Platelet α-Granule Release in Cocaine Users

Henry M. Rinder, MD; Kenneth A. Ault, MD; Peter I. Jatlow, MD; Thomas R. Kosten, MD; Brian R. Smith, MD

Background Cocaine use has been associated with arterial occlusion resulting from platelet-rich thrombi and with an accelerated, often atypical atherosclerotic lesion that could be ascribed to platelet activation and platelet α-granule release.

Methods and Results Using a flow cytometric method to quantitate the percent of circulating activated platelets in whole blood (those that express the α-granule membrane protein P-selectin), we found that 5 of 25 samples from 12 long-term cocaine users had a baseline level of circulating activated platelets >3 SD (range, 19% to 60%) above the mean (4.4±3.7%, mean±1 SD) for 85 nonusers (sample n=130). This subset resulted in a significantly higher mean baseline level of circulating activated platelets (11.8±14.4%) for all cocaine users (P=.01). By contrast, cocaine and its metabolites, at concentrations documented as obtainable during in vivo cocaine use (10⁻⁷ to 10⁻⁶ mol/L), had no effect on in vitro platelet activation or aggregation, either directly or in concert with platelet agonists. However, in experiments in which cocaine users received blinded infusions of placebo or cocaine, the mean percent of circulating activated platelets rose significantly (P<.05) after infusion of either placebo (peak 77±31%) or cocaine (peak 65±28%), the latter at doses resulting in peak plasma cocaine levels averaging <10⁻⁴ mol/L.

Conclusions Long-term cocaine use in some subjects is intermittently associated with high basal levels of circulating platelets that have undergone α-granule release. The inability of cocaine and its metabolites at concentrations of 10⁻⁷ to 10⁻⁵ mol/L to cause platelet P-selectin expression in vitro in this study, coupled with the acute increase in circulating activated platelets observed in vivo after either cocaine or placebo infusion, suggests that in vivo platelet α-granule release associated with cocaine use may occur through indirect rather than direct effects of the drug. (Circulation. 1994;90:1162-1167.)

Key Words • cocaine • platelets • flow cytometry • thrombosis • atherosclerosis

Cocaine use has been associated with sudden cardiac death and, more uncommonly, acute myocardial infarction. In autopsy studies of cardiac death associated with cocaine, several pathological findings have been demonstrated, including platelet-rich coronary thrombi,1,2 (sometimes in otherwise normal vessels) and accelerated atheromatous lesions, often with atypical features.1-4 Both findings might logically result from enhanced platelet activation leading to platelet aggregate formation and release of platelet α-granule contents, which include platelet-derived growth factor and transforming growth factor-β. These latter mediators have been implicated in the pathophysiology of atherosclerosis and in vascular remodeling of smooth muscle cells.5,6

We and others have previously used a very sensitive assay of platelet α-granule release (flow cytometric determination of expression of P-selectin on the platelet surface) to measure circulating activated platelets in cardiopulmonary bypass,7 vascular injury,8 and other clinical settings.9,10 P-selectin (CD62P) is an integral membrane protein that is translocated from the platelet α-granule and the endothelial cell Weibel-Palade body to the external cell membrane after cellular activation and release.11 P-selectin functions as a receptor for activated platelet and endothelial cell binding to monocytes and neutrophils12 and likely induces functional changes in those leukocytes.13 Thus, P-selectin not only serves as a marker for circulating activated platelets but also may be an important receptor linking the inflammatory and coagulation processes.7,14 Our goal in the present study was to examine the characteristics of platelet P-selectin expression in long-term cocaine users and the direct in vitro effects of cocaine and its metabolites on platelets from healthy controls.

Methods

In Vitro Studies

To determine the direct effects of cocaine and its metabolites on P-selectin expression and function in whole blood (WB), blood from healthy volunteers (n=10) who were taking no medications was drawn into heparin anticoagulant (14 U/mL final concentration). WB was then incubated with diluent (5% dextrose in water), cocaine hydrochloride (NIDA), ecgonine methyl ester (Sigma Chemical Co), benzoylcgonine (Sigma), or cocaethylene (synthesized in this laboratory)15 at 10⁻⁷ to 10⁻⁵ mol/L final concentration for 15 minutes at 37°C. Samples from each incubation were then stimulated with diluent, epinephrine (Parke-Davis), ADP, or epinephrine followed by ADP for 5 minutes at 22°C and then fixed in paraformaldehyde (PFA) as described below. We have previously shown that P-selectin expression is maximal at this time point.16

For aggregometry studies, citrated (0.38% final concentration) WB was drawn from healthy volunteers; platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared in the standard manner.17 Platelet counts were adjusted in the PRP to 200×10⁶ cells/L, and PRP was then incubated with diluent, cocaine, or metabolites as above. Platelet aggregometry in response to diluent, epinephrine, ADP, and arachi-
donic acid (Biodata) was performed in the standard manner using a dual-sample DP-247E aggregometer (Sienco). For determination of platelet α-granule release in PRP, PRP prepared as above was incubated with diluent, cocaine, or cocaine metabolites for 15 minutes at 37°C and then stimulated with diluent, epinephrine, or ADP for 5 minutes at room temperature and fixed in PFA, as previously described.\(^7\)

**Patient Studies**

After approval by the Yale Human Investigations Committee and written informed consent, volunteers identified as frequent users of freebase cocaine with no history of vascular disease were admitted to the clinical research center.\(^8\) Urine for benzoylecgonine content was obtained to verify recent abstinence from cocaine.

**Part A**

Eight subjects had a single blood sample drawn for determination of the baseline level of circulating activated platelets. Baseline blood samples were immediately drawn from a peripheral vein into PFA in phosphate-buffered saline (1% final concentration).\(^9\) For baseline controls, 85 age-matched healthy individuals who were not taking any medications similarly had venous blood drawn for baseline values (total sample n=130).

**Part B**

As part of a separate ongoing study,\(^1^0\) four additional subjects who were receiving double-blinded infusions of cocaine or placebo (5% dextrose in water) were also studied for baseline and postinfusion levels of circulating activated platelets. All four subjects had normal 12-lead ECGs before and at the completion of the study. Continuous ECG and Holter monitoring was performed during all infusions. Any ECG evidence for ischemia was a criterion for immediate termination of the study; this did not occur in any of the study subjects. The four subjects received a total of 20 infusions. Venous blood samples were drawn from an indwelling catheter and transferred immediately into PFA just before and at various time points after receiving an intravenous injection over 5 minutes of either cocaine (0.25 or 0.50 mg/kg, n = 7 for each dose) or placebo (5% dextrose in water, n = 6). The placebo solution was then infused in all subjects to maintain the patency of the venous catheter between samplings. Two healthy volunteers received an identical intravenous bolus and infusion of the placebo solution with venous catheter samples drawn identically over 6 hours for measurement of percent P-selectin–positive platelets.

**Antibodies**

The monoclonal antibody (MAb) 1E3 has been shown to recognize P-selectin on the surface of activated platelets.\(^2^0\) The MAb P2 (AMAC) is directed against the platelet integrin GP IIb/IIIa and has been previously used in WB studies to identify platelets by flow cytometry.\(^2^1\) The MAb HLe (anti-CD45, Becton-Dickinson) recognizes a determinant on neutrophils, monocytes, and lymphocytes but not on platelets or erythrocytes.\(^2^1\)

**Fluorescence Labeling and Flow Cytometry**

To determine the percentage of platelets expressing P-selectin, fixed WB and PRP specimens were kept at 4°C for 60 minutes, washed with Tyrode’s-HEPES buffer,\(^2^2\) and labeled with saturating amounts of FITC/anti-GP IIb/IIIa and biotin/anti-P-selectin at 4°C. Samples were then washed and incubated with phycoerythrin (PE)-streptavidin at 4°C, washed, and resuspended for flow cytometric analysis.\(^2^2\) For control labeling, an irrelevant isotype-specific control MAb was used to set a threshold for P-selectin–positive platelets as previously described.\(^2^2\) The determination of the percentage of neutrophil-platelet and monocyte-platelet conjugates in WB after epinephrine/ADP was performed as previously described.\(^7\) In brief, neutrophils and monocytes were identified on the flow cytometer using a combination of size and expression of the leukocyte-specific marker CD45. Using size and right-angle scatter, the neutrophils and monocytes were analyzed separately for platelet-marker (GP IIb/IIIa) fluorescence (again using a threshold set with an irrelevant isotype-specific MAb). Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson).

**Cocaine Levels**

Plasma samples from blood drawn identically as above but into Vacutainer tubes containing sodium fluoride (to inhibit plasma cholinesterase activity) were prepared immediately and snap-frozen at −70°C. Cocaine concentrations in these samples were quantified as previously reported using high-performance liquid chromatography.\(^2^3\),\(^2^4\)

**Statistical Analysis**

Comparisons between groups were performed using twotailed Student’s t test. All values are expressed as the mean±SD.

**Results**

**In Vitro Studies of Cocaine and Its Metabolites on Platelets From Healthy Controls**

**P-Selectin Expression and Function in WB**

Cocaine and its metabolites in concentrations ranging from 10\(^{-7}\) to 10\(^{-5}\) mol/L did not increase platelet P-selectin expression in WB after incubation for as long as 30 minutes compared with incubation with the diluent solution (5% dextrose in water) or with blood immediately fixed after phlebotomy. Moreover, there was no synergy or inhibition of P-selectin expression after addition of platelet agonists (ADP and/or epinephrine) to WB preincubated with cocaine and its metabolites. A representative series of experiments is detailed in the Table. Even at the highest dose (10\(^{-5}\) mol/L, Table), there was no enhancement by cocaine or its metabolites of platelet P-selectin expression. We have previously shown that stimulation of WB with epinephrine followed by ADP results in increased monocyte-platelet and neutrophil-platelet adhesions that are P-selectin dependent.\(^1^9\) This assay provides a functional assessment of P-selectin activity rather than a quantitative evaluation. Cocaine (10\(^{-5}\) mol/L) incubation of WB followed by stimulation with 1 μmol/L epinephrine and 5 μmol/L ADP did not affect the percentage increase in the formation of monocyte-platelet conjugates (37±4% in diluent versus 41±4% in cocaine, \(P>.10\)). The increase in neutrophil-platelet binding after epinephrine/ADP stimulation, which is quantitatively lower in WB compared with monocyte-platelet binding, was also not affected by cocaine (data not shown).

**Platelet Aggregometry and P-Selectin Expression in PRP**

Cocaine and its metabolites at concentrations of 10\(^{-6}\) and 10\(^{-5}\) mol/L did not cause spontaneous platelet aggregation. Cocaine and its metabolites were also examined for a synergistic effect on platelet aggregation using platelet agonists at subthreshold concentrations, ie, agonist doses that did not cause second-wave aggregation in diluent-incubated PRP. Potential inhibition of platelet aggregation by cocaine and its metabolites was also examined using agonists at threshold and higher concentrations. To summarize the results of 10 experi-
ments, cocaine and its metabolites showed no in vitro effects (neither synergy nor inhibition) on platelet aggregation in response to epinephrine (0.1 to 5.0 μmol/L), ADP (0.5 to 10 μmol/L), or arachidonic acid (100 to 500 μg/mL). Similar to the findings in WB, incubation of PRP with cocaine or its metabolites at concentrations of 10⁻³ and 10⁻⁵ mol/L did not cause increased P-selectin expression compared with diluent-treated samples, nor was there any synergistic increase in P-selectin after cocaine/metabolite incubation before epinephrine or ADP addition to PRP.

**Patient Studies**

**Baseline Platelet Activation In Vivo**

Cocaine users and age-matched healthy controls were assessed for the baseline percent of circulating P-selectin-positive platelets. The resting level of P-selectin-positive platelets in the 8 subjects from study part A (sample n=8) and the 4 subjects from study part B (sample n=17) was 11.8±14.4% (mean±SD); this was significantly (P=.01) higher than the value in 85 healthy controls (4.4±3.7%; range, 1% to 14%; sample n=130). Five samples from cocaine users (2 subjects in study part A and 3 separate subjects in study part B) had levels ranging from 19% to 60% (36.2±15.9%); all five levels were >3 SD above the mean of the healthy controls and more than the highest normal level observed.

**Platelet Activation In Vivo After Blinded Infusion of Cocaine or Placebo**

For the 4 subjects from study part B, the time course of platelet activation after cocaine and placebo infusion is shown in the Figure. Values shown are the mean for all subjects (n=20), for both cocaine doses combined (n=14), and for the placebo infusion (n=6). The average peak level for all cocaine infusions (n=14) was 65±28%; the peak percent of P-selectin-positive platelets after infusion of 0.25 mg/kg (n=7) and 0.50 mg/kg (n=7) cocaine was similar: 72±31% and 59±26%, respectively (P>.10). The peak percent of circulating activated platelets after placebo (n=6) was 77±31%, which is not significantly different from the peak levels for the cocaine infusions (P>.10 compared with either cocaine dose alone or the combination of both cocaine doses). At 30 minutes after the infusion, both the placebo and cocaine groups demonstrated a statistically significant rise in the percent P-selectin-positive platelets when compared with the preinfusion time point, and at 60 minutes after the infusion, both groups continued to demonstrate a significant increase compared with before the infusion and with 30 minutes after the infusion (P<.01 for all pairwise comparisons). Mean levels peaked at 150 minutes after the infusion and remained significantly elevated over the preinfusion values for the next 5 hours (P<.03 for all pairwise comparisons).

Peak intravenous cocaine concentrations were reached within 5 to 15 minutes after the infusion and declined rapidly thereafter; the mean peak cocaine concentrations in the high- and low-dose cocaine infusion groups were 8.1×10⁻⁷ and 2.6×10⁻⁷ mol/L, respectively. Only one subject had a peak level >10⁻⁷ mol/L (1.2×10⁻⁶ mol/L). Continuous ECG and Holter monitoring revealed no evidence for myocardial ischemia during any of the 20 infusion studies. One cocaine subject was chronically receiving desipramine before and during the infusion studies; the baseline and peak platelet activation values for the 4 studies in this subject did not differ significantly from those for the other 16 infusion studies. In healthy controls receiving the intravenous placebo bolus and infusion, the level of activated platelets remained <10% during the entire 6 hours.

In 20 studies, cocaine users received a blinded infusion of either cocaine (0.25 or 0.50 mg/kg) or placebo; blood samples were then drawn over the next 6 hours to determine the percent of P-selectin-positive platelets. Plot of mean percent of activated platelets at all sampling points is shown for all patients, for the subset who received cocaine (either dose), and for the subset who received placebo. There was no significant difference between the cocaine and placebo groups, but there was a significant rise (P<.01) in percent P-selectin-positive platelets at 30 and 60 minutes from preinfusion values in both cocaine and placebo groups that persisted for the next 5 hours.
Discussion

This pilot study found that some long-term cocaine users had baseline levels of circulating activated platelets that were intermittently higher than those for any healthy control; one subject had a level four times greater than that of the highest healthy control. It is not surprising that basal levels of circulating activated platelets would be abnormally high in only a subset of samples. Because P-selectin on platelets mediates adhesion to neutrophils and monocytes, it is likely that these activated platelets may be bound quickly to leukocytes and/or cleared from the circulation; there is evidence that transfused activated platelets are cleared more rapidly from the circulation than unactivated platelets. In addition, the abnormally high levels could reflect recent cocaine use, other drug use, or vascular disease that was not clinically detectable. The frequency of acute thrombotic events during cocaine use is similarly variable. Even in autopsy studies of death associated with cocaine use, significant coronary thrombotic events were found in only 10% to 13% of cases; the remaining deaths were attributed to respiratory arrest and arrhythmias.

Despite this finding of increased basal activation in some samples from cocaine users, we determined that cocaine and its metabolites at concentrations of 10^{-7} to 10^{-5} mol/L had no direct in vitro effect on platelet aggregation or on platelet P-selectin expression and function. Cocaine inhibition of serotonin reuptake might be expected to potentiate platelet activation, but we found no synergistic effect of cocaine with any agonist. The local anesthetic actions of cocaine are known to occur through blockade of sodium channels. We have reported that blockade of the platelet Na^+/H channel by such potent methods as incubation with amiloride or extracellular acidification inhibits P-selectin expression to agonists. However, at the cocaine concentrations used in this study, we found no inhibition of platelet activation to agonists. The cocaine concentrations used in vitro (10^{-7} to 10^{-5} mol/L) were chosen because they incorporate and exceed the peak cocaine concentrations that produce the cocaine “high” in long-term users (analogous to our patient population). These peak levels occasionally reach 10^{-5} mol/L; the highest reported cocaine level in a short-term, repeated-dose smoking study of long-term users was 2.8 \times 10^{-5} mol/L. The highest fatal level reported (derived from autopsy studies) was 8 \times 10^{-5} mol/L. In the present study, there was only one instance in which peak cocaine levels exceeded 1 \times 10^{-5} mol/L after intravenous infusion. Although it is possible that local vascular concentrations of cocaine immediately after bolus are transiently higher than the levels reported above, these concentrations would have to be more than 10-fold higher than the levels at 5 minutes after injection to exceed 10^{-5} mol/L. Thus, our in vitro studies suggest that cocaine’s effect on platelet activation in vivo is not immediate and direct.

Data from the infusion study similarly do not illustrate a direct effect of cocaine on platelets in the circulation. When cocaine users received an infusion of placebo or cocaine, the percentage of circulating activated platelets increased significantly after infusion in a time-dependent fashion. The mean peak percentages of P-selectin-positive platelets in the cocaine challenge study (59% to 77%) were much higher than the mean levels of circulating activated platelets observed previously in patients undergoing cardiopulmonary bypass (18% to 28%). A procedure that results in significant platelet α-granule release. Furthermore, these high levels of P-selectin expression persisted for nearly 6 hours after the infusion. As noted above, there is evidence for increased clearance of activated platelets and adhesion of activated platelets to leukocytes in vivo. We speculate that the persistently high levels of P-selectin—expressing platelets in these subjects could be due to defects in either leukocyte (eg, tissue macrophage) adhesion or overall platelet clearance. Another possibility is that the cocaine users responded to the infusion with an elevated, sustained release of platelets from the marrow that then became activated. The data presented here do not specifically implicate any of these explanations.

Coupled with our in vitro findings, these data suggest that there is an indirect effect of cocaine operative in the promotion of platelet α-granule release. Other studies have implicated catecholamines and increased sympathetic activity in promoting platelet activation and possibly promoting vascular thrombosis, and we have previously found that epinephrine in vitro acts synergistically with ADP in inducing platelet P-selectin expression. Because cocaine is known to augment the action of various monoamines through blockade of reuptake at the monoamine transporter, catecholamine physiology might be related to P-selectin expression associated with cocaine use. In several studies of cocaine users, significant elevations in epinephrine and norepinephrine levels have been found after recent use. In the present infusion study, each of the patients expected to receive cocaine, and all patients had a positive chronotropic response in the first 30 minutes after infusion, regardless of whether they received placebo or cocaine. This conditioned-cue sympathomimetic response has been noted in other cocaine studies. Based on our in vitro data, it is unlikely that increased α-granule release in these individuals is related solely to the combination of short-term cocaine and catecholamine increases. By contrast, long-term cocaine use may indirectly “prime” platelets to have a lower threshold response to “weak” physiological platelet agonists, a mechanism that is consistent with cocaine’s known pharmacological effects. At the current time, however, the exact mechanism underlying these observations remains unknown.

Recent work by Kugelmass et al using cocaine at 10^{-3} to 10^{-2} mol/L determined that in vitro incubation of isolated platelets (PRP or gel-filtered platelets) at these concentrations had no effect on P-selectin expression, a finding that we confirmed in the present study using 10^{-3} mol/L cocaine as our highest in vitro concentration. In contrast to our in vitro WB findings, however, Kugelmass et al found that incubation of WB with cocaine at a concentration of 10^{-3} mol/L directly caused an increase in platelet P-selectin in 50% of donors and, when followed by epinephrine or ADP, caused a synergistic increase in P-selectin expression. Because Kugelmass found that cocaine directly induced platelet P-selectin in WB in only half of donors, it is possible that our donors were in the subset who do not respond to...
cocaine. It is also possible that differences in methodology may partly explain the discrepancy in our findings; we used different fixative concentrations and different anti-P-selectin MAbs. Our in vitro platelet aggregation results can also be contrasted with the two other in vitro aggregation studies in the literature.41,42 Because we examined human platelets, it is difficult to fully compare our results with those of the study of Togna et al.,41 which reported that cocaine treatment of rabbit platelets had both inhibitory and aggregatory effects to a variety of platelet agonists. Rezkalla and coworkers42 found that aggregation to a single dose of ADP (1 μmol/L) was enhanced by cocaine; however, they used a cocaine dose (1.5×10⁻⁵ mol/L) greater than the highest dose (1×10⁻⁵ mol/L) in our in vitro studies.

Several caveats apply to this work. Our results may reflect drug use other than cocaine. Although all of the study patients were screened by history and urine examination for recent opiate and alcohol use, it is possible that prior use of these substances or other drugs of abuse, alone or in combination with cocaine, may be responsible for these platelet findings. However, we could not discern any difference in self-reported drug use between the 5 patients with very high baseline levels of activated platelets and the remaining 7 cocaine users. The high levels of platelet P-selectin after cocaine or placebo infusion could reflect an emotional response to an intravenous injection, ie, a conditioned-cue response to the infusion itself and not specifically to the possibility of receiving cocaine. However, in an ongoing study of long-term cocaine users with an indwelling intravenous line placed solely for sampling and injection of 5% dextrose solution, we have found that the percent of platelets expressing P-selectin remained <10% immediately after infusion and for 6 hours after the infusion (H. Rinder, E. McCance-Katz, unpublished observations); these cocaine users were aware that they were receiving intravenous dextrose. These data, coupled with the lack of platelet activation in healthy controls receiving intravenous dextrose and after in vitro incubation with placebo solution, mitigate against the possibility that either the intravenous placement or the placebo solution was responsible for the high levels of P-selectin expression.

In autopsy and angiographic studies of selected cocaine users, between 30% and 40% of individuals had significant (≥70% occlusion) coronary artery disease that appeared more severe than expected for the age of the patients.43,44 Coronary disease in cocaine users may be characterized by features that are somewhat atypical of the atherosclerosis found in individuals who do not use cocaine, including medial and intimal smooth muscle cell infiltration, intimal proliferation of smooth muscle cells, and lack of complications lesions (plaque rupture or hemorrhage) in acute thrombosis.1-4 Some studies describe occlusive platelet-rich coronary thrombi complicating the long-term obstruction.1,2 The ability of cocaine to cause vasoconstriction in animals45 and humans46 might promote platelet interaction with the endothelium and platelet aggregation leading to platelet-rich thrombi. In our study, no ECG changes indicative of myocardial ischemia were detected in subjects during the infusion protocol; this finding is consistent with reports that platelet α-granule release alone is insufficient to cause platelet aggregation necessary for thrombosis.8 Thus, the primary effect of platelet α-granule release in long-term cocaine users may be the elaboration of platelet-derived growth factor and transforming growth factor-β from the α-granule. These vascular cytokines have been implicated in smooth muscle cell proliferation and vascular remodeling in atherosclerosis47 and could be involved in the accelerated atherogenesis found in some cocaine users.

In summary, our results suggest that cocaine use is associated with intermittent but markedly increased levels of platelet α-granule release and that this activation is unlikely to be due to a direct short-term effect of cocaine or its metabolites. These preliminary studies deserve further examination to determine if vascular complications associated with cocaine use are related to platelet α-granule release.

Acknowledgments

This work was supported by National Institutes of Health grants HL-02668 (Dr Rinder), DA-04060 (Dr Jatlow), DA-04060, DA-06190, DA-0112 (Dr Kosten), and HL-47193 (Dr Smith). Dr Smith is a Scholar of the Leukemia Society of America.

References


Platelet alpha-granule release in cocaine users.
H M Rinder, K A Ault, P I Jatlow, T R Kosten and B R Smith

Circulation. 1994;90:1162-1167
doi: 10.1161/01.CIR.90.3.1162

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/90/3/1162

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/