Morning Increase in Platelet Aggregability

Association With Assumption of the Upright Posture

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The frequencies of onset of myocardial infarction and sudden cardiac death are increased between 6 AM and 12 noon. Platelet aggregability, which may play a role in the cause of these disorders, has been observed to increase after the normal morning activities of awakening, arising, and ambulating. To determine which morning activity or activities are responsible for this aggregability increase, we measured platelet aggregation in 16 normal subjects on a control day of delayed arising (i.e., subjects remained supine until 12:30 PM) and on a day in which normal morning activities were divided into three isolated components of awakening (8 AM), assumption of upright posture (9:30 AM), and ambulating (11 AM). Blood samples to assess platelet aggregability were drawn at 8 AM before activity and 90 minutes after the initiation of each activity (i.e., at 9:30 AM, 11 AM, and 12:30 PM). For the group, in vitro platelet responsiveness to adenosine diphosphate and epinephrine increased only after assumption of the upright posture. The lowest concentration of agonist required to produce biphasic platelet aggregantion decrease (aggregability increased) between 9:30 and 11 AM (90 minutes after assumption of the upright posture) from 3.3±0.3 to 2.4±0.2 μM for adenosine diphosphate (p<0.05) and from 2.1±0.5 to 1.0±0.4 μM for epinephrine (p<0.05). During the same interval, plasma epinephrine increased from 34±7 to 55±9 pg/ml (p<0.05), and plasma norepinephrine increased from 169±19 to 298±25 pg/ml (p<0.01). There was no significant change in aggregability or catecholamine concentrations on the control day. Thus, the simple assumption of upright posture is capable of causing the morning increase of platelet aggregability. Recognition of the effect of the assumption of upright posture on platelet aggregability will assist in identification of the biochemical cause of the platelet aggregability increase in the morning and may facilitate design of measures to eliminate potentially harmful surges in platelet aggregability. (Circulation 1988;78:35-40)

Platelet aggregability increases in the morning, as do the frequencies of onset of myocardial infarction and sudden cardiac death.1-4 In an earlier study of 15 healthy volunteers,1 we reported that a complex of normal morning activities (forced awakening and exposure to natural light, assumption of the upright posture, showering, drinking one cup of coffee, and walking up and down three flights of stairs) was associated with a significant increase of in vitro platelet responsiveness to epinephrine or adenosine diphosphate (ADP). The study also showed that there was no morning increase in platelet responsiveness if the subjects remained inactive. However, the specific morning activity (or activities) responsible for the increase in platelet aggregability was not identified. The purpose of the present study, therefore, was to determine which of the morning activities was associated with the platelet aggregability increase.

Subjects and Methods

The study was conducted in 16 healthy male volunteers aged 20–35 years who were nonsmokers, had taken no aspirin or other medication in the previous 2 weeks, and had given informed consent. Subjects were admitted to the Clinical Research
Center of the Brigham and Women's Hospital on the night before the study. On the day of experimental activity, they adhered to a standardized protocol in which the complex of normal morning activities was divided into three components separated by 90-minute intervals (Figure 1). Venipunctures were performed at 90-minute intervals between 8 AM and 12:30 PM. On the study day, the subjects were awakened at 8 AM for the first venipuncture, which was performed with the subjects supine in bed and in a dark room. After the sample was taken, the shade of the window was raised, permitting sunlight to enter the room, and the subjects were required to remain awake. However, they remained supine and inactive until after the 9:30-AM venipuncture when they rose from bed and stood upright for 1 minute; they then remained seated upright at minimal activity until after the 11-AM venipuncture. After the 11-AM sample, the subjects first showered and then walked up and down three flights of stairs. The final venipuncture was performed at 12:30 PM with the subjects in a seated position.

On an alternate control day, the room remained dark and the subjects were permitted to sleep between blood sampling and did not rise until after the 12:30-PM sample. The order of the experimental and control days was randomly assigned. No coffee or food was consumed before 12:30 PM on either day.

**Blood Sample Collection**

All blood samples were drawn without stasis from an antecubital vein with a 21-gauge butterfly needle and a two-syringe technique. A 27-ml sample of blood for platelet aggregation studies was collected in a plastic syringe containing 3 ml 3.8% sodium citrate (9:1, vol/vol). A 3-ml sample of blood was collected in a second syringe for catecholamine analysis, and an 8-ml sample was collected in a third syringe for the determination of plasma renin activity and angiotensin II levels. A final 5-ml sample was collected in a tube containing EDTA for hematocrit and whole blood platelet count determination.

**Platelet Aggregation Studies**

Blood samples were centrifuged at 160g for 10 minutes to obtain platelet-rich plasma (PRP). The PRP was transferred by pipette to a polystyrene tube, which was then tightly capped. Blood samples were centrifuged at 2,500g for 20 minutes to obtain platelet-poor plasma (PPP). Platelet aggregation studies were performed according to the method of Born with a Bio/Data Pap-4 Aggregometer (Haltbo, Pennsylvania) at 37°C with 1-ml siliconized cuvettes and siliconized stirring bars (1,200 rpm). The volume of PRP added to each cuvette was 450 μl. The aggregometer was adjusted before each experiment so that PRP gave 0% and PPP gave 100% light transmittance. ADP (Sigma Chemical,
St. Louis, Missouri) and epinephrine (Sigma Chemical) were used as aggregating agents. ADP was used in concentrations of 0.25–10.0 μM, and epinephrine was used in concentrations of 0.01–10 μM. Six concentrations, which included concentrations above and below the threshold concentration, were then selected for each subject and used for each timepoint in a stepwise manner from highest to lowest concentration. Aggregation studies using ADP and epinephrine were initiated 45 minutes and 1 hour, respectively, after venipuncture and were completed within 90 minutes of venipuncture.

The tracings were then analyzed by one of the authors, who was blinded with regard to the time and day at which the individual aggregation studies were performed. The lowest concentration of aggregating agent sufficient to produce biphasic aggregation was identified for each timepoint.

Plasma epinephrine and norepinephrine concentrations were determined by a single radioenzymatic method based on a modification of the method of Peuler and Johnson.8 Plasma renin activity and angiotensin II concentration were determined by the method of Emanuel et al.7 Hematocrit and platelet count were determined in whole blood by a Coulter counter (Coulter Electronics, Hialeah, Florida). Platelet count in PRP was determined with a Model R1 Coulter Counter (Coulter Electronics).

Statistical Analysis

Comparisons between mean values at 8 AM, 9:30 AM, 11 AM, and 12:30 PM were made with two-way analysis of variance and Duncan’s test for post hoc differences. Because earlier studies have established that renin and angiotensin II increase within 90 minutes of assumption of upright posture,8–10 a paired t test was used to compare the renin and angiotensin II changes from 9:30 AM to 11 AM. Correlation studies between aggregability changes and plasma endocrine concentration changes were performed with linear regression techniques. Data organization and analysis were performed with the aid of the CLINFO system of the General Clinical Research Center at Brigham and Women’s Hospital.

Results

Platelet Aggregability

There was no increase in platelet aggregability after exposure to light and awakening (Figure 2A). However, after assumption of the upright posture, the minimum concentration of ADP required to produce biphasic aggregation fell significantly (aggregability increased) from 3.3 ± 0.3 to 2.4 ± 0.2 μM (p<0.05). Similar changes were observed in the minimum concentration of epinephrine required to produce biphasic aggregation (2.1 ± 0.5 to 1.0 ± 0.4 μM, respectively; p<0.05). There was no further increase in aggregability at 12:30 PM after showering and walking up and down three flights of stairs.

On the control day with no activity, there were no significant changes over time in platelet responsiveness to ADP or epinephrine. The concentrations of ADP required to produce biphasic aggregation at 8 AM, 9:30 AM, 11 AM, and 12:30 PM were 2.7 ± 0.3, 3.4 ± 0.3, 3.7 ± 0.4, and 3.7 ± 0.3 μM, respectively. Corresponding values for the amount of epinephrine required to produce biphasic aggregation were 2.7 ± 0.7, 2.1 ± 0.5, 3.3 ± 0.9, and 2.2 ± 0.4 μM.

The minimum concentration of ADP required to produce biphasic aggregation was significantly less (aggregability was significantly greater) at 11 AM after assumption of the upright posture on the day of experimental activity as compared with 11 AM on the day of no activity (2.4 ± 0.2 vs. 3.7 ± 0.4 μM, respectively; p<0.05).

There was considerable interindividual variation in the change in response to either ADP or epinephrine after assumption of the upright posture (Figure 3). Aggregability increased after assumption of the upright posture in 11 of 16 subjects in response to ADP and remained unchanged in three of 16 subjects. The response to epinephrine increased in 10 of 16 subjects and remained unchanged in five of 16 subjects. The increased response to epinephrine for the group was in large part due to increases in the six individuals who exhibited the least aggregability to epinephrine in the supine position. However, the
increased response to ADP was more uniformly observed among 11 of 16 subjects. Tracings in a subject with an increase in aggregability after assumption of upright posture are shown in Figure 4. The type of response demonstrated in Figure 4 (i.e., the production of irreversible aggregation by ADP after upright posture by concentrations that produced only reversible aggregation when the subjects were supine) occurred in 11 of the 16 subjects.

Plasma Catecholamines, Renin Activity, and Angiotensin II Concentrations

On the day of activity, plasma epinephrine and norepinephrine concentrations increased significantly after posture change (from 9:30 AM to 11 AM; 34 ± 7 vs. 55 ± 9 pg/ml, p < 0.05, and 169 ± 19 vs. 298 ± 25 pg/ml, p < 0.01, respectively; Figure 2B). As seen in Table 1, plasma renin activity and angiotensin II concentration increased significantly from 9:30 AM to 11 AM after posture change (from 1.7 ± 0.3 to 2.3 ± 0.4 ng/ml/hr and from 21 ± 2 to 25 ± 3 ng/ml/hr, respectively; p < 0.05). On the day of no activity, there were no significant increases of plasma catecholamines, renin activity, or angiotensin II concentration.

A weak but statistically significant correlation existed between the magnitude of the 9:30-to-11-AM increase in platelet response to epinephrine and ADP and the magnitude of the increase of plasma epinephrine concentrations (r = 0.41 and 0.46, respectively; p < 0.02 for both). There were no significant correlations between the morning changes in platelet responsiveness and changes in plasma norepinephrine concentration, plasma renin activity, or angiotensin II concentration.

Hematocrit and Platelet Count

The hematocrit showed a small but statistically significant increase after assumption of upright posture (from 41.2 ± 0.7% to 43.3 ± 0.8%; p < 0.05; Table 2). No significant differences were observed between the platelet count of either whole blood or PRP after arising (Table 2). There was no significant correlation between morning changes in platelet responsiveness and changes in hematocrit or platelet count.

Discussion

The results indicate that for the group of subjects studied, assumption of the upright posture in the morning caused an increase in platelet aggregability. The posture change (1 minute standing followed by sitting for the remainder of the 90-minute period) was also associated with a significant increase in plasma epinephrine and norepinephrine concentrations, renin activity, and angiotensin II concentration. The changes in plasma epinephrine concentrations correlated weakly with the changes in platelet aggregability.

<p>| Table 1. Posture-Related Renin and Angiotensin II Changes |
|---------------------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Time of day</th>
<th>Plasma renin activity (ng/ml/hr)</th>
<th>Plasma Angiotensin II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 AM</td>
<td>2.1 ± 0.3</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>After awakening (9:30 AM)</td>
<td>1.7 ± 0.3</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>After assuming upright posture (11 AM)</td>
<td>2.3 ± 0.4</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>After ambulating (12:30 AM)</td>
<td>2.2 ± 0.3</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

On day of activity, subjects were awoken and lights turned on after the 8-AM sample. After the 9:30-AM sample, subjects assumed upright posture. After the 11-AM sample, subjects showered and walked three flights of stairs. On control day, subjects remained supine and in darkness until after the 12:30-PM sample. *p < 0.05 for two-tailed paired t test.
TABLE 2. Posture-Related Hematologic Changes

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Hematocrit (%)</th>
<th>Platelet count in whole blood (10^3/mm^3)</th>
<th>Platelet count in PRP (10^3/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>Control</td>
<td>Activity</td>
</tr>
<tr>
<td><strong>8 AM</strong></td>
<td>41.6±0.8</td>
<td>41.2±0.8</td>
<td>251±13</td>
</tr>
<tr>
<td>After awakening</td>
<td></td>
<td></td>
<td>116±4</td>
</tr>
<tr>
<td>(9:30 AM)</td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>After assuming</td>
<td></td>
<td></td>
<td>143±10</td>
</tr>
<tr>
<td>upright posture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11 AM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After ambulating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12:30 PM)</td>
<td>41.3±0.6</td>
<td>41.4±0.7</td>
<td>255±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>145±12</td>
</tr>
</tbody>
</table>

On day of activity, subjects were awoken and lights turned on after 8-AM sample. Subjects assumed upright posture after 9:30-AM sample. Subjects showered and walked up and down three flights of stairs after 11-AM sample. On control day, subjects remained supine and in darkness until after 12:30-PM sample.

* p<0.05 for Duncan’s test for post hoc differences between consecutive samples (two-way analysis of variance).

We initiated studies of platelet aggregability in an effort to follow the lead provided by the observation that myocardial infarction and sudden cardiac death are more frequent during the morning period. Our previous study demonstrated a morning increase in platelet aggregability but did not isolate the individual roles of the various morning activities. The finding in the present study that assumption of upright posture is the activity associated with the increase in platelet aggregability is, to our knowledge, the first report of the effect of postural change on platelet aggregability. Earlier studies have reported the stimulatory effect of epinephrine and norepinephrine on platelet activation, but postural change was not isolated as the stimulus for the catecholamine increase.

Assumption of upright posture after a period of recumbency produces a major challenge to the cardiovascular system and triggers many compensatory homeostatic mechanisms. The initial decrease in central blood volume and stroke volume is countered by increases in heart rate and peripheral vascular resistance. Plasma renin activity, angiotensin II concentrations, and plasma epinephrine and norepinephrine concentrations increase. An increase in angiotensin II may be of importance for platelet aggregability because angiotensin II has been shown to potentiate epinephrine-induced platelet aggregability. Although increases in plasma levels of these hormones occurred with posture change, the mechanism of the aggregability increase remains unknown; only a weak correlation existed between changes in platelet aggregability and plasma epinephrine and no significant correlation was found between changes in platelet aggregability and plasma renin activity or angiotensin II. The concentrations of epinephrine alone required to produce biphasic aggregation in vitro are at least two orders of magnitude greater than in vivo levels observed after assumption of the upright posture (10^-8 vs. 3×10^-10 mol/l, respectively); however, synergistic interaction may enable epinephrine to potentiate ADP-induced aggregation at significantly lower concentrations.

The physiological effects of assumption of upright posture are similar to those occurring during acute hemorrhage. In both situations, vasoconstriction and catecholamine release occur as the circulatory system attempts to maintain adequate perfusion pressure. The similarities are of significance for the present study because platelet aggregability has previously been shown to increase during acute hemorrhage. This may be considered a compensatory process because such an increase in aggregability might help to seal the source of acute blood loss.

It is of note that the percent increase in aggregability after the assumption of upright posture observed in the present study is of a similar magnitude to that observed in our previous study, in which a complete complex of normal morning activities was performed. This indicates that the assumption of upright posture (i.e., standing for a short period and then sitting) in the morning may, by itself, account for the entire morning increase in platelet aggregability observed during normal morning activities. In the present study, the physical activity of walking up and down stairs did not produce an increase in platelet aggregability beyond that caused by assumption of the upright posture. In the previous study, there was a statistically significant increase in platelet aggregability after physical activity accompanied by the assumption of upright posture. It is possible that such physical activity, which in this protocol occurred 3 hours after awakening, might have been more of a powerful stimulus to increasing platelet aggregability than the assumption of upright posture if performed closer to the time of awakening.

As in the previous study, there was considerable interindividual variation in platelet aggregability after the assumption of upright posture. There were no predictors of which individuals would show an increase in platelet aggregability, indicating that as yet undetermined factors play a role in producing this increase in platelet aggregability.

Further investigation of the cause of the morning increase in platelet aggregability can now focus on...
the events accompanying assumption of upright posture. Studies should be conducted to determine how soon after assumption of upright posture the platelet aggregability increase occurs and what duration of previous supine posture is required to precondition the body for the platelet aggregability increase. Clarification of the mechanisms of the increase of platelet aggregability with assumption of upright posture may suggest therapeutic approaches to eliminate undesirable surges in platelet aggregability. Studies in patients with hypertension or coronary artery disease are also needed to assess whether their platelets show a pattern of increase similar to that of healthy volunteers. Basal reactivity of platelets, as well as surges in platelet aggregability, must be studied in a population to determine whether either or both identify patients with a biochemical lesion that increases their risk of subsequent cardiac events. Finally, epidemiological studies are now needed to determine the relation between the assumption of upright posture and the onset of myocardial infarction and sudden cardiac death.

Acknowledgments

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References


Key Words • epinephrine • ADP • myocardial infarction • sudden cardiac death
Morning increase in platelet aggregability. Association with assumption of the upright posture.

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