Activation of Human Platelets by Cocaine

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Background. Cocaine ingestion has been associated with thrombosis of coronary as well as peripheral arteries, but the mechanism by which cocaine promotes thrombus formation is unknown. Accordingly, we determined whether cocaine activates human platelets by flow cytometric analysis of whole blood to which cocaine was added.

Methods and Results. Activated platelets were detected by “two-color” flow cytometric analysis of the binding of fluorescently labeled antibodies directed against either platelet-associated fibrinogen or P-selectin, which are found on the surface of platelets only after stimulation. Platelets were distinguished from other constituents of whole blood by their ability to bind an anti-glycoprotein Ib antibody bound to both activated and resting platelets. Incubation of whole blood with cocaine, in concentrations of 10 μM to 13 mM, induced significant increases in both platelet-associated fibrinogen (range of increase, 45±12% to 125±40%) and P-selectin expression (36±15% to 112±24%). In platelets suspended in either buffer or plasma, however, P-selectin expression was detected only at the highest cocaine concentration (85±13% increase in plasma and 59±7% in buffer). Neither aspirin nor the ADP scavenger apyrase inhibited cocaine-induced P-selectin expression. Cocaine inhibited the uptake of 14C-radiolabeled serotonin by platelets (IC50, 8.7 μM). P-selectin expression and fibrinogen binding were found after the addition of cocaine alone to blood taken from some but not all donors; however, platelet activation in response to submaximal concentrations of the agonists ADP or epinephrine was enhanced by a low concentration of cocaine added to blood from every donor.

Conclusions. Cocaine, in concentrations similar to those found clinically, induces activation of individual platelets studied in whole blood from some but not all donors, and platelet response to physiological agonists is enhanced by cocaine. Thus, cocaine-induced platelet activation may contribute to thrombosis following cocaine ingestion. (Circulation. 1995;88:876-883.)

KEY WORDS • cocaine • thrombosis • platelets • P-selectin • fibrinogen

Of the many and varied cardiovascular disorders that have been associated with cocaine ingestion, most attention has been generated by the cases of myocardial infarction temporally related to cocaine consumption, because they occur most often in patients much younger than those in whom coronary artery disease usually is manifested clinically. Moreover, more than one third of the patients who have sustained a cocaine-related myocardial infarction and subsequently have undergone either angiographic or pathological examination did not have significant coronary artery stenosis.1 The pathophysiological basis for cocaine-induced myocardial infarction is not yet clear; multiple contributing mechanisms have been postulated, including adrenergic-mediated increases in myocardial oxygen demand, coronary vasospasm, endothelial dysfunction, and accelerated atherosclerosis.1,2 It is also possible that cocaine promotes the formation of coronary thrombosis; such a mechanism could account not only for those infarctions in which a thrombus is demonstrated but also for those patients with angiographically "normal" coronary arteries, as the absence of discernible thrombus may reflect the endogenous thrombolytic capacity of an otherwise normal artery, particularly in younger patients. The possibility that cocaine exerts a prothrombotic effect is bolstered by reports of cocaine-induced pulmonary infarction and thrombosis of peripheral arteries and veins.3-6

An attractive hypothesis to explain the association of thrombosis with cocaine ingestion is that cocaine either directly or indirectly induces activation of platelets. The demonstration of thrombi comprising platelet aggregates in the coronary arteries of patients with fatal cocaine-associated myocardial infarction supports this theory. Although other local anesthetics, including lidocaine8 and dibucaine,9,10 have been shown to inhibit platelet aggregation to standard agonists in vitro, no effect of cocaine on aggregation of human platelets has been reported. The purpose of this study, therefore, was to determine if cocaine activates human platelets. To test this hypothesis, we used a sensitive assay modified from that described by Shattil and colleagues11 to detect activation of individual platelets in whole blood, thus allowing study of platelet activation while preserving cellular communication with erythrocytes and leukocytes, both of which can affect platelet function.12,13 This flow cytometric assay detects fluorochrome conjugated antibodies directed against epitopes expressed on platelets only after activation, thus permitting the assessment...
of activation of individual platelets in either platelet-rich plasma, gel-filtered platelets, or whole blood. In this study, platelet activation was determined by the detection of P-selectin (previously known as GMP-140 or PADGEM), which is a protein contained in the platelet α-granule membrane that becomes expressed on the surface when this membrane fuses with the platelet surface during the release reaction, and association of fibrinogen with the platelet surface, an event that is an activation-dependent precursor of secondary platelet aggregation.

Methods

Preparation of Whole Blood and Platelet-Rich Plasma

Blood was obtained from healthy, nonfasting volunteers who denied cocaine ingestion and who had not taken aspirin within 2 weeks of donation. Blood was collected from a clean antecubital vein via a 19-gauge needle with the use of a light tourniquet. The initial 5 mL of sample was discarded, and the remainder was anticoagulated with 3.8% trisodium citrate (Sigma Chemical, St Louis, Mo) (1:9 dilution). Platelet-rich plasma and platelets washed by gel filtration and suspended in buffer were prepared using standard techniques described previously.

Platelet Stimulation

Aliquots of whole blood, gel-filtered platelets, or platelet-rich plasma (500 μL) were immediately transferred to polystyrene tubes. Standard agonists ADP (10 μM) (Sigma) and phorbol 12,13-dibutyrate (PdBu) (0.2 μM) (Sigma) and equal volumes of appropriate solvents 0.9% NaCl and dimethyl sulfoxide (DMSO) (Sigma) were mixed without stirring in aliquots of blood, gel-filtered platelets, or platelet-rich plasma so that the effect of cocaine might be compared with that of known platelet stimulators. Cocaine solution (provided by the Beth Israel Hospital Pharmacy as a 4% solution with 0.5% chlorobutanol as preservative) was mixed without stirring in aliquots of blood or platelet-rich plasma to provide the following final cocaine concentrations: 13 mM, 1.3 mM, 130 μM, and 10 μM. Stock 0.5% chlorobutanol solution was mixed with aliquots of control blood, gel-filtered platelets, or platelet-rich plasma. Samples were incubated for 2 minutes at room temperature for ADP, PdBu, DMSO, or NaCl or for 5 minutes for cocaine and chlorobutanol solutions. Times were chosen by preliminary experiments that demonstrated the time course at which peak effect was achieved. After incubation, samples were fixed with a 1:1 dilution of 1.5% paraformaldehyde in phosphate-buffer solution (PBS) at 4°C and processed that day.

Preparation of Antibodies and Controls

The murine monoclonal antibody 6D1 (a kind gift of Barry Coller, MD), which binds to the platelet-specific glycoprotein Ib (GpIb), was used to distinguish platelets from erythrocytes and leukocytes, to which there is minimal binding of this antibody. Goat anti-mouse immunoglobulin (IgG) conjugated to phycoerythrin (GAM-PE) (Sigma) was used to detect 6D1 binding to platelets. S12, a murine monoclonal antibody that binds to human P-selectin, was kindly provided by Dr Rodger McEver, Oklahoma Medical Research Foundation. S12 was conjugated to fluorescein isothiocyanate (FITC) by standard methods. Rabbit anti-human fibrinogen antibody (RAHFg) was raised in rabbits and purified, as previously described. This antibody was then conjugated to FITC (RAHFg-FITC). Because the limited supplies of this antibody were exhausted, a second IgG anti-fibrinogen antibody, raised in goats against human fibrinogen (GAHFg), was used in later experiments to assess platelet fibrinogen binding. GAHFg was also conjugated to fluorescein (GAHFg-FITC) (Atlantic Antibody, Stillwater, Minn). Although both anti-fibrinogen antibodies bound to platelets only after their activation, as described below, their binding characteristics were not identical; therefore, they were used individually in separate sections of this study. No attempt was made to combine results on fibrinogen binding obtained using these different antibodies.

To confirm that antibody binding was specific to platelet activation and did not reflect an artifact of sample processing or microaggregate formation, fluorescence from paired fluorophore-conjugated isotype antibodies that did not bind blood cell antigens were compared with that associated with the activation-dependent antibodies. Samples treated with standard agonists and cocaine were labeled, as below, with FITC conjugated anti-fibrinogen antibodies and S12-FITC in parallel with FITC conjugated control antibodies with equal or greater fluorescence-to-protein ratios. Mouse IgG-FITC (Sigma) served as a control for S12-FITC, and rabbit IgG-FITC (Sigma) was the control for RAHFg-FITC. Goat anti-human haptoglobin-FITC (IgG) (Atlantic Antibody) was used to control for GAHFg-FITC.

Flow Cytometric Evaluation of Platelet Activation

Blood cells were labeled with the appropriate antibody using a technique similar to that previously reported. After cell fixation with paraformaldehyde, which was used to prevent time-dependent expression of P-selectin and fibrinogen, 5 μL aliquots of blood or platelet-rich plasma were diluted in 50 μL aliquots of PBS in duplicate. Although fixation of cells before the addition of antibody has been shown to decrease the amount of epitope detected using this assay, preliminary experiments revealed improved consistency of antibody binding to platelets if fixation preceded the addition of antibodies, and thus this sequence was adopted for these experiments. These samples were then incubated with 6D1 at a final concentration of 10 μg/mL for 20 minutes at room temperature. Next, samples were centrifuged at 800g for 1 minute and washed with 50 μL PBS. The duplicate samples were similarly incubated with GAM-PE (24 μg/mL), spun, and washed with PBS. Individual samples were then incubated with S12-FITC (30 μg/mL) or an anti-fibrinogen antibody, RAHFg-FITC (30 μg/mL), or GAHFg-FITC (30 μg/mL) for 20 minutes at room temperature. Samples then were diluted with PBS to a final volume of 600 μL and immediately examined by flow cytometry. Processed samples of blood, gel-filtered platelets, and platelet-rich plasma were analyzed in a Becton Dickinson FacStar Plus flow cytometer (Becton Dickinson, Braintree, Mass). Fluorescent chromophores were excited with a 5-W argon laser at 200 mW power with a
wavelength of 488 nm. FITC and PE fluorescence were detected using 530±11- and 575±11-nm band-pass filters, respectively. To distinguish platelets from other cells and debris, two complementary techniques were used, as previously described.11 A fluorescent threshold was set to analyze only those cells that bound 6D1-GAM-PE, effectively excluding erythrocytes and leukocytes from platelets. The platelet population was further defined by their forward and right-angle light scatter characteristics, and a gate was set around these particles, as has been previously described.11 S12 and RAHFg binding were then determined by analyzing 5000 platelets per sample at a rate of less than 1,000 cells per second. Light scatter and fluorescence data were obtained with gain settings in a logarithmic mode, and data were analyzed with a Hewlett-Packard Consort 30 software package (Palo Alto, Calif). Fluorescence was expressed as mean log fluorescence per cell. Mean log fluorescence was converted to a linear scale and calibrated with fluorescent standard beads (Flow Cytometry Standards Corporation, Research Triangle Park, NC), as previously described.24

Enhancement Studies

To assess whether cocaine could enhance platelet activation induced by standard agonists, whole blood was incubated with cocaine, ADP, epinephrine (Abbott Laboratories, North Chicago, Ill), or a combination of cocaine and either ADP or epinephrine. Aliquots of citrated whole blood (500 μL) were incubated for 5 minutes at room temperature without stirring with ADP (0.2 to 20 μM), epinephrine (1 μM), cocaine (10 or 130 μM), or combinations of epinephrine and cocaine or ADP and cocaine. Platelets were then fixed with paraformaldehyde, as described above. P-selectin expression and fibrinogen binding to the platelet were assayed using S12-FITC and GAHFg-FITC, respectively.

Inhibition of Thromboxane A2, Formation and ADP

To investigate if cocaine-induced release of α-granule products required cyclooxygenase metabolites of platelet arachidonic acid, citrated whole blood was pretreated with aspirin (1 mM) (Sigma) for 30 minutes at room temperature. Donor-paired, citrated whole blood that had been standing at room temperature for the same period of time served as a control for time-dependent platelet activation. Thereafter, blood was incubated with standard agonists or cocaine, as described above. S12 and GAHFg binding in the aspirin-treated samples were compared with paired controls. Similarly, to investigate if ADP, derived from either platelets themselves or erythrocytes, was necessary for cocaine to induce platelet activation, citrated whole blood was pretreated with the ADP scavenger apyrase (Sigma) (2 units/mL) for 5 minutes. Preliminary experiments using flow cytometry demonstrated that apyrase completely eliminated the ability of ADP to increase P-selectin expression of platelets in whole blood, thus confirming and extending findings with apyrase in whole blood aggregometry studies reported previously.25-27 Subsequently, incubation with cocaine and standard agonists followed by flow cytometric determination of S12 binding was performed.

5-Hydroxytryptamine Uptake

Studies of 14C-radiolabeled serotonin (14C-5HT) were performed in plasma to assess whether cocaine prevented uptake of serotonin. Aliquots of platelet-rich plasma, which were first incubated with control (chlorobutanol solution), cocaine (13 mM), and imipramine (CIBA-GEIGY) (2 μM) for 20 minutes at room temperature, and 14C-5HT (Amersham) were then added for 30 minutes at room temperature. Total available 14C-5HT was determined by scintigraphy of a 100 μL sample. The treated platelet-rich plasma then was centrifuged, and scintigraphy was performed on the platelet-free supernatant. 14C-5HT uptake was then calculated as 1− (free cpm/total cpm). Samples were performed in duplicate.

Statistical Analysis

All data are given as the mean±SEM. Statistical significance was assessed by Student’s t test.

Results

Addition of cocaine to whole blood resulted in a rightward shift of the platelet population fluorescence curve with the appearance of a subpopulation of activated platelets, as shown in representative histograms for RAHFg and S12 binding, which reflect detection of
platelet-associated fibrinogen and surface expression of P-selectin (Fig 1). Binding of S12, expressed as fluorescence per platelet, increased by 112±24% (P<.001) when blood was exposed to cocaine at 13 mM, 77±12% (P<.001) at a cocaine concentration of 1.3 mM, and 16±16% (P=NS) at a cocaine concentration of 130 μM (Fig 2, top). A cocaine concentration of 10 μM induced a 36±15% (P<.04) increase in S12 binding. For comparison, incubation with the phorbol ester PdBu (0.2 μM), which promotes marked release of the contents of α-granules, resulted in a 62±22% increase. Cocaine-induced S12 binding achieved a maximum after 5 minutes incubation with the drug (Fig 3). Platelet-associated fibrinogen binding also increased in a typical concentration-response fashion on exposure to cocaine (Fig 2, bottom). Addition of cocaine (10 μM) resulted in a 45±12% (P<.01) increase in RAHF binding. Treatment with higher concentrations of cocaine (130 μM and 13 mM) induced 61±19% (P<.01) and 125±40% (P<.03) increases in RAHF binding, respectively. Treatment with ADP (10 μM), a potent stimulus of platelet fibrinogen binding, induced a 166±28% increase in RAHF binding. Thus, incubation of whole blood with cocaine induced significant increases in platelet fibrinogen binding as well as release of the contents of α-granules.

To determine if cocaine-induced platelet activation occurred with a similar frequency in our population of normal donors tested as described, we designated a positive response, or "platelet activation," as mean fluorescence per platelet of more than 3 SDs above the mean fluorescence of unstimulated platelets to which saline and 0.5% chlorobutanol in water had been added. By this criterion, samples from three of six donors demonstrated platelet activation at cocaine concentrations of 10 and 130 μM, as determined by fibrinogen binding, whereas a cocaine concentration of 13 mM resulted in an increased fibrinogen binding response in four of five donors. Similar results are seen for S12 binding, as platelets from 89% of donors responded to a cocaine concentration of 13 mM. For cocaine concentrations of 10 and 130 μM, fewer donors were positive (36% and 27%, respectively). Thus, there was notable heterogeneity of response to cocaine among donors, in both quantitative change in S12 or RAHF binding and the threshold drug concentration to which platelets responded.

To determine whether constituents of whole blood other than plasma contributed to the increased expression of P-selectin on the surface of cocaine-treated platelets, the above results were compared with the stimulation by cocaine of platelets suspended in platelet-rich plasma. Addition of the highest concentration of cocaine (13 mM) resulted in a significant increase in S12 binding, 85±13% (P<.002); at the lower cocaine concentrations, however, no significant difference from
control was observed. Likewise, treatment of gel-filtered platelets with cocaine resulted in a significant rise in S12 binding, 59±7% (P<.001), only at high concentrations (13 mM cocaine). When these results are directly compared with the results in whole blood (Fig 2, top) it is apparent that P-selectin expression of platelets suspended in buffer (gel-filtered platelets) or plasma (platelet-rich plasma) that results from exposure to cocaine parallels that of platelets in whole blood but that the latter is enhanced. Thus, although very high concentrations of cocaine can directly stimulate P-selectin expression in platelets, other constituents of whole blood enhance the stimulatory effect of submaximal concentrations of cocaine.

As platelets are activated in vivo under circumstances of multiple stimuli, we sought to determine whether cocaine could enhance the stimulatory effects of agonists that mimic physiological mediators likely to be present after cocaine ingestion. Whole blood was incubated with ADP, epinephrine, submaximal concentrations of cocaine, or the combination of cocaine and ADP or epinephrine, as described in “Methods.” Enhancement was defined as an increase in mean fluorescence per antibody-labeled platelet induced by the combination of cocaine and agonist that was more than the sum of the increases in mean fluorescence per platelet separately induced by cocaine and the agonist. In all samples (n=4), enhancement in S12 and/or GAHfg binding could be demonstrated. We found variability among donors regarding specific agonists and concentrations; however, the effect of a standard agonist could be reproducibly enhanced by cocaine at submaximal concentrations (10 and 130 μM). The mean values of these determinations are shown in Fig 4, top and bottom, for P-selectin expression and platelet-bound fibrinogen, respectively.

To determine if cocaine-induced α-granule secretion was dependent on metabolites of cyclooxygenase or ADP, whole blood was pretreated with aspirin or the ADP scavenger apyrase and then incubated with cocaine, followed by determination of S12 binding. In no sample (n=4 for each agent) was there a significant difference in S12 binding between aspirin or apyrase-treated platelets and paired controls. Thus, cocaine-induced platelet activation does not depend on ADP or arachidonic acid metabolites.

Cocaine is known to inhibit presynaptic uptake of the amine serotonin in neural tissue. As platelets also imibbe, store, and release significant amounts of serotonin, we sought to establish if cocaine also inhibited its uptake by platelets. Cocaine inhibited 14C-5HT uptake in a concentration-dependent manner (Fig 5) with an IC50 of 8.7 μM. By comparison, imipramine completely inhibited 14C-5HT uptake, and the chlorobutanol control demonstrated no significant effect (84±6% maximum uptake).

**Discussion**

The results of this study indicate that cocaine induces release of platelet α-granule contents and the binding of fibrinogen to the platelet surface and thereby implicate cocaine as an agonist capable of activating platelets in whole blood. Control experiments using matched antibodies that do not bind to platelets confirm that the increased fluorescence of platelets is specific for the activated state and is not the result of microaggregates. Significant increases in platelet-associated fibrinogen were demonstrated at a cocaine concentration of 10 μM and increased further with higher drug concentrations. Similarly, release of platelet α-granule contents, as measured by S12 binding, revealed a concentration-dependent response to cocaine added to whole blood. Neither platelet cyclooxygenase metabolites nor ADP, whether released by proximate platelets or by erythrocytes, are essential for cocaine-induced platelet activation. Cocaine, however, does inhibit serotonin (SHT) reuptake, as shown by these and previous studies.26Thus, cocaine may increase the local concentration of this endogenous agonist at the surface of the platelet, an effect that might also enhance the degree of activation induced by other agonists, such as epinephrine, when present in subthreshold concentrations.

![Bar graphs of enhancement of epinephrine- and ADP-induced platelet activation in whole blood by cocaine. Bars represent the mean±SEM response for near-threshold concentrations of cocaine only (10 μM or 130 μM), the sum of the activation induced by these concentrations of cocaine and that induced by either ADP or epinephrine added to a separate aliquot of blood, and the observed combination of these concentrations of cocaine added together with epinephrine or ADP alone, as determined by S12 binding. Bottom, Percent increase ×100 in P-selectin expression above that induced by submaximal concentrations of epinephrine or ADP alone, as determined by goat anti-human fibrinogen antibody binding, above that induced by submaximal concentrations of epinephrine or ADP alone. Bars represent mean±SEM values of four separate determinations.](http://circ.ahajournals.org/doi/figure/10.1161/01.CIR.88.3.880.f004)
Cocaine, at lower concentrations, enhances the effect of ADP and epinephrine, as do other standard platelet agonists; similar results have been reported in rabbit platelets suspended in plasma and stimulated by low concentrations of cocaine and arachidonic acid. Our laboratory previously has demonstrated that enhancement of platelet activation by pairs of agonists, each in subthreshold concentration, has characteristic features regardless of the identity of the agonists. These features include an initial rise in intracellular Ca\(^{2+}\) and phosphorylation of intracellular protein substrates that correlate closely to fibrinogen binding to the platelet surface. Our observation that the binding of fibrinogen can be enhanced by combinations of cocaine and either ADP or epinephrine suggests that the effect of cocaine on platelets may be mediated through mechanisms of activation common to other platelet agonists.

Comparisons of the effects of cocaine on platelets in whole blood, gel-filtered platelets, and platelet-rich plasma provide further insight into the interaction of platelets with other blood cells. The extent of P-selectin expression induced by cocaine in our studies is similar to that detected by George and colleagues using radiolabeled S12 antibody to detect platelet activation by standard agonists added to human whole blood. Flow cytometric determination of P-selectin expression in washed platelets following maximal stimulation with thrombin as reported by this group has, however, demonstrated a sevenfold increase in epitope expression, which is more than that induced by either cocaine or PdBu in our studies. Differences in the platelet medium, as well as variance in the flow cytometric method, including differences in fixation techniques, may account for the lesser degree of P-selectin expression in our experiments. In the direct comparisons reported in this study, the response of platelets to cocaine in whole blood was greater than that of platelets washed by gel filtration or of those suspended in plasma, suggesting that erythrocytes, leukocytes, or other blood cells may amplify platelet activation, a finding that is consistent with platelet activation induced by other stimulants. The presence of erythrocytes can enhance platelet response to standard agonists, and neutrophils can also promote platelet activation, an effect that apparently is mediated by leukotriene B\(_4\). The association of activated platelets with monocytes both in vitro and in vivo has also been described, although whether either cell type enhances the other has not been established. Which, if any, of the cells existing in whole blood enhance the effect of cocaine on blood platelets might be established by selective addition of isolated blood components.

This potential of blood constituents to amplify the effect of an agonist emphasizes the usefulness of the method used in these experiments to investigate the effect of cocaine on platelet activation. Previous studies by our laboratory and others found that cocaine could not directly cause aggregation of platelets suspended in platelet-rich plasma, as assessed by turbidometric methods. The platelet stimulation by cocaine as detected by flow cytometric analysis of individual platelets in whole blood may reflect both greater sensitivity and preservation of intercellular interaction.

The concentration range of cocaine that induces fibrinogen binding and the release of the contents of \(\alpha\)-granules and prevents platelet uptake of serotonin is likely to be relevant to the clinical problem of cocaine abuse. Although the most pronounced effect of cocaine on platelet function in these studies is with millimolar concentrations of the drug, our data indicate that significant increases in \(\alpha\)-granule release, fibrinogen binding, and inhibition of serotonin uptake are evident at a cocaine concentration of 10 \(\mu\)M.

In addition, this submaximal concentration of cocaine enhances epinephrine- and ADP-induced platelet activation. Cocaine has been shown to increase the systemic concentrations of catecholamines, particularly epinephrine, in animal models. Thus, platelet activation that may result from this increase in catecholamine activity is likely to be enhanced by cocaine, and together with the direct platelet activation induced by cocaine may represent a potent prothrombotic effect. Serum cocaine levels of 80 \(\mu\)M have been reported in autopsy studies of patients suffering cocaine-associated sudden death. As the drug is rapidly metabolized by serum cholinesterases in vivo, it is probable that premortem serum concentrations in these patients were manyfold higher. Experimental studies of cocaine ingestion in volunteers have revealed serum cocaine levels as high as 50 \(\mu\)M. Moreover, it is also likely that the local blood concentrations of cocaine in local capillary networks at the site of ingestion, through which platelets circulate, may be manyfold higher than those found in the peripheral circulation.

Because this study demonstrates that cocaine induces platelet activation, one may question why apparently only a minority of those who ingest the drug develop thrombotic consequences. Our data indicate that there is marked individual variability in cocaine’s effect on platelets, as samples from fewer than 50% of donors met our criteria for significant release of \(\alpha\)-granule products at a cocaine concentration of 10 \(\mu\)M. Thus, one might advance the hypothesis that only a subset of those who ingest cocaine in “lower” amounts will develop significant platelet activation and sustain clinically apparent thrombosis, perhaps facilitated by concomitant states that predispose to activation of platelets or soluble factors of clotting.
Cocaine-induced platelet activation may contribute to cardiovascular disorders other than acute thrombosis. The release of platelet-derived mitogens, including platelet-derived growth factor, epidermal growth factor, and transforming growth factor-β, from platelet α-granules may explain the premature atherosclerosis associated with cocaine abuse, particularly if the endothelium is altered by such use. Similarly, the elevation of local concentrations of vasoactive substances such as serotonin, as a result of diminished uptake, might contribute to the coronary vasospasm described with cocaine administration. Thus, it is likely that acute vascular disorders associated with cocaine represent the interplay of several pathophysiological pathways rather than resulting from a single cause. Cocaine-induced platelet activation potentially can contribute to many of these processes and may represent a final event leading to the clinical presentation of acute thrombotic occlusion.

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