Role of Cyclic Nucleotides in Rapid Platelet Adhesion to Collagen

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Adhesion of human platelets to type I collagen under arterial flow conditions is extremely fast, being mediated primarily by the $\alpha_{IIb/IIIa}$ integrin (glycoprotein IIb/IIIa). We have investigated the involvement of cyclic nucleotides in platelet adhesion to soluble native collagen immobilized on Sepharose beads using a new microadhesion assay under arterial flow conditions. To prevent platelet stimulation by thromboxanes and adenosine diphosphate (ADP), experiments were performed with aspirin-treated platelets in the presence of ADP-removing enzymes such as creatine phosphate/creatinine phosphokinase or apyrase. Rapid reciprocal changes in platelet adenosine 3'5'-cyclic monophosphate (cAMP) and guanosine 3'5'-cyclic monophosphate (cGMP) occurred during adhesion. cAMP levels in adherent platelets were 2.4-fold lower than in effluent platelets or in static controls, whereas cGMP levels were increased 2.4-fold.

ADHESION OF blood platelets to a damaged vessel wall is an essential early step in both hemostasis and thrombosis.1 This process must occur almost immediately, as suggested by the theoretical calculations of Born and Richardson2 and by studies using fluorescent video microscopy.3 Depending on the type and depth of injury, different components of the subendothelial tissue may interact with circulating blood platelets.4,4 In most instances, platelets adhere before the release reaction occurs, with the subsequent triggering of local aggregation. Collagen present in the vessel wall is considered to be one of the primary physiologic stimuli of this process.5 In vitro, collagen is a potent stimulant of platelet aggregation and secretion and there have been many studies of the biochemical responses that occur during collagen-induced activation.6,10 Collagen differs from other physiologic stimuli in that it exists in a nonsoluble state and can adhere to the collagen surface before secretion and aggregation take place. This initial step in the platelet-collagen interaction has been little studied and there are few data showing biochemical changes associated with adhesion.

Recently, we described a novel system to study platelet adhesion to collagen in the absence of secretion and aggregation under flow conditions similar to those observed in the microcirculation.11 In plasma, this process involves at least two platelet integrin receptors: $\alpha_{IIb/IIIa}$ (glycoprotein [GP] IIb/IIIa complex) and $\alpha_{IIb}$ (GP/IIa/IIIa complex).11,12 In plasma-free media containing $\text{Mg}^{2+}$, adhesion to collagen is mediated mainly by the $\alpha_{IIb/IIIa}$ integrin complex11,13 and is extremely rapid, with exponential half-times as short as 0.2 seconds.11

It is well known that platelet responses to various agonists can be regulated by the adenylate and guanylate cyclase pathways.14 In particular, it has been shown that collagen fibers can lower adenosine 3'5'-cyclic monophosphate (cAMP) levels in intact platelets7,11 and inhibit adenylate cyclase in platelet membranes.19,20 It has been also shown that collagen-induced aggregation is associated with increases in platelet guanosine 3'5'-cyclic monophosphate (cGMP).15,21 Physiologically, these two pathways can be stimulated by factors produced by vascular endothelium, namely, prostacyclin (PGI$_2$)22 and endothelium-derived relaxing factor, the most important component of which is nitric oxide (NO).23,24 These two agents can inhibit platelet function by increasing the concentrations of intracellular cAMP and cGMP, respectively.

Studies of the effect of PGs on platelet adhesion to collagen have yielded conflicting results.16-18,25-32 Higgs et al.25 and Weiss and Turitto27 observed that, under flow conditions, PGI$_2$ inhibits platelet adhesion to rabbit arterial subendothelium in whole blood in a dose-dependent manner. This inhibitory effect was more pronounced at higher shear rates.27 High doses of PGI$_2$ infused into rabbits reduce surface coverage and platelet attachment to the aortic subendothelium after balloon injury.28 On the other hand, several studies performed under stirred or static conditions in the presence of EDTA, ADP-removing enzymes, and cyclooxygenase and lipooxygenase inhibitors suggest that PGs either do not influence adhesion or affect it only slightly.25,29-31 Radomski et al.31 showed that adhesion of washed platelets in the presence of EDTA is inhibited only by 25% with 1 $\mu$mol/L PGI$_2$, whereas Lapetina et al.29 observed no inhibition by PGE$_1$. Similarly, Santoro,32 using a microtitre-plate assay, found that PGE$_1$ did not affect adhesion to collagen-coated plastic dishes. Smith et al.30 reported that the increase of cAMP in platelets by PGD$_2$ or forskolin did not block adhesion but, surprisingly, potentiated it. As an explanation of this behavior, it was suggested that cAMP inhibits only secondary effects, such as increases in cytoplasmic calcium induced by the released ADP, thromboxane A$_2$, or serotonin, and not
the primary response to collagen. Several investigators argue that PGs do not affect the initial (contact) phase of adhesion, but do inhibit subsequent steps related to platelet activation such as spreading, aggregation, and the release reaction. However, subsequent studies showed that cGMP may inhibit platelet function. Stimulation of soluble guanylate cyclase by NO or by sodium nitroprusside (SNP) blocks platelet aggregation and causes disaggregation of platelet aggregates. An increase in cGMP via the soluble guanylate cyclase is believed to increase cAMP levels by inhibition of the cGMP-dependent cAMP phosphodiesterase. The latter increase might then lead to the inhibition of platelet function by activation of the cAMP-dependent protein kinase. Radomski et al reported that platelet adhesion to collagen under stirred conditions is totally blocked by NO. Recently, the shear conditions, endothelial cells under flow conditions in whole blood. NO reduced platelet adhesion to the matrix of cultured endothelial cells under flow conditions in whole blood.

Most previous investigations on the role of cyclic nucleotides in collagen-platelet interactions have been performed under stirred or low shear conditions. The adhesion (contact) times being studied were usually of the order of minutes or even hours. However, in vivo platelet adhesion to the exposed subendothelium must be extremely fast. Also, because the life-times of regulators such as prostacyclin and nitric oxide in circulation are short, this suggests that regulation or changes in cyclic nucleotides during adhesion have to occur very rapidly. To our knowledge, there are no reports about the role of cyclic nucleotides for short (<1 minute) adhesion times and whether rapid changes in cyclic nucleotides may regulate adhesion.

In the present study, using our microadhesion column under conditions of arterial flow, we have found that attachment to collagen leads to reciprocal changes in cAMP and cGMP in adhering aspirin-treated platelets. We also found that rapid adhesion is inhibited up to 80% by high doses of PGE, and may be completely blocked by SNP within 5 seconds.

MATERIALS AND METHODS

Materials. PGE, PGI, SNP, aspirin, indomethacin, apyrase (grade VII; from potato), hirudin, bovine serum albumin (BSA; fatty acid free), creatine phosphokinase (CPK), creatine phosphate (CP), nordihydroguaiaretic acid (NDGA), and Sepharose 4B-200 were from Sigma Chemical Co (St Louis, MO). BrCN was from Aldrich (Milwaukee, WI). GRGDSP peptide was from Peninsula Labs (Belmont, CA). P2 (anti-GPIIb/IIIa complex) monoclonal antibody (MoAb) and Z2 (anti-GPIIb) MoAb were purchased from Amgen (Westbrook, ME). 6F15 McAb reactive with platelet membrane GPIIb/IIIa complex (a,b) was a kind gift from Dr Barry S. Coller (State University of New York, Stony Brook). Native salt-soluble type I collagen from rat skin was kindly donated by Dr Garyalian (University of Virginia, Charlottesville).

Preparation of human platelets. Human venous blood was anti-coagulated with 0.10 mol of citric acid-citrate-dextrose (ACD) buffer to a final citrate concentration of 11.5 mmol/L in whole blood, and centrifuged at 350g twice for 3 minutes and once for 5 minutes. The resultant platelet-rich plasma was gently mixed with 0.05 vol of ACD, apyrase (7.5 U/mL ADPase activity), indomethacin (1 mg/mL), and PGH2 (0.3 mg/mL) and centrifuged at 620g for 20 minutes. The platelet pellet was then washed in ACD buffer containing 0.3% BSA and apyrase (7.5 U/mL), and centrifuged again as previously described. Finally, the platelets were resuspended in a modified HEPES-Tyrode medium (137 mmol/L NaCl, 2.7 mmol/L KCI, 12 mmol/L NaHCO3, 0.42 mmol/L NaH2PO4, 5.5 mmol/L glucose, 10 mmol/L HEPES, pH 7.4) containing 2 mmol/L Mg2+, 0.15% BSA, and inhibitors of aggregation 7.5 U/mL apyrase (ADP-removing enzyme), 0.01 U/mL hirudin (thrombin inhibitor), and 500 mmol/L GRGDSP peptide (inhibitor of fibrinogen binding to activated platelets). The platelets were kept under a mixture of 5% CO2 and 95% air at room temperature and used within 4 to 5 hours after final resuspension. To help eliminate the effect of collagen-induced generation of thromboxane A2, experiments were performed with platelets treated with 100 mmol/L aspirin at 37°C. In most experiments, adhesion studies were performed by incubation of aspirin-treated platelets with ADP-removing enzyme system 40 U/mL creatine phosphokinase plus 10 mmol/L creatine phosphate or with 20 mmol/L NDGA (lipoxigenase inhibitor). Platelets were incubated with different MoAbs for 10 minutes at 37°C.

Rapid cyclic nucleotide formation by PGE or SNP under flow conditions. Stimulation of synthesis of platelet cAMP and cGMP by different concentrations of PGE1 and SNP for short reaction times (1 to 4 seconds) was tested using a quenched-flow approach. It involves pumping washed platelets and an inducing agent at 37°C through a narrow diameter tube of precise length, the distance for a given flow rate corresponds to an exact reaction time. The internal diameter of the reaction tube used in these experiments was about 0.3 mm and gave wall shear rates of 2.520 s-1 for a pumping speed of 6.7 mL s-1. The reaction was stopped with 10% (w/vol) trichloroacetic acid (TCA) in isotonic saline solution.

Adhesion assay. The continuous-flow adhesion approach was essentially as described previously. BrCN-activated Sepharose 4B beads were coated either with native salt-soluble collagen type I from rat skin or with BSA and were used as adhesive substrates in our microadhesion column. A two-syringe system using 1-mL syringes was used. One syringe containing washed platelets and the second syringe containing SNP or PGE, in isotonic NaCl or buffer were connected via teflon tubing to the microadhesion column. Their contents were immediately mixed for a maximum pre-exposure time of 3.5 seconds and pumped through the collagen beads. Flow rates were regulated by a variable-speed syringe pump from 6.7 to 2.0 μL s-1 and gave shear rates at the surface of the beads from 3,400 to 1,020 s-1. The different contact (adhesion) times were obtained by changing the amount of beads or the flow rates in the microcolumn and were calculated from the formula Q = μVf, where Q (μL s-1) represents the flow rate through the column. Adhesion to collagen was determined by counting single platelets in the suspension before and after exposure to the collagen-coated beads using a resistive particle counter (Particle Data, Elmhurst, IL), and was expressed as the percentage of platelets bound to collagen. Because nonspecific adhesion to the BSA-coated beads in an Mg2+-containing medium was 3% to 5%, the extent of platelet adhesion to collagen was corrected for this contribution at each experimental point.

To analyze the changes in concentrations of cyclic nucleotides during adhesion, we usually pumped 2 to 3 × 108 platelets through a microcolumn of 50 μL of collagen-coated beads at 3.35 μL s-1 for about 2.5 minutes. This corresponded to an average adhesion time of 1.25 minutes. The shear rate generated by this condition was
Platelets in the column effluents were treated with that had adhered to the beads resulting from both the ether extraction added as internal standards to both TCA-quenched platelets attached concentration of platelets pumped into the microcolumn.

Briefly, known amounts of radiolabeled CAMP and cGMP were varied from 1,020 to age

to 1,700

the percentage of adhesion (usually

about 1,700 s⁻¹ and gave a contact time of 1.35 seconds. Determination of the number of platelets attached to the beads was based on the percentage of adhesion (usually 50% to 60%) relative to the concentration of platelets pumped into the microcolumn.

Extraction of cyclic nucleotides for assay of cGMP and cAMP content. Both platelets attached to the collagen-coated beads and platelets in the column effluents were treated with 0.6 mL of 10% (wt/vol) ice-cold TCA in isotonic saline solution. The quenched materials were kept on ice for 30 minutes and then the platelet lysates were separated from the beads and cell debris by centrifugation for 10 minutes at 6,000g. The supernatants were extracted 4 times with anhydrous ether to remove TCA. The extent of cAMP and cGMP recovery in our experimental samples was described as described.³³ Briefly, known amounts of radiolabeled cAMP and cGMP were added as internal standards to both TCA-quenched platelets attached to collagen-coated beads and to TCA-quenched platelets in the column effluents. The samples were centrifuged and supernatants extracted with ether. The recovery of cAMP and cGMP for platelets that had adhered to the beads resulting from both the ether extraction and from cAMP or cGMP trapping by the beads was 85%. The cAMP and cGMP recovery for platelets in suspension was 90%. cGMP concentrations were determined in the resultant aqueous extracts by commercially available radioimmunoassay kits (Advanced Magnetics, Cambridge, MA) after acetylation of samples. For cAMP determination, the aqueous extracts were mixed with equal volumes of 50 mmol/L acetate buffer, pH 6.5, and the cAMP content was then measured by radioimmunoassay as described.³⁴ Values for platelet cyclic nucleotides were corrected for the recovery of cAMP and cGMP.

Statistics. Values are given as means ± SD with the number of observations given in parentheses. Means were compared by the Student’s t-test for unpaired data.

RESULTS

Basic adhesion kinetics and effects of antibodies. The adhesion kinetics of aspirin-treated platelets in plasma-free medium containing Mg²⁺, GRGDSP peptide, hirudin, and apyrase in the presence of different MoAbs are shown in Fig 1. Both the initial rate and extent of adhesion were unchanged when CP/CPK was used as ADP-removing enzyme system with or without lipoxygenase inhibitor NDGA. Platelet adhesion to collagen was inhibited by the anti-GPId/Ia antibody 6F1 (5 to 50 μg/mL) in a dose-dependent manner. Major inhibition (about 80%) occurred at 25 μg/mL of the 6F1 antibody. We did not observe any inhibition of adhesion by either anti-GPib (SZ2) or anti-GPib/Ila (P2) antibody at concentrations up to 25 μg/mL. This finding indicates that adhesion in our system was mediated primarily by the GPIa/Ila (αβ₁) receptor.

Effects of platelet adhesion on cAMP levels. The cAMP content of unstimulated static platelets was 10.78 ± 1.40 pmol/10⁹ platelets. Platelets that adhered to the collagen-coated beads had a 2.4-fold lower cAMP level (P < .001) compared with resting platelets (static control, Table 1). Platelets in the column effluent, which were in short contact with the collagen beads, had only slightly (18%) but not significantly (P > .05) reduced cAMP levels. Another flowing (dynamic) control was provided by pumping platelets through a column filled with BSA-coated beads. The cAMP concentration in the effluent from this column (11.8 ± 2.8 pmol/10⁹ platelets) was not significantly different from the static controls (P > .05). The cyclic AMP content of platelets adhering to the collagen-coated beads in the presence of another ADP-removing enzyme system CP (10 mmol/L)/CPK (40 U/mL) and 20 μmol/L NDGA (lipoxygenase inhibitor) was 4.75 ± 0.95 pmol/10⁹ platelets (3 independent experiments), which is not significantly different from values

<table>
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<tr>
<th>Cyclic Nucleotide (pmol/10⁹ platelets)</th>
<th>Static Control</th>
<th>Flowing Control</th>
<th>Effluent Platelets</th>
<th>Adherent Platelets</th>
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<tbody>
<tr>
<td>cAMP</td>
<td>10.78 ± 1.40</td>
<td>11.80 ± 2.80</td>
<td>8.79 ± 2.80</td>
<td>4.55 ± 1.08</td>
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<tr>
<td>cGMP</td>
<td>2.52 ± 0.34</td>
<td>2.72 ± 0.41</td>
<td>3.11 ± 0.42</td>
<td>6.16 ± 1.87</td>
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Washed aspirin-treated platelets in the presence of Mg²⁺, apyrase, hirudin, and GRGDSP peptide were pumped through the collagen-coated beads for 2.5 minutes at a contact time (tₚ) of 1.35 seconds (37°C). Adhesion was terminated by the addition of 10% (wt/vol) ice-cold TCA and concentrations of cAMP and cGMP were determined by radioimmunoassay. The “flowing control” represents platelets pumped through BSA-coated beads. Data shown represent means ± SD of five separate platelet preparations.
seen in the presence of apyrase (4.55 ± 1.08 pmol/10⁹ platelets).

Effects of platelet adhesion on cGMP levels. The level of cGMP in unstimulated platelets was 2.52 ± 0.34 pmol/10⁹ cells. Platelets attached to collagen showed a 2.4-fold increase in cGMP compared with static controls (P < .001, Table 1). Platelets emerging in the column effluent had slightly (23%) but not significantly (P > .05) increased concentrations of cGMP. The increase in cGMP content was not observed in the effluent from the control BSA column.

Effects of PGE₁ and SNP on rapid changes in platelet cyclic nucleotides. Before platelets reached the collagen-coated beads, they were mixed in a connecting tube with different concentrations of PGE₁ or SNP solutions for about 3.5 seconds. The kinetics of changes in cyclic nucleotides induced by these agents within 1.5 to 3.8 seconds are shown in Fig 2A and B. For a mixing time of 3.8 seconds, 10 μmol/L PGE₁ induced a 16.4 ± 1.4-fold increase in cAMP. PGE₁ was specific for increasing cAMP and did not influence cGMP levels (data not shown). However, SNP increased not only platelet cGMP but also caused a dose-dependent increase in cAMP. SNP at 50 μmol/L caused about a ninefold increase in cGMP and a 3.2-fold increase in cAMP. The cyclic nucleotides levels were increased in a concentration-dependent manner (data not shown).

Inhibition of adhesion by PGE₁ and SNP. Platelets were pumped through the collagen-coated beads together with increasing concentrations of these agents after a brief pre-exposure for about 3.5 seconds. Both PGE₁ and SNP strongly and rapidly reduced adhesion in a dose-dependent manner, with about 80% inhibition caused by 20 μmol/L PGE₁ at a contact time (t₀) of 1.35 seconds (Fig 3A). The extent of inhibition was correlated with the increase in cAMP of the flowing platelets (Fig 3B). Almost complete inhibition (90%) was observed for 100 μmol/L SNP at t₀ = 1.35 seconds (Fig 4A). At lower concentrations, the extent of inhibition was dose-dependent and correlated with the increases in cAMP and cGMP levels (Fig 4B).

Platelets attached to the collagen beads in the presence of PGE₁ had lower levels of cAMP compared with static controls treated with this drug or with platelets emerging in the column effluent (Fig 5). The cAMP content of PGE₁-treated platelets attached to collagen was significantly higher than in adherent platelets that had not been exposed to PGE₁. Platelets bound to collagen in the presence of SNP also had lower levels of cAMP than did SNP-treated static controls and effluent platelets. However, we observed that adhesion to collagen caused no significant changes in cGMP levels when SNP-treated platelets were pumped through the collagen-coated beads (data not shown).

DISCUSSION

We have found that adhesion of aspirin-treated platelets to collagen causes levels of cAMP to decrease compared with static controls (Table 1). Nonadherent platelets that may have had only brief contact with the collagen surface and platelets pumped through control, BSA-coated beads (flowing control) had unchanged cAMP levels. The decrease in cAMP concentration during adhesion is not associated with secretion from platelet dense granules as monitored by serotonin release⁸ or with secretion from α-granules as monitored by expression of platelet α-granule membrane protein GMP-140.⁹ In addition, changes in cAMP could not be attributed to the released ADP, because the decrease in cAMP occurred in the presence of the ADP-removing enzyme systems CP/CPK and apyrase. The use of PGI₂ for an intermediate step during the preparation of the washed platelets might have suggested increased cAMP levels greater than the basal levels seen at the beginning of the experiment. The subsequent decrease in cAMP concentration could then be caused by lowering the previously elevated cAMP. However, we consider this possibility to be unlikely based on recent studies. In particular, it has been shown that

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**Fig 2.** Rapid kinetics of cyclic nucleotides synthesis in platelets stimulated with (A) PGE₁ or (B) SNP. Washed platelets were pumped together with 10 μmol/L PGE₁, or with 50 μmol/L SNP through the reaction tube of 0.3 mm ID at 10 to 30 dynes/cm² at 37°C. At the various times indicated the stimulations were terminated by addition of 10% 10% TCA and then the concentrations of cAMP and cGMP were determined by radioimmunoassay. Values are expressed as the fold increase (means ± SD of three different platelet preparations) over resting levels, which were 10.24 pmol/10⁹ platelets for cAMP and 2.24 pmol/10⁹ platelets for cGMP.
Fig 3. Inhibition of rapid platelet adhesion to collagen by PGE₁. (A) Time course of PGE₁-induced inhibition. Platelets were pumped through collagen-coated beads together with buffer (○) or with different concentrations of PGE₁: 1 μmol/L (●), 10 μmol/L (□), and 20 μmol/L (▲) at 37°C. Preexposure time (time during which platelets were mixed with inhibitor) was about 3.5 seconds. (B) Percentage of PGE₁-induced inhibition at a contact time (tₙ) of 1.35 seconds as a function of concentration. Data shown represent means ± SD of six independent platelet preparations.

Fig 4. Inhibition of rapid platelet adhesion by SNP. (A) Time course of SNP-induced inhibition. Platelets were pumped through collagen-coated beads together with buffer (○) or with SNP at 1 μmol/L (●), 12.5 μmol/L (□), 50 μmol/L (▲), and 100 μmol/L (△). Preexposure time was about 3.5 seconds. (B) Percentage of inhibition as a function of SNP concentration at a contact time (tₙ) of 1.35 seconds. Data shown represent means ± SD of six independent experiments.
The increase in cGMP content preceded aggregation measured by light scattering and reached a maximum when aggregation began. This suggested that the change in cGMP was not an effect of release reaction but rather a result of intracellular synthesis of cGMP induced by collagen. Recently, Radomski et al. showed that platelets stimulated with collagen synthesize NO from L-arginine, which activates the soluble guanylate cyclase. Our results support and extend these observations, indicating that direct binding of platelets to collagen via the \( \alpha_{\beta_\beta} \) integrin complex stimulates cGMP synthesis in the absence of platelet aggregation and secretion. The changes in cGMP may, in turn, influence the platelet cytoskeleton organization, similar to findings in neuroblastoma cells. This may represent part of the major events in the subsequent phases of the adhesion process.

In our studies, PGE\(_1\) induced a very fast, dose-dependent inhibition of platelet adhesion to collagen that was correlated with the increase of cAMP (Fig 3A). The concentrations of PG required to inhibit adhesion were much higher than those necessary to block completely platelet aggregation, and no more than 80% reduction was seen with 20 \( \mu \)mol/L PGE\(_1\). The residual adhesion (about 10% to 15% of total platelet number pumped through the collagen-coated beads) was mediated by a mechanism that was insensitive to an increase in platelet cAMP. Our results agree with the data of Weiss and Turitto, who found similar inhibition of adhesion to collagen with 1 \( \mu \)mol/L PGI\(_2\) at a shear rate of 800 s\(^{-1}\), and the data of Karniguan et al., who noted a strong inhibition of adhesion both for normal and thrombasthenic platelets to coated plastic dishes under these conditions.

Although these data are qualitatively similar to data obtained using a microtiter plate assay, a major difference is that, under flow conditions, almost 90% of pumped platelets adhered to collagen within 5 seconds, whereas, under static conditions, only 10% of cells adhered within 30 minutes. It is possible that in systems in which only a low degree of adhesion is observed, changes in cyclic nucleotides are not easily detectable and platelets that adhere to the collagen-coated plastic dishes under these conditions are not sensitive to prostacyclin. We have recently found that large, heavy platelets that adhere to collagen faster and more efficiently than small light platelets are less sensitive to inhibition by prostacyclin. This might explain the slight inhibition or lack of inhibition observed in other studies. It is also possible that the contradictory results in the literature are caused by different collagen preparations. Most earlier studies on platelet interaction with collagen indicate that various types of collagen differ in their ability to initiate the platelet release reaction and aggregation. A recent report also suggests that platelets possess different receptors for collagen type I and III. Thus, adhesion and adhesion-induced platelet biochemical responses may depend on the collagen type. This issue requires further study with highly purified different collagen substrates.

Blocking the \( \alpha_{\beta_\beta} \) collagen receptor with MoAb 6F1 also failed to cause complete inhibition of adhesion, the extent of which (about 80%) was very similar to that caused by high doses of PGE\(_1\). This finding suggests that the adhesion inhibited by the increase of cAMP may depend on collagen binding to the \( \alpha_{\beta_\beta} \) integrin.

We found rapid and almost complete inhibition of platelet adhesion by 100 \( \mu \)mol/L SNP (Fig 4A). This result agrees with the studies of Radomski et al., who found that NO totally blocks adhesion to collagen fibers in suspension, and the observations of de Graff et al., who found that NO strongly blocked adhesion to the extracellular matrix produced by endothelial cells in whole blood under flow conditions. SNP (50 \( \mu \)mol/L) increased both cGMP (up to 9-fold) and cAMP levels (up to 3-fold) under rapid flow conditions (Fig 2B). It is unlikely that the threefold increase in cAMP was the only cause of the observed inhibition, because an approximate 20-fold increase in cAMP was required to cause a similar inhibition when PGE\(_1\) was used. A threefold increase in cAMP induced by PGE\(_1\) resulted in less than 20%
inhibition of adhesion. Therefore, it appears that the cGMP increase is primarily responsible for the inhibition of the adhesion to collagen caused by SNP. Similar conclusions were reached by Maurice and Haslam in their study of inhibition of platelet-activating factor-induced aggregation by SNP. A threefold increase in cAMP induced by SNP was also observed by Maurice and Haslam in rabbit platelets, although some other studies in human platelets failed to detect any changes in cAMP by SNP. The increase in cAMP may result from inhibition by cGMP of the low-Km cAMP-cGMP inhibitable phosphodiesterase. The mechanism for the inhibition of platelet adhesion to collagen by SNP is not clear. It is possible that it is caused by synergistic effect of cAMP and cGMP or by a cGMP-independent mechanism, such as stimulation of ADP-ribosylation.

Intriguingly, the inhibition of platelet adhesion by increased levels of cGMP appears to contradict our observations that an increase in cGMP is induced by attachment to collagen (Table 1). It is possible that relatively small increases in cGMP potentiate adhesion to collagen or result from adhesion, whereas higher levels inhibit both adhesion and aggregation. This issue requires further study.

The results of our study indicate that platelet cAMP and cGMP undergo significant and reciprocal changes during the α2β1-mediated platelet-collagen interaction. The initial phase of platelet attachment to collagen under flow conditions may be blocked within seconds by PGE2 and SNP. Therefore, intravascular release of PGI2 and NO under arterial flow conditions may have powerful regulatory effects on the initial interaction of platelets with the exposed vessel wall.

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