Responses of Muscle Sympathetic Nerve Activity and Cardiac Output to the Cold Pressor Test

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Abstract We measured muscle sympathetic nerve activity (MSA) to clarify the mechanisms of the blood pressure rise during cold pressor test (CPT), simultaneously with impedance cardiography and blood pressure wave measurement in 10 healthy subjects. MSA remained unchanged during the initial period of 0–30s of the CPT and increased remarkably during the later period of 30–90s of the CPT, while cardiac output exhibited a slight increase during the initial period but not during the later period. Mean blood pressure increased significantly throughout the entire period of CPT and reached the maximal level during 90–120s of the CPT. The mean blood pressure and total peripheral resistance during the CPT showed a linear correlation with MSA. In conclusion, an increase of cardiac output elevates blood pressure at the initial period of the CPT with little increase in MSA, while an increase of MSA plays an essential role to elevate the blood pressure at the later period of the CPT.

Key words: cold pressor test, muscle sympathetic nerve activity, cardiac output, total peripheral resistance.

A strong rise in blood pressure is observed as reported by Hines and Brown [1] on the immersion of one hand in ice water for some moments, i.e., the cold pressor test (CPT) [1, 2]. CPT has been, therefore, used as a method to measure the function of the neural control of the cardiovascular system [2–5]. The underlying mechanism of the blood pressure elevation in this test was widespread sympathetic activation [2–4]. Indeed the sympathetic nervous system seems to contribute to this pressor response, judging from the studies of serum catecholamines [6–8]. Recently, microneurographical studies directly revealed that muscle sympathetic nerve activity (MSA) was strongly activated by the CPT [9, 10]. The increase in MSA correlated linearly with the rise of blood pressure in the CPT, and thus the rise of the blood pressure might be attributed to enhancement of MSA [9].

On the other hand, there has been controversy about which increase, cardiac output, and/or peripheral resistance, contributes to the blood pressure rise during
the CPT. Some reports have indicated an increase in cardiac output [3] while others have suggested an increase in total peripheral resistance [2], or both [5], as causative factors for elevation of blood pressure. However, their data lacked the measurement of cardiac output and continuous blood pressure monitoring, and they could not clarify time-dependent change of each parameter. This study aimed to 1) examine the time course of cardiac output and MSA during the CPT, 2) reveal the contributing factor of blood pressure elevation in the CPT, and 3) clarify the mechanism of blood pressure elevation during the CPT.

SUBJECTS AND METHODS

Subjects were 10 healthy, normotensive males, aged 26–35 (29.8 ± 3.5, mean ± SD), who gave informed consent before the experiment. This study was approved by Human Research Committee of Research Institute of Environmental Medicine, Nagoya University, and was carried out under the guidelines of Japan Micro-neurography Society.

The subjects were laid on the bed, and a tungsten microelectrode with the tip diameter of approximately 1 μm and impedance 3–5 MΩ was inserted percutaneously without anesthesia into the muscle fascicle of the tibial nerve at popliteal fossa. The microelectrode was connected to a differential preamplifier (WPI, DAM-6A, gain 60 dB) and a buffer amplifier. The neural activity was then fed through a band-pass filter with a band width of 500 to 5,000 Hz. For monitoring during the examination, the filtered neurogram was observed on a storage oscilloscope (Tektronix 5113) and a sound amplifier with a loudspeaker and stored to a data recorder for later analysis. For analysis, the neurogram was fed through a full wave rectifying and integrating circuit (time constant, 0.1 s) to obtain a mean voltage display of the neural activity.

Discharges of sympathetic nerve fibers were recognized from the temporal patterns of spontaneous activity, which show a difference in skin and muscle nerve fascicles. MSA occurs as distinct heartbeat synchronous burst discharges whereas skin sympathetic nerve activity consists of more irregular bursts of varying amplitude and duration, being independent of the heartbeat. Thus the following maneuvers and criteria were applied for identification of MSA: 1) simultaneously observable afferent discharge as the result of tapping or squeezing the muscle, but not from light touch of the skin, 2) spontaneous and grouped burst discharges with synchronized heartbeat, 3) activity increased with apnea and Valsalva's maneuver, and 4) rhythmic grouped discharges that did not change with electrical or arousal stimuli [11].

Blood pressure waves were monitored by a mass compensatory photoplethysmography (Ohmeda, Finapres 2300) in the third finger of the right hand kept at the level of the left ventricle. Electrocardiogram for heart rate, pneumogram by an impedance method for respiration monitoring, and neurogram of MSA were recorded continuously on an FM magnetic tape recorder (Sony, Magnescale KS-
The mean voltage neurogram, electrocardiogram, respiratory movements, blood pressure, differentiated impedance cardiogram, and phonocardiogram were monitored on a thermal pen recorder (NEC-San-ei, Recti-Horiz).

The cold pressor test (CPT) was performed by immersing the subject's left hand up to the wrist in ice water (4°C) for 2 min. Experimental protocol was: resting for 10 min, the CPT for 2 min, and recovery for 5 min. Inadvertent performance of a Valsalva's maneuver or holding respiration was prohibited and natural respiration was ordered during the experiment because such maneuvers have been reported to activate MSA [12]. After the experiment, subjects were asked how and when sensation occurred and when sensation of pain reached the maximum during the CPT.

The mean voltage neurogram was used for analysis. The quantification of MSA was conducted as the number of bursts per minute (Burst rate) and as the number of bursts per 100 heartbeats (burst incidence). The amplitude of each integrated burst was measured to determine total MSA, which was calculated as bursts per minute multiplied by mean MSA burst amplitude and was expressed in arbitrary units. Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure. Cardiac output was calculated for each 10 s along with Cubicek's method [13]; average stroke volume of 10 consecutive heart beats multiplied by heart rate. The mean value of total MSA for the initial 5 min during resting period was taken as the control and total MSA were expressed as fractional change. Cardiac output was measured for 1 min before the CPT, for 2 min during the CPT, and for 1 min after the CPT. Total peripheral resistance was calculated as mean blood pressure divided by cardiac output and expressed as fractional change. The mean value of stroke volume, cardiac output, and total peripheral resistance for 1 min before the CPT was taken as control value and changes were expressed as fractional change. The mean values of each parameter for 30 s during the CPT and the recovery were used for analysis. The parameters concerning MSA (burst rate, burst incidence, total MSA), and mean blood pressure and heart rate obtained for the first 5 min of the control period were averaged as the control value. Since all the parameters changed markedly during the CPT and for 1 min after the CPT, their mean values of each 30 s were calculated. For statistical analysis, Student's t-test was employed. The results were shown as mean ± standard error and p values below 0.05 were considered significant.

RESULTS

1. The response of mean blood pressure and heart rate to the cold pressor test

Mean blood pressure and heart rate significantly increased during the CPT. However, the time course of changes in these parameters was different. Mean blood pressure had been increasing during the CPT at the 90–120 s period up to 1.31 ± 0.03 times. On the other hand, heart rate reached the maximum at the 30–60 s period. It increased from 59.6 ± 2.2 to 72.9 ± 5.3 beats/min at this period and then
decreased (Table 1, Figs. 1 and 2).

2. The response of muscle sympathetic nerve activity to the cold pressor test

MSA was activated remarkably by the CPT with the latency of 10 to 20 s. Burst rate increased from $18.2 \pm 2.8$ to $57.1 \pm 12.9$ bursts/min at the 60–90 s period of the CPT. Burst incidence increased from $30.5 \pm 4.6$ bursts/100 beats to $74.7 \pm 13.0$ bursts/100 beats in the same period. Both parameters began to decrease after 90 s of the CPT. Total MSA peaked at the 60–90 s period of the CPT, when the mean value increased by $7.66 \pm 2.51$ (Table 1, Figs. 3–6).

3. The response of cardiac output to the cold pressor test

Although heart rate increased, stroke volume decreased during the CPT. Stroke volume reached the minimum at the 60–90 s period of the CPT, which showed a 14% decrease in stroke volume. The cardiac output exhibited a slight increase during the CPT, the maximum increase of which was 5% at the 0–30 s period of the CPT (Table 2, Fig. 7).

4. The change of total peripheral resistance during the cold pressor test

Total peripheral resistance elevated during the CPT. It peaked at the 90–120 s period of the CPT, the mean value of which increased by $1.3 \pm 0.08$ times. The time course was almost the same as that of mean blood pressure (Table 2).

5. The relationship between total muscle sympathetic nerve activity and total peripheral resistance, and between total muscle sympathetic nerve activity and mean arterial pressure

A significant positive correlation was observed between the mean value during every 30 s of total MSA and total peripheral resistance ($n = 7, r = 0.87, p < 0.05$). A significant positive correlation was also seen between the mean value during every 30 s of total MSA and mean blood pressure ($n = 7, r = 0.81, p < 0.05$). The curve of these plots drawn following time course showed hysteresis. (Figs. 8 and 9).

6. Pain sensation and muscle sympathetic nerve activity during the cold pressor test

During the CPT, local cold sensation occurred immediately in the immersed hand and it turned into the sensation of pain within 30 s. The sensation of pain reached the maximum around 1 min of the CPT. The grade of this painful sensation was almost proportional to that of total MSA.

DISCUSSION

Heines et al. [2] explained that the cause of the rise of blood pressure in the CPT is a widespread vasopressor reaction initiated though a neurogenic reflex arc. Heji [14] measured cardiovascular function and reported that the pressor response in most normotensive subjects was due to a rise in cardiac output. On the other
Table 1. The response of muscle sympathetic nerve activity, heart rate, and mean blood pressure to the cold pressor test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cold pressor test</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5 min 5-10 min</td>
<td>0-30 s 30-60 s 60-90 s 90-120 s</td>
<td>0-30 s 30-60 s 1-5 min</td>
</tr>
<tr>
<td>Burst rate (bursts/min)</td>
<td>18 ± 3 17 ± 3</td>
<td>24 ± 6* 48 ± 10** 55 ± 11** 48 ± 8**</td>
<td>36 ± 8** 25 ± 4* 18 ± 2</td>
</tr>
<tr>
<td>Burst incidence (bursts/100 beats)</td>
<td>29 ± 5 26 ± 4</td>
<td>35 ± 6* 63 ± 13** 70 ± 14** 67 ± 11**</td>
<td>55 ± 11** 42 ± 7* 22 ± 5</td>
</tr>
<tr>
<td>Total MSA (A.U.)</td>
<td>1 0.97 ± 0</td>
<td>2.5 ± 0.5** 7.5 ± 2.1** 7.7 ± 1.7** 7.1 ± 1.6**</td>
<td>5.2 ± 0.8* 2.9 ± 0.3 1.1 ± 0.1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>61 ± 2 62 ± 2</td>
<td>72 ± 4** 76 ± 6** 75 ± 6** 70 ± 4**</td>
<td>67 ± 4* 63 ± 2 63 ± 2</td>
</tr>
<tr>
<td>Mean blood pressure (A.U.)</td>
<td>1 0.96 ± 0.1</td>
<td>1.06 ± 0.03* 1.21 ± 0.04** 1.31 ± 0.05** 1.35 ± 0.04**</td>
<td>1.22 ± 0.02** 1.18 ± 0.03* 1.03 ± 0.03</td>
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Data are shown as mean ± standard error (n = 10). * ** Indicates significant difference compared to control value (1-5 min): p<0.05, p<0.01, respectively.

Table 2. The response of cardiac output, stroke volume, and total peripheral resistance to the cold pressor test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cold pressor test</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0-60 s</td>
<td>0-30 s 30-60 s 60-90 s 90-120 s</td>
<td>0-30 s 30-60 s</td>
</tr>
<tr>
<td>Cardiac output (A.U.)</td>
<td>1</td>
<td>1.05 ± 0.03* 1.04 ± 0.05 1.03 ± 0.03 1.02 ± 0.06</td>
<td>0.098 ± 0.05 1.02 ± 0.02</td>
</tr>
<tr>
<td>Stroke volume (A.U.)</td>
<td>1</td>
<td>0.90 ± 0.03 0.86 ± 0.07* 0.84 ± 0.05* 0.88 ± 0.05</td>
<td>0.89 ± 0.04 1.02 ± 0.03</td>
</tr>
<tr>
<td>Total peripheral resistance (A.U.)</td>
<td>1</td>
<td>1.01 ± 0.01 1.16 ± 0.03* 1.27 ± 0.05** 1.32 ± 0.05**</td>
<td>1.24 ± 0.03** 1.15 ± 0.03*</td>
</tr>
</tbody>
</table>

Data are shown as mean ± standard error (n = 10). * ** Indicates significant difference compared to control value: p<0.05, p<0.01, respectively.
Fig. 1. The change of mean blood pressure. The mean blood pressure increased during entire period of the cold pressor test and reached maximum during the 90-120s period.

Fig. 2. The change of heart rate. The heart rate reached maximum during the 30-60s period, which showed different time course from that of blood pressure.
Fig. 3. A representative record of muscle sympathetic nerve activity (MSA). MSA increased markedly during the cold pressor test, but there was about 20s latency of activation of MSA.
Fig. 4. The change of burst rate during the cold pressor test. Burst rate increased maximally during the 60–90 s period.

Fig. 5. The change of burst incidence during the cold pressor test. Burst incidence reached the maximum during the 60–90 s period.
Fig. 6. The change of total MSA. During the cold pressor test, total MSA increased only slightly during the 0–30s period but increased markedly afterward.

Fig. 7. The change of cardiac output and stroke volume. Cardiac output increased significantly only during the 0–30s period. Stroke volume decreased during the cold pressor test.
Fig. 8. The correlation between total MSA and total peripheral resistance. There was a significant positive correlation between total MSA and total peripheral resistance. The curve following the plots according to the time course showed hysteresis.

Fig. 9. The correlation between total MSA and mean arterial pressure. There was a significant positive correlation between total MSA and mean arterial pressure. The curve following the plots according to the time course showed hysteresis.
hand, Green et al. [15] described that both total peripheral resistance and cardiac output increased during the CPT. However, what happens in the time course of the CPT and which factors contribute to the blood pressure rise incorporated during the CPT have been so far unsolved. To clarify this problem, we used simultaneous monitoring of muscle sympathetic nerve activity, cardiac output, and blood pressure.

In our result, during the initial 30s of the CPT, the activation of MSA was little and the increase in total peripheral resistance was only 3%. On the contrary, the increase in cardiac output in this period was 5%. Therefore, in the initial 30s of the CPT, the increase in cardiac output followed by the rise of heart rate seemed to contribute to the elevation of blood pressure much more than the activation of MSA.

On the contrary, MSA was remarkably enhanced with proportional elevation of total peripheral resistance in the 30–120s period of the CPT. During this period, cardiac output did not increase, and thus seemed to contribute little to the rise of blood pressure. Therefore the increase in total peripheral resistance caused by the activation of MSA played an essential role in responding to the CPT in this terminal part of 2 min CPT. Victor et al. [9] reported that the arterial pressure response to the CPT provides an approximate index of response of muscle sympathetic nerve activity. Fagius et al. [10] assumed that MSA contributes an important role to the rise of blood pressure during the CPT. Our results in the 30–120s period were compatible with their results, demonstrating that the factors in the rise of blood pressure were MSA and total peripheral resistance during the CPT. This dissociation of time-dependent changes of cardiac output and total peripheral resistance, i.e., MSA, might make previous controversy complicated.

Muscle sympathetic nerve activity is principally controlled by arterial baroreflexes [16]. It is described that the decrease of blood pressure activates MSA and blood pressure rise suppresses MSA [17]. However, MSA had been highly activated during the CPT in spite of the increase in blood pressure. This suggests that MSA is activated during the CPT beyond baroreflex control, presumably enhanced by a command from the higher center through afferent input from sensory receptors of the immersed hand. In this situation, baroreflex inhibitory influence was not completely overridden, since the cardiac rhythmicity of MSA which follows baroreceptor regulation was preserved during the procedure [10]. The increase in MSA by this mechanism may constrict peripheral vessels and contribute to the blood pressure elevation.

How much percentage MSA contributes hemodynamic control has not yet been clarified. However, radioisotope studies have revealed that 20% of noradrenaline is produced by muscles, the kidneys share 22% and liver-splanchnicus is 9% [18]. Since the activation mode of MSA resembles that of renal sympathetic activity [19], it is likely that peripheral vasoconstriction in the muscles as well as in the kidneys plays an important role in the hemodynamic control in humans. Renin-angiotensin-aldosterone system [20] and adrenal medulla as well as secreted
catecholamines [8], seemed not to be associated with this pressor response.

The cold sensation occurred during the initial 0–30s period during the CPT. Among cutaneous nociceptors, this sensation is considered to be transmitted from polymodal nociceptors, mechanothermal nociceptors, and thermal nociceptors which react to cold skin temperature below 5 to 20°C [21]. It showed time-dependent increase, becoming maximal at the period around 1 min, as Wolf and Hardy reported [22]. This may correspond to the maximal activation of MSA during the CPT at the period around 1 min. These findings may indicate that the reflex pathway to activate MSA during the CPT is originated from cutaneous nociceptors, and that it conducts the afferent volleys by unmyelinated C and Aδ fibers and involves central vasomotor centers which control MSA. However, more investigation is necessitated to clarify the receptor and reflex pathway during the CPT.

This initial pressor response during the 0–30s period might be contributed by the cardiac response due to psychological effect. This assumption can be explained by the fact that this initial heart rate rise as well as pressor response was significantly abolished by hypnotic analgesia [23].

The later pressor response during the 30–120s period of CPT is postulated to be caused via neural projection of the pain sensation to the cerebral cortex because the total MSA was well correlated to the subjective pain sensation. Or it might be via the direct stimulation on the vasoactive sympathetic center in the medulla oblongata. The latter hypothesis is based on the report by Jänig that this activation of sympathetic nervous system can be observed in the decerebrated cat [24].

MSA reached the maximum during the 60–90s period. The immersion time for the CPT, therefore, shall be 2 min, which is necessary and sufficient. Mean blood pressure and total peripheral resistance increased maximally at the 90–120s period. There were significant correlations between total MSA and mean arterial pressure, and MSA and total peripheral resistance, which indicated that MSA is essential for this pressor response. The hysteresis of this phenomenon signified the latency from MSA activation to the increases in peripheral resistance and blood pressure. Spectral analysis of this delay [25] is compatible with our result.

In conclusion, the initial pressor response might be contributed by the cardiac response due to the cold sensation via the cerebral cortex, and the later response after 30s was well attributed by the vasoconstriction, which was induced by the sympathetic activation via the nociceptive stimuli.

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