Platelet activation and secretion associated with emotional stress

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ABSTRACT Platelets are believed to play a role in the pathogenesis of atherosclerosis and of the vascular obstruction that causes the acute complications of coronary artery disease. Since specific behavioral patterns appear to be related to the development of coronary artery disease and since emotional stress may predispose an individual to acute cardiovascular ischemia, it was hypothesized that platelet activation by catecholamines might be involved in these events. To study emotional stress, plasma samples were obtained from 61 senior medical residents immediately before they were to speak in public. There were significant increases in the plasma concentrations of the platelet-secreted proteins platelet factor 4 and b-thromboglobulin and epinephrine and norepinephrine immediately before speaking, which demonstrates that platelet activation and secretion occur in association with this type of emotional stress. Four trials were carried out to study the mechanism for this observed platelet secretion: (1) phenoxybenzamine, (2) propranolol, (3) 650 mg aspirin, and (4) 80 mg aspirin were given several hours before the public speaking engagement. Neither phenoxybenzamine nor propranolol in doses that blocked the hemodynamic effects of a1- and b1-adrenergic stimulation modified platelet secretion. Aspirin also did not block platelet secretion, which suggests that platelets were not being stimulated through a cyclooxygenase-dependent pathway. This study provides direct evidence of platelet secretion in vivo in association with emotional stress, and underscores the potential importance of platelet activation and secretion in the acute events that occur in patients with vascular disease. Circulation 71, No. 6, 1129–1134, 1985.

Emotional stress has long been thought to be associated with coronary artery disease, both as a risk factor for the development of atherosclerosis1, 2 and as a precipitating factor for sudden events.3–6 Multiple responses to emotional stress can affect cardiac function, with a common factor being adrenergic stimulation.7–9 Catecholamine secretion is increased during emotional stress and this response may be exaggerated in subjects with type A behavior patterns who are at greater risk of development of coronary artery disease.10 Both epinephrine and norepinephrine are potent stimuli for platelets in vitro.11 The recent demonstration that platelet membranes contain a2-adrenergic receptors suggests that catecholamines may activate platelets directly.12, 13 If platelets are activated directly by catecholamines in vivo, secretion of platelet-derived growth factor could contribute to the smooth muscle hyperplasia of atherosclerotic lesions14, 15 and platelet aggregate formation could play a role in the thrombosis of acute myocardial infarction and sudden death.16 Most of the experimental data that suggest a role for platelets in atherosclerosis come from platelet survival studies17, 18 or tests of platelet function in vitro, including studies of aggregation.14 With the development of radioimmunoassays for the measurement of two platelet-secreted proteins, platelet factor 4 (PF4) and b-thromboglobulin (bTG), it has become possible to assess platelet secretion in vivo. These assays currently represent the most sensitive method for detection of the activation of circulating platelets. Many investigative groups have reported that plasma concentrations of PF4 and/or bTG are elevated in patients with coronary artery disease, and this confirms the platelet survival data.19–21

Can circulating concentrations of catecholamines stimulate platelet activation and secretion during be-
havioral stress, thereby providing a mechanism by which platelets may play a role in the development of atherosclerosis or coronary artery obstruction? To study this question, plasma concentrations of catecholamines and the platelet-secreted proteins PF4 and \( \beta TG \) were measured in response to public speaking as a model for emotional stress. Public speaking has previously been demonstrated to be associated with both an increase in heart rate and an increase in plasma catecholamine levels that is most pronounced during the minute preceding or the first minute of speaking.\(^8\)\(^9\) These studies now demonstrate that public speaking is also associated with significant increases in the plasma concentrations of the platelet-secreted proteins PF4 and \( \beta TG \).

Methods

Experimental design. Sixty-one housestaff members of the Department of Medicine of our institution participated in this study over a 3 year period. Several subjects participated in more than one part of the study. All subjects gave informed consent for the protocol, which was approved by the Institutional Review Board of the University of Texas Health Science Center, San Antonio. Venous blood samples were obtained from each subject at three different times and were specified as control 1, stress, and control 2. The first blood sample, control 1, was obtained at the beginning of the academic year, up to 11 months before the stress sample. Stress blood samples were drawn at approximately 8:15 A.M., immediately before public speaking (60 min lecture), and control 2 samples were obtained 5 days to 3 weeks later. All blood samples were obtained through a butterfly needle with the subjects seated. Not all subjects were fasting, but none had taken additional antiplatelet agents or adrenergic agonists or antagonists for 1 week before blood collection. In each of the studies in which placebo or drug was administered, subjects were given these medications just for the stress period. Placebo and drugs were prepared in capsules of identical appearance by our hospital pharmacy and provided to us with numbered codes. The randomized protocol included tests of the following: (1) placebo vs 10 mg of the \( \alpha \)-adrenergic antagonist phenoxybenzamine (given at 6 P.M. and 12 midnight the night before, and 6 A.M. the morning of the lecture), (2) placebo vs 40 mg of the \( \beta \)-adrenergic antagonist propranolol, (3) placebo vs 650 mg aspirin, and (4) placebo vs 80 mg aspirin. The last three medications were taken at between 6 and 7 A.M. on the morning of the lecture.

Assays. Venous blood was collected and PF4 was measured by our previously published method.\(^7\) \( \beta TG \) was measured with radioimmunoassay kits purchased from Amersham (Arlington Heights, IL). Platelet counts were performed in EDTA-anticoagulated platelet-rich plasma with a Coulter Model C Thrombocounter and Thromboglu. Platelet aggregation studies were performed on platelet-rich plasma from blood anticoagulated with 3.8% sodium citrate (9:1 vol/vol) adjusted to a standard platelet count of 200,000/µl with platelet-poor plasma. Epinephrine and ADP, in final concentrations ranging from 0.625 to 20 µM, were used as platelet-aggregating agents. The percent of aggregation 3 min after the addition of the aggregating agent was determined for each concentration. Circulating platelet aggregates were assayed by the method of Wu and Hoak.\(^23\) Malondialdehyde (MDA) generation was determined as follows: Platelets were treated with either warmed bovine thrombin (2 U/ml) or 0.9% NaCl and the MDA generated was calculated with a molar extinction coefficient of 1.55 \( \times \) 10\(^4\) and expressed in nanomoles of MDA/10\(^8\) platelets. MDA production was calculated by subtracting the MDA produced by the saline-treated platelets from that produced by the thrombin-treated platelets.\(^23\)\(^24\) Plasma fibrinopeptide A (FPA) concentrations were determined by RIA-Quan FPA Test Kits purchased from Mallinkrodt, St. Louis. Plasma norepinephrine and epinephrine concentrations were determined by the modified radioenzymatic method of Passon and Peuler (Upjohn Co., Kalamazoo, MI).\(^25\)

Statistical analysis. All PF4, \( \beta TG \), epinephrine, and norepinephrine concentrations were converted to natural logarithms to normally distribute the data before statistical analysis. Data from the pilot study were analyzed by paired t test. Values from the combined placebo groups for control 1, stress, and control 2 periods were compared by one-way analysis of variance (ANOVA). If there was a statistical difference among these three measurements, they were further analyzed by paired t test. Results of all four drug studies were analyzed by two-way ANOVA. Probability values were obtained for the differences among control and stress measurements and for the differences between placebo- and drug-treated groups of subjects.

Results

In an initial pilot study eight male subjects (mean age 28 years) were evaluated immediately before giving their lectures (stress) and 1 week later (control). Platelet counts, plasma PF4, circulating platelet aggregates, platelet aggregation with ADP and epinephrine, and plasma catecholamines were measured. There was a significant increase in both plasma PF4 and epinephrine levels when stress measurements were compared with control by paired t test (data not shown). There were no differences in platelet count, circulating platelet aggregates, plasma norepinephrine, or percent aggregation to six different concentrations of ADP and epinephrine at 3 min. Circulating platelet aggregates and platelet aggregation were measured because an earlier study reported abnormal aggregation under similar conditions.\(^22\) Neither of these parameters were measured in subsequent studies.

The remaining four studies were performed to evaluate possible mechanisms for stress-associated platelet secretion. In each study the subjects were randomly assigned to drug or placebo, which was taken before the time of stress.

The data from the 34 subjects in the placebo groups (mean age 29 years; 32 men, two women) of the four studies were combined for statistical analysis (table 1). There was a statistically significant increase in plasma PF4, norepinephrine, and epinephrine at the time of stress when compared with either control 1 or control 2 (one-way ANOVA and paired t test). Plasma \( \beta TG \) was increased at the time of stress when compared with control 1, but not control 2. Platelet counts were not significantly different at the three time points.
TABLE 1
The effect of public speaking on platelet counts and plasma concentrations of PF4, βTG, and catecholamines

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Stress</th>
<th>Control 2</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF4 (ng/ml)</td>
<td>9.8 (2.0)</td>
<td>26 (3.6)</td>
<td>16 (2.5)</td>
<td>.001</td>
</tr>
<tr>
<td>βTG (ng/ml)</td>
<td>33 (4.7)</td>
<td>70 (7.9)</td>
<td>63 (9.8)</td>
<td>.001</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>60 (6.4)</td>
<td>107 (13)</td>
<td>53 (5.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>337 (15)</td>
<td>415 (30)</td>
<td>349 (22)</td>
<td>.046</td>
</tr>
<tr>
<td>Platelet count (× 10⁹/µl)</td>
<td>293 (12)</td>
<td>297 (9)</td>
<td>318 (11)</td>
<td>NS</td>
</tr>
</tbody>
</table>

The data are the values from 34 subjects representing the placebo groups in the studies presented in tables 2 to 4. Numbers in parentheses represent the SEM.

<sup>a</sup>Determined by one-way ANOVA.

Effect of α-adrenergic blockade. Twelve subjects were randomly assigned to a phenoxybenzamine or placebo group. Both substances were taken before the time of public speaking to evaluate the importance of platelet α-adrenergic receptors in stress-associated platelet secretion (table 2). When levels of PF4, βTG, epinephrine, and norepinephrine were analyzed at all three time points (by two-way ANOVA), there were significant differences detected between control 1, stress, and control 2. However, no differences in levels of PF4, βTG, or epinephrine were detected between the placebo- and phenoxybenzamine-treated groups. Norepinephrine concentrations were significantly higher in the subjects treated with phenoxybenzamine. These data suggest that phenoxybenzamine does not modify the platelet secretion that occurs with emotional stress, although it is associated with an effect on norepinephrine.

Effect of β-adrenergic blockade. In the second study 26 subjects were randomly assigned to receive a single dose of placebo or propranolol taken 1 hr before speaking in public to determine if blocking the β-adrenergic hemodynamic responses would modify platelet secretion (table 3).

There were significant differences in levels of PF4, βTG, epinephrine, and norepinephrine at control 1, stress, and control 2. However, there were no differences in these four parameters between the placebo- and propranolol-treated groups, even though the heart rate and systolic blood pressure were significantly lower during stress (p < .05) in propranolol- than in placebo-treated subjects.

Effect of aspirin on platelet activation. To determine if platelet secretion was occurring in response to a prostaglandin-dependent stimulus, two groups of subjects were randomly assigned to receive aspirin or placebo. In the initial study, 650 mg of aspirin or placebo was given 1 to 2 hr before public speaking to allow time for complete inhibition of platelet cyclooxygenase. In the second study, 80 mg of aspirin or placebo was administered. This low dose was selected to try to ensure that platelet prostaglandin synthetase was inhibited while prostacyclin synthesis in the endothelial cells was partially spared.

Fourteen subjects were randomly assigned to a 650 mg aspirin or placebo group. The results are shown in table 4. There were significant differences among PF4 and epinephrine concentrations at control 1, stress, and control 2 in both groups. There were no differences in norepinephrine (p = .14) or βTG (p = .06) levels. There was a significant difference between the MDA concentrations in the placebo- and aspirin-treated groups immediately before stress. In spite of this effect on MDA concentration, which is a measure of prostaglandin production, there were no differences in PF4 secretion when the data for the placebo- and aspirin-

TABLE 2
The effect of phenoxybenzamine on platelet activation and plasma catecholamines during the emotional stress of public speaking

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Phenoxybenzamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Stress</td>
</tr>
<tr>
<td>PF4 (ng/ml)</td>
<td>5.6 (1.9)</td>
<td>24 (8.3)</td>
</tr>
<tr>
<td>βTG (ng/ml)</td>
<td>24 (9.8)</td>
<td>59 (11)</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>47 (11)</td>
<td>70 (59)</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>288 (16)</td>
<td>386 (29)</td>
</tr>
<tr>
<td>Platelets (× 10⁹/µl)</td>
<td>340 (32)</td>
<td>336 (17)</td>
</tr>
</tbody>
</table>

The data are the values from six subjects in each group. Numbers in parentheses represent the SEM.
treated groups were compared. There were no significant differences in platelet count in either treatment group at any of the time points.

Twelve subjects were randomly assigned to an 80 mg aspirin or placebo group (table 4). PF4 and epinephrine were significantly different when analyzed at control 1, stress, and control 2, but were not different in the placebo- and aspirin-treated groups. There were no significant changes in plasma βTG (p = .075), platelet count, norepinephrine, or FPA concentrations associated with stress. Unlike in the previous study, there was not a significant decrease in MDA generation in the aspirin-treated as compared with the placebo-treated group. This group of subjects also received 650 mg of aspirin immediately after the first control blood sample was obtained and they demonstrated a decrease of from 2.7 to 0.16 nmol MDA/10⁹ platelets after the higher dose.

**Discussion**

This study demonstrates that platelet secretion of PF4 and βTG occurs in association with a rise in catecholamine levels in healthy volunteers immediately before speaking in public. This is the same time at which cardiovascular catecholamine responses are marked. In a comparable study of medical residents undergoing the emotional stress of public speaking, Moss and Wyner documented tachycardia in all subjects during the minute before and at the beginning of speaking. The occurrence of platelet activation at the same time suggests a role for platelets in the cardiovascular events that are related to stress. This role may be

**TABLE 3**
The effect of propranolol on platelet activation and plasma catecholamines during the emotional stress of public speaking

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th></th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Stress</td>
<td>Control 2</td>
</tr>
<tr>
<td>PF4 (ng/ml)</td>
<td>15 (4.7)</td>
<td>32 (5.2)</td>
<td>24 (5.4)</td>
</tr>
<tr>
<td>βTG (ng/ml)</td>
<td>44 (10)</td>
<td>102 (13)</td>
<td>94 (21)</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>62 (9)</td>
<td>101 (13)</td>
<td>64 (10)</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>335 (16)</td>
<td>414 (48)</td>
<td>361 (36)</td>
</tr>
<tr>
<td>Platelet count (× 10⁵/μl)</td>
<td>294 (16)</td>
<td>291 (13)</td>
<td>334 (13)</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>ND</td>
<td>89 (4.5)</td>
<td>ND</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>ND</td>
<td>136 (2.7)</td>
<td>ND</td>
</tr>
</tbody>
</table>

The data are values for 13 subjects in each group. Numbers in parentheses represent the SEM; ND = not determined; BP = blood pressure.

**TABLE 4**
The effect of aspirin on platelet activation and plasma catecholamines during the emotional stress of public speaking

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th></th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Stress</td>
<td>Control 2</td>
</tr>
<tr>
<td>650 mg aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF4 (ng/ml)</td>
<td>6.3 (1.6)</td>
<td>27 (11)</td>
<td>14 (3.7)</td>
</tr>
<tr>
<td>βTG (ng/ml)</td>
<td>26 (7.0)</td>
<td>43 (12)</td>
<td>33 (9.1)</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>59 (8.5)</td>
<td>88 (15)</td>
<td>49 (9.1)</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>324 (40)</td>
<td>368 (24)</td>
<td>318 (35)</td>
</tr>
<tr>
<td>Platelets (× 10⁵/μl)</td>
<td>290 (25)</td>
<td>284 (18)</td>
<td>321 (36)</td>
</tr>
<tr>
<td>MDA (nmol/10⁹ platelets)</td>
<td>1.9 (0.4)</td>
<td>2.7 (0.9)</td>
<td>2.5 (0.8)</td>
</tr>
<tr>
<td>80 mg aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF4 (ng/ml)</td>
<td>8.2 (1)</td>
<td>18 (4)</td>
<td>10 (1.5)</td>
</tr>
<tr>
<td>βTG (ng/ml)</td>
<td>31 (4.4)</td>
<td>56 (16)</td>
<td>58 (9.7)</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>73 (23)</td>
<td>127 (30)</td>
<td>54 (13)</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>397 (48)</td>
<td>491 (108)</td>
<td>414 (65)</td>
</tr>
<tr>
<td>Platelets (× 10⁵/μl)</td>
<td>255 (27)</td>
<td>290 (30)</td>
<td>288 (18)</td>
</tr>
<tr>
<td>MDA (nmol/10⁹ platelets)</td>
<td>2.3 (0.4)</td>
<td>2.9 (0.6)</td>
<td>2.3 (0.9)</td>
</tr>
<tr>
<td>FPA (ng/ml)</td>
<td>1.6 (0.2)</td>
<td>1.4 (0.1)</td>
<td>1.3 (0.1)</td>
</tr>
</tbody>
</table>

The data are values from seven subjects in each group in the 650 mg aspirin study and six subjects in each group in the 80 mg aspirin study. Numbers in parentheses represent the SEM.
chronic and recurrent, as in the genesis of atherosclerosis in subjects with "coronary prone" behavior who have exaggerated catecholamine responses. In these subjects repeated platelet activation and secretion of platelet-derived growth factor may enhance the arterial smooth muscle proliferation in developing atherosmas.15 Also, activated platelets that have secreted their α-granule proteins have an altered plasma membrane surface that facilitates platelet-platelet and platelet--vessel wall interactions.31 The platelet activation that is associated with emotional stress may enhance accumulation of platelets in the turbulent blood flow at sites of arterial damage and partial obstruction32 and this could trigger acute ischemia.

When data from all of the subjects receiving drugs or placebo in the four separate studies were grouped and then analyzed, PF4 and βTG were both significantly increased in plasma samples obtained immediately before speaking in comparison with both control 1 and control 2 samples. The correlation coefficient for PF4 and βTG concentrations in the same plasma samples was .649 (p < .001). Therefore, since no reservoir for βTG other than platelet α-granules is known, the increased plasma concentrations of PF4 and βTG must indicate platelet secretion rather than an increased plasma PF4 concentration derived from the endothelial surface.33

The stimulus provoking platelet secretion in our studies is not known. The plasma concentration of epinephrine measured during stress in our subjects (100 pg/ml or 0.6 nM) is 1/10,000 of the standard epinephrine concentration used for platelet aggregation in vitro. However, studies in vitro in which combinations of platelet agonists were used have demonstrated a potentiation effect of epinephrine and norepinephrine at concentrations achieved in the circulation during stress.34 In addition, our sampling procedure (only one sample was obtained from each subject) probably missed the peak plasma concentrations of epinephrine since drawing of blood required a minimum of 5 min and had to be completed before the subjects began their lectures. The lack of effect of phenoxybenzamine suggests that platelets are not being directly stimulated by circulating catecholamines through their α2-adrenergic membrane receptors. However, α2-adrenergic blockade may not have been maximal, even though this dose of phenoxybenzamine was able to block a 40 mm Hg blood pressure rise in response to an adjusted dose of intravenous phenylephrine in healthy volunteers (data not shown).

Propranolol was administered to our subjects to determine if blocking the hemodynamic response to emotional stress would also modify platelet secretion. It has recently been demonstrated that the well-known physiologic responses to emotional stress, including tachycardia, sweating, tremor, and dry mouth, can be minimized by β-blockade of peripheral receptors.35 Platelet membranes contain few β-receptors,36 and β-antagonists appear to have a membrane-stabilizing effect on platelets only at high doses.37 Therefore, we did not expect that propranolol would block a direct stimulation of platelets. However, changes in blood vessel diameter and cardiac output may modify blood flow characteristics and decrease platelet activation. However, there was no effect on platelet activation and secretion observed when the effects of propranolol were compared with those of placebo.

Aspirin blocks platelet activation and secretion in response to arachidonic acid and low doses of ADP, epinephrine, and collagen, but platelet stimulation by thrombin and higher doses of collagen is not inhibited.30 Our studies were performed with two different doses of aspirin to determine if platelet secretion, but not endothelial cell production of prostacyclin, could be modified. Although 650 mg of aspirin blocks both platelet and endothelial cell prostaglandin synthetase, Weksler et al.39 have recently demonstrated that a dose of 80 mg blocked platelet prostaglandin synthesis, but allowed vessel endothelial cells to generate prostacyclin in vitro in response to a stimulus. In our studies, neither aspirin dose affected the platelet secretion that occurred with stress.

Thrombin is another potential agonist that could trigger platelet secretion during emotional stress, since catecholamine infusions are associated with accelerated blood coagulation and increased plasma activities of factors V and VIII.38, 39 We measured the plasma concentration of FPA as an indirect assay of thrombin activity and found no change during the period of stress when platelet secretion occurred. However, our earlier studies have demonstrated that platelet secretion of PF4 is stimulated by concentrations of thrombin lower than those required for fibrin formation.40 Therefore, the absence of an increased FPA concentration does not rule out thrombin as a mediator of this effect on platelets.

We have demonstrated that platelet activation and secretion occurs in association with an increase in plasma catecholamine levels immediately before speaking in public. Although the stimulus is unknown, this represents a mechanism that might partially explain how behavioral stress, catecholamines, and platelets may play a role in the development of atherosclerosis and the acute complications of coronary artery disease.
We thank Ms. Catherine DeLea, Dr. Alexander Shepherd, and Dr. Gilbert C. White II for their advice and criticism, Ms. Judy Anderson for her technical assistance, and Mrs. Beverly Blann and Mrs. Joyce Laderer for their secretarial assistance.

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