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# Altered Interaction Between Plasminogen Activator Inhibitor Type 1 Activity and Sympathetic Nerve Activity With Aging

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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  $5.2 \pm 0.5$  to  $7.3 \pm 1.2$  IU/ml and from  $2.85 \pm 0.68$  to  $4.06 \pm 0.73$  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.<sup>1</sup> Recent studies have demonstrated that altered interaction between several plasma coagulation and fibrinolytic factors plays an important role in age-associated thrombosis.<sup>2,3</sup> 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.<sup>4,5</sup>

Recently, in an animal model, stimulation of sympathetic nerve activity (SNA) was demonstrated to release tPA through sympathetic axons<sup>6–8</sup> and age-related increase of SNA has been reported by many investigators.<sup>9–12</sup> 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).<sup>13–15</sup> In fact, previous epidemiological studies have suggested that high catecholamine levels are associ-

ated with major cardiovascular risk factors and thus may contribute to the long-term development of atherosclerosis.<sup>16–18</sup> Some studies have reported that  $\beta$ -blockers are very effective in preventing the process of plaque disruption, thrombosis, and sudden cardiac death.<sup>19,20</sup>

With regard to stress, previous studies have examined mental and physical stress in humans after infusion of catecholamine agents.<sup>13–15</sup> 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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**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/m <sup>2</sup>	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.

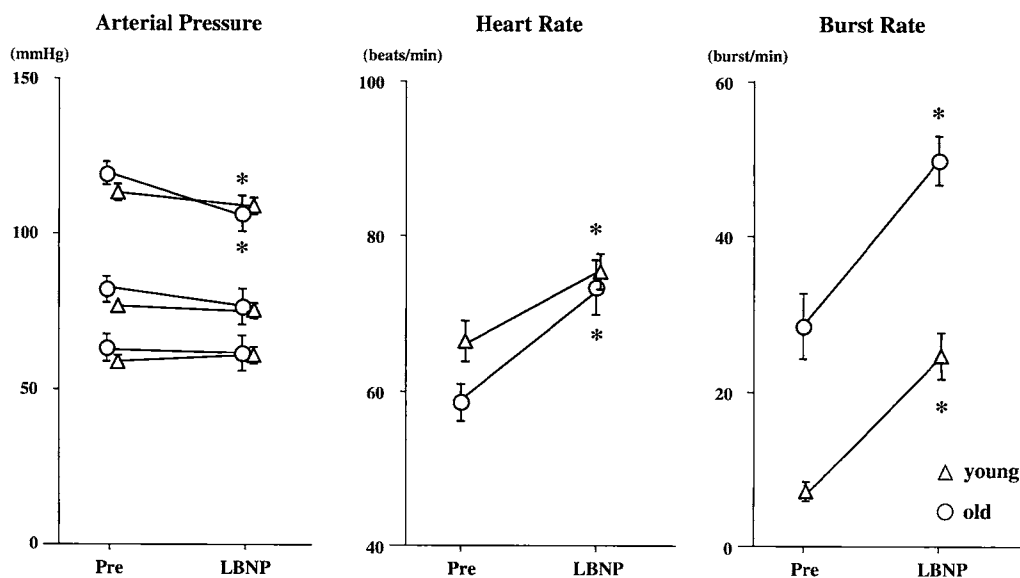


Fig 1. Hemodynamic changes in the young and aged groups. Values are means±SE. \*p<0.01 compared with Pre vs lower body negative pressure (LBNP).

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<sup>21,22</sup> In brief: the electrodes were connected to a pre-amplifier 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 neu-

rogram. 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 bio-functional immunosorbent assay kit (Chromolize™ tPA Assay Kit, Biopool AB).

#### Study Protocol

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

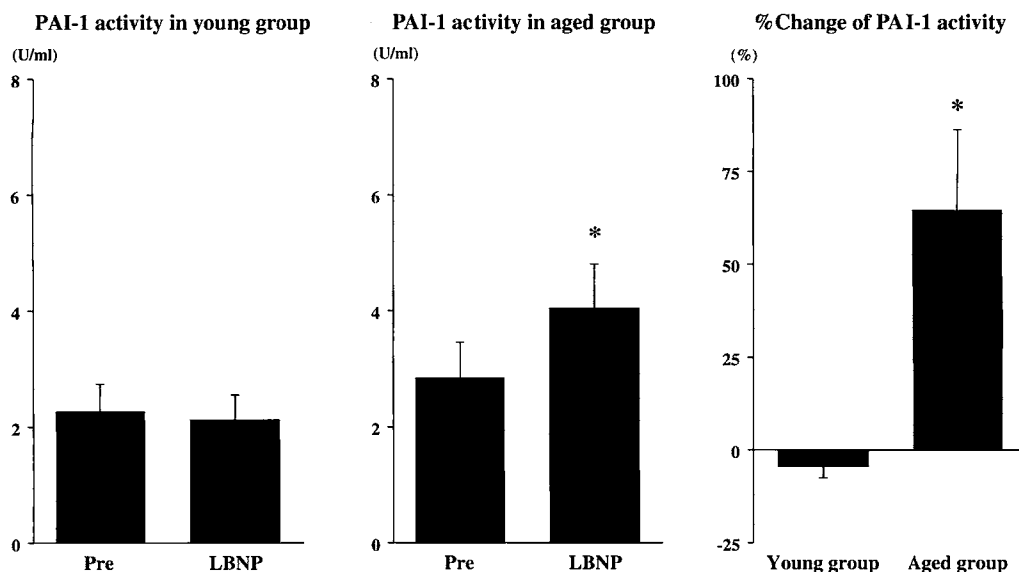


Fig 2. Plasminogen activator inhibitor type 1 (PAI-1) activity in the young and aged groups and % change of PAI-1 activity. Values are means  $\pm$  SE. \* $p < 0.01$  compared with Pre vs lower body negative pressure (LBNP); \* $p < 0.01$  compared with young vs aged groups.

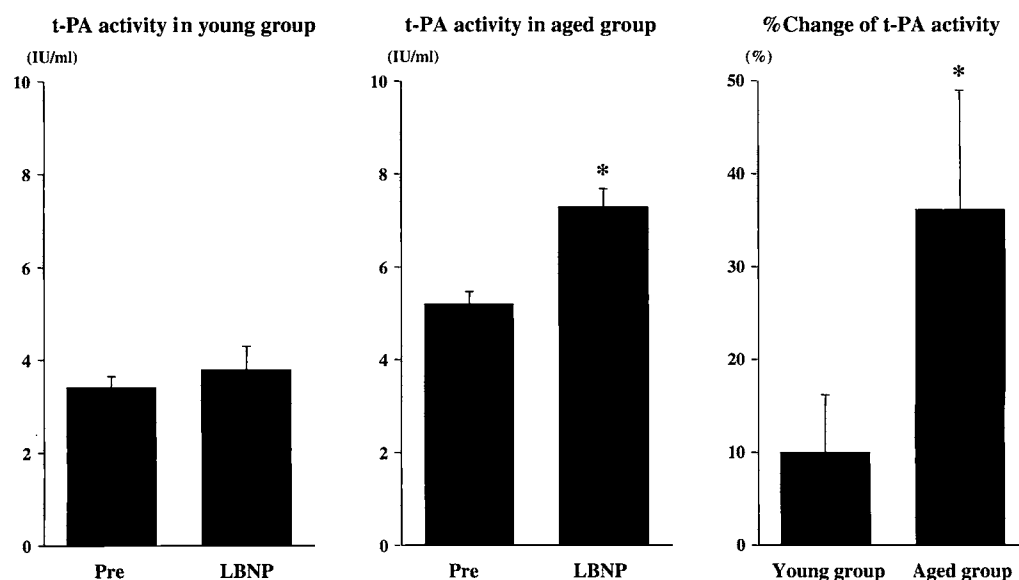


Fig 3. Tissue plasminogen activator (tPA) activity in the young and aged groups and % change of tPA activity. Values are means  $\pm$  SE. \* $p < 0.01$  compared with Pre vs lower body negative pressure (LBNP); \* $p < 0.01$  compared with young vs aged groups.

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  $\pm$  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  $5.2 \pm 0.5$  to  $7.3 \pm 1.2$  IU/ml, and from  $2.85 \pm 0.68$  to  $4.06 \pm 0.73$  U/ml,  $p < 0.01$ , respectively) in the aged group. In the young group, tPA activity had a tendency of increase during LBNP (from  $3.4 \pm 0.3$  to  $3.8 \pm 0.5$  IU/ml), but the increase in PAI-1 activity was not exaggerated during LBNP (from  $2.27 \pm 0.47$  to  $2.11 \pm 0.43$  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.

## 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.<sup>23</sup> 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.<sup>24–26</sup> 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.<sup>27–31</sup> 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.<sup>14,32,33</sup> Recent observations are that fibrinolysis with tPA activity, tPA antigen and PAI-1 activity depend on catecholamine levels.<sup>24,34–37</sup> A previous report suggested that non-selective  $\beta$ -blockers abolished fibrinolytic activity mediated by epinephrine.<sup>38</sup> Our results show that SNA is significantly related to PAI-1 activity during LBNP, which

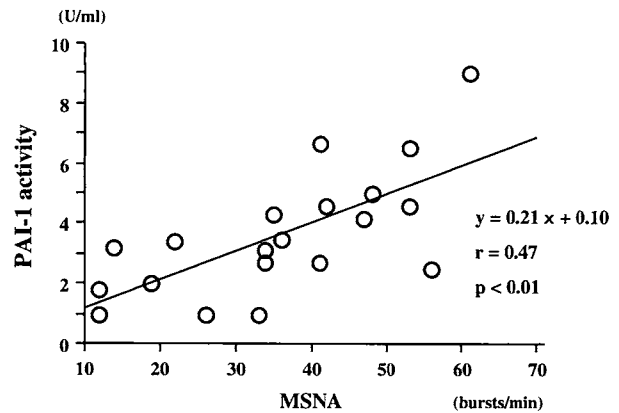


Fig 4. Relationship between muscle sympathetic nerve activity (MSNA) and plasminogen activator inhibitor type 1 (PAI-1) activity in the young and aged groups.

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.<sup>39</sup> Contrary to the situation for PAI-1, the release of tPA might be caused by increased catecholamines, 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.<sup>40</sup> 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.<sup>41,42</sup> Previous results suggest that dose-dependent stimulation with an infusion of epinephrine increases PAI-1 activity within 15–40 min,<sup>35</sup> 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.

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