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Background It has been reported that sympathetic nerve activity (SNA) is associated with fibrinolysis, but the interaction between SNA and the fibrinolytic system with aging has not been elucidated in humans. The purpose of this study was to examine the effect of age-related SNA on the activity of plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator (tPA) using muscle SNA (MSNA).

Methods and Results This study included 16 young subjects (mean age 26.1 years) and 10 aged subjects (mean age 56.9 years). Lower body negative pressure (LBNP) was performed at –40 mmHg for 30 min. LBNP significantly increased both tPA and PAI-1 activity (from 5.2±0.5 to 7.3±1.2 IU/ml and from 2.85±0.68 to 4.06±0.73 U/ml, $p<0.01$, respectively) in the aged group. In the young group, tPA activity tended to increase, whereas PAI-1 activity was unchanged. There was a correlation between MSNA and PAI-1 activity in the aged group ($r=0.47$, $p<0.01$).

Conclusions SNA in an aging subject leads to an increase in the activity of PAI-1, which indicates that an altered interaction between SNA and PAI-1 activity contributes to increased cardiovascular events in the elderly population. (*Circ J* 2008; **72:** 458–462)

Key Words: Fibrinolysis; Lower body negative pressure; Muscle sympathetic nerve activity; Plasminogen activator inhibitor type 1

It is well known that aging increases the risk of atherosclerosis and cardiovascular events[1] Recent studies have demonstrated that altered interaction between several plasma coagulation and fibrinolytic factors plays an important role in age-associated thrombosis[2,3] The fibrinolytic system is mainly regulated by the activity of plasminogen activator inhibitor type 1 (PAI-1) and of tissue plasminogen activator (tPA). The interaction between PAI-1 and tPA is reported to be regulated by multifactorial mechanisms, such as neurohormonal factors[4,5]

Recently, in an animal model, stimulation of sympathetic nerve activity (SNA) was demonstrated to release tPA through sympathetic axons[6–8] and age-related increase of SNA has been reported by many investigators[9–12] Because aging *is predisposed* to thrombosis, together with various stress factors, it is likely that alteration of the fibrinolytic system with aging contributes to the sympathetic nervous system (SNS)[13–15] In fact, previous epidemiological studies have suggested that high catecholamine levels are associated with major cardiovascular risk factors and thus may contribute to the long-term development of atherosclerosis[16–18] Some studies have reported that β-blockers are very effective in preventing the process of plaque disruption, thrombosis, and sudden cardiac death[19,20]

With regard to stress, previous studies have examined mental and physical stress in humans after infusion of catecholamine agents[13–15] However, it is unknown whether central hypovolemic stress affects the activity of coagulation and fibrinolytic factors. Moreover, in previous studies, measurements of catecholamine concentration were performed to evaluate this sympathetic hyperactivity, but to our knowledge, there has not been a report evaluating the relationship between direct recording of the SNS (using a microneurogram) and the activity of coagulation and fibrinolytic factors in humans.

Therefore, the purpose of this study was to examine whether the response of the fibrinolytic system to hypovolemia is altered with aging. Additionally, we investigated any differences in young and aged subjects, and evaluated whether the activation of the SNS is related to the activity of coagulation and fibrinolytic factors.

Methods

Subjects

The study population comprised 16 young healthy subjects (all men: aged between 21 and 37 years, mean age 26.1 years) and 10 aged subjects (9 men, 1 woman; aged between 46 and 71 years, mean age 56.9 years). All aged subjects

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*Department of Disease Control and Homeostasis, Graduate School of Medical Science, Kanazawa University, *Department of Cardiology, Kanazawa City Hospital, Kanazawa, Japan*

Mailing address: Kanichi Otowa, MD, Department of Disease Control and Homeostasis, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. E-mail: otowanomori@gmail.com

Table 1 Baseline Characteristics of the Young and Aged Subjects

	Young (n=16)	*Aged (n=10)*	*p value*
Age, years	*26.1±1.3*	*56.9±1.9*	*<0.01*
Male, n	*16*	*9*	*NS*
Weight, kg	*65.8±2.4*	*60.9±2.4*	*NS*
Height, m	*172.6±1.2*	*164.1±2.4*	*<0.01*
Body mass index, kg/m²	*22.0±0.6*	*22.5±0.5*	*NS*
Heart rate, beats/min	*66.2±2.6*	*58.5±2.4*	*NS*
Systolic arterial pressure, mmHg	*113.3±2.6*	*119.3±3.8*	*NS*
Diastolic arterial pressure, mmHg	*58.8±2.3*	*63.3±4.4*	*NS*
Mean arterial pressure, mmHg	*77.0±2.1*	*81.9±4.1*	*NS*
Burst rate, burst/min	*7.1±1.8*	*28.4±4.3*	*<0.01*
Burst incidence, burst/100 beats	*10.2±2.3*	*48.4±7.4*	*<0.01*

Values are mean±SE.

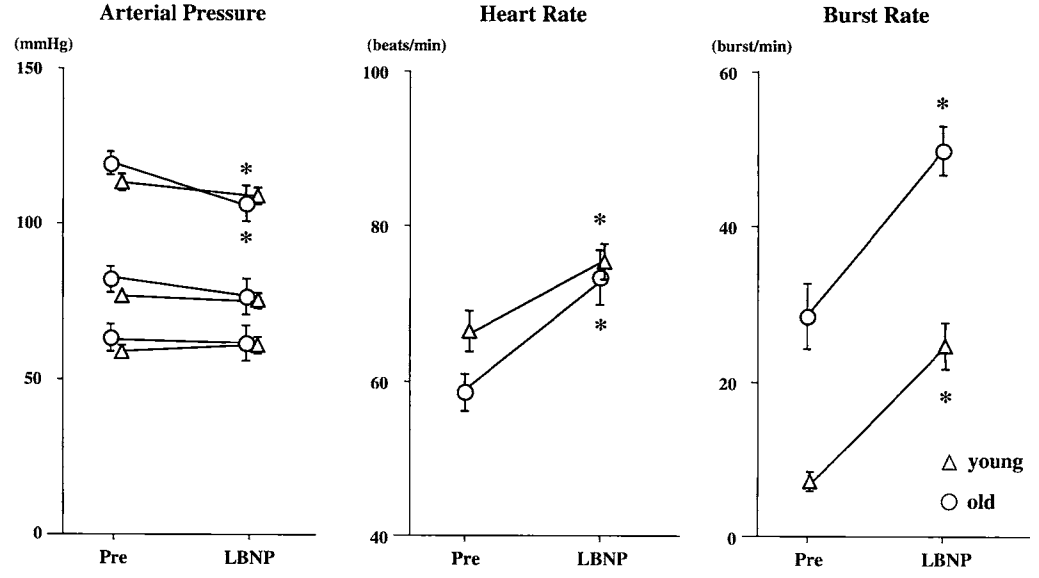

Fig 1. Hemodynamic changes in the young and aged groups. Values are means±SE. *p<0.01 compared with Pre vs lower body negative pressure (LBNP).

had not had any anginal episodes or a myocardial infarction in the past 1 year at least. Patients diagnosed with congestive heart failure, atrial fibrillation, and neurological diseases were excluded. The experimental protocol and purpose were explained in detail to each subject and informed consent, approved by the Ethics Committee of Kanazawa University, was given by all patients.

Measurement of Hemodynamic Change

Heart rate (HR) was recorded by continuous ECG monitoring and arterial pressure (AP) in the radial artery was non-invasively monitored using the JENTOW® system (Nihon Korin, Tokyo, Japan). Muscle SNA (MSNA) was recorded from a muscle nerve fascicle in the peroneal nerve using tungsten microelectrodes and the micro-neurographic technique. All experiments were carried out with subjects lying supine.

MSNA

Measurement of MSNA was performed as previously described[21,22] In brief: the electrodes were connected to a preamplifier with a gain of 1,000 and an amplifier with a gain of 70. The signal was fed through a bandpass filter (700–2,000 Hz) and a resistance–capacitance integrating circuit with a time constant of 0.1 s, producing a mean voltage neurogram. The signal was also monitored through a loudspeaker and on an oscilloscope, and recorded with a paper chart recorder (Nihon Koden, Tokyo, Japan). MSNA was identified based on internal cardiac and respiratory activity without evoking of arousal stimuli. The Valsalva maneuver was performed to confirm the increase in multiunit (burst) firing frequency. Sympathetic bursts were identified by inspection of the filtered and mean voltage neurograms. Nerve activity is expressed as bursts per min (burst rate: BR) and bursts per 100 heartbeats (burst incidence: BI).

Blood Sampling and Laboratory Method

Before and during lower body negative pressure (LBNP), an initial 30 ml of blood was drawn from an antecubital vein using a 21-gauge needle while the patient was recumbent. These samples were centrifuged at 3,000 rpm for 10 min and after centrifugation, they were immediately stored at −70°C until analysis. PAI-1 activity was measured using a chromogenic assay kit (Spectrolyse®/pL PAI, Biopool AB, Umeå, Sweden) and that of tPA was measured using a biofunctional immunosorbent assay kit (Chromolize™ tPA Assay Kit, Biopool AB).

Study Protocol

A LBNP protocol was used for this study. The subject's

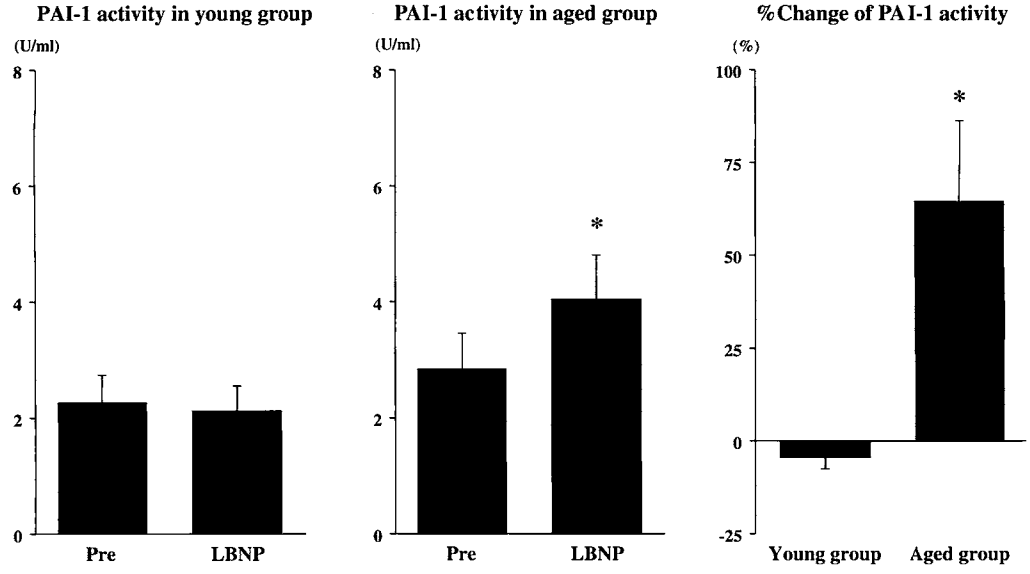

Fig 2. Plasminogen activator inhibitor type 1 (PAI-1) activity in the young and aged groups and % change of PAI-1 activity. Values are means ± SE. *p<0.01 compared with Pre vs lower body negative pressure (LBNP); *p<0.01 compared with young vs aged groups.

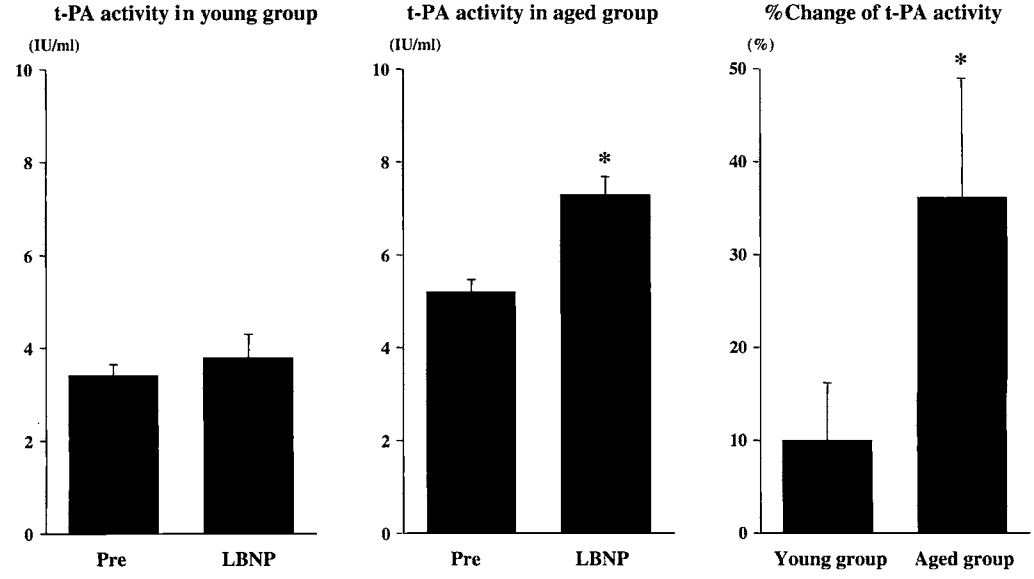

Fig 3. Tissue plasminogen activator (tPA) activity in the young and aged groups and % change of tPA activity. Values are means ± SE. *p<0.01 compared with Pre vs lower body negative pressure (LBNP); *p<0.01 compared with young vs aged groups.

lower body (below the iliac crest), excluding the left leg, was enclosed in a chamber that was sealed and connected to an adjustable vacuum. All subjects rested supine for 30 min. HR, AP, and MSNA were determined from stationary continuous data. After 10-min segments of the time series data had been measured during the baseline period, LBNP was applied at −40 mmHg for 30 min. The data during LBNP were measured for 25–30 min. Blood samples for measuring hematological parameters were obtained from the upper limbs at baseline and at the end of LBNP. This study was performed between 15.00 h and 17.00 h.

Statistical Analysis

All results are expressed as mean ± SE. The significance of differences in the hemodynamic and MSNA variables, and other parameters, before and after LBNP was assessed by Wilcoxon single-ranked test. The Mann-Whitney U test was performed to compare parameters between young and aged subjects. Correlations between MSNA and hematological parameters were analyzed by Pearson's correlation coefficient. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Baseline characteristics of the subjects are shown in Table 1. The young group was significantly taller, but weight and body mass index were not significantly different be-

tween the 2 groups. HR and AP were not significantly different between the groups, but the BR of the young group was significantly lower than that of the aged group.

The effects of LBNP on the hemodynamics and MSNA are summarized in Fig 1. LBNP significantly increased HR, and decreased systolic AP in both the young and aged group. Diastolic AP and mean AP were unchanged. As expected, in both groups BR and BI were significantly increased during LBNP.

The effects of LBNP on coagulation and fibrinolytic activity are presented in Figs 2 and 3. After LBNP, the activity of both tPA and PAI-1 was significantly increased (from 5.2±0.5 to 7.3±1.2 IU/ml, and from 2.85±0.68 to 4.06±0.73 U/ml, $p<0.01$, respectively) in the aged group. In the young group, tPA activity had a tendency of increase during LBNP (from 3.4±0.3 to 3.8±0.5 IU/ml), but the increase in PAI-1 activity was not exaggerated during LBNP (from 2.27±0.47 to 2.11±0.43 U/ml).

The correlation between MSNA and PAI-1 activity is shown in Fig 4. There was a significant correlation between BR and PAI-1 activity in the aged group, but not in the young group. tPA activity did not correlate with MSNA in either group.

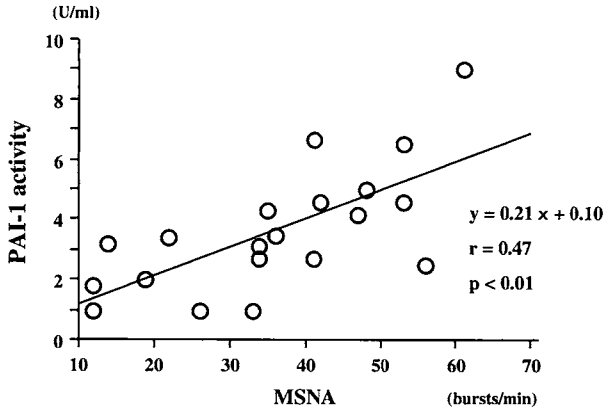

Fig 4. Relationship between muscle sympathetic nerve activity (MSNA) and plasminogen activator inhibitor type 1 (PAI-1) activity in the young and aged groups.

Discussion

This is the first study to demonstrate that activation of the SNS with LBNP leads to increased tPA and PAI-1 activity in the aged, whereas in the young group, LBNP increased MSNA but had no effect of either t-PA or PAI-1 activity. Additionally, there was a significant correlation between PAI-1 activity and MSNA in the aged group. These findings suggest that the aging-related response of PAI-1 would contribute to cardiovascular events.

In animals, Miskin et al reported enhancement of PAI-1 mRNA in cardiovascular cells after injection of kainite, an analog of glutamate, into the SNS.[23] They reported that elevation of PAI-1 mRNA levels was detected 3 h after systemic administration of kainite. Additionally, some reports have shown that SNA induced enhancement of PAI-1 mRNA, and increased PAI-1 activity.[24–26] More importantly, PAI-1 expression is dramatically enhanced in aged mice. In humans, elevation of PAI-1 levels occurs in several thrombotic conditions in atherosclerotic patients and aged subjects.[27–31] These results are consistent with our findings in the present aged subjects, but not in the young subjects. The acute reflex of the SNS during LBNP in the young subjects appeared to decrease PAI-1 activity or change it slightly, which might contribute to the defense mechanism against hypercoagulation in the central hypovolemic state. On the other hand, hyperactivity of the SNS in a hypovolemic state increased the activity of PAI-1 in aged group. These results indicate that in the aging population an altered response of PAI-1 to LBNP might trigger a hypercoagulable state and enhance overt thrombosis.

We found a significant correlation between PAI-1 activity and MSNA in the aged group. In humans, previous studies have reported that activation of SNS with mental and physical stress augmented PAI-1 activity and reduced fibrinolytic activity.[14,32,33] Recent observations are that fibrinolysis with tPA activity, tPA antigen and PAI-1 activity depend on catecholamine levels.[24,34–37] A previous report suggested that non-selective β-blockers abolished fibrinolytic activity mediated by epinephrine.[38] Our results show that SNA is significantly related to PAI-1 activity during LBNP, which suggests that PAI-1 activity would be increased by enhanced sympathetic efferent neuronal activity.

We also found that tPA activity tended to increase in the young group, but was actually increased in the old group, whereas PAI-1 activity was increased in aged subjects only. A previous study reported that tPA might be increased in plasma in 3 ways: (1) hemoconcentration, which increases the concentration of most proteins in blood, (2) reduced hepatic blood flow, which decreases clearance of tPA, and (3) release of epinephrine and other factors during stress, which stimulates increased secretion of tPA.[39] Contrary to the situation for PAI-1, the release of tPA might be caused by increased cathecholamines, which contribute to the prevention of cardiovascular events. In this study, we used LBNP to replicate the central hypovolemic state, such as spending a long time having a bath, because many older people die from cardiovascular events occurring while in the bathtub.[40] We emphasize that this difference between young and older subjects may be explained by our results.

Our study differs from previous ones in that the acute reflex of hyperactivity of the SNS was induced by LBNP. LBNP is well known to increase SNA and catecholamine concentration by reducing baroreceptor restraint.[41,42] Previous results suggest that dose-dependent stimulation with an infusion of epinephrine increases PAI-1 activity within 15–40 min,[35] which suggests that elevation of catecholamine levels or orthostatic stress induces a hypercoagulable state. However, the measurement of catecholamine levels is largely influenced by factors such as posture and mental stress, so we evaluated direct recording of efferent SNA, which enables an exact response of SNA to be measured during physiological stress. Accordingly, our findings demonstrate that augmentation of SNA with aging is associated with disorders of the fibrinolytic system.

We did not measure other coagulation markers, which may affect cardiovascular events in humans, but our aim was to examine the interaction between SNA and the fibrinolysis systems. The present results provide additional information about therapy for cardiovascular events, but future study is needed to identify the relationship between SNA and other coagulation factors.

In conclusion, we found that an augmented response of SNA with aging leads to increased PAI-1 activity, which suggests that an altered interaction between SNA and PAI-1 activity contributes to increased cardiovascular events in

the elderly.

References

1. Tracy RP, Bovill EG. Thrombosis and cardiovascular risk in the elderly. *Arch Pathol Lab Med* 1992; **116:** 1307–1312.
2. Franchini M. Hemostasis and aging. *Crit Rev Oncol Hematol* 2006; **60:** 144–151.
3. Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: Implication in the pathogenesis of thrombotic disorders in the elderly. *Cardiovasc Res* 2005; **66:** 276–285.
4. Kruithof EK. Plasminogen activator inhibitors: A review. *Enzyme* 1988; **40:** 113–121.
5. Prowse CV, Cash JD. Physiologic and pharmacologic enhancement of fibrinolysis. *Semin Thromb Hemost* 1984; **10:** 51–60.
6. Schaefer U, Machida T, Vorlova S, Strickland S, Levi R. The plasminogen activator system modulates sympathetic nerve function. *J Exp Med* 2006; **203:** 2191–2200.
7. Jiang X, Wang Y, Hand AR, Gillies C, Cone RE, Kirk J, et al. Storage and release of tissue plasminogen activator by sympathetic axons in resistance vessel walls. *Microvasc Res* 2002; **64:** 438–447.
8. Wang Y, Jiang X, Hand AR, Gilles C, Kirk J, Cone RE, et al. Additional evidence that the sympathetic nervous system regulates the vessel wall release of tissue plasminogen activator. *Blood Coagul Fibrinolysis* 2002; **13:** 471–481.
9. Sverrisdottir YB, Johannsson G, Jungersten L, Wallin BG, Elam M. Is the somatotropic axis related to sympathetic nerve activity in healthy ageing men? *J Hypertens* 2001; **19:** 2019–2024.
10. Ebert TJ, Morgan BJ, Barney JA, Denahan T, Smith JJ. Effects of aging on baroreflex regulation of sympathetic activity in humans. *Am J Physiol* 1992; **263:** H798–H803.
11. Ng AV, Callister R, Johnson DG, Seals DR. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension* 1993; **21:** 498–503.
12. Jones PP, Davy KP, Alexander S, Seals DR. Age-related increase in muscle sympathetic nerve activity is associated with abdominal adiposity. *Am J Physiol* 1997; **272:** E976–E980.
13. Lecomte D, Fornes P, Nicolas G. Stressful events as a trigger of sudden death: A study of 43 medico-legal autopsy cases. *Forensic Sci Int* 1996; **79:** 1–10.
14. Jern C, Eriksson E, Tengborn L, Risberg B, Wadenvik H, Jern S. Changes of plasma coagulation and fibrinolysis in response to mental stress. *Thromb Haemost* 1989; **62:** 767–771.
15. Camejo G, Hurt E, Wiklund O, Rosengren B, Lopez F, Bondjers G. Modifications of low-density lipoprotein induced by arterial proteoglycans and chondroitin-6-sulfate. *Biochim Biophys Acta* 1991; **1096:** 253–261.
16. Goldstein DS. Plasma catecholamines and essential hypertension: An analytical review. *Hypertension* 1983; **5:** 86–99.
17. Peles E, Goldstein DS, Akselrod S, Nitzan H, Azaria M, Almog S, et al. Interrelationships among measures of autonomic activity and cardiovascular risk factors during orthostasis and the oral glucose tolerance test. *Clin Auton Res* 1995; **5:** 271–278.
18. Abe M, Iwaoka M, Nakamura T, Kitta Y, Takano H, Kodama Y, et al. Association of high levels of plasma free dopamine with future coronary events in patients with coronary artery disease. *Circ J* 2007; **71:** 688–692.
19. Nakamura M, Lee DP, Yeung AC. Identification and treatment of vulnerable plaque. *Rev Cardiovasc Med* 2004; **5**(Suppl 2)**:** S22–S33.
20. Shah PK. Plaque disruption and coronary thrombosis: New insight into pathogenesis and prevention. *Clin Cardiol* 1997; **20**(Suppl)**:** II-33–II-44.
21. Yuasa T, Takata S, Maruyama M, Yasuma K, Yoshizawa H, Kontani M, et al. Low-dose atropine attenuates muscle sympathetic nerve activity in healthy humans. *Hypertens Res* 2000; **23:** 213–218.
22. Murai H, Takata S, Maruyama M, Nakano M, Kobayashi D, Otowa K, et al. The activity of a single muscle sympathetic vasoconstrictor nerve unit is affected by physiological stress in humans. *Am J Physiol Heart Circ Physiol* 2006; **290:** H853–H860.
23. Miskin R, Abramovitz R. Enhancement of PAI-1 mRNA in cardiovascular cells after kainate injection is mediated through the sympathetic nervous system. *J Mol Cell Cardiol* 2005; **38:** 715–722.
24. Larsson PT, Wiman B, Olsson G, Angelin B, Hjemdahl P. Influence of metoprolol treatment on sympatho-adrenal activation of fibrinolysis. *Thromb Haemost* 1990; **63:** 482–487.
25. Yamamoto K, Takeshita K, Shimokawa T, Yi H, Isobe K, Loskutoff DJ, et al. Plasminogen activator inhibitor-1 is a major stress-regulated gene: Implications for stress-induced thrombosis in aged individuals. *Proc Natl Acad Sci USA* 2002; **99:** 890–895.
26. Konkle BA, Schuster SJ, Kelly MD, Harjes K, Hassett DE, Bohrer M, et al. Plasminogen activator inhibitor-1 messenger RNA expression is induced in rat hepatocytes in vivo by dexamethasone. *Blood* 1992; **79:** 2636–2642.
27. Yamamoto K, Saito H. A pathological role of increased expression of plasminogen activator inhibitor-1 in human or animal disorders. *Int J Hematol* 1998; **68:** 371–385.
28. Hamsten A, Wiman B, de Faire U, Blomback M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med* 1985; **313:** 1557–1563.
29. Wiman B, Hamsten A. The fibrinolytic enzyme system and its role in the etiology of thromboembolic disease. *Semin Thromb Hemost* 1990; **16:** 207–216.
30. Pralong G, Calandra T, Glauser MP, Schellekens J, Verhoef J, Bachmann F, et al. Plasminogen activator inhibitor 1: A new prognostic marker in septic shock. *Thromb Haemost* 1989; **61:** 459–462.
31. Naya M, Tsukamoto T, Inubushi M, Morita K, Katoh C, Furumoto T, et al. Elevated plasma plasminogen activator inhibitor type-1 is an independent predictor of coronary microvascular dysfunction in hypertension. *Circ J* 2007; **71:** 348–353.
32. Malyszko J, Urano T, Takada Y, Takada A. Stress-dependent changes in fibrinolysis, serotonin and platelet aggregation in rats. *Life Sci* 1994; **54:** 1275–1280.
33. Takada Y, Urano T, Takahashi H, Nagai N, Takada A. Effects of electric footshock and water immersion restraint stresses on fibrinolytic parameters in the plasma of rats. *Thromb Res* 1998; **89:** 107–114.
34. van der Poll T, Levi M, Dentener M, Jansen PM, Coyle SM, Braxton CC, et al. Epinephrine exerts anticoagulant effects during human endotoxemia. *J Exp Med* 1997; **185:** 1143–1148.
35. Wallen NH, Larsson PT, Broijersen A, Andersson A, Hjemdahl P. Effects of an oral dose of isosorbide dinitrate on platelet function and fibrinolysis in healthy volunteers. *Br J Clin Pharmacol* 1993; **35:** 143–151.
36. Chandler WL, Levy WC, Veith RC, Stratton JR. A kinetic model of the circulatory regulation of tissue plasminogen activator during exercise, epinephrine infusion, and endurance training. *Blood* 1993; **81:** 3293–3302.
37. von Kanel R, Dimsdale JE. Effects of sympathetic activation by adrenergic infusions on hemostasis in vivo. *Eur J Haematol* 2000; **65:** 357–369.
38. Yamazaki H, Sano T, Odakura T, Takeuchi K, Matsumura T, Hosaki S, et al. Appearance of thrombogenic tendency induced by adrenaline and its prevention by adrenergic blocking agent, nialamide and pyridinolcarbamate. *Thromb Diath Haemorrh* 1971: **26:** 251–263.
39. Chandler WL, Levy WC, Stratton JR. The circulatory regulation of TPA and UPA secretion, clearance, and inhibition during exercise and during the infusion of isoproterenol and phenylephrine. *Circulation* 1995; **92:** 2984–2994.
40. Kido M, Hitosugi M, Yokoyama T, Tokudome S. Sudden death while bathing. *Nippon Rinsho* 2005; **63:** 1239–1242 (in Japanese).
41. Baily RG, Sinoway LI. Insight into human baroreceptor function using multiple indices of neural activity. *Heart Fail* 1990; **6:** 33–41.
42. Sander-Jensen K, Mehlsen J, Stadeager C, Christensen NJ, Fahrenkrug J, Schwartz TW, et al. Increase in vagal activity during hypotensive lower-body negative pressure in humans. *Am J Physiol* 1988; **255:** R149–R156.