Muscle Sympathetic Nerve Activity During Cold Pressor Test in Patients With Cerebrovascular Accidents

Takashi Mizushima, MD; Fumihiro Tajima, MD; Takeshi Nakamura, MD; Mitsuru Yamamoto, MD; Kyu-Ha Lee, MD; Hajime Ogata, MD

Background and Purpose—Autonomic dysfunction is frequently present in patients with cerebrovascular accidents (CVA). However, the pathophysiological mechanisms of these disorders are not clear. The purpose of the study was to assess the effects of CVA on the autonomic nervous system.

Methods—In eight male patients with a history of CVA with damage of the cortical or subcortical structures, we measured the cold pressor response during recording of muscle sympathetic nerve activity (MSNA) from the peroneal nerve on the hemiplegic side. We also studied 10 age-matched male control subjects. Tests were performed before, during, and after immersion of the nonhemiplegic hand in ice water for a period of 3 minutes in each phase. We also recorded changes in heart rate (HR), arterial blood pressure, skin temperature of the middle finger, and perception of pain using the Borg’s score.

Results—During the control period, the mean burst count of MSNA in CVA (57.2±3.9 beats/100 HR) was higher than in control subjects (36.3±3.2 beats/100 HR) (P<.05). Total MSNA (the mean burst amplitude per minute times burst rate) increased significantly in CVA and control during the immersion period by 79.9±18.4% and 133.1±25.6%, respectively. The percent change in total MSNA in CVA was attenuated during immersion compared with control subjects. The HR and skin temperature responses as well as the Borg’s score were similar in both groups during control, hand immersion, and recovery periods.

Conclusions—The present results suggest that increased MSNA in CVA may be due to damage of cortical or subcortical structures or stroke-related changes in other areas or nonspecific changes that cause continuous increase in basal MSNA.

Key Words: autonomic nervous system • blood pressure • cerebrovascular disorders • microneurography

The technique of microneurography has enabled researchers to record efferent MSNA in humans by direct intraneural recording of postganglionic sympathetic efferent fibers.1–3 MSNA consists of sympathetic vasconstrictor nerve impulses that innervate skeletal blood vessels and thus plays an important role in the regulation of regional blood flow and systemic BP.4,5 Microneurography has contributed to our understanding of normal and abnormal function of the autonomic nervous system in several clinical disorders, such as diabetic polyneuropathy,6 congestive heart failure,7 and hypertension.8

Autonomic dysfunction, eg, shoulder-hand syndrome,9 supraventricular tachycardia,10 and other cardiovascular diseases,11–13 is frequently encountered in cerebrovascular disease. BP is controlled by a group of neurons in the medulla oblongata collectively termed the vasomotor area or vasomotor center.14 Descending tracts from the cerebral cortex (particularly the limbic cortex) connect to the vasomotor center and relay in the hypothalamus and possibly in the mesencephalon.14 Consequently, central lesions that lead to autonomic dysfunction may result in myocardial damage, cardiac arrhythmia, and disturbances of the mechanisms regulating arterial BP.

Clinically, the cold pressor test has been used to detect autonomic disorders and measures the response of arterial BP and HR, to hand immersion in cold water.15 A marked increase in MSNA during the cold pressor test has been described during microelectrode recordings from the peroneal nerve in healthy subjects.16,17 Thus, one could gain insight into the regulatory mechanisms that control MSNA during localized cooling of the skin.1

In the present study we recorded MSNA in the lower leg (peroneal microneurography) during cold pressor tests in patients who had subcortical or cortical lesions after more than 3 months of CVA. We measured simultaneously HR, BP, skin temperature of the third finger, and pain rating in eight male poststroke patients and 10 sex- and age-matched control subjects before, during, and after 3 minutes of hand immersion in ice water. The purpose of this study was to characterize the response of MSNA in poststroke patients during localized cooling of the skin and to assess quantitatively the effects of CVA on the autonomic nervous system.

Subjects and Methods

Subjects
Peroneal nerve recordings were performed in eight male patients with a history of CVA and 10 age- and resting BP–matched male subjects aged 48 to 67 years (mean±SEM age, 57.5±2.5 years) and 43 to 68
Selected Abbreviations and Acronyms
- BP = blood pressure
- bpm = beats per minute
- CVA = cerebrovascular accident
- HR = heart rate
- MSNA = muscle sympathetic nerve activity

years (mean±SEM age, 57.8±2.4 years), respectively. Systolic BP, diastolic BP, and mean BP of the control subjects were similar to those in poststroke patients (Tables 1 and 2). None of the control subjects was on any medication.

Stoke occurred at least 3 months before the present study and was confirmed by CT scan and classified according to the classification of cerebrovascular disease III of the National Institute of Neurological Disorders and Stroke.2 Patient characteristics and type of CVA are shown in Table 3. Patients and control subjects were free of diabetes and neurological and cardiovascular disorders with the exception of hypertension and CVA. In addition, patients were not on medications that may affect the cardiovascular system at least 36 hours before the study. Other medications are shown in Table 4. A number of patients were on purgatives to prevent constipation that may potentially alter sympathetic nerve activity. Each patient gave signed informed consent, and the experimental protocol was approved by the Human Investigation Committee of the University of Occupational and Environmental Health, Fukuoka, Japan.

Nerve Recordings
Previous studies have demonstrated that the burst rate of MSNA on the hemiplegic side in hemiplegic stroke patients is similar to that on the normal side.19 We confirmed this observation in a series of preliminary studies in six poststroke patients which demonstrated that the mean burst count of MSNA on the hemiplegic side (63.1±3.8 beats/100 HR) was similar to that recorded on the normal side (61.5±3.1 beats/100 HR), and we accordingly recorded MSNA from the peroneal nerve on the hemiplegic side in all subjects. A tungsten microelectrode (Iowa Doppler Products) with a noninsulated tip approximately 5 μm in diameter was inserted at the location of the head of the fibula through intact skin into the underlying peroneal nerve. A reference surface electrode was attached 15 mm from the recording electrode. The nerve was localized by means of electrical stimulation delivered through the recording electrode. The position within a muscle-nerve fascicle was identified by muscle twitches after electrical stimulation. After identification of the fascicle, the electrode was carefully adjusted until the characteristic multiunit bursts of MSNA were encountered. The nerve signal was amplified in two steps, with a total gain of 50 000, and fed through a 500- to 1700-Hz band-pass filter. A resistance-capacitance integrating network with a time constant of 100 milliseconds allowed the recording of the mean voltage neurogram. The amplitude of individual bursts was measured, and the total MSNA was calculated by multiplying the mean burst amplitude per minute by burst rate and expressed as units per minute. For analysis of the responses of MSNA in poststroke patients and control subjects, the mean amplitude of the sympathetic burst during immersion and recovery periods was expressed as a percentage of the mean burst amplitude recorded during the control period. MSNA was expressed as the total burst area per minute. Basal levels of MSNA, BP, and HR were determined during the 3-minute control period.

Physiological Measurements
HR was determined from the ECG. After we confirmed the absence of laterality of BP in the upper extremities, a continuous recording of BP was obtained with the use of photoplethysmography (Finapres, NO2300). The sensor was placed on a finger of the hand on the hemiplegic side. The skin temperature of the third finger of the immersed hand was measured with a thermocouple (AM-7001 ANRITISU). To assess the subjects’ perception of pain during skin cooling, a 15-point modified Borg’s score was used, as described previously.28 The score accommodated the full range of cooling-associated human pain perception from nonpainful cold discomfort to intensely painful sensation. Subjects identified the descriptive phrase that best described their perception and gave the most closely associated numerical value.

### Table 1. Resting Arterial BP in CVA Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Mean BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115.0</td>
<td>71.0</td>
<td>86.0</td>
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<tr>
<td>2</td>
<td>117.2</td>
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<td>3</td>
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<td>80.5</td>
<td>101.1</td>
</tr>
<tr>
<td>4</td>
<td>98.8</td>
<td>41.7</td>
<td>60.7</td>
</tr>
<tr>
<td>5</td>
<td>119.2</td>
<td>67.7</td>
<td>84.8</td>
</tr>
<tr>
<td>6</td>
<td>129.5</td>
<td>89.0</td>
<td>102.5</td>
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<tr>
<td>7</td>
<td>106.2</td>
<td>69.8</td>
<td>81.9</td>
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<tr>
<td>8</td>
<td>154.3</td>
<td>72.2</td>
<td>99.9</td>
</tr>
<tr>
<td>Mean</td>
<td>122.8</td>
<td>68.3</td>
<td>86.4</td>
</tr>
</tbody>
</table>

Values are expressed in millimeters of mercury.

### Table 2. Resting Arterial BP in Control Subjects

<table>
<thead>
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<th>Diastolic BP</th>
<th>Mean BP</th>
</tr>
</thead>
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<td>73.9</td>
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<td>101.7</td>
<td>66.0</td>
<td>77.9</td>
</tr>
<tr>
<td>3</td>
<td>103.8</td>
<td>55.7</td>
<td>71.7</td>
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<td>4</td>
<td>114.3</td>
<td>76.5</td>
<td>89.1</td>
</tr>
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<td>93.6</td>
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<td>65.4</td>
</tr>
<tr>
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<td>151.3</td>
<td>87.5</td>
<td>108.8</td>
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<tr>
<td>7</td>
<td>144.3</td>
<td>85.7</td>
<td>105.2</td>
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<tr>
<td>8</td>
<td>156.5</td>
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<tr>
<td>Mean</td>
<td>123.3</td>
<td>68.8</td>
<td>86.9</td>
</tr>
</tbody>
</table>

Values are expressed in millimeters of mercury.

**Experimental Protocol**
Subjects reported to the laboratory at 3 PM to 4 PM wearing short pants and T-shirts. They were studied in the supine position in a quiet room (ambient temperature, 26°C to 27°C). Each test consisted of 9 minutes of recording. After basal recording for 3 minutes (control period), the subject immersed the unaffected hand to the wrist into a 0°C water bath for a period of 3 minutes (immersion period), followed by removal of the hand from the bath and continuation of recording for another 3 minutes (recovery period).

**Data Analysis**
All signals were stored on tape and recorded on a chart paper for subsequent analysis. The number of bursts of MSNA was determined by visual inspection of the mean voltage neurogram; all recordings were analyzed by the same investigator. The total outflow of MSNA (total MSNA) was identified by inspection of the mean voltage neurogram. The amplitude of individual bursts was measured, and the total MSNA was calculated by multiplying the mean burst amplitude per minute by burst rate and expressed as units per minute. For analysis of the responses of MSNA in poststroke patients and control subjects, the mean amplitude of the sympathetic burst during immersion and recovery periods was expressed as a percentage of the mean burst amplitude recorded during the control period. MSNA was expressed as the total burst area per minute. Basal levels of MSNA, BP, and HR were determined during the 3-minute control period.

**Statistical Analysis**
We determined the significant changes in each parameter from the control to the immersion period within the test and changes from control between different tests within a given time period using repeated-measures ANOVA. Differences between groups were exam-
ined for statistical significance with the use of Fisher’s protected least significant difference analysis. A value of \( P < 0.05 \) was considered statistically significant. Data were reported as mean \( \pm \) SEM.

### Results

#### Changes in MSNA

During the control period, the mean burst rate of MSNA in poststroke patients (37.6 \( \pm \) 2.8 bpm) was significantly higher than in control subjects (24.2 \( \pm \) 1.7 bpm) \( P < 0.05 \). The mean burst count of MSNA in poststroke patients (57.2 \( \pm \) 3.9 beats/100 HR) was also higher \( P < 0.05 \) than that in control subjects (36.3 \( \pm \) 3.2 beats/100 HR) during the control period. The mean burst count of MSNA increased significantly during the immersion period in poststroke patients and control subjects to 67.7 \( \pm \) 5.0 and 61.1 \( \pm \) 3.5 beats/100 HR \( P < 0.05 \), respectively (Fig 1). Surprisingly, the mean absolute value of burst count of MSNA during immersion in the control subjects was almost similar to that in poststroke patients.

Immersion of the hand in cold water increased the total MSNA by 79.9 \( \pm \) 18.4\% in poststroke patients (relative to the control period; \( P < 0.05 \)) (Fig 2) and by 133.1 \( \pm \) 25.6\% in control subjects. During the recovery period, the total MSNA immediately decreased in both groups; however, it remained at a level significantly higher than the control period in control subjects. The percent change in total MSNA during the immersion period was significantly less in poststroke patients than in control subjects \( P < 0.05 \).

#### Changes in Cardiovascular Parameters

HR increased significantly during the cold pressor test from a mean control value of 64.0 \( \pm \) 2.8 and 65.8 \( \pm \) 2.4 bpm to 69.4 \( \pm \) 3.1 and 72.3 \( \pm \) 3.3 bpm in the control and poststroke patients, respectively, and rapidly returned to control levels during the recovery period in both groups (Fig 3). BP in both groups increased significantly during the immersion period but returned to the control level immediately after immersion (Fig 4). There were no differences in BP between control and poststroke patients throughout the entire test.

#### Changes in Skin Temperature

The skin temperature of the third finger abruptly fell to 19.3 \( \pm \) 0.8°C at 30 seconds of immersion and progressively decreased to 12.8 \( \pm \) 0.6°C at 3 minutes of immersion in control subjects. In poststroke patients, the skin temperature of the third finger was 17.1 \( \pm \) 0.8°C at 30 seconds of immersion and 10.6 \( \pm \) 0.8°C at 3 minutes of immersion. On removal from water, the skin temperature of the third finger immediately rose toward the baseline level in both the control and poststroke patients, although the temperature was lower in the latter group at the end of the test compared with the control. Interestingly, the decrease in the skin temperature of the third finger in poststroke patients was greater than that in control subjects during the period extending from 90 seconds after immersion.

### Table 3. Patient Characteristics and Type of Cerebrovascular Lesion

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Hemiplegia</th>
<th>Type of Lesion</th>
<th>Location of Lesion</th>
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<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>M</td>
<td>R</td>
<td>B</td>
<td>L basal ganglion</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>M</td>
<td>R</td>
<td>I</td>
<td>L basal ganglion</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>M</td>
<td>R</td>
<td>B</td>
<td>L basal ganglion</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>M</td>
<td>L</td>
<td>I</td>
<td>R basal ganglion and thalamus</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>M</td>
<td>L</td>
<td>B</td>
<td>R basal ganglion and thalamus</td>
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<tr>
<td>6</td>
<td>56</td>
<td>M</td>
<td>L</td>
<td>B</td>
<td>R basal ganglion and thalamus</td>
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<tr>
<td>7</td>
<td>55</td>
<td>M</td>
<td>R</td>
<td>B</td>
<td>L basal ganglion and thalamus</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>M</td>
<td>L</td>
<td>B</td>
<td>R basal ganglion and thalamus</td>
<td>3</td>
</tr>
</tbody>
</table>

R indicates right; L, left; B, bleeding; and I, infarction.

### Table 4. Medications Used by Poststroke Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Medication</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Purgative</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>Purgative</td>
</tr>
</tbody>
</table>
immersion in cold water to 90 seconds after removal of the hand (Fig 5). This finding suggests that the magnitude of stimulation in poststroke patients was enhanced during immersion.

Changes in Perceived Pain
During ice water immersion, there was a sensation of intense pain that was perceived almost immediately and persisted throughout immersion in poststroke patients and control subjects. The Borg’s score during immersion in poststroke patients was not different from that in control subjects (Fig 6).

Discussion
The major findings of the present study were the following: (1) total MSNA in the supine resting condition in poststroke patients was higher than in control subjects; (2) the percent change in total MSNA (MSNA response) during ice water immersion was attenuated in poststroke patients compared with control subjects; and (3) there were no differences between poststroke patients and control subjects in the skin temperature of the third finger and ratings of perceived pain during immersion. MSNA could probably be influenced by a variety of nonspecific stimuli. However, since our control subjects were selected so as to be closely similar to the poststroke patients, we could rule out the possible effects of age, sex, hypertension, and the use of medications. Accordingly, our results suggest that stroke is directly associated with increased MSNA in poststroke patients compared with control subjects at baseline resting conditions.

It is well known that immersion of the hand in ice water induces a high pressor response, including a significant rise in BP and HR. The test also induces a powerful stimulation of MSNA. BP, HR, and MSNA responses in the control subjects and poststroke patients in the present study were consistent with those reported in the above earlier studies. However, the percent increase of MSNA in poststroke patients during the cold pressor test was attenuated compared with the control subjects.

The medullary vasomotor area receives input from the cerebral cortex (particularly the limbic cortex) that is relayed in the hypothalamus and possibly also in the mesencephalon.

Figure 2. Percent change in total MSNA in control (n=10) and poststroke patients (n=8) during 3 minutes of the cold pressor test (hand immersion in 0°C water). *P<.05 compared with control period; #P<.05 control vs poststroke patients. Data are mean±SEM.

Figure 3. Changes in HR in control (n=10) and poststroke patients (n=8) during 3 minutes of the cold pressor test (hand immersion in 0°C water). *P<.05 compared with control period. Data are mean±SEM.

Figure 4. Changes in mean arterial BP in control (n=10) and poststroke patients (n=8) during 3 minutes of the cold pressor test (hand immersion in 0°C water). *P<.05 compared with control period. Data are mean±SEM.

Figure 5. Changes in skin temperature of the immersed finger in control (n=10) and poststroke patients (n=8) during 3 minutes of the cold pressor test (hand immersion in 0°C water). *P<.05 compared with control period; #P<.05 control vs poststroke patients. Data are mean±SEM.
These fibers are responsible for the rise in BP and tachycardia during emotions such as fear, sexual excitement, and anger. In the present study, afferent stimuli to the cortex from thermoreceptors in the hand and thermal information into the vasomotor area from the hands were similar in both groups of subjects, since the skin temperature of the third finger and the ratings of perceived pain in poststroke patients were not different from those in control subjects. These results suggest that stimuli from the finger to the cortex and hypothalamus in poststroke patients were similar to those in control subjects.

The mechanisms of augmented MSNA during the control period in poststroke patients may be explained by the following mechanisms: (1) impulses generated in the cerebral cortex might inhibit the tonic discharge of sympathetic nerve activity, and the loss of such tonic inhibitory influences after cortical or subcortical lesions in poststroke patients might increase MSNA, and (2) poststroke patients may be physically uncomfortable or stimulated by nonspecific stimuli during the test, resulting in a continuous augmentation of MSNA. It is possible that the augmented basal MSNA in poststroke patients may directly or indirectly contribute to the attenuated sympathetic response during the cold pressor test.

In contrast, the HR response in poststroke patients tended to be higher than in control subjects during the experimental period, although the difference was not statistically significant. Although the basal MSNA in poststroke patients was augmented during the control period, the difference in HR between the two groups was not statistically significant. In this regard, previous studies by Victor et al demonstrated that changes in MSNA do not always parallel those of HR. The present study does not provide a sufficient explanation for the difference between augmented MSNA and lack of change in HR during the control period between control subjects and poststroke patients. On the other hand, the mean BP during the experimental period in poststroke patients was almost similar to that of control subjects. The tendency for increased HR in poststroke patients may result in a similar increase in mean BP despite the presence of attenuated MSNA during the cold pressor test.

Previous studies reported that age, sex, and resting BP could influence the behavior of MSNA. Therefore, to eliminate the effect of these factors on our results, we selected control subjects who were similar to poststroke patients with regard to age, BP (systolic, diastolic, and mean BP), and sex.

Clinical observation indicates that a proportion of patients with hemispheric stroke may develop the shoulder-hand syndrome, orthostatic hypotension, ECG abnormalities, or other cardiovascular diseases. These disorders might be induced by damage of cortical and subcortical structures. However, no reports have shown direct evidence of disorders of regulation of MSNA in such patients. Most reports describing sympathetic disorders in patients with CVA have used either indirect measurements of sympathetic nerve activity or systemic measurement of sympathetic nerve activity (such as blood catecholamines). The present results demonstrating the presence of disorders of MSNA regulation during the cold pressor test will help us to understand these clinical aspects of poststroke patients.

In conclusion, the present study showed that total MSNA in supine resting patients with a history of CVA was higher than in control subjects and that the percent change in total MSNA (MSNA response) during the cold pressor test was attenuated in these patients compared with control subjects. This behavior of MSNA may be due to damage of cortical or subcortical structures or other direct or indirect stroke-related associated changes that produce tonic activation of MSNA.

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References
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