Published in final edited form as:

Thromb Haemost. 2013 January ; 109(1): 85–92. doi:10.1160/TH12-03-0202.

Hyperreactive platelet phenotypes: Relationship to altered serotonin transporter number, transport kinetics and intrinsic response to adrenergic co-stimulation

Jeffrey S. Berger¹, Richard C. Becker², Cynthia Kuhn², Michael J. Helms², Thomas L. Ortel², and Redford Williams²

¹New York University Medical Center, New York, New York, USA

²Duke University Medical Center, Durham, North Carolina, USA

Summary

The mechanism underlying a hyperreactive platelet phenotype remains unknown. Since serotonin has been shown to influence platelet biology and atherothrombosis, we sought to investigate the association of platelet serotonin transporter number, binding affinity, and uptake kinetics to platelet aggregation. A total of 542 healthy volunteers had light transmittance platelet aggregometry measured in response to varying concentrations of epinephrine, serotonin, epinephrine plus serotonin, ADP and collagen. Transporter-dependent serotonin uptake rate was determined (V_{max}), as were serotonin transporter number (B_{max}) and binding affinity (K_d) using ³H paroxetine binding in a homologous displacement assay, nonlinear regression and validated algorithms for kinetic modeling. Stimulation with submaximal (2 μ M) epinephrine concentration elicited a distinct, bimodal pattern of platelet aggregation in this population. In contrast, subjects exhibited minimal aggregation in response to serotonin alone. Co-stimulation with submaximal epinephrine and serotonin induced platelet aggregation to a level beyond that observed with either agonist alone and maintained a bimodal response distribution. Subjects with heightened (>60%) platelet aggregation to both epinephrine alone and epinephrine plus serotonin exhibited increased platelet serotonin uptake, and transporter number and affinity. In a population of healthy subjects, co-stimulation with submaximal concentrations of epinephrine and serotonin identifies a subset of individuals with a hyperreactive platelet aggregation profile that is associated with changes in platelet serotonin function.

Keywords

Platelets; platelet activity; serotonin; epinephrine; transporter

Introduction

Pathological and clinical studies have consistently demonstrated that platelets play a major role in the pathogenesis of atherosclerosis and coronary thrombosis (1–3). Prior clinical studies have demonstrated the importance of increased platelet aggregation in coronary

© Schattauer 2013

Correspondence to: Jeffrey S. Berger, MD, MS, FAHA, FACC, Director of Cardiovascular Thrombosis, New York University School of Medicine, 530 First Avenue, Skirball 9R, New York, NY 10016, USA, Tel.: +1 212 263 4004, Fax: +1 212 263 3988, Jeffrey.berger@nyumc.org.

Conflicts of interest

Redford Williams holds a U.S. patent on the 5HTTLPR L allele as risk marker for CVD in persons exposed to chronic stress. There are no other conflicts of interest to disclose.

artery atherosclerosis and thrombotic disorders, including myocardial infarction, stroke and cardiovascular death (4–6). Although screening subjects for increased platelet activity (or reactivity in response to biologically relevant stimuli) may identify individuals at high-risk for cardiovascular events, it remains a research tool, yet to be validated and subsequently implemented in standard clinical care (7, 8).

Data suggest that *in vitro* aggregation assays can reliably and reproducibly assess increased platelet reactivity (9). Stimulation with submaximal concentrations of adrenergic agonists (epinephrine) as well as ADP and collagen, reveals a hyperreactive platelet phenotype in a bimodal distribution (9, 10). The mechanistic basis for this finding remains uncertain. However, in biological systems platelet agonists act synergistically with one another (11–13), and platelet activation *in vivo* involves a combination of agonists. In addition to the adrenergic system, the serotonergic and purinergic systems are known to influence platelet homeostasis (14). Serotonin may augment platelet activity (14, 15) through the release of serotonin stored within granule repositories, causing vasoconstriction, further aggregation (16, 17), and the release of chemokines that induce production and release of inflammatory cytokines (18–20). A synergistic interaction between serotonergic and adrenergic stimuli has been proposed to explain the relationship between stress and thrombotic events, including myocardial infarction and sudden cardiac death (21, 22). Because platelets are exposed simultaneously to multiple agonists *in vivo*, the investigation of biological responses to dual stimulation may better identify physiological effects that have implications for the pathogenesis of thrombotic disorders.

We investigated platelet serotonin transporter expression, including platelet serotonin transporter number, binding affinity, and uptake kinetics and platelet aggregation in response to single and dual agonists, representing the serotonergic, adrenergic and purinergic systems in a large group of healthy subjects without cardiovascular disease.

Methods

Study design and population

Subjects were recruited to take part in the Family Heart Study, a single-centre investigation designed to examine the relationship between psychosocial behaviours and cardiovascular risk factors. The sample was recruited between August 2004 to September 2008, and was comprised of 542 volunteers without cardiovascular disease. The study was conducted at Duke University Medical Center, and all subjects gave informed consent prior to their participation using a form approved by the Duke University Medical Center Institutional Review Board. Men and women between the ages of 18 and 55 years were selected for inclusion. Sibling pairs were recruited via community based advertisements. Exclusions included pregnancy, diabetes, hypertension that required medication(s), and the use of psychoactive medications, anti-inflammatory agents including non-steroidal anti-inflammatory drugs and use of aspirin. All subjects were required to read and write English.

Procedures

All subjects were asked to refrain from eating or drinking after midnight and to avoid smoking after 8 PM on the day preceding the data collection. The physiological assessment incorporated an anthropometric evaluation, including height and weight measurements. Blood samples were drawn in the morning between 8 and 9 AM.

Sample collection

Blood samples were collected after a 30-minute (min) resting baseline period following catheter insertion following a non-traumatic venipuncture. During that time, subjects sat

quietly and were undisturbed. Blood was collected into tubes containing heparin and glutathione for anticoagulation and preservation of catecholamines, respectively, into 3.8% sodium citrate solution (9:1) for platelet aggregation studies, and into acid-citrate-dextrose (ACD) for serotonin assays. Blood for platelet activation was collected from the latter part of the sample, after samples for neuroendocrine and inflammatory markers had been obtained.

Platelet aggregometry

Blood samples were obtained in the morning to avoid circadian variation in platelet response (23). Platelet-rich plasma (PRP) was separated by centrifugation for 10 min at 160 g and adjusted to 250,000 platelets/ μ l with autologous platelet-poor plasma for use in a Chronolog 4-channel platelet aggregometer (Chronolog, Havertown, PA, USA). One hundred percent transmittance was the optical density obtained with platelet-poor plasma (PPP). Epinephrine, ADP and collagen were purchased from Chronolog. Serotonin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Aggregation triggered by epinephrine (2, 4 and 10 μ M), serotonin (10 μ g/ml), collagen (2, 4 and 10 μ g/ml) and ADP (1, 2 and 5 μ M) as well as each agonist combined with serotonin (10 μ g/ml) was evaluated. All samples were processed and the platelet aggregation studies performed by the same experienced technician using one instrument and an identical lot of agonists. Maximum percent aggregation within 10 min was recorded.

Other platelet assays

Platelet serotonin transporter number was quantitated with [3 H]-paroxetine binding as described by Nemeroff (24) and modified by Slotkin et al. (25). PRP was obtained by centrifugation of whole blood at 100 g for 30 min. The PRP was centrifuged ($39,000 \times g$ for 10 min at 4°C) and the platelet-rich pellet was lysed by suspension in 5 mM Tris buffer (pH 7.5) containing 5 mM EDTA, pH 7.5, sedimented at 39,000 g suspended in 70 mM Tris (pH 7.5), resedimented, and finally resuspended in the assay buffer (50 mM Tris (pH 7.5) containing 120 mM NaCl and 5mM KCl). Aliquots were taken for protein determination and the suspension frozen at -70°C until assay at a concentration of 1 mg protein/ml. Binding of [3 H]-paroxetine to platelet membranes was accomplished by incubating aliquots of platelet membranes in six different concentrations of [3 H]-paroxetine (25, 50, 100, 250, 500, 1,000 pM) in triplicate using 100 μ g of platelet protein/tube in a final volume of 250 μ l assay buffer. Tubes were incubated on ice for 60 min after which 5 ml of ice-cold buffer were added and labelled membranes harvested by vacuum filtration on Whatman GF/C glass fiber papers pre-soaked in 0.05% polyethyleneimine. Filters were washed three times with 5 ml of ice-cold buffer, and filter papers counted by liquid scintillation spectrometry using a non-toluene based fluor (Safety Solv). Binding was analysed by nonlinear regression algorithm for sigmoid curves with Prism 3.0 (Graphpad, San Diego, CA, USA) to determine the K_d and B_{max} of binding. Non-specific binding was determined as the binding in the presence of 0.22 mM serotonin.

Platelet serotonin uptake assays were conducted as described by Slotkin et al. (25). Aliquots of platelets were diluted in modified Krebs-Henseleit buffer (calcium free containing 2 mM EDTA and pargyline, 100 μ M and incubated with 3 H serotonin [1,2- 3 H(N)] with or without imipramine. Samples were incubated with a range of serotonin concentrations (5 nM- 1 μ M). Samples were incubated for 10 min at 37°C. Platelets were trapped on Whatman GF/C filters and washed by vacuum filtration with ice-cold phosphate-buffered saline and counted by liquid scintillation spectrometry with a non-toluene based fluor. Uptake rate (V_{max}) and affinity (K_d) determined by a nonlinear regression algorithm for sigmoid curves with Prism 3.0 (Graphpad).

Statistical analysis

SAS (Cary, NC, USA) software was used to conduct all analyses. All data were examined using histograms and scatter plots. Results are reported as the mean $\pm$ standard error of the mean. Because two or more subjects were often present from within the same family in our sample, a mixed-model analysis of variance was utilized for testing the differences of means between aggregation groups defined as <60% vs. 60% aggregation (9). In these models, family was treated as a source of random replication error, with additional covariate adjustment for age, body mass index (BMI), smoking status, race and sex. The means between aggregation groups are thus least-square means, adjusted for all other effects in the model and controlling for family membership. The 60% cut-off for calling a hyperreactive platelet phenotype is consistent with previous data (9, 10). Kappa statistics were calculated to measure the strength of correlation between aggregation to epinephrine (2 μ M) and to ADP and to collagen, respectively. A p-value <0.05 was considered statistically significant.

Results

Under uniform conditions, we performed platelet aggregation in 542 subjects to assess platelet phenotypes. Baseline characteristics of this study group are detailed in Table 1.

Adrenergic agonists and platelet aggregation

Subjects exhibited little spontaneous aggregation (median, 1%; inter-quartile range, 1–2%) (Figure 1A). We sought to determine if there were differences in platelet aggregation according to varying concentrations of epinephrine. At a concentration of 2 μ M epinephrine a bimodal distribution of platelet aggregation was observed. The majority of individuals (61%) exhibited low levels of platelet aggregation (<40%), very few subjects (5%) exhibited moderate platelet aggregation (40–60%), and a significant minority (34%) of participants displayed a hyperreactive phenotype (>60% aggregation). With increasing epinephrine concentrations, this bi-modal response became blunted and less apparent, with a larger proportion exhibiting >60% aggregation (Figure 1B–D), a pattern consistent with prior work (9).

Purinergic and other agonists

To confirm a concentration-dependent loss of a bimodal response, and to determine if aggregation was agonist specific, we next measured platelet aggregation to several other agonists including ADP and collagen at various concentrations. The distribution patterns observed for ADP and collagen were similar to those demonstrated for epinephrine (data not shown). As with epinephrine, increasing concentrations blunted the bimodal response observed with the lowest tested concentrations. Subjects with increased platelet aggregation (>60%) to low concentration of epinephrine (2 μ M) were also more likely to have increased platelet aggregation in response to low concentrations of ADP and collagen. The *kappa* was 0.65 and 0.28 for the association of epinephrine to ADP and to collagen, respectively.

Serotonin and platelet aggregation

As shown in Figure 2, in contrast to the 34% of subjects with >60% aggregation in response to epinephrine (2 μ M) alone, no subjects exhibited >60% platelet aggregation in response to serotonin (10 μ M) alone (median, 0%; inter-quartile range, 0–1%). When serotonin was added to the lowest concentration of epinephrine, however, 62% of the subjects had platelet aggregation >60% while still maintaining a bimodal distribution (p <0.001 comparing the response from EPI vs. EPI + serotonin). When serotonin was added to ADP and collagen, a similar shift to increased percent aggregation was observed.

Platelet aggregation and serotonin biology

We next compared platelet serotonin uptake rate (V_{max}), serotonin transporter number (B_{max}) and binding affinity (K_d) in groups of subjects with low (< 60% response; $n=365$) and high (>60% response; $n=179$ subjects) levels of platelet aggregation to 2 μ M epinephrine. Seven subjects with missing data on platelet serotonin uptake and affinity were excluded from this analysis. While no difference in platelet serotonin uptake was observed between groups, subjects with >60% platelet response to 2 μ M epinephrine exhibited a higher serotonin transporter number and a stronger binding affinity (Table 2), suggesting that serotonin kinetics may play a significant role in increased platelet aggregation in response to epinephrine.

We further investigated whether the measured serotonin parameters differed with respect to the effect of serotonin on the platelet aggregation response to 2 μ M epinephrine. Subjects with a low level of platelet aggregation to 2 μ M epinephrine were subdivided into those with decreased aggregation in response to 2 μ M epinephrine plus serotonin (< 60% response; $n=201$ subjects; Group A) and those with increased aggregation in response 2 μ M epinephrine plus serotonin (>60% response; $n=155$ subjects; Group B). For subjects with a high level of aggregation to 2 μ M epinephrine, almost all showed an elevated level of aggregation to 2 μ M epinephrine plus serotonin (>60% response; $n=177$ subjects; Group C). Two subjects exhibited high aggregation to 2 μ M epinephrine alone but low aggregation in response to 2 μ M epinephrine plus serotonin; these two individuals were excluded from further analysis. We hypothesised that individuals in Group C would exhibit enhanced platelet serotonin uptake, transporter number, and binding affinity compared to individuals in Group A, whereas individuals in Group B would exhibit an intermediate pattern of platelet serotonin dynamics. In support of our hypothesis, subjects in Group C exhibited higher platelet serotonin uptake (V_{max} , $t=2.34$; $p=0.02$), stronger binding affinity (K_d ; $t=2.44$; $p=0.015$), and higher serotonin transporter number (B_{max} ; $t=5.78$; $p<0.001$) than subjects in Group A (Table 3). In addition, Group B showed the expected intermediate pattern: higher platelet serotonin uptake rate ($t=2.87$; $p=0.005$) than group A, but weaker binding affinity ($t=3.42$; $p=0.001$) and serotonin transporter number ($t=4.50$; $p<0.001$) than group C (Table 3).

We next examined levels of platelet aggregation to varying concentrations of collagen and ADP as a function of the three groups defined above in terms of > vs. < 60% aggregation to both 2 μ M epinephrine alone and 2 μ M epinephrine + serotonin. As shown in Figure 3A, those subjects in Group A showed reduced platelet aggregation to 2 μ M collagen compared to those in Groups B and C ($p<0.0001$ for each comparison). Percent aggregation did not differ between groups B and C ($p=0.23$). All three groups differed from each other at collagen concentrations of 4 μ M and 10 μ M ($p<0.0001$ for each comparison). Group A also showed a larger increase than Groups B and C in platelet aggregation to increasing collagen concentrations. As shown in Figure 3B, the pattern of aggregation to varying concentrations of ADP was more uniform than that for collagen, reflecting the stronger *kappa* for associations between aggregation to low epinephrine and ADP concentrations (0.65) than the *kappa* between low epinephrine and collagen (0.28) concentrations. Groups A, B and C all differed from each other ($p<0.0001$) at all three ADP concentrations and also showed increasing within-group increases in aggregation that followed a more dose-response pattern as a function of increasing ADP concentrations.

Discussion

The results of the present study suggest a role for the serotonin transporter in platelet hyperreactivity in certain individuals. This is the first study to show that individual differences in platelet aggregation to serotonergic and non-serotonergic stimuli are

associated with individual differences in measures of platelet serotonergic function (both expression and function of the transporter). Additionally, we replicated previous findings (9, 10) that submaximal concentrations of epinephrine identified a small but distinct population of subjects with increased (>60%) platelet aggregation, and that this hyperreactivity appears to be a “global phenomenon”, wherein individuals with increased platelet aggregation in response to epinephrine were also likely to have a heightened response to other platelet agonists.

In the present study, we found that submaximal agonist concentrations identify platelet hyperresponders with a clear bimodal response to stimulation. The majority of subjects exhibited relatively modest platelet aggregation (<40%), whereas a significant number demonstrated more than 60% aggregation, consistent with a hyperreactive platelet phenotype. Few subjects demonstrated aggregation in the 40% to 60% aggregation range at any agonist concentration, suggesting that less than 40% and more than 60% aggregation represent appropriate criteria for distinguishing between either minimal or absent aggregation and full or complete aggregation. Similar to Yee et al. (10), we also observed a global or generalised response to adrenergic and non-adrenergic stimulation. Individuals with increased platelet aggregation to epinephrine were also more likely to have increased platelet aggregation in response to collagen and ADP stimulation, though the relationship was stronger for ADP. This finding supports a converging signal transduction pathway leading to a physiological response, such as platelet aggregation, that is similar across different agonists (26).

Serotonin and submaximal concentration of EPI induced aggregation in a larger proportion of subjects than EPI alone, although the bimodal distribution was maintained (Figure 2). When serotonin was employed as the sole agonist, subjects exhibited a modest response, reflecting the well-known phenomenon that platelets are reactive to serotonin only if already activated by other means – e.g. epinephrine (15). The construct of dual agonist stimulation, as observed previously, may reflect a more physiological environment for platelets (12, 15) and has been reported previously with epinephrine and serotonin. The present findings suggest that serotonin transporter (SERT) function may contribute to increased platelet aggregation in this physiologic context. Platelet serotonin uptake, transporter number and affinity were significantly increased in subjects who showed exaggerated platelet aggregation to both EPI 2 μ M alone and EPI 2 μ M + serotonin compared to those with 60% aggregation to both EPI 2 μ M alone and EPI 2 μ M + serotonin (Table 2). These results demonstrate that increased capacity to accumulate serotonin is associated with increased platelet reactivity to stimuli for aggregation. Serotonin contributes to platelet aggregation after its release via activation of 5 HT_{2a} receptors on platelets (27).

The increased platelet serotonin transporter number and uptake we observed in subjects with increased platelet aggregation to both EPI 2 μ M and EPI 2 μ M + serotonin could have implications for the pathophysiology of atherosclerotic cardiovascular disease. Increased serotonin availability to vascular endothelial cells and smooth muscle cells has been shown *ex vivo* and *in vitro* to provoke cellular proliferation and vasoconstriction - potentially contributing to atheromatous plaque growth and development [pic](28, 29). Individuals with the lower function allele of a 5-HT transporter promoter polymorphism (5HTTLPR) have decreased platelet uptake of serotonin (30) and a lower risk of myocardial infarction (31–33). Consistent with these findings, the interaction between the integrin IIb/beta 3 and the serotonin transporter is associated with platelet aggregation (34). It is therefore not surprising that increased concentration of platelet serotonin has been associated with an increased risk of coronary artery disease and adverse cardiovascular events (29). This association could be partly explained by the synergistic effect of serotonin on platelet aggregation. Further evidence for pathologic sequelae of serotonin effects on platelet

function comes from epidemiological research, suggesting that treatment of depressed CHD patients with selective serotonin reuptake inhibitors (SSRIs) is associated with decreased mortality and recurrent myocardial infarction (35, 36). Recent research aimed at identifying the mechanism(s) responsible for SSRI protective effects has examined effects of platelet serotonin transporter blockade on agonist-stimulated *in vitro* platelet aggregation. Galan et al. documented that ADP-induced platelet aggregation is enhanced by serotonin and that this augmented aggregation is blocked by treatment with the SSRI citalopram (37). In a similar study Tseng et al. found that citalopram also inhibited collagen-induced platelet aggregation, P-selectin expression, and thromboxane A₂ release (38). By documenting in a large, healthy sample that exaggerated platelet aggregation is associated with increased platelet serotonin uptake function, the present study complements these extensive reports of serotonergic involvement in the pathogenesis of atherosclerotic cardiovascular disease by suggesting persons with such increased platelet serotonin uptake function are at increased disease risk and might be targeted for preventive interventions that interfere with platelet serotonin uptake functions. Ongoing studies such as the National Institutes of Health-sponsored REMIT study examining the effect of serotonin specific reuptake inhibitors on mental stress induced myocardial ischaemia, will shed additional light on the relationship between platelet aggregation and serotonin transporter biology (39).

In addition to our novel findings, this study is noteworthy for the direction of investigation. While many studies proceed from receptor discovery or count to function (platelet aggregation in this case), in this study we began by characterising a hyperreactive platelet phenotype (platelet aggregation >60% to epinephrine 2 μ M) in a normal young adult sample. As part of a research program aimed at understanding the mechanisms underlying cardiovascular risk factors like platelet aggregation in response to adrenergic and serotonergic stimulation, we were able to identify a bimodal response to both adrenergic and serotonergic platelet stimulation that was similar to that found by other investigators and found enhanced platelet serotonin dynamics in those subjects who exhibited platelet hyperaggregation in response to adrenergic and serotonergic stimulation. Our findings suggest that a strategy beginning with a hyperreactive platelet phenotype may be a useful approach to identifying underlying pathogenic mechanisms.

Limitations

An important limitation of the current study is its cross-sectional design. Nevertheless, the evidence that increased platelet aggregation in response to both epinephrine alone and dual stimulation with adrenergic and serotonergic agonists is associated with increased platelet serotonin uptake, transporter number and binding affinity makes a biologically plausible case that the association reflects causality. Future studies are warranted to validate our results and examine the reported association prospectively, and determine whether this hyperreactive platelet phenotype is associated with increased risk of developing coronary atherosclerosis and/or incidence of clinical events. Our measurements of platelet aggregation were at a single time point, and the function of platelets within an individual may change over time. However, previous data with slightly different concentrations demonstrated excellent reproducibility for platelet aggregometry using submaximal concentrations of adrenergic agonists which persisted for up to three years.(9) Preliminary data from our laboratory demonstrates good reproducibility of platelet aggregation induced by epinephrine 2 μ M measured weekly for three weeks by demonstrating a coefficient of variance of 11% at 5 min of aggregation and 6.5% at maximum aggregation.

Conclusions

Co-stimulation with submaximal concentrations of epinephrine and serotonin identifies a distinct profile of platelet aggregation (hyperreactive phenotype) with accompanying

alterations in serotonin binding and transport biology. The underlying mechanism(s) and its relationship to atherothrombotic events remain unknown; however, genetic and/or environmental influences on platelet serotonin transporter function, possibly coupled with a heightened serotonin receptor-mediated response to extracellular serotonin concentration following epinephrine (sympathetic, adrenergic and possibly purinergic) stimulation is a biologically-plausible hypothesis worthy of further investigation. Studies are currently in progress to investigate the relationship among functional serotonin gene polymorphisms, increased platelet aggregation to agonist co-stimulation, altered platelet serotonin kinetics and cardiovascular events.

Acknowledgments

Supported by grant P01-HL036587 from the National Heart, Lung and Blood Institute. Dr Berger was partially funded by an American Heart Association Fellow to Faculty Award (0775074N).

References

1. Davi G, Patrono C. Platelet activation and atherothrombosis. *New Engl J Med*. 2007; 357:2482–2494. [PubMed: 18077812]
2. Ruggeri ZM. Platelets in atherothrombosis. *Nature Med*. 2002; 8:1227–1234. [PubMed: 12411949]
3. Vorchheimer DA, Becker R. Platelets in atherothrombosis. *Mayo Clin Proc*. 2006; 81:59–68. [PubMed: 16438480]
4. Elwood PC, Renaud S, Sharp DS, et al. Ischemic heart disease and platelet aggregation. The Caerphilly Collaborative Heart Disease Study. *Circulation*. 1991; 83:38–44. [PubMed: 1984896]
5. Gurbel PA, Becker RC, Mann KG, et al. Platelet function monitoring in patients with coronary artery disease. *J Am Coll Cardiol*. 2007; 50:1822–1834. [PubMed: 17980247]
6. Trip MD, Cats VM, van Capelle FJ, et al. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. *New Engl J Med*. 1990; 322:1549–1554. [PubMed: 2336086]
7. Michelson AD. Platelet function testing in cardiovascular diseases. *Circulation*. 2004; 110:e489–493. [PubMed: 15533872]
8. Sharma G, Berger JS. Platelet activity and cardiovascular risk in apparently healthy individuals: a review of the data. *J Thromb Thrombolysis*. 2011; 32:201–208. [PubMed: 21562837]
9. Yee DL, Sun CW, Bergeron AL, et al. Aggregometry detects platelet hyperreactivity in healthy individuals. *Blood*. 2005; 106:2723–2729. [PubMed: 15972447]
10. Yee DL, Bergeron AL, Sun CW, et al. Platelet hyperreactivity generalizes to multiple forms of stimulation. *J Thromb Haemost*. 2006; 4:2043–2050. [PubMed: 16961612]
11. Yoshida N, Aoki N. Potentiation by collagen or epinephrine of platelet responsiveness to aggregation. The possible role of substance(s) released from platelet membranes. *J Lab Clin Med*. 1977; 89:603–614. [PubMed: 839118]
12. Huang EM, Detwiler TC. Characteristics of the synergistic actions of platelet agonists. *Blood*. 1981; 57:685–691. [PubMed: 7470619]
13. Kinlough-Rathbone RL, Packham MA, Mustard JF. Synergism between platelet aggregating agents: the role of the arachidonate pathway. *Thrombosis Res*. 1977; 11:567–580.
14. De Clerck F. The role of serotonin in thrombogenesis. *Clin Physiol Biochem*. 1990; 8 (Suppl 3): 40–49.
15. Li N, Wallen NH, Ladjevardi M, et al. Effects of serotonin on platelet activation in whole blood. *Blood Coagul Fibrinol*. 1997; 8:517–523.
16. Eidt JF, Ashton J, Golino P, et al. Thromboxane A2 and serotonin mediate coronary blood flow reductions in unsedated dogs. *Am J Physiol*. 1989; 257:H873–882. [PubMed: 2782444]
17. Ashton JH, Benedict CR, Fitzgerald C, et al. Serotonin as a mediator of cyclic flow variations in stenosed canine coronary arteries. *Circulation*. 1986; 73:572–578. [PubMed: 2936531]
18. Flad HD, Brandt E. Platelet-derived chemokines: pathophysiology and therapeutic aspects. *Cell Mol Life Sci*. 2010; 67:2363–2386. [PubMed: 20213276]

19. Otterdal K, Smith C, Oie E, et al. Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. *Blood*. 2006; 108:928–935. [PubMed: 16861346]
20. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev*. 2007; 21:99–111. [PubMed: 16987572]
21. Walker RF, Codd EE. Neuroimmunomodulatory interactions of norepinephrine and serotonin. *J Neuroimmunol*. 1985; 10:41–58. [PubMed: 3902887]
22. Thompson NT, Scrutton MC, Wallis RB. Synergistic responses in human platelets. Comparison between aggregation, secretion and cytosolic Ca²⁺ concentration. *Eur J Biochem*. 1986; 161:399–408. [PubMed: 3490977]
23. Tofler GH, Brezinski D, Schafer AI, et al. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N Engl J Med*. 1987; 316:1514–1518. [PubMed: 3587281]
24. Nemeroff CB, Knight DL, Krishnan RR, et al. Marked reduction in the number of platelet-³H-imipramine binding sites in geriatric depression. *Arch Gen Psychiatry*. 1988; 45:919–923. [PubMed: 2844132]
25. Slotkin TA, McCook EC, Ritchie JC, et al. Serotonin transporter expression in rat brain regions and blood platelets: aging and glucocorticoid effects. *Biol Psych*. 1997; 41:172–183.
26. Chatterjee MS, Purvis JE, Brass LF, et al. Pairwise agonist scanning predicts cellular signaling responses to combinatorial stimuli. *Nat Biotechnol*. 2010; 28:727–732. [PubMed: 20562863]
27. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res*. 2006; 99:1293–1304. [PubMed: 17158345]
28. Ishida T, Hirata K, Sakoda T, et al. Identification of mRNA for 5-HT₁ and 5-HT₂ receptor subtypes in human coronary arteries. *Cardiovasc Res*. 1999; 41:267–274. [PubMed: 10325974]
29. Vikenes K, Farstad M, Nordrehaug JE. Serotonin is associated with coronary artery disease and cardiac events. *Circulation*. 1999; 100:483–489. [PubMed: 10430761]
30. Greenberg BD, Tolliver TJ, Huang SJ, et al. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Am J Med Genet*. 1999; 88:83–87. [PubMed: 10050973]
31. Fumeron F, Betoulle D, Nicaud V, et al. Serotonin transporter gene polymorphism and myocardial infarction: Etude Cas-Temoins de l'Infarctus du Myocarde (ECTIM). *Circulation*. 2002; 105:2943–2945. [PubMed: 12081984]
32. Arinami T, Ohtsuki T, Yamakawa-Kobayashi K, et al. A synergistic effect of serotonin transporter gene polymorphism and smoking in association with CHD. *Thromb Haemost*. 1999; 81:853–856. [PubMed: 10404755]
33. Coto E, Reguero JR, Alvarez V, et al. 5-Hydroxytryptamine 5-HT_{2A} receptor and 5-hydroxytryptamine transporter polymorphisms in acute myocardial infarction. *Clin Sci*. 2003; 104:241–245. [PubMed: 12605580]
34. Carneiro AM, Cook EH, Murphy DL, et al. Interactions between integrin α IIb β 3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J Clin Invest*. 2008; 118:1544–1552. [PubMed: 18317590]
35. Lesperance F, Frasere-Smith N, Koszycki D, et al. Effects of citalopram and interpersonal psychotherapy on depression in patients with coronary artery disease: the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy (CREATE) trial. *J Am Med Assoc*. 2007; 297:367–379.
36. Taylor CB, Youngblood ME, Catellier D, et al. Effects of antidepressant medication on morbidity and mortality in depressed patients after myocardial infarction. *Arch Gen Psychiatry*. 2005; 62:792–798. [PubMed: 15997021]
37. Galan AM, Lopez-Vilchez I, Diaz-Ricart M, et al. Serotonergic mechanisms enhance platelet-mediated thrombogenicity. *Thromb Haemost*. 2009; 102:511–519. [PubMed: 19718472]
38. Tseng YL, Chiang ML, Huang TF, et al. A selective serotonin reuptake inhibitor, citalopram, inhibits collagen-induced platelet aggregation and activation. *Thromb Res*. 2010; 126:517–523. [PubMed: 20961601]
39. Jiang W, Velazquez EJ, Samad Z, et al. Responses of mental stress-induced myocardial ischemia to escitalopram treatment: background, design, and method for the Responses of Mental Stress

Induced Myocardial Ischemia to Escitalopram Treatment trial. Am Heart J. 2012; 163:20–26.
[PubMed: 22172432]

Figure 1. Distribution of platelet aggregation response to control and increasing epinephrine concentrations (a-0 μ M [control], b-2 μ M, c-4 μ M, d-10 μ M).

Figure 2. Distribution of platelet aggregation response to submaximal epinephrine, serotonin and serotonin plus submaximal epinephrine.

Figure 3. Platelet aggregation to collagen (A) and ADP (B)

A) Percent aggregation (means $\pm$ SEM) to varying collagen concentrations in Group A (<60% aggregation to Epi 2 μ M and Epi 2 μ M + 5HT), Group B (>60% aggregation only to Epi 2 μ M + 5HT) and Group C (>60% to both Epi 2 μ M and Epi 2 μ M + 5HT); B) Percent aggregation to varying ADP concentrations in Group A, Group B and Group C.

Table 1

Baseline characteristics.

Age, mean years $\pm$ SD	30.4 $\pm$ 0.4
Women N (%)	320 (59)
Race N (%)	
African American	179 (33)
Caucasian	363 (67)
Body mass index, kg/m ² $\pm$ SD	26.1 $\pm$ 0.25
Cigarette smokers N (%)	
Never	423 (78)
Former	66 (12)
Current	50 (9)

N, number, SD, standard deviation

Table 2

Platelet serotonin dynamics as a function of aggregation to submaximal epinephrine alone.

	60% aggregation in response to EPI 2 μM (n=356)	>60% aggregation to EPI 2 μM (n=179)	P-value
Platelet Serotonin Uptake, Vmax (SEM) (pmoles 5HT/107Plts/5 min)	338 (14)	362 (20)	NS
Transporter Number, Bmax (SEM) (pmoles/mg protein)	133(3.4)	165 (4.6)	<0.0001
Binding Affinity, KD (SEM) (nM)	537 (15)	466 (17) *	0.0010

EPI, epinephrine; SEM, standard error of the mean; NS, not significant.

* A lower Kd indicates higher binding affinity.

Table 3

Platelet serotonin dynamics as a function of aggregation to submaximal epinephrine alone and submaximal epinephrine + serotonin.

	Group A (n=201)	Group B (n=155)	Group C (n=177)	P value (A vs C)	P value (A vs B)	P value (B vs C)
Platelet serotonin uptake, V_{max} (SEM) (pmoles 5HT/107 Plts/5min)	307 (16)	380 (22)	364 (20)	0.02	0.005	NS
Transporter number, B_{max} (SEM) (pmoles/mg protein)	130 (4.5)	137 (4.9)	165 (4.6)	<0.001	NS	<0.001
Binding affinity, K_D (SEM) (nM)	524 (18)	553 (21)	467 (17) *	0.015	NS	<0.001

SEM, standard error of the mean; NS, not significant ($p>0.05$). Group A – 60% aggregation in response to *both* EPI 2 μ M alone and EPI 2 μ M + serotonin. Group B – >60% aggregation *only* to EPI 2 μ M + serotonin. Group C – >60% aggregation to *both* EPI 2 μ M and EPI 2 μ M + serotonin.

* A lower K_D indicates higher binding affinity.