Effects of the Cold Pressor Test on Muscle Sympathetic Nerve Activity in Humans

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SUMMARY The purpose of this study was to determine the effects of the cold pressor test on sympathetic outflow with direct measurements of nerve traffic in conscious humans and to test the strength of correlation between sympathetic nerve discharge and the changes in arterial pressure, heart rate, and plasma norepinephrine. In 25 healthy subjects, arterial pressure, heart rate, and muscle sympathetic nerve activity were measured with microelectrodes inserted percutaneously into a peroneal muscle nerve fascicle in the leg during immersion of the hand in ice water for 2 minutes. Arterial pressure rose steadily during the first and second minutes of the cold pressor test. Muscle sympathetic activity (burst frequency × amplitude) did not increase in the first 30 seconds of the test but increased from 230 ± 27 to 386 ± 52 units (mean ± SE, p<0.05) by the end of the first minute of the test and to 574 ± 73 (p<0.01) during the second minute. In contrast, heart rate increased maximally during the first 30 seconds of the cold pressor test and returned to control during the second minute. The increases in heart rate were abolished by β-adrenergic blockade. The increases in muscle sympathetic activity during the cold pressor test were correlated with the increases in both mean arterial pressure (r=0.86, p<0.01) and peripheral venous norepinephrine (r=0.72, p<0.05); however, large changes in nerve traffic were associated with small changes in plasma norepinephrine.

The major new conclusions from this study are that 1) stimulation of sympathetic neural outflow to skeletal muscle is an important component of the sympathetic response to the cold pressor test, 2) the cold pressor test appears to produce differential effects on sympathetic outflow to the heart and to the skeletal muscles, and 3) the arterial pressure response to the cold pressor test provides an approximate index of muscle sympathetic activity in this setting. (Hypertension 9: 429-436, 1987)

KEY WORDS • cold pressor test • sympathetic nerve activity • heart rate • plasma norepinephrine

In most healthy human subjects, cutaneous application of ice water, the cold pressor test (CPT), increases arterial pressure, heart rate, and vascular resistance.1 For many years, the CPT has been used to evaluate sympathetic neural control of the peripheral and coronary circulations in humans. The CPT has been reported to produce exaggerated pressor responses in hypertension-prone persons2-7 and augmented coronary vasoconstrictor responses in patients with ischemic heart disease.8-10 In contrast, cold pressor responses are impaired in patients with orthostatic hypotension caused by efferent sympathetic failure.11 Furthermore, in experimental studies of neurocirculatory regulation in humans, the CPT has been used as a nonspecific stimulus to sympathetic neural outflow.12-14 In both clinical and experimental settings, the underlying assumption has been that the CPT evokes generalized sympathetic activation.

The CPT, therefore, would be expected to markedly stimulate neural release of norepinephrine. However, increases in plasma norepinephrine levels during the CPT have been inconsistent and often surprisingly small and have shown little, if any, correlation with the changes in arterial pressure and heart rate.15-22 These
previous studies do not permit definitive conclusions regarding effects of the CPT on sympathetic nerve traffic since plasma norepinephrine levels are influenced by other factors besides central sympathetic outflow.

In the present study, we recorded sympathetic nerve traffic directly with microelectrodes inserted into a peroneal muscle nerve fascicle (microneurography) in awake, unanesthetized human subjects. Nerve traffic in the leg was recorded during immersion of the hand in ice water (CPT). The purpose of this study was 1) to examine effects of the CPT on muscle sympathetic nerve activity (MSNA); 2) to determine if the CPT produces parallel responses in arterial pressure, heart rate, and muscle sympathetic outflow; and 3) to test the strength of correlation between the sympathetic nerve response and the changes in plasma norepinephrine.

Subjects and Methods

Twenty-one men and four women (ages 18 to 30 years) participated in this study after providing written, informed consent. All subjects were normotensive (supine blood pressures < 140/90 mm Hg), were taking no medications, and had no evidence of cardiopulmonary disease, based on history and physical examination at the time of the study. The studies were approved by the institutional committee on human investigation.

Measurements

All experiments were performed with the subjects supine. Efferent MSNA in the leg, blood pressure, and heart rate were recorded during immersion of the left hand in ice water. Heart rate (electrocardiogram), respiration (pneumograph), and MSNA (microneurography) were recorded continuously on a Gould physiological recorder (Model 2800S; Oxnard, CA, USA). Respiration was monitored to detect inadvertent performance of a Valsalva maneuver or prolonged exhalation since these respiratory maneuvers markedly stimulate MSNA. No such maneuvers were detected during the CPT. Blood pressure was measured by sphygmomanometry in the right arm. In 10 experiments, 60 ml of blood was withdrawn from a right forearm vein for plasma catecholamine determinations. Plasma venous norepinephrine was measured by high performance liquid chromatography.

Microneurography

Multiunit recordings of postganglionic sympathetic nerve activity were obtained from a muscle nerve fascicle in the right peroneal nerve posterior to the fibular head. The recordings were made with tungsten microelectrodes 200 μm in diameter in the shaft, tapering to an uninsulated tip of 1 to 5 μm. A reference electrode was inserted subcutaneously 1 to 3 cm from the recording electrode. The electrodes were connected to a preamplifier with a gain of 1000 and an amplifier with a gain of 50-fold. The neural activity was then fed through a bandpass filter with a bandwidth of 700 to 2000 Hz. For monitoring during the experiment, the filtered neurogram was routed through an amplitude discriminator to a storage oscilloscope and a loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network (time constant, 0.1 seconds) to obtain a mean voltage display of the neural activity.

There were three criteria for an acceptable recording of MSNA. First, weak electrical stimulation (1–3 V; 0.2 msec; 1 Hz) through the electrode in the peroneal nerve elicited involuntary muscle contraction (muscle nerve fascicle) but not paresthesias (cutaneous nerve fascicle). Second, tapping or stretching the muscles or tendons supplied by the impaled fascicle elicited afferent mechanoreceptor discharges, whereas stroking skin in the distribution of the peroneal nerve did not evoke afferent discharges. Third, the neurogram revealed spontaneous, intermittent, pulse-synchronous bursts that increased during held expiration and Phases 2 and 3 of a Valsalva maneuver, characteristic of MSNA. Evidence that such activity represents efferent sympathetic activity has derived from earlier studies and includes 1) interruption of the activity by local nerve block proximal but not distal to the recording site, 2) elimination of the activity by ganglionic blockade, and 3) conduction velocity approximating 1 m/sec. Neurograms that revealed spontaneous activity characteristic of cutaneous sympathetic activity were not accepted. Inadvertent contraction of the leg muscles adjacent to the recording electrode produces electromyographic activity, which causes a sudden rise in baseline noise level on the mean voltage neurogram and produces a characteristic repetitive firing that is evident on both the filtered neurogram and the audio display. These electromyographic artifacts were readily distinguished from sympathetic bursts. Resting nerve activity was measured for 6 to 10 minutes before the experiments were begun to ensure that a stable baseline level of nerve activity had been obtained.

Cold Pressor Test

The CPT was performed by immersing the subject’s left hand up to the wrist in ice water for 2 minutes. Subjects avoided isometric contraction and performance of a Valsalva maneuver or held expiration during the CPT. Measurements were obtained during control, intervention (CPT), and recovery for 2 minutes each.

β-Adrenergic Blockade

In nine experiments we used β-adrenergic blockade to examine sympathetic drive to the sinus node during the CPT. Heart rate responses to the CPT were measured before and 15 minutes after intravenous infusion of propranolol, 0.15 mg/kg.

Plasma Norepinephrine

In 10 experiments we obtained venous blood samples for norepinephrine from an indwelling cannula in a right forearm vein. Ten-milliliter aliquots were with-
drawn during the last 30 seconds of each minute of control, CPT, and recovery periods while we performed simultaneous measurements of nerve traffic, heart rate, and arterial pressure. The samples were collected in chilled, heparinized tubes and promptly centrifuged at 4°C. Norepinephrine levels were assayed using high performance liquid chromatography with an electrochemical detector (SmithKline Biotechnology, Van Nuys, CA, USA). This assay was sensitive to 10 pg/nl with a coefficient of variation of 10%.

Data Analysis

The mean voltage neurogram, electrocardiogram, and respiratory movements were recorded at a paper speed of 5 mm/sec. Sympathetic bursts were identified by inspection of the mean voltage neurogram and expressed as bursts per minute (burst frequency) and as bursts per 100 heartbeats. The latter provided a heart rate-independent measure of central sympathetic outflow. The amplitude of each burst was determined by inspection and calculated using a light pen and digitizing tablet. Total MSNA was calculated as bursts per minute × mean burst amplitude and expressed in arbitrary units. We have previously determined that the intraobserver variability in identifying bursts is less than 5% while interobserver variability is less than 10%.28

Mean arterial pressure was calculated as diastolic pressure plus one third of pulse pressure. The measurements of blood pressure were obtained during the last 30 seconds of each minute. Values for MSNA and heart rate were calculated as the mean for the first 30 seconds and for the entire first and second minute of the CPT. For the comparisons of arterial pressure and heart rate were calculated as the mean for the first 30 seconds and for the entire first and second minute of the CPT. For the comparisons of arterial pressure and sympathetic nerve responses to the CPT, neurograms were analyzed with the investigator blinded to the increases in arterial pressure and sympathetic nerve responses to the CPT and returned toward the control values during the second minute (see Figure 2). In contrast, MSNA peaked during the second minute. Total MSNA (burst frequency × amplitude) increased from 230 ± 27 to 386 ± 52 units (p < 0.05) during the first minute of the CPT and to 574 ± 73 units (p < 0.0001 compared with control) during the second minute (see Table 1). The peak responses in arterial pressure also occurred in the second minute of the CPT. MSNA and arterial pressure returned toward control values during the recovery period.

There was a significant positive correlation between the increases in mean arterial pressure and the increases in total MSNA from rest to the second minute of CPT (r = 0.86, p < 0.01; Figure 3). There was also a statistically significant correlation between increases in arterial pressure and sympathetic nerve responses from rest to the first minute of CPT (r = 0.59, p < 0.05). In contrast, there was no significant correlation between increases in heart rate and MSNA during the first minute of the CPT (r = 0.02) or with respect to peak responses (r = 0.07).

β-Adrenergic blockade with propranolol decreased resting heart rate from 57 ± 3 to 48 ± 2 beats/min (p < 0.05) and abolished the increases in heart rate during the CPT (p < 0.01; Figure 4). In contrast, resting values of mean arterial pressure were similar before and after propranolol (92 ± 2 vs 90 ± 4 mm Hg), as were the maximal increases in arterial pressure during the CPT: +20 ± 2 mm Hg before vs +18 ± 2 mm Hg after propranolol.

Unlike MSNA, plasma norepinephrine did not increase in the first minute of the CPT (Figure 5). There was a small but significant (p < 0.05) increase in plasma norepinephrine in the second minute of the test, but the peak increase in norepinephrine occurred in the first minute of the recovery period. Figure 5 shows a positive correlation between the peak change in total

### Table 1. Responses to the Cold Pressor Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control period</th>
<th>Cold pressor test</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st min</td>
<td>2nd min</td>
<td>1st min</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94 ± 2</td>
<td>106 ± 2*</td>
<td>113 ± 2*</td>
<td>100 ± 2*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>63 ± 2</td>
<td>63 ± 2</td>
<td>70 ± 3*</td>
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<tr>
<td>Muscle sympathetic nerve activity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bursts/min</td>
<td>18 ± 2</td>
<td>17 ± 2</td>
<td>24 ± 2*</td>
</tr>
<tr>
<td>Bursts/100 heartbeats</td>
<td>30 ± 3</td>
<td>28 ± 3</td>
<td>36 ± 4*</td>
</tr>
<tr>
<td>Total activity (bursts/min × mean burst amplitude)</td>
<td>242 ± 32</td>
<td>230 ± 27</td>
<td>386 ± 52*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 25 subjects. Values for total muscle sympathetic nerve activity are expressed in arbitrary units.

*p < 0.05, compared with control values.
FIGURE 1. Segments from original records in two subjects (A and B) of arterial pressure (systolic/diastolic, mean), mean voltage neurogram of muscle sympathetic nerve activity (MSNA), and electrocardiogram (ECG). Data represent the last 30 seconds of each 2-minute measurement period. Subject A showed typical increases in arterial pressure and MSNA during the cold pressor test. Subject B showed more dramatic responses in both arterial pressure and nerve traffic, the latter represented by the striking increase in frequency and amplitude of the neural bursts. These sympathoexcitatory responses provide direct evidence that the cold pressor test is a potent reflex sympathetic stimulus.

MSNA and the peak change in plasma norepinephrine in response to the CPT ($r = 0.72$, $p < 0.05$). However, large changes in MSNA were associated with only small changes in norepinephrine levels.

Discussion
Most previous studies of sympathetic responses to the cold pressor test have employed indirect indices of sympathetic nervous activity. In this study, we used intraneural microelectrode recordings to provide direct measurements of muscle sympathetic nerve discharge during cold pressor stimulation in conscious humans. The findings provide direct evidence for increased sympathetic drive to skeletal muscle during the CPT and demonstrate the complex relationships between the changes in muscle sympathetic outflow, arterial pressure, heart rate, and plasma norepinephrine during this stimulus.

Effects of the Cold Pressor Test on Muscle Sympathetic Nerve Activity and Arterial Pressure
Under resting conditions, there is normally an inverse relationship between acute changes in arterial pressure and MSNA. Thus, in humans sympathetic outflow to muscle is inhibited during arterial systole and occurs during spontaneous decreases in arterial pressure. In addition, MSNA is inhibited by sudden increases in carotid baroreceptor activity produced by neck suction. These observations indicate that MSNA is normally under arterial baroreceptor reflex control; however, during the CPT, this inhibitory influence of the arterial baroreceptor reflex on sympathetic outflow was overridden, since the increases in MSNA occurred in spite of substantial increases in arterial pressure.

We found a positive correlation between the increases in MSNA and the increases in arterial pressure during the CPT. This was unexpected since previous studies have shown no correlation between changes in arterial pressure and MSNA during static handgrip, during lower body negative pressure, during clonidine administration, or between resting levels of arterial pressure and basal levels of MSNA in normotensive and hypertensive subjects. In contrast, there is a positive correlation between increased levels of MSNA and arterial pressure in patients with the Guillain-Barré syndrome. The present correlation appears to indicate that activation of sympathetic vasoconstrictor outflow to skeletal muscle is an important component of the pressor response to the CPT. These findings lend support to the use of the arterial pressure response to the CPT as an approximate index of sympathetic neural function in studies of hypertension.
Effects of the cold pressor test (CPT) on mean arterial pressure (MAP), heart rate (HR), and muscle sympathetic nerve activity (MSNA). Nerve activity is expressed in total activity (units = burst frequency x amplitude) and in bursts per 100 heartbeats. Measurements were taken during 2 minutes each of control (C1, C2), cold pressor test (CPT1, CPT2), and recovery (R1, R2). Values are means ± SE for 25 subjects. Asterisk indicates values significantly different (p<0.05) from control. The maximal rise in HR occurred in the first minute of the CPT, whereas the maximal response in MAP occurred in the second minute when there was a striking increase in sympathetic nerve traffic.

Comparison of Effects of Cold Pressor Test on Sympathetic Nerve Activity to Skin and Skeletal Muscle

Previous studies have used microneurography to examine effects of cutaneous cold stimulation on sympathetic activity in humans. Delius et al.24 found that MSNA did not increase during brief application of ice to the abdomen. The discrepancy between their results and our findings of increased MSNA during the CPT mostly likely relate to differences in the duration of the cold stimulus (i.e., a few seconds vs 2 minutes). More recently, Fagius and Blumberg35 reported that the CPT produced abrupt increases in skin sympathetic activity. In contrast to our study in which muscle sympathetic activity increased only after the first 30 seconds of the CPT, skin sympathetic activity in the median nerve increased immediately upon immersion of the contralateral hand in ice water.35 This finding is not surprising since skin sympathetic activity is extremely responsive to noxious or arousal stimuli.23 In contrast, MSNA does not increase and sometimes tends to decrease in response to arousal stimuli.23 Thus, the increases in MSNA in our study cannot be explained as a nonspecific response to arousal.

Contrasting Effects of Cold Pressor Stimulation on Muscle Sympathetic Nerve Activity and Heart Rate: Possible Mechanisms of Cold Pressor-Induced Sympathetic Neural Activation

The increases in heart rate during the CPT appear to be mediated by sympathetic activation rather than by parasympathetic withdrawal, since these increases were abolished by propranolol. In contrast to the pressor response, the magnitude of the chronotropic response did not correlate with the change in MSNA during the CPT. This finding indicates that heart rate cannot be used as a global index of sympathetic activation during this test. Furthermore, the temporal dissociation of heart rate and MSNA responses suggests that sympathetic outflow to the heart (sinoatrial node)
Comparison of Muscle Sympathetic Nerve Activity and Plasma Norepinephrine Responses

Previous studies have used plasma norepinephrine levels to assess sympathetic neural activation during the CPT. Cuddy et al., using a fluorometric assay, reported that the CPT did not produce significant increases in plasma norepinephrine. Even with the use of radioenzymatic catecholamine assays, some investigators have failed to detect significant changes in plasma norepinephrine during the CPT. Other investigators have reported that the CPT increases plasma norepinephrine by amounts ranging from +20%, +23%, +64%, +67%, +152%, to +240%. In addition, previous studies have shown little or no correlation between the increases in norepinephrine and in arterial pressure during cold pressor stimulation. Thus, the CPT has been considered to be a less reliable stress test of cardiovascular autonomic function than submaximal dynamic exercise, in which hemodynamic responses are closely associated with consistently large increases in plasma catecholamines.

It is important to consider the duration of the CPT in the interpretation of the plasma norepinephrine responses. Stratton et al. found that norepinephrine levels did not increase during the first 2 minutes of cold pressor testing but did increase during continued application of the CPT for up to 6 minutes. We also observed a small but significant rise in plasma norepinephrine after 2 minutes of cold pressor testing. In addition, we found a significant positive correlation between the peak changes in plasma norepinephrine and MSNA. The strength of this correlation is similar to that reported between resting values of norepinephrine and MSNA in normal subjects and in patients with congestive heart failure. In spite of this correlation, our findings suggest two limitations in the use of plasma norepinephrine as an index of MSNA during the CPT. First, the increases in norepinephrine in venous blood lag behind the increases in MSNA. Thus, the maximal increase in plasma norepinephrine in venous blood lag behind the increases in MSNA. Second, and more importantly, plasma norepinephrine is an insensitive measure of MSNA; large changes in MSNA during the CPT are associated with small changes in plasma norepinephrine.

We suggest that the propranolol-dependent increases in heart rate during the CPT are related to the sensation of pain, since pain and heart rate during the CPT vary similarly as a function of water temperature, since maximal heart rate and pain responses both occur in the first minute of stimulus with subsequent cold adaptation, and since heart rate and pain during the CPT have been modified in parallel with biofeedback. We speculate that activation of cutaneous afferents mediates the increases in MSNA, but further studies are needed to determine the relative contributions of central neural and peripheral reflex mechanisms in determining sympathetic nerve discharge during the CPT.
Limitations of the Present Study and Possible Clinical Implications

In conclusion, this study indicates that the CPT in healthy humans is a potent stimulus to MSNA but appears to exert differential effects on sympathetic outflow to the heart and skeletal muscles. The mechanisms responsible for the dissociation in the increases in MSNA and the propranolol-dependent increases in heart rate were not elucidated in the present study. The findings in healthy subjects suggest that in the clinical setting increases in cardiac sympathetic activity should be reflected by the heart rate response in the first 30 seconds of the test, whereas increases in skeletal muscle sympathetic activity should be estimated by measuring the increases in arterial pressure in the second minute of the cold pressor stimulus. Further studies are needed to determine if sympathetic nerve responses to the CPT are altered in patients with hypertension.

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