

Epinephrine Augments von Willebrand Factor–Dependent Shear-Induced Platelet Aggregation

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Background. Shear-induced platelet aggregation (SIPA) is an important mechanism in thrombogenesis. von Willebrand factor (vWF) binding to platelet glycoprotein Ib (GP Ib) has been found to be crucial for platelet aggregation under the high shear force probably generated in stenosed coronary artery. The physiological significance of vWF-dependent SIPA has not been clarified.

Methods and Results. Blood samples were collected from 23 normal volunteers. SIPA was continuously monitored using a modified cone-plate viscometer adapted for measuring the transmitted light intensity of the material. The effects of low concentrations of epinephrine, ADP, and collagen on SIPA under both low shear (12 dyne/cm²) and high shear (108 dyne/cm²) force were investigated. All agonists tested enhanced SIPA under low shear force, whereas only epinephrine augmented SIPA under high shear force. The maximum extents of SIPA under high shear force in the absence and presence of epinephrine (10 ng/ml) were 37.9±11.5% and 59.7±13.9%, respectively. The antagonist of the α_2 -adrenergic receptor yohimbine (1 μ g/ml) antagonized the effects of epinephrine. The monoclonal antibody NMC-4 against vWF, which was shown to inhibit its binding to GP Ib, completely abolished SIPA under high shear force, even in the presence of epinephrine. However, this antibody only partially inhibited SIPA under low shear force.

Conclusions. Our findings suggest that epinephrine is the agonist that enhances SIPA mediated by vWF through its specific receptor. This may be clinically important because occlusion of the coronary artery often occurs in stenosed atherosclerotic vessels under sympathetic stimulation. (*Circulation* 1992;86:1859–1863)

KEY WORDS • epinephrine • α_2 -receptor • von Willebrand factor • platelets

Platelet aggregation induced by shear forces has been accepted as an important contributor to thrombogenesis in certain pathological conditions.¹ Currently, the mechanism of shear stress-induced platelet aggregation (SIPA) has been studied by several investigators, including ourselves.^{2–7} SIPA is different than agonist-induced platelet aggregation in certain aspects. Aspirin does not inhibit SIPA,⁸ indicating that the cyclooxygenase pathway does not play a role. Distinct observations have been made that different adhesive proteins and platelet membrane glycoproteins are involved in aggregation depending on the shear stress conditions.² Under low shear force (12 dyne/cm²), platelet aggregation can be induced by fibrinogen binding to the glycoprotein (GP) IIb/IIIa complex. In contrast, aggregation occurring under shear

force >80 dyne/cm² is mediated by von Willebrand factor (vWF), even in the presence of a physiological concentration of fibrinogen, by interacting with its platelet-binding sites, both GP Ib and GP IIb/IIIa. The latter mechanism is particularly important because it may occur in the stenosed coronary artery in the presence of external ionized calcium within normal plasma levels.² vWF is, therefore, considered to play a crucial role in the formation of platelet aggregates in the rheological condition of high shear stress, which may occur in partially occluded arteries or arterioles.^{9,10}

Some investigators have suggested a correlation between sympathetic activities and coronary occlusion.¹¹ Other reports have suggested that epinephrine augmented ADP-induced platelet aggregation.^{12,13} However, no study has investigated the effects of epinephrine on vWF-mediated platelet aggregations, which could play an important role in thrombotic occlusion of stenotic coronary artery. In the present study, we investigated the effects of low concentrations of various agonists on SIPA under both low and high shear forces. We tried to characterize the effects of a low concentration of epinephrine on vWF-mediated SIPA compared with the effects of other agonists. The results may be clinically relevant because epinephrine has been shown

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to play a role in the pathogenesis of coronary occlusive disease.¹⁴⁻¹⁶

Methods

Sample Collection

Blood was drawn from 23 healthy male volunteers (age range, 26–31 years) who abstained from drugs known to interfere with platelet functions for the week preceding the studies. Blood was mixed with 1:10 vol of 3.8% sodium citrate solution. Platelet-rich plasma (PRP) was prepared by centrifugation at 100g for 15 minutes at 22–25°C; the platelet count was adjusted to $3 \times 10^5 \mu\text{l}^{-1}$ using homologous platelet-poor plasma (PPP) obtained by centrifugation of blood at 3,000g for 20 minutes.

Measurement of Shear Stress-Induced Platelet Aggregation

The method used to measure SIPA with a cone-plate viscometer has been described.^{2,17} Although whole blood viscosity varies with shear condition, especially at shear rates $<10 \text{ sec}^{-1}$, plasma and PRP containing $3 \times 10^5 \mu\text{l}^{-1}$ of platelets are known to act as a Newtonian fluid.¹⁸ The cone is rotated with a rotor motor regulated by a computer. With this instrument, the cone could be rotated at a maximum rotation speed of 2,000 rpm to generate a constant shear stress without turbulent flow. Helium-neon laser light at 633 nm was passed through the streaming samples, and the transmitted light intensity was continuously recorded. The percent platelet aggregation was calculated according to Lambert-Beer's equation.²

To measure SIPA, 400 μl of PRP was applied to the surface of a polymethylmethacrylate plate and exposed to shear stress at 24°C for 6 minutes. The rotation rate of the cone was 10 rpm for the first 50 seconds; then it was increased to 200 rpm (the corresponding shear stress was 12 dyne/cm²) or 1,800 rpm (the corresponding shear stress was 108 dyne/cm²) within 45 seconds. The concentrations of intrinsic epinephrine in autologous PPP were measured by high-performance liquid chromatography using electrochemical detection as previously described.¹⁹

Study Protocol

In eight subjects, the effects of various concentrations of epinephrine (1, 5, 10, and 20 ng/ml) and ADP (1, 5, and 10 nM) on low and high SIPA were tested. In the remaining 15 subjects, the effects of low concentrations of various agonists (epinephrine, ADP, and collagen) on SIPA were measured with or without preincubation with the α_2 -receptor blocker yohimbine. The effects of monoclonal antibody NMC-4, which reacts with the amino terminal 97-kd fragment of vWF, were also tested. NMC-4 was proved to completely inhibit vWF binding to platelets in the presence of both ristocetin and bitorcetin at an immunoglobulin G concentration of approximately 10 $\mu\text{g/ml}$.²⁰ Epinephrine, ADP, and yohimbine were obtained from Daiichi Seiyaku, Sigma, and Tokyo Kasei Kougyou Co., Ltd., respectively.

Data Analysis

All data are expressed as mean \pm SD. Differences between the concentrations of epinephrine were analyzed using one-way ANOVA for the effects of epineph-

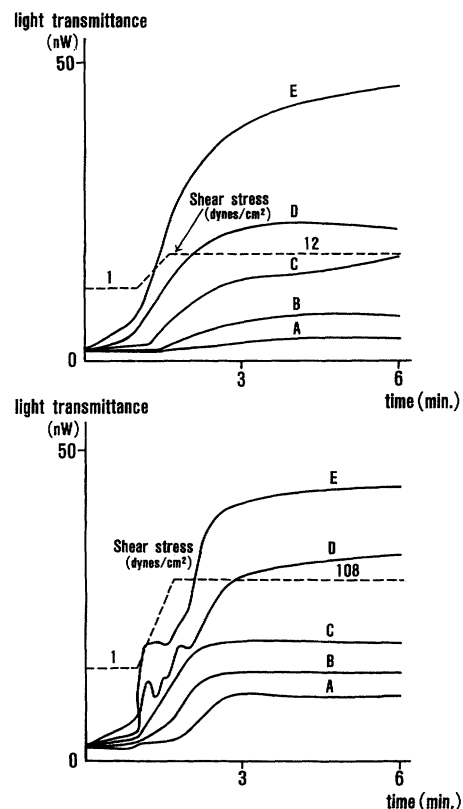


FIGURE 1. Tracings of change in transmitted light intensity of platelet-rich plasma under low (upper panel) and high (lower panel) shear stress with or without exogenously added epinephrine. Shear force applied to platelet-rich plasma is shown by the dotted line. A, no addition; B, epinephrine 1 ng/ml; C, epinephrine 5 ng/ml; D, epinephrine 10 ng/ml; E, epinephrine 20 ng/ml.

rine on SIPA. Student's paired *t* test was used to compare the two groups of data. A value of $p < 0.05$ was considered statistically significant.

Results

Dose-Dependent Augmentation of SIPA by Exogenous Epinephrine

Figure 1 shows the representative tracings of transmitted light intensity of PRP under both low and high shear stress. The transmitted light intensity of PRP increased with shearing time. The extent of platelet aggregation induced by both low and high shear stress was augmented by exogenously adding epinephrine in a dose-dependent fashion. Figure 2 shows the effects of exogenously added epinephrine on the maximum extent of platelet aggregation induced by low and high shear stress in eight subjects. The maximum extent of platelet aggregation induced by low shear stress was $22.8 \pm 10.1\%$ without epinephrine. It increased to $34.1 \pm 10.1\%$, $45.3 \pm 13.1\%$, $55.9 \pm 13.5\%$, and $68.3 \pm 11.9\%$ in the presence of epinephrine added at concentrations of 1, 5, 10, and 20 ng/ml, respectively ($p < 0.05$ between all groups of data). Under high shear stress, the extent was $37.6 \pm 5.7\%$ without epinephrine, which was greater than that induced by low shear stress ($p < 0.05$). Exogenously added epinephrine at concentrations of 1, 5, 10, and 20 ng/ml augmented platelet aggregation to

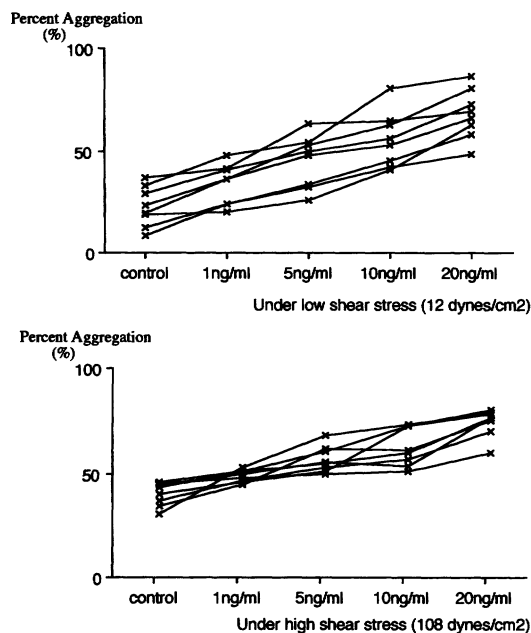


FIGURE 2. Tracings of the maximum extent of platelet aggregation induced by low (upper panel) and high (lower panel) shear stress. Exogenous epinephrine added at various concentrations augmented platelet aggregation induced by both low and high shear stress in a dose-dependent manner. Results of experiments on eight samples are shown.

46.0±2.8%, 54.2±6.2%, 60.1±8.9%, and 71.9±6.6%, respectively ($p<0.05$ between all groups of data except between 5 and 10 ng/ml). Adding 1, 5, or 10 nM ADP also enhanced SIPA under low shear stress to 32.5±8.5%, 38.0±10.6%, and 50.2±14.2% ($p<0.05$ between all groups of data). However, these concentrations of ADP had no effect on SIPA induced by high shear stress.

Effects of α_2 -Receptor Blockade and Anti-vWF Antibody

Table 1 shows the effects of various agonists on the maximum extent of platelet aggregation induced by low and high shear stress in the remaining 15 cases. The concentration of epinephrine in plasma obtained from each individual at rest was 0.04±0.01 ng/ml. At a low shear force corresponding to 12 dyne/cm², ADP (10 nM), collagen (200 ng/ml), and epinephrine (10 ng/ml) all enhanced SIPA ($p<0.05$). In contrast, SIPA under a high shear force corresponding to 108 dyne/cm² was

TABLE 1. Effects of Low Concentration of Agonists on Shear-Induced Platelet Aggregation

Agonist	Percent of aggregation	
	Low shear (12 dyne/cm ²)	High shear (108 dyne/cm ²)
No addition	32.5±7.2	37.9±11.5
Epinephrine (10 ng/ml)	75.2±6.1*	59.7±13.9*
ADP (10 nM)	51.8±7.8*	34.8±11.5
Collagen (200 ng/ml)	51.5±8.5*	38.5±10.5
Epinephrine (10 ng/ml) +yohimbine (1 µg/ml)	31.4±4.6	35.2±15.5

* $p<0.05$ vs. no addition.

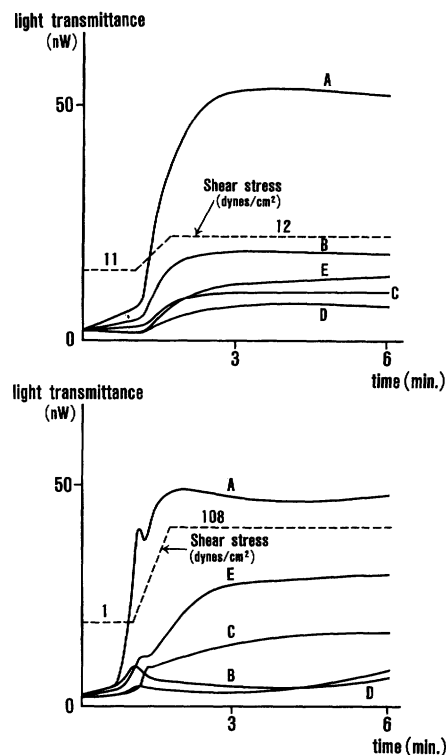


FIGURE 3. Tracings of effects of yohimbine and monoclonal antibody NMC-4 on relatively high shear-induced platelet aggregation. Yohimbine (1 µg/ml) or NMC-4 (12.3 µg/ml) was preincubated with platelet-rich plasma containing exogenously added epinephrine (10 ng/ml). Shear-induced platelet aggregation then was continuously recorded. The dotted line indicates the level of shear stress applied during 6-minute periods. A, exogenously added epinephrine (10 ng/ml) only; B, A plus NMC-4 (12.3 µg/ml); C, A plus yohimbine (1 µg/ml); D, NMC-4 only; E, no addition.

potentiated only by epinephrine ($p<0.05$). Neither ADP nor collagen had an effect on SIPA under high shear force. This augmentation of SIPA by epinephrine under low or high shear stress was blocked by preincubation of PRP with the α_2 -adrenergic antagonist yohimbine at a concentration of 1 µg/ml ($p<0.05$). The enhancing effects of ADP and collagen on SIPA induced by low shear stress were not affected by preincubation at this dose of yohimbine. Figure 3 shows the effects of the anti-vWF monoclonal antibody NMC-4, which inhibited vWF binding to platelet GP Ib on SIPA, in comparison with the effects of yohimbine. NMC-4 totally abolished high SIPA in both the presence and absence of exogenously added epinephrine, showing that epinephrine caused potentiation of vWF-mediated SIPA. In the case of low shear stress, NMC-4 only partially inhibited SIPA, with or without epinephrine.

Discussion

Two distinct mechanisms of platelet aggregation have been recognized—agonist-induced platelet aggregation and SIPA. In the former, the cyclooxygenase pathway plays a central role, and aspirin is able to block this process.²¹ After binding of the agonists to the respective platelet receptors, GP IIB/IIIa becomes activated to allow fibrinogen to bind. In contrast, SIPA is insensitive

to aspirin.⁸ Different adhesive proteins and platelet membrane glycoproteins are involved in aggregation depending on the shear stress conditions and the concentration of divalent cations.² When blood is collected with trisodium citrates as the anticoagulant, platelet aggregation can be induced under low shear stress (12 dyne/cm²). This was mediated by fibrinogen binding to the GP IIb/IIIa complex. However, this aggregation was absent in plasma containing hirudin as the anticoagulant and having a normal divalent cation concentration.² On the other hand, platelet aggregation was prominent under high shear stress (108 dyne/cm²), even under the condition in which external ionized calcium was within normal plasma levels. Aggregation apparently is mediated by binding of vWF to both GP Ib and GP IIb/IIIa.² This aggregation is unique because fibrinogen essentially plays no role. This pathway of aggregation may be considered to play a role in coronary thrombogenesis because animal studies demonstrated that vWF binding to GP Ib played an important role in thrombogenesis in stenosed coronary artery where estimated shear stress (200–400 dyne/cm²) was greater than that used in our study.^{9,10,22}

Enhancement of high SIPA by epinephrine was also demonstrated in PRP containing hirudin as the anticoagulant (data not shown). Therefore, the potentiation of vWF-mediated SIPA by epinephrine may be important. The fact that NMC-4 partially inhibited low SIPA in the presence of exogenous epinephrine indicated that epinephrine made low-sheared platelets reactive to vWF (data not shown). Ardlie et al¹² first reported that epinephrine not only caused platelet aggregation but also enhanced the aggregation induced by other agonists such as ADP. Since these observations, many studies have suggested the role of α_2 -adrenergic stimulation, although the mechanism and the clinical implications have yet to be clarified.^{23–27} We speculated that increased calcium influx through α_2 -receptor stimulation may play a role because calcium influx was essential for SIPA mediated by vWF.²⁸

Sudden occlusion of the coronary artery usually occurs at sites of stenosed arterial lesions and may be triggered by sympathetic stimulation. Epinephrine is usually present in the circulation at a concentration of 0.03 ± 0.09 ng/ml and may increase to 0.5–1 ng/ml with sympathetic stimulation.²⁹ The increase in epinephrine concentration has been suggested to play a role in arterial thrombosis. Folts et al³⁰ reported that an increase in the plasma concentration of epinephrine to 1–10 ng/ml triggered the formation of thrombus mainly composed of platelets in constricted canine coronary artery. Our present results may provide an explanation for these experimental observations.

It is concluded that epinephrine potentiated vWF-mediated SIPA through α_2 -adrenergic stimulation. This may play an important role in the pathogenesis of arterial thrombosis of stenosed coronary artery.

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