations of azo-PGH2 (up to 100 μmol/L) were able to overcome the inhibition of shape change by FSBA (Fig 6). In contrast, platelets preincubated with FSBA (100 μmol/L 20 min.) in the presence of adenosine deaminase and refractory to ADP failed to aggregate with even 1 μmol/L azo-PGH2 (Table 1) in agreement with a previous report.15

**DISCUSSION**

The data in this study emphasize the complexity of the interaction of agonists, intracellular autacoids, and intercel-

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**Table 2. Effect of Increasing Concentrations of Collagen on Inhibition of Platelet Shape Change by FSBA**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration</th>
<th>Maximum Rate of Shape Change (mm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMF FSBA Indomethacin</td>
</tr>
<tr>
<td>ADP</td>
<td>(μmol/L)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>2.9 0 2.65</td>
</tr>
<tr>
<td>Collagen</td>
<td>(μg/mL)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>6.4 0 0</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>7.5 0 0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>8.25 0.1 0</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>8.0 0.1 0</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>8.0 0.2 0</td>
</tr>
</tbody>
</table>

Washed platelets were incubated with 100 μmol/L FSBA in DMF or DMF alone for 30 minutes or 10 μmol/L indomethacin for 30 seconds at 37 °C. ADP or collagen at various concentrations was added, and the maximum rate of shape change recorded. Each point is the mean of duplicate determinations.

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**Fig 4.** Concentration-dependent effect of FSBA on collagen-induced shape change. Washed platelets were incubated at 37 °C with various concentrations of FSBA for 40 minutes. At the end of the incubation, 0.5 ml was placed in an aggregometer. 4 μg collagen was added, and the rate of shape change as indicated by the deflection of the chart recorder was measured. Inhibition was virtually complete at concentrations of 30 μmol/L or more.

**Fig 5.** Time-dependent effects of FSBA on azo-PGH2-induced shape change. Washed platelets were incubated with 40 μmol/L FSBA (○) or with DMF (×). Aliquots (0.5 ml) were removed at various times, and 10 nmol/L azo-PGH2 was added in the aggregometer. After 60 minutes the azo-PGH2-induced shape change was completely inhibited.

**Fig 6.** Concentration-dependent effects of azo-PGH2-induced shape change on FSBA-labeled platelets. Washed platelets were incubated with 40 μmol/L FSBA at 37 °C. After 80 minutes, 0.5-ml aliquots were removed, and increasing concentrations of azo-PGH2 were completely overcome by an azo-PGH2 concentration at 100 nmol/L.
INHIBITION OF PLATELET ACTIVATION BY FSBA

The results with collagen imply a role for ADP in those collagen-induced shape change.2829 The inhibition of collagen-induced shape change shown that covalent incorporation of SBA into platelets after incubation with FSBA produces a time-dependent inhibition of collagen-induced platelet aggregation (Fig 1) and prevents collagen-induced fibrinogen binding sites (Fig 2); both effects are a function of the FSBA concentration. Since FSBA under the same conditions inhibits these same functions when stimulated by ADP, the results with collagen imply a role for ADP in those responses. FSBA also inhibits ADP-induced shape change.1819 The inhibition of collagen-induced shape change by FSBA in both a time-(Fig 3) and concentration- (Fig 4) dependent fashion implies that ADP is also required for collagen-induced shape change.

Previous studies of collagen-induced platelet aggregation have yielded controversial results as to whether ADP is required. Geratz et al20 attributed the action of collagen on platelets entirely to ADP, whereas Kinlough-Rathbone et al21 felt that ADP was only partly responsible. However, Nunn22 concluded that ADP does not play an essential role in collagen-induced aggregation. One method used to arrive at the latter conclusion was the use of adenosine to inhibit ADP effects on platelets. High concentrations of collagen (20 to 30 μg/mL) were unaffected by adenosine. However, considerable evidence exists that suggests that adenosine inhibition of platelet function is mediated through a mechanism separate from ADP stimulation.23 The characteristics of an adenosine receptor are distinct from those of any ADP receptors as evidenced by the different pattern of agonist and antagonist effects.24 In general, the inhibitory effects of adenosine seem to be mediated by stimulating adenylyl cyclase and increasing cyclic adenosine monophosphate levels,2526 and these effects can be enhanced by phosphodiesterase inhibitors.27

Nunn22 also observed that the amount of ADP released by collagen at the onset of aggregation cannot alone account for the extent of aggregation. This observation supported the theory of Packham et al,2021 which attributed collagen-induced aggregation to the effects of released ADP and newly synthesized prostaglandin endoperoxides. By combining indomethacin (to block cyclooxygeynase-catalyzed endoperoxide synthesis) and creatinine phosphate/creatine phosphokinase (to convert ADP to adenosine triphosphate [ATP], an antagonist of ADP), Packham et al2021 completely inhibited collagen-induced platelet activation. Neither agent alone completely blocked platelet activation by high concentrations of collagen. FSBA completely inhibited aggregation even by high concentrations of collagen (Table 1). The difference from the results of Packham et al2021 may have been due to small concentrations of ADP that are not removed by the ADP-depleting enzymes. In contrast, FSBA inhibited TXA2 formation induced by collagen only minimally, and its effects were mainly due to its ability to inhibit ADP binding.1820 Endoperoxide-induced platelet aggregation and fibrinogen binding was shown to require ADP by Morinelli et al,23 and this was supported by the studies of Rao et al24 using platelets with the storage pool deficiency. Thus, FSBA exerts its effect on collagen-induced platelet aggregation and fibrinogen binding by a direct effect on blocking an ADP receptor.

The effect of collagen on platelet shape change is more complex. After a short lag, both prostaglandin endoperoxides and TXA2 are produced, and ADP is released after adding the collagen. Either FSBA or indomethacin can totally inhibit shape change even at very high collagen concentrations (Table 2). This suggests that collagen-induced shape change is dependent on a synergistic role of both these autacoids. In contrast, shape change induced by very high concentrations of azo-PGH2 (100 nmol/L) could not be inhibited by FSBA,25 implying a direct effect of TXA2 and/or prostaglandin endoperoxides independent of ADP. However, in this study, we show that platelets activated by low concentrations of azo-PGH2 (10 nmol/L) are inhibited by FSBA in a time-dependent manner (Fig 5). By increasing the concentrations of azo-PGH2, FSBA inhibition was overcome (Fig 6) at a concentration of 100 nmol/L azo-PGH2. It thus appears that at low concentrations of azo-PGH2 potentiation of the response by ADP is necessary for induction of shape change.

It is interesting to note that collagen-induced TXB2 synthesis was partially inhibited by FSBA. These preliminary findings suggest that secreted ADP may play a potentiating role in thromboxane synthesis as well. Such an explanation is supported by the observations of Siess et al25 showing that ATP and enzyme systems that deplete ADP inhibit not only aggregation but also phospholipase C activation and arachidonic acid metabolism induced by endoperoxide analogues. The effects of FSBA on these pathways require further exploration.

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Inhibition of collagen-induced platelet activation by 5'-p-fluorosulfonylbenzoyl adenosine: evidence for an adenosine diphosphate requirement and synergistic influence of prostaglandin endoperoxides

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