Cocaine-Induced Platelet Defects

Lisa K. Jennings, PhD; Melanie M. White, BS; Curtis M. Sauer, MD; Alvin M. Mauer, MD; James T. Robertson, MD

Background and Purpose: Numerous studies have demonstrated an association between acute cardiac events, cerebrovascular accidents, and cocaine use. The underlying mechanisms leading to these complications have not been well defined. Using various in vitro model systems, it has been reported that cocaine, up to or greater than an order of magnitude of the lethal dose, causes either inhibitory or proaggregatory effects on platelet function.

Methods: To address these reported discrepancies, we examined the effect of cocaine and its carrier on the activation and aggregation of human platelets in vitro.

Results: We found that cocaine inhibited platelet aggregation when platelets were challenged with ADP, collagen, or arachidonic acid. This inhibition was due to a direct effect on fibrinogen binding to the activated platelet. Cocaine also caused the dissociation of preformed platelet aggregates. At these same concentrations, cocaine did not inhibit agonist-mediated increases in cytosolic calcium or inhibit platelet shape change, suggesting that its effect on platelet aggregation was a selective process and not due to a total destruction of platelet function. Interestingly, the organization of the cytoskeleton of activated platelets, a secondary effect critical to cell receptor clustering and clot retraction, was disrupted by cocaine treatment. In addition, alterations in platelet protein electrophoretic patterns were observed on preincubation of platelets with cocaine.

Conclusions: We conclude that cocaine may have a direct inhibitory effect on the ability of platelets to participate in thrombus formation. The contribution of this effect as an underlying mechanism of sudden death in cocaine abusers is unknown. (Stroke. 1993;24:1352-1359.)

Key Words • cocaine • platelet activation • platelet aggregation

Coincident with the recent dramatic increase in cocaine abuse in the United States, an increasing number of strokes temporally related to cocaine use have been reported.1 In most but not all series, the majority of these strokes were hemorrhagic.1–4 Underlying vascular lesions, especially aneurysm and arteriovenous malformation, have been identified in three fourths and one half of the cases of cocaine-related subarachnoid and intracerebral hemorrhages, respectively.3 It is in those cases that lack an identifiable etiology that cocaine may have been directly responsible for the stroke. Further strengthening this association between cocaine and cerebral hemorrhage are cases in which bleeding occurred in multiple and unusual locations.3,5 Postulated mechanisms for cocaine-related cerebral hemorrhage include acute hypertension and profound cerebral vasodilation followed by reperfusion.2,3,5 These mechanisms, however, remain unproven. Coagulation disorders, especially those related to platelet dysfunction after cocaine use, have, until recently, received little attention.

O’Brien and Boullin6 were the first to report that local anesthetics at various concentrations inhibited platelet adhesion to foreign surfaces and platelet aggregation initiated by ADP and thrombin. Additional studies have demonstrated that exposure of blood platelets to tertiary amine local anesthetics such as dibucaine, procaine, and tetracaine results in the inhibition of platelet activation and aggregation.6–18 Treatment of blood platelets with dibucaine caused decreased fibrinogen receptor exposure on the activated platelet and decreased calcium uptake and exchange across the platelet membrane.15 Feinstein et al19 reported that local anesthetics directly inhibited aggregation, and this inhibition was partially antagonized by calcium; the platelet release reaction was also blocked by local anesthetics independent of external calcium concentrations or the aggregation response.

With regard to cocaine, it has been demonstrated that, in addition to human platelet aggregation, rabbit platelet aggregation induced by ADP and thrombin was inhibited by pretreatment with cocaine.20 On the other hand, it has been shown that cocaine enhances the response of rabbit platelets to arachidonic acid, possibly potentiating thromboxane generation and platelet aggregation.20 In view of these conflicting reports, a critical analysis of the effect of various concentrations of cocaine on human platelet activation and aggregation is needed. We found that cocaine, up to an order of magnitude greater than predicted systemic concentrations of lethal doses, inhibited human platelet aggregation induced by the agonists ADP, collagen, and arachidonic acid and also caused disaggregation of platelets. Interestingly, at these same concentrations cocaine had
no measurable effect on increases in platelet cytosolic calcium, a primary event associated with platelet activation.

Materials and Methods

Materials

We used the following reagents: cocaine hydrochloride (10%, Roxane Laboratories, Inc, Columbus, Ohio); carrier for cocaine (Roxane Labs); ADP (Sigma Chemical Co, St Louis, Mo); collagen (Hormon-Chemie, Munich, Germany); arachidonic acid (Bio Data Corp, Hatboro, Pa); indo-1 AM (Molecular Probes, Inc, Eugene, Ore); Coulter Immunoprep (Hialeah, Fla); anti-human fibrinogen, fluorescein conjugate, fluorescein isothiocyanate (FITC) (TAGO, Inc, Burlingame, Calif); Sequester-Sol (Cambridge Chemical Products, Inc, Detroit, Mich); an anti-glycoprotein (GP) IIB-IIIa polyclonal antibody (Dr D.R. Phillips, COR Therapeutics, South San Francisco, Calif).

Methods

Platelet preparation. Blood was drawn into a plastic syringe and mixed in plastic centrifuge tubes with 0.129 mol/L buffered citrate anticoagulant at a ratio of 9 parts blood to 1 part anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifugation at 135g for 15 minutes at room temperature. The PRP was diluted to 2.5×10^6/mL with autologous platelet-poor plasma (PPP).

Platelet aggregation. Aggregation was performed using a Payton Lumiaggregometer (Buffalo, NY) as previously described.21 To determine the effect of cocaine on platelet aggregation, 450 μL PRP was preincubated for 2 minutes with 50 μL of various concentrations of cocaine (diluted in carrier) or carrier control. Agonist (5 μL of either ADP, collagen, or arachidonic acid) was added, and aggregation was monitored for 5 minutes or until maximum aggregation response was observed, whichever occurred first. Aggregation was measured as percent change in light transmission.

To examine the effect of cocaine on platelet aggregate stability, 50 μL of agonist was added to 450 μL PRP, and aggregation was measured for 3 minutes. At this point varying concentrations of cocaine or carrier control were added, and the effect was monitored for an additional 5 minutes. Disaggregation was measured as percent change in light transmission from maximal aggregation to maximum disaggregation.

Fibrinogen binding. To measure fibrinogen binding to activated versus resting platelets, 15 μL of 100 μmol/L ADP or saline was added to 150 μL PRP (7.5×10^7/mL) and incubated at room temperature for 10 minutes. Next, 15 μL of anti-fibrinogen antibody (FITC conjugated) was added and the mixture incubated for an additional 20 minutes.22 After this period, the specimen was prepared for flow cytometry by adding 10 μL of the above mixture to 75 μL phosphate-buffered saline (PBS), 125 μL solution B, and 50 μL solution C (Coulter Immunoprep). The sample was stored at 4°C until analyzed. For cocaine inhibition studies, 0.2% cocaine, carrier, or buffer was added to the PRP and incubated for 5 minutes before the addition of agonist. For fibrinogen dissociation studies, 0.4% cocaine, carrier, or buffer was added after the 10-minute incubation of PRP with agonist and allowed to incubate for 5 minutes before the addition of anti-fibrinogen antibody. Samples were processed as described for the fibrinogen-binding studies.

Flow cytometric analysis. Flow cytometric analysis of platelets was performed as previously described.23 A Coulter EPICS 753 flow cytometer with laser adjusted to deliver 800 mW at 488 nm was used to analyze platelets. Cells in suspension were analyzed at a flow rate of approximately 400 cells per second. The forward angle light scatter and fluorescence signals from each cell were collected and stored by the Coulter MDADS minicomputer analysis system as two-parameter 64×64 channel histograms. Routinely, fluorescence signals were collected from that portion of the light scatter profile representing single platelets so as to exclude
contributions from debris and other blood cells. Data from 25,000 platelets were usually collected, which required 2 minutes or less of instrument run time.

Platelet cytoskeletal preparations: Triton X-100-insoluble cytoskeletons of cocaine- and carrier-treated platelets in plasma were prepared according to the previously published methods. Cytoskeletal proteins were identified by their electrophoretic properties through sodium dodecyl sulfate (SDS)–polyacrylamide gels compared with protein standards and by their solubility properties in Triton X-100–containing buffers. Platelets were either pretreated with 0.1% cocaine for aggregation studies or treated with 0.2% cocaine for disaggregation studies as described in the “Methods” section entitled “Platelet aggregation.” PRP was removed directly from the aggregometer cuvette and immediately lysed with 0.5 mL of ice-cold 2% Triton X-100 extraction buffer containing 100 mmol/L tris(hydroxymethyl)aminomethane (Tris) HCl, 10 mmol/L ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid, 2 mmol/L 2-mercaptoethanol, and 2 mmol/L leupeptin, pH 7.4 (2X extraction buffer). The sample was vortexed for 10 seconds and centrifuged at 10,000 g for 5 minutes. The supernatant was discarded and the pellet washed two times in 1X extraction buffer. Reducing sample buffer (0.5 mL) consisting of 2% SDS, 2% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue, and 50 mmol/L Tris HCl, pH 6.8, was added to the final pellet. The sample was then heated for 10 minutes at 100°C. Solubilized whole platelet proteins were prepared by adding two drops of Sequester-Sol to citrated PRP and then centrifuging at 1600g for 10 minutes. The supernatant was removed, and the pellet was washed once in buffer containing 140 mmol/L NaCl, 20 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and 1 mmol/L ethylenediaminetetraacetic acid, pH 7.1, and resuspended in buffer containing 137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L MgCl₂, 3.3 mmol/L NaH₂PO₄, and 20 mmol/L HEPES, pH 7.4, to the original volume. An equal volume of sample buffer was then added for SDS–polyacrylamide gel electrophoresis analysis.

Gel electrophoresis. Samples were electrophoresed through 5% to 20% SDS–polyacrylamide exponential gradient gels overnight at a constant voltage of 25 V. Proteins were visualized by staining with Coomassie brilliant blue.

Immunoblotting. An LKB 2005 Transfer Electrophoretter was used to transfer polypeptides from SDS–polyacrylamide gels to nitrocellulose sheets. The transfer buffer contained 25 mmol/L Tris, 10 mmol/L glycine, and 20% methanol, pH 8.3. Proteins were electrotransferred for 1 hour at 0.7 mA. After transfer, the nitrocellulose sheets were incubated overnight with 10 mmol/L Tris, 0.9% NaCl, and 3% bovine serum albumin (BSA), pH 7.4 (immune stain buffer). Primary antibody (1:500) was added to the immune stain buffer, and nitrocellulose sheets were incubated for 16 hours. The membrane was washed four times with 10 mmol/L Tris, 0.9% NaCl, and 1% BSA, pH 7.4 (immune incubation buffer). Visualization of bound antibody was achieved by incubating the membrane with species-specific anti-

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**TABLE 1. Effect of Cocaine on Arachidonic Acid–Mediated Platelet Aggregation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg/mL AA</td>
<td>&lt;5</td>
</tr>
<tr>
<td>100 µg/mL AA+0.2% carrier undiluted</td>
<td>85</td>
</tr>
<tr>
<td>100 µg/mL AA+0.2% carrier diluted in saline</td>
<td>≤5</td>
</tr>
<tr>
<td>100 µg/mL AA+0.2% cocaine diluted in saline</td>
<td>≤5</td>
</tr>
<tr>
<td>100 µg/mL AA+0.02% cocaine diluted in carrier</td>
<td>83</td>
</tr>
<tr>
<td>100 µg/mL AA+≥0.04% cocaine diluted in carrier or saline</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are an average of three separate determinations. AA indicates arachidonic acid.
immunoglobulin G horseradish peroxidase conjugate for 2 hours. The sheets were washed four times in immune incubation buffer and developed by 4-chloro-1-naphthol color development reagent.

Platelet cytosolic calcium. Platelets were loaded with indo-1, and agonist-induced changes in cytosolic free calcium were analyzed by ratio cytofluorimetry by the method of Jennings et al.²⁸ PRP was loaded with indo-1 for 30 minutes at 37°C at the concentration that resulted in maximal response to agonist for that donor (15 or 20 μmol/L). An aliquot was diluted in a buffer consisting of 2 mmol/L CaCl₂, 150 mmol/L NaCl, and 10 mmol/L Tris, pH 7.4. Cocaine or carrier was added at a final concentration of 0.2%. Samples were then stimulated with either 100 μmol/L ADP or 0.2 U/mL thrombin.

Results

To evaluate the effect of cocaine on platelet aggregation, platelets from four medication-free donors were preincubated with increasing concentrations of cocaine or carrier and challenged with the agonists ADP (10 μmol/L) or collagen (2 μg/mL) in the aggregometer (Fig 1). We found that the aggregation response to both agonists was inhibited by cocaine in a concentration-dependent manner. The addition of carrier had no effect on the aggregation response to either ADP or collagen (data not shown). When ADP was the agonist, the platelet aggregation responses were inhibited completely by 0.2% cocaine (Fig 1A). With respect to collagen, the aggregation responses were completely inhibited by 0.1% cocaine (Fig 1B). This inhibition of aggregation appeared to be specifically related to the ability of platelets to bind fibrinogen because shape change in response to ADP addition was observed (data not shown). Behnke⁷ previously showed that platelets treated with cocaine at a concentration of 3000 μg/mL (0.3%) inhibited shape change in response to 1 μg/mL ADP. Thus, it is possible that higher concentrations of anesthetics are required to inhibit this response.

Previous reports have suggested that low-dose cocaine (0.005% to 0.05%) enhances the response of rabbit platelets to nonaggregating levels of arachidonic acid, thus leading to increased platelet reactivity rather than inhibition.²⁰ When we pretreated platelets with 0.2% cocaine, the aggregation response to 500 μg/mL arachidonic acid was totally inhibited (Table 1). In addition, cocaine was able to induce disaggregation of preformed platelet aggregates (data not shown). Since we observed inhibitory effects of cocaine similar to those seen when platelets were stimulated with ADP and collagen, we investigated the reported enhanced platelet response to subthreshold levels of arachidonic acid in the presence of cocaine (Table 1). First, we established our subthreshold level of arachidonic acid. When platelets were challenged with 100 μg/mL arachidonic acid, they did not aggregate; however, treatment with greater than 100 μg/mL elicited a measurable aggregation response. Based on these experiments 100 μg/mL was selected as our subthreshold concentration. In the process of setting up our assay system we found that, in contrast to all our other aggregation assays in which carrier controls caused no effect on aggregation, the addition of the carrier (0.2%) to platelets caused a marked aggregation response (82% to 89%, n=3) to subthreshold amounts of arachidonic acid. However, when the platelets were treated with 0.02% carrier diluted in saline, the platelets had an aggregation response of 5% or less when challenged with 100 μg/mL arachidonic acid. As expected, platelets preincubated with 10-fold less cocaine (0.02%) diluted in saline rather than carrier did not aggregate in response to subthreshold amounts of arachidonic acid; however, platelets incubated with 0.02% cocaine diluted in carrier rather than saline showed a 75% to 93% aggregation response to 100 μg/mL of arachidonic acid. As the concentration of cocaine in the carrier was increased (greater than or equal to 0.04%), the aggregatory effect of the carrier was abolished. Thus, cocaine does inhibit arachidonic acid–mediated platelet aggregation, and it appears that carrier and not cocaine is responsible for an enhanced response of human platelets to subthreshold levels of this agonist. Based on our studies it is difficult to support the hypothesis that cocaine causes an enhanced aggregatory response of human platelets to agonists in vivo. We would suggest that cocaine may inhibit platelet function particularly when high local concentrations are achieved.

To determine whether cocaine had the capacity to disaggregate platelets and possibly affect preformed aggregates in the circulation in vivo, cocaine was added at increasing concentrations 3 minutes after the initiation of aggregation induced by ADP or collagen (Fig 2A and 2B). We found that disaggregation due to the presence of cocaine was dependent on the concentration of drug added. Maximal disaggregation was seen at approximately 0.4% cocaine with both agonists. Concentrations of 0.05% or lower did not appear to affect platelet aggregate stability.

### Table 2. Fibrinogen Binding to Carrier- and Cocaine-Treated Platelets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of Total Platelets with Bound Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.0±2.3</td>
</tr>
<tr>
<td>Carrier</td>
<td>5.5±2.7</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td>ADP</td>
<td>83.6±7.4</td>
</tr>
<tr>
<td>Carrier+ADP</td>
<td>84.0±6.1</td>
</tr>
<tr>
<td>Cocaine+ADP</td>
<td>4.8±1.5</td>
</tr>
<tr>
<td>ADP+carrier</td>
<td>80.8±6.4</td>
</tr>
<tr>
<td>ADP+cocaine</td>
<td>1.6±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=5. ADP concentration, 100 μmol/L.

### Table 3. Effect of Cocaine on Agonist-Induced Increases in Platelet Cytosolic Calcium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Maximum Mean Fluorescent Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>19.6±0.2</td>
</tr>
<tr>
<td>Carrier+ADP</td>
<td>29.9±2.2</td>
</tr>
<tr>
<td>Cocaine+ADP</td>
<td>29.2±1.4</td>
</tr>
<tr>
<td>Carrier+thrombin</td>
<td>38.8±3.4</td>
</tr>
<tr>
<td>Cocaine+thrombin</td>
<td>40.2±2.7</td>
</tr>
</tbody>
</table>

Values are mean of three separate experiments±SD. ADP concentration, 100 μmol/L; thrombin concentration, 0.2 U/mL.
Once platelets are activated, it is the binding of fibrinogen that supports platelet aggregation. When platelets were preincubated with 0.2% cocaine, fibrinogen binding to ADP-activated platelets dropped to approximately 5% of the total platelets analyzed compared with that of 84% when platelets were preincubated with saline or with carrier (Table 2, n=5). When platelets were activated with ADP and then 0.4% cocaine added, the percentage of platelets having bound fibrinogen dropped from 81% (in the presence of saline or carrier) to 2% when cocaine was added. Thus, the inhibition of aggregation or the disaggregation of platelets mediated by cocaine is due at least in part to the inhibition of fibrinogen binding or to the dissociation of fibrinogen bound to agonist-activated platelets. Similar results were found when platelets were treated with 1 mmol/L dibucaine.15

The results from the above experiments suggest that cocaine dramatically affects fibrinogen binding to activated platelets. The following experiments were designed to determine whether cocaine specifically altered ligand binding to GPIIb-IIIa, the human platelet fibrinogen receptor, or if cocaine at the same concentration inhibited other calcium-dependent pathways that would normally lead to platelet activation, fibrinogen binding, and platelet aggregation. It has been suggested that cocaine, along with other local anesthetics such as dibucaine, tetracaine, and lidocaine, may perturb cell membrane phospholipid organization by displacing calcium from the membrane and modifying cellular calcium homeostasis.9

Since platelet cytoplasmic free calcium plays a central role in platelet activation, we examined whether cocaine altered agonist-induced increases in platelet cytosolic calcium. Indo-1-loaded platelets were preincubated with 0.2% cocaine and then challenged with either ADP or thrombin (Table 3). Response was compared with that obtained when platelets were preincubated with the drug vehicle. We found that the addition of cocaine did not inhibit increases in cytosolic free calcium levels normally observed when platelets are challenged with ADP or thrombin. Thus, it appears that cocaine at the concentrations used in this study does not have a general effect on platelet reactivity by altering all physiological responses dependent on the availability of calcium ions but does specifically alter fibrinogen binding, thereby preventing platelet aggregation.

Following platelet activation, alterations in the platelet cytoskeleton include a net polymerization of monomeric actin into filaments and an increased association of these filaments with other cytoskeletal proteins.24,29,30 Platelet secretion, shape change, aggregation, and clot retraction require the forces generated by the interaction of these contractile proteins. Further examination of the mechanism by which cocaine affects platelet reactivity was carried out by examining the organization of the cytoskeletal core of platelets pretreated with cocaine or the carrier control. In addition, the composition of the cytoskeleton when cocaine-treated platelets were challenged with the agonists ADP or collagen was analyzed. Cytoskeletal cores of platelets in plasma were isolated by solubilization with Triton X-100 extraction buffer and centrifugation at 10 000g. The cores were analyzed for protein content by SDS–polyacrylamide gel electrophoresis. Fig 3 is a representative photograph of electrophoresed samples after staining with Coomassie brilliant blue. Three separate cytoskeletal isolations were conducted, and identical results were obtained. Lanes 1 and 2 show that platelets stimulated with collagen or ADP had an increased incorporation of actin-binding protein (ABP), talin, α-actinin, myosin, and actin into the Triton-insoluble residue when compared with platelets treated with cocaine or carrier alone (lanes 3 and 4). When platelets were pretreated with cocaine and then challenged with agonist, the incorporation of these cytoskeletal elements did not increase.
treated with 2 mmol/L tetracaine had major reductions of bands 1 and 2 (presumably ABP and talin) and increases in the staining intensity of some bands in the lower-molecular-weight regions. Our studies show that the decreased ABP incorporation in the residues from cocaine-treated platelets was not due to its proteolysis by platelet calcium-dependent proteases in the intact platelet because (1) cocaine does not stimulate calcium influx and (2) ABP and talin in intact platelets were not degraded by these proteases during a 5-minute incubation period with cocaine (Fig 4). It is possible that increased susceptibility of ABP and talin to proteolysis occurred once the platelets were lysed by Triton X-100.

Interestingly, as reported earlier with lidocaine-treated platelets,13 we did find subtle differences in the protein content of solubilized platelets depending on whether they were exposed to cocaine or carrier. These alterations may in part explain the inhibitory effect of cocaine on platelet function. Fig 4 shows the Coomassie blue–stained gels of SDS-solubilized whole platelet proteins that were electrophoresed through a gradient polyacrylamide gel. At concentrations of 0.02% (lanes 1 and 2), the protein staining pattern of either the cocaine- or carrier-treated platelets appeared identical and was indistinguishable from that of PBS-treated platelets (data not shown). However, when 0.2% cocaine was incubated with the platelets (lane 3), the staining pattern of three regions was affected in the apparent molecular weight ranges of 170 to 180 kD, 75 kD, and 27 kD. The identification of these altered proteins has not been carried out, but it is possible that their alteration may in part contribute to the profound effects that cocaine and possibly other anesthetics have on platelet function. The appearance of the new 27-kD protein in the cocaine-treated samples may represent a degradation product(s) of one or more higher-molecular-weight proteins.

Studies by Phillips et al39 showed that after thrombin-stimulated platelet aggregation, a subpopulation of GPIIb-IIIa became associated with the Triton X-100–insoluble cytoskeleton. Further studies suggested that GPIIb-IIIa association with the cytoskeleton was dependent on both the degree of aggregation and the formation of the pseudopodial cytoskeletal assembly.29 Because both of these processes are affected by cocaine treatment, we examined the effect of cocaine exposure on GPIIb-IIIa incorporation into the Triton X-100 residue. Immunoblotting was used to demonstrate that GPIIb-IIIa was incorporated into the Triton X-100–insoluble cytoskeletons of ADP- and collagen-aggregated platelets (Fig 5, lanes 1 and 2). In contrast, GPIIb-IIIa was not detected in the insoluble cytoskeletons of platelets that had been preincubated with cocaine (0.2%) before agonist addition (lanes 3 and 4) or disaggregated by 0.4% cocaine (lanes 5 and 6). These results confirmed our previous findings that aggregation of platelets is required for the retention of GPIIb-IIIa in the 10 000g sedimentable Triton-insoluble residue and that cocaine, through its ability to cause platelet dissociation, reduced incorporation of not only the cytoskeletal elements but also GPIIb-IIIa (lanes 9 and 10) when compared with those platelets treated with carrier (lanes 11 and 12).

![Figure 4: Photograph shows alteration of the electrophoretic mobility of platelet proteins by incubation of intact platelets with cocaine. Washed platelets were treated with two concentrations of cocaine and isolated by centrifugation (n=3). Platelet proteins were solubilized with reducing sample buffer, analyzed by sodium dodecyl sulfate–gel electrophoresis, and stained by Coomassie blue. Lane 1, 0.02% carrier; lane 2, 0.02% cocaine; lane 3, 0.2% carrier; lane 4, 0.2% cocaine; and lane 5, molecular weight standards. Three regions on the gel (<) have altered protein staining patterns when platelets are preincubated with 0.2% cocaine.](http://stroke.ahajournals.org/)

(lanes 5 and 6) but was similar to the levels observed when treated with carrier or cocaine in the absence of agonist. As anticipated, treatment with carrier before agonist addition had no effect on the composition of the cores (lanes 7 and 8).

When platelet aggregates were dissociated by cocaine addition (lanes 9 and 10), the retention of ABP, talin, α-actinin, myosin, and actin in the cytoskeletal cores was reduced when compared with those residues isolated from aggregated platelets in the presence (lanes 11 and 12) or absence (lanes 1 and 2) of carrier. Therefore, our results suggest that cocaine not only affected organization of the contractile cytoskeletal assembly composed of primarily actin and myosin but also disrupted the platelet pseudopodial cytoskeleton previously suggested to be composed primarily of ABP and α-actinin.29 The levels of cytoskeletal elements remaining in the isolated cores reflected the extent of disaggregation achieved by cocaine treatment.

One possible explanation for alterations in cytoskeletal organization is cocaine-induced activation of the calcium-dependent protease that hydrolyzes ABP and talin, primary components of the cytoskeletal network. Previous studies by Nachmias et al12 showed that low-salt precipitates isolated from human platelets pre-
Discussion

The deleterious effects of cocaine on the cerebrovascular system have been well documented. Much less is understood about the direct effect of cocaine on human platelet function and the possible contribution of this effect to cocaine-mediated stroke. To elucidate further the potential effects of cocaine on hemostasis, we examined the effect of cocaine on human platelet aggregation and platelet activation responses such as platelet cytosolic free calcium levels, the organization of the platelet cytoskeleton, and fibrinogen binding. Our data support the following conclusions: (1) cocaine prevents the binding of fibrinogen to agonist-stimulated platelets and can bring about the dissociation of fibrinogen bound to platelets incorporated into a platelet aggregate, thus causing platelet disaggregation; (2) cocaine does not inhibit agonist-mediated increases in platelet cytosolic calcium, and therefore a central mechanism that governs the reactivity of the human platelet is still intact; and (3) cocaine affects the organization of the platelet cytoskeleton and inhibits the increased association of ABP, myosin, α-actinin, and actin as well as GPIIb-IIIa in the Triton X-100-insoluble residue of ADP- or collagen-aggregated platelets. Our in vitro data suggest that cocaine has a dramatic effect on mechanisms responsible for mediating both adhesive and cohesive properties of platelets.

It has been difficult to determine the systemic levels of cocaine in patients admitted to the hospital for cocaine abuse due to its short half-life (0.8 to 1.25 hours). The fatal dose of cocaine has been approximated to be 1.2 g, although severe toxic effects have been reported from doses as low as 20 mg. Thus, based on blood volumes of an average-sized person (5 L), the concentrations of cocaine that caused platelet inhibition in our in vitro experiments are within an order of magnitude of the predicted systemic concentration of lethal doses of cocaine. An earlier attempt was made to examine an ex vivo effect of cocaine on platelet function by studying platelets from patients with documented cocaine abuse and cerebral hemorrhage. While depressed platelet function was observed, interpretation of the direct effects of cocaine abuse on platelet function was not possible since each individual had also ingested other drugs (eg, aspirin, alcohol) known to depress platelet function. Whether depressed platelet function due to cocaine abuse or due to a combination of drugs is clinically relevant to the incidence of cerebral hemorrhage or to the management of these patients remains to be determined.

Acknowledgments

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References


27. Burnette WN. 'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radiolabeled protein A. Anal Biochem. 1981;112:195-203.


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