Norepinephrine, but not epinephrine, enhances platelet reactivity and coagulation after exercise in humans

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Ikarugi, Hideo, Tomomi Taka, Shoko Nakajima, Takanori Noguchi, Sadahiro Watanabe, Yasuto Sasaki, Shukoh Haga, Takashi Ueda, Junji Seki, and Junichiro Yamamoto. Norepinephrine, but not epinephrine, enhances platelet reactivity and coagulation after exercise in humans. J. Appl. Physiol. 86(1): 133–138, 1999.—The effects of exercise and catecholamines on platelet reactivity or coagulation and fibrinolysis appear to be inconsistent. This may be partly due to the methods employed in previous studies. In the present study, we investigated the effects of acute aerobic exercise and catecholamines on the thrombotic status by a novel in vitro method, shear-induced hemostatic plug formation (hemostatometry), using nonanticoagulated (native) blood. Aerobic exercise (60% maximal O2 consumption) was performed by healthy male volunteers for 20 min, and the effect on platelet reactivity and coagulation was assessed by performing hemostatometry before and immediately after exercise. Exercise significantly increased shear-induced platelet reactivity, coagulation, and catecholamine levels. The effect of catecholamines on platelet reactivity and coagulation was assessed in vitro by adding catecholamines to blood collected in the resting state. The main findings of the present study are that elevation of circulating norepinephrine at levels that are attained during exercise causes platelet hyperreactivity and a platelet-mediated enhanced coagulation. This may be a mechanism of an association of aerobic exercise with thrombotic risk.

platelet aggregation; catecholamine; shear stress; hemostatometry

ANGINA, MYOCARDIAL INFARCTION, and sudden cardiac death are associated with thrombus formation in the coronary arteries. It is generally believed that these conditions can be inhibited by long-term exercise (27, 31, 37, 38, 41). However, evidence for such prevention is inconclusive, and the mechanism through which long-term exercise exerts a beneficial effect on various ischemic conditions is unclear. The effects of exercise on platelet reactivity, blood coagulation, and fibrinolysis have been studied extensively, but the findings are inconsistent (3, 5, 20, 33), possibly due to the methods employed in previous studies. Platelets respond to a variety of agonists, and the doses of coagulation and fibrinolysis variables make it difficult to assess platelet function, coagulation, and fibrinolysis as a whole.

Tests of shear-induced platelet reactivity seem to be physiologically relevant to arterial thrombosis (8, 21, 23, 26, 35). A novel technique, hemostatometry, uses nonanticoagulated blood to simultaneously measure shear-induced platelet reactivity and coagulation and is also physiologically relevant to arterial thrombosis (26). This technique is very important because arterial thrombosis is a multicellular event, involving not only platelets but also erythrocytes, leukocytes, and interaction of these cells (19, 30), and such interaction occurs in plasma at physiological calcium ion concentration (4, 29). This in vitro system, which employs unadulterated blood, thus allowing thrombin generation, has relevance in vivo (14, 42).

It has been reported that epinephrine augments shear-induced platelet aggregation (12, 22, 36). However, this has only been observed at supraphysiological epinephrine concentrations. Norepinephrine is another physiologically important catecholamine that increases more markedly than epinephrine under various stresses (28), especially light-to-moderate exercise (7, 17). The effects of norepinephrine on shear-induced platelet reactivity and coagulation have not been studied previously.

Accordingly, the purpose of the present study was to examine the effects of acute aerobic exercise, epinephrine, and norepinephrine on platelet reactivity and coagulation by using hemostatometry to assess shear-induced thrombosis.

METHODS

Hemostatometry. The hemostatometer was invented by Gorog and Kovacs (9, 10, 26). On the basis of their published data, a three-channel hemostatometer was constructed in the physiology laboratory at the Faculty of Nutrition of Kobe Gakuin University (Kobe, Japan) for research purposes. A diagram of the instrument is shown in Fig. 1. A syringe containing nonanticoagulated blood with or without catecholamines at 37°C is used to deliver blood into specially manufactured polyethylene tubing (OD 1.00 ± 0.02 mm; ID 0.50 ± 0.01 mm), which is punctured with a needle of 0.18-mm diameter, and then a hemostatic plug is formed by shear forces (375 dyn/cm² immediately after puncture). A reservoir detects pressure changes in the system. The tubing is punctured at 2.5 min after blood withdrawal, resulting in bleeding, hemostatic plug formation, and coagulation. The process of plug formation and coagulation is measured by using the...
changes in pressure, which are recorded and analyzed by computer.

A typical hemostatogram is shown in Fig. 2. The pressure falls after puncture and recovers to the initial level (60 mmHg) as the hemostatic plug is formed, followed by a gradual pressure drop caused by subsequent coagulation. The areas until 30% (H1, mmHg·s) and 90% (H2) recovery from the maximal pressure drop are used as indexes of hemostatic plug formation. The time until a 10-mmHg pressure drop from baseline is used as an index of the onset of coagulation of flowing blood (CT1, min), and the time until the pressure is maintained at 10 mmHg for 1 min is an index of completed coagulation (CT2).

The reproducibilities of our hemostatometer, the coefficients of variation for the variables, were as follows: human blood (n = 54): H1 = 21%, H2 = 15%, CT1 = 11%, CT2 = 13%; rat blood (n = 124): H1 = 25%, H2 = 18%, CT1 = 6%, CT2 = 6%. These values were similar to those published by Ratunatunga et al. (26).

Morphology of the hemostatic plug. When the pressure recovered to the initial level after puncture of the polyethylene tubing, it was perfused with phosphate-buffered saline for 5 min and then with 2.5% glutaraldehyde. The tubing with the hemostatic plug was cut out and further fixed with 2.5% glutaraldehyde and 1% OsO4. Ultrathin sections were cut and observed with a transmission electron microscope (JEM 2000EX).

Exercise. Male volunteers, aged 19–34 yr (all nonsmokers taking daily physical exercise), were enrolled, and the experiment was performed after informed consent was given. The volunteers had taken no medications for at least 2 wk. Volunteers were divided into two groups, morning and afternoon. Blood was collected in the morning group while subjects were fasting and in the afternoon group after subjects had eaten a light breakfast but no lunch. Maximal oxygen consumption (V\textsubscript{O}2\textsubscript{max}) was measured during exercise on a bicycle ergometer (Monark 818E), according to the procedure that the workload was given by 30-W increments every minute until the volunteer was exhausted or that the respiratory exchange ratio (CO\textsubscript{2} production-to-O\textsubscript{2} consumption ratio) exceeded 1.0 (2). Individual V\textsubscript{O}2\textsubscript{max} was 51.2 ± 1.1 (SE) ml·kg\textsuperscript{-1}·min\textsuperscript{-1}. And 60% V\textsubscript{O}2\textsubscript{max} exercise was done for 20 min. Blood was collected from the antecubital vein immediately before and after exercise. The first 3 ml of blood were used for catecholamine and blood cell measurements, and the subsequent 3 × 3 ml were used for hemostatometry.

Catecholamines. Blood was collected from the antecubital vein at rest. The first 3 ml were set aside, and the subsequent 10 ml were used for the experiment. The first 3 ml of blood were placed in a syringe containing 30 µl of catecholamine (final concentrations: epinephrine 0.5, 1.0, or 2.0 nM, Sigma Chemical, St. Louis, MO; norepinephrine: 3.75 or 7.5 nM, Nacalai Tesque, Kyoto, Japan) or saline, and measurement was performed after gentle mixing.

Plasma catecholamine and other assays. Plasma was prepared from EDTA-anticoagulated blood (final concentration: 10 mmol/l) and stored at −80° until use. Plasma catecholamine concentrations were measured by high-performance liquid chromatography. Blood cell counts and hematocrit were measured with an automated cell counter (Sysmex Microcellcounter SF-3000, Toa Medical Electronics).

Statistical analysis. Hemostatometry measurements were performed in triplicate by using three channels, and the mean values were calculated. Data are expressed as means ± SE.
H1 and H2 were converted to logarithmic values. Statistical analysis was performed with Student’s paired t-test (2-tailed) or one-way, repeated-measures ANOVA followed by a contrast test to identify differences between two groups. \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Electron microscopy of the hemostatic plug. An electron micrograph of the plug is shown in Fig. 3. The typical hemostatic plug was composed of packed platelets, and fibrin was rarely visible. Degranulation of platelets was observed. Scattered fibrin was also seen at a higher magnification, but our findings did not suggest that fibrin played an important role in shear-induced hemostatic plug formation during hemostatometry.

| Table 1. Effect of acute aerobic exercise on blood cell counts and hematocrit |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Before Exercise | After Exercise | \( P \) Value |
| Erythrocytes, \( \times 10^9/\mu l \) | 478 ± 8 | 499 ± 9 | <0.0001 |
| Leukocytes, \( \times 10^9/\mu l \) | 64.2 ± 5.3 | 84.8 ± 5.4 | <0.0001 |
| Platelets, \( \times 10^9/\mu l \) | 24.6 ± 1.3 | 27.6 ± 1.4 | <0.0001 |
| Hematocrit, % | 44.8 ± 0.6 | 46.7 ± 0.6 | <0.0001 |

Values are expressed as means \( \pm \) SE.

Effect of exercise on blood cells and hematocrit. As shown in Table 1, exercise for 20 min significantly increased the erythrocyte, leukocyte, and platelet counts as well as the hematocrit \( (P < 0.0001) \), indicating that hemoconcentration had occurred.

Effect of exercise on plasma catecholamines. Exercise for 20 min significantly increased both plasma epinephrine and norepinephrine levels \( (P < 0.0005 \text{ and } P < 0.0001, \text{ respectively}) \) (Fig. 4). Epinephrine increased from \( 0.35 \pm 0.05 \) nM immediately before exercise to

Fig. 3. Microscopic features of thrombus formed in needle hole. A: light micrograph of plug induced by shear force (magnification \( \times 260 \)). Bar, 10 \( \mu m \). B: transmission electron micrograph of plug (magnification \( \times 7,400 \)). Bar, 1 \( \mu m \). C: transmission electron micrograph of plug (magnification \( \times 10,000 \)). Arrow, fibrin; bar, 1 \( \mu m \).

Fig. 4. Effect of acute aerobic exercise on plasma catecholamines. Individual and mean values (\( \pm \) SE) are shown. \( *P < 0.0005, **P < 0.0001 \).
0.77 ± 0.12 nM immediately after exercise, and norepinephrine increased from 2.20 ± 0.16 to 6.18 ± 0.62 nM.

Effects of exercise and catecholamines on shear-induced platelet reactivity. The effects of exercise, epinephrine, norepinephrine, and the combination of both catecholamines on shear-induced platelet reactivity (platelet adhesion and/or aggregation: H1 and H2) are shown in Table 2. Exercise significantly reduced both H1 and H2, which meant enhanced platelet reactivity. A physiological epinephrine concentration (2 nM) did not significantly alter H1 or H2. However, H1 and H2 were significantly reduced by 7.5 nM norepinephrine, which was the physiological maximum concentration after exercise. H1 and H2 were also significantly reduced by a combination of the 50% maximal concentrations of epinephrine and norepinephrine.

Effects of exercise and catecholamines on coagulation. The effects of exercise, epinephrine, norepinephrine, and the combination of both catecholamines on the coagulation of flowing blood (CT1 and CT2) are also shown in Table 2. Exercise significantly reduced CT1 and CT2, which meant enhanced coagulation. A physiological level of norepinephrine (7.5 nM) also significantly enhanced coagulation.

**DISCUSSION**

The number of patients suffering from angina, myocardial infarction, and stroke has been increasing in developed countries. Prevention and cure of arterial thrombosis associated with these diseases are thus important medical and social problems. Aerobic exercise is thought to be beneficial as is medication. However, exercise sometimes results in sudden cardiac death. Studies on the mechanism of the effect of aerobic exercise on arterial thrombosis are essential, not only for the prevention of such cardiac events but also for the safe enjoyment of sports.

Arterial thrombi are mainly composed of platelets. Platelet reactivity in vivo can be assessed from agonist-induced platelet aggregation, the platelet-release reaction, and the ratio of prostanoid metabolites. Accumulated evidence suggests that recently developed methods for measuring platelet reactivity by activating platelets solely with shear forces seem to be useful (8, 18, 21, 23, 26, 35). Hemostatometry employs solely shear forces to induce formation of a platelet-rich hemostatic plug. Despite the lack of blood vessel wall, physiological relevance of this in vivo technique is superior to any other in vitro platelet function tests. Because a native blood sample is tested, various biologically active substances secreted by the vascular endothelium into the bloodstream are still in the withdrawn blood sample, and, although these substances have short half-lives, the test that starts <150 s after blood withdrawal measures their contribution to hemostasis.

The hemostatic plug formed in nonanticoagulated blood under high-shear forces was mainly composed of platelets, and there was little fibrin (Fig. 3). This indicates that H1 and H2 can be used as indexes of platelet reactivity. The present study demonstrated that exercise enhanced platelet reactivity. This was consistent with the results of our previous study using another in vitro test, the Thrombotic Status Analyzer (16). Hemostatometry is very sensitive and has reasonably good reproducibility, as shown in METHODS. Therefore, variations in platelet reactivity among individuals can be assessed by this method. Relatively large variations in H1 and H2 shown in Table 2 are due to the individual differences, not artifacts originating from this method. The effect of exercise on platelet reactivity in the present study differed from that shown in a number of other studies (3, 5, 20, 33), and this may have been due to the methods used. Shear-induced platelet aggregation is physiologically relevant to arterial thrombosis and should be useful for assessing the effect of exercise on platelet reactivity.

A hypercoagulable state after exercise was observed in the present study (Table 2). There are a number of papers on the coagulation state after exercise. The changes in coagulation and fibrinolysis parameter con-

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**Table 2. Effects of exercise and catecholamines on shear-induced thrombosis and dynamic coagulation**

<table>
<thead>
<tr>
<th>Exercise</th>
<th>n</th>
<th>H1, mmHg·s</th>
<th>H2, mmHg·s</th>
<th>CT1, min</th>
<th>CT2, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>13</td>
<td>3.51 ± 439</td>
<td>13.72 ± 1.700</td>
<td>16.84 ± 0.94</td>
<td>22.24 ± 1.26</td>
</tr>
<tr>
<td>After</td>
<td>13</td>
<td>2.710 ± 425*</td>
<td>10.378 ± 1.577*</td>
<td>15.61 ± 1.03*</td>
<td>19.91 ± 1.73*</td>
</tr>
<tr>
<td>Epi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>3.066 ± 253</td>
<td>10.630 ± 896</td>
<td>15.16 ± 0.56</td>
<td>19.87 ± 0.70</td>
</tr>
<tr>
<td>2 nM</td>
<td>12</td>
<td>2.839 ± 251</td>
<td>9.712 ± 911</td>
<td>16.08 ± 0.51</td>
<td>20.44 ± 0.65</td>
</tr>
<tr>
<td>NE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>3.308 ± 447</td>
<td>15.466 ± 1.929</td>
<td>17.38 ± 0.97</td>
<td>22.55 ± 1.67</td>
</tr>
<tr>
<td>3.75 nM</td>
<td>10</td>
<td>2.223 ± 555*</td>
<td>10.686 ± 2.359*</td>
<td>15.73 ± 0.98</td>
<td>19.66 ± 1.30*</td>
</tr>
<tr>
<td>7.5 nM</td>
<td>10</td>
<td>1.489 ± 454</td>
<td>7.366 ± 1.822</td>
<td>14.77 ± 1.17*</td>
<td>19.01 ± 1.33*</td>
</tr>
<tr>
<td>Epi + NE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>2.944 ± 688</td>
<td>11.592 ± 2.277</td>
<td>15.75 ± 0.93</td>
<td>19.76 ± 1.37</td>
</tr>
<tr>
<td>0.5 nM + 3.75 nM</td>
<td>10</td>
<td>1.404 ± 429*</td>
<td>6.522 ± 1.400*</td>
<td>14.05 ± 1.18</td>
<td>19.47 ± 1.26</td>
</tr>
<tr>
<td>1 nM + 7.5 nM</td>
<td>10</td>
<td>1.705 ± 477*</td>
<td>7.662 ± 1.745*</td>
<td>15.17 ± 1.48</td>
<td>18.88 ± 1.63</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of subjects; H1, H2, areas of hemostatogram until 30 and 90% recovery from maximal pressure drop, respectively; CT1, CT2, time until 10-mmHg pressure drop from baseline and time until 10 mmHg is maintained for 1 min, respectively; Epi, epinephrine; NE, norepinephrine. Catecholamine concentrations are the final concentrations. *P < 0.05; †P < 0.01 vs. before exercise or control.
centrations were measured in these studies (1, 6, 39), and the authors have speculated that exercise increases fibrinolysis rather than coagulation, resulting in a hypocoagulable state. However, speculating about the coagulation state by measuring these parameters needs attention. The processes of blood coagulation and fibrinolysis are complex and involve enzymes, inhibitors, and many types of blood cells. Therefore, it is very difficult to speculate about coagulation state as a whole from the changes in these parameters measured in relatively purified fractions. The hypercoagulable state after exercise is assessed by a whole blood clotting-time method by using nonanticoagulated blood (24). Also, a hyperfibrinolytic state after exercise is reported by using a whole blood clot lysis-time method (13). Exercise may induce a hypercoagulable state as well as a hyperfibrinolytic one. These two phenomena are not contradictory because it is well known that the fibrinolytic reaction is a relatively slow process compared with coagulation. That is, a hypercoagulable state may be dominant in the early stage, and then a hypocoagulable or fibrinolytic state follows.

An elevated, but still physiological, concentration of norepinephrine enhanced coagulation. The effect on coagulation could be a consequence of enhanced platelet reactivity. In vivo, platelets are known to play a pivotal role in the coagulation response by adhering and aggregating at the site of vessel injury. Activated platelets provide substantial phospholipid surface for the assembly of membrane-dependent procoagulant enzyme complexes, such as prothrombinase complex consisting of factor Xa, factor Va, calcium ions, and phospholipid bilayer (32). Except for the clotting time of whole blood in plastic tubes (24), other overall coagulation tests performed in stagnant blood samples (tube tests) cannot detect platelet-mediated enhanced coagulation. In contrast to stagnant tube tests, hemostatometry measures dynamic coagulation and detects the contribution of activated platelets to coagulation of the flowing blood (11).

Studies on the circadian rhythms of myocardial infarction, sudden cardiac death, and platelet susceptibility to catecholamines have suggested that catecholamines may enhance platelet reactivity (34, 40). The present in vitro study demonstrated that a physiological level of norepinephrine, but not epinephrine, induced platelet hyperreactivity (Table 2). It has been reported that epinephrine enhances platelet aggregation, but the concentrations used in previous studies were supraphysiological (12, 22, 36). Hemocoagulation was observed after exercise in the present study (Table 1), and this might be partly responsible for the observed platelet hyperreactivity and hypercoagulable state.

Catecholamines increase under various circumstances (15), but norepinephrine does so more readily compared with epinephrine (28). Norepinephrine increases remarkably even with mild exercise, but epinephrine only does so with strenuous exercise (7, 17). In patients with a cardiac pacemaker, plasma norepinephrine increased up to 10 nM (20 nM in the coronary arteries) during exercise, but the epinephrine concentration was only 0.7 nM (25). Plasma epinephrine rarely reaches 1 nM (15). A higher norepinephrine concentration was confirmed after aerobic exercise in the present study, so the plasma norepinephrine level may be more important during mild aerobic exercise.

An increase in norepinephrine could be critically important in patients who have platelet hyperaggregability and arterial stenosis. However, it has been reported that regular aerobic exercise reduces platelet reactivity and decreases the thrombotic tendency (27, 31, 37, 38, 41). Therefore, aerobic exercise may be useful for prevention and cure of arterial thrombosis. It is, however, important to assess the acute thrombotic and platelet response to exercise in patients and healthy persons before the onset of long-term exercise. Shear-induced platelet aggregation tests such as hemostatometry may be useful to monitor exercise.

In conclusion, the present study demonstrated that acute aerobic exercise induced platelet hyperreactivity and a hypercoagulable state. It was suggested that a physiological increase of norepinephrine may be related to these responses.

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