Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans

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THE COLD PRESSOR TEST is typically performed by immersing a subject's hand into ice water for a short period of time and is a potent stimulus for eliciting large elevations in blood pressure (10). For many years, the cold pressor test has been used both clinically and experimentally to evaluate non-baroreflex-mediated sympathetic neural control in humans (6, 14, 22, 23). A number of studies have been performed to identify the mechanisms leading to elevations in blood pressure during this procedure. For example, the cold pressor test increases plasma norepinephrine (9, 27) and muscle sympathetic nerve activity (MSNA) (5, 21, 26). The increase in MSNA correlates linearly with increases in both mean arterial blood pressure and peripheral venous norepinephrine concentration (26). Moreover, Kregel et al. (12) suggested that the increase in MSNA during a cold pressor test is driven by high-threshold nociceptive fibers in the hand.
METHODS

Subjects. Ten subjects (7 men and 3 women) participated in this study. The subjects' average age was 34 ± 2 (SE) yr, and all were of normal height (173 ± 3 cm) and weight (74 ± 3 kg). All subjects were normotensive (supine blood pressures < 140/90 mmHg), were not taking medications, and did not have any cardiopulmonary diseases. A written informed consent from each subject was obtained before participation in this institutionally approved study.

Measurements. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in the peroneal nerve. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which muscle sympathetic bursts were clearly identified using previously established criteria (25). The nerve signal was amplified (50,000–90,000 times), passed through a band-pass filter with a bandwidth of 500–5,000 Hz, and integrated with a time constant of 0.1 s (Iowa Bioengineering; Iowa City, IA). The mean voltage neurograms were displayed together with blood pressure on a chart recorder. The nerve signal was also routed to an oscilloscope and a loudspeaker for monitoring throughout the study.

Heart rate was obtained from the electrocardiogram signal (SpaceLabs; Redmond, WA) interfaced with a cardiometer (1,000-Hz sampling rate, CWE; Ardmore, PA). Blood pressure was recorded on a beat-by-beat basis from a finger not exposed to the cold water (Finapres, Ohmeda; Louisville, CO). Resting blood pressures obtained from the Finapres were verified during the experiment by auscultation of the brachial artery (SunTech, Medical Instruments; Raleigh, NC). To ensure that subjects avoided Valsalva maneuvers during the cold pressor test, respiratory frequency was monitored using pieoelectric pneumography.

Protocols. All studies were conducted with the subject in a supine position and in a room with an ambient temperature of 24–26°C. To assess baroreflex sensitivity, changes in arterial blood pressure were induced by bolus injections of sodium nitroprusside and phenylephrine HCl (4, 8) during both a control condition and a cold pressor test. These drugs were administered intravenously via a catheter placed in the opposite arm relative to the hand placed in the water. For the control trials, after a 5-min baseline period, 100 μg sodium nitroprusside was administered, followed ~60 s later by 150 μg phenylephrine HCl. These doses decreased arterial pressure 10–15 mmHg below baseline levels and then increased blood pressure 5–10 mmHg above baseline levels, respectively.

After a sufficient period to allow the hemodynamic affects of the previously administered vasoactive drugs to subside, a hand was immersed to the wrist in an ice-water slurry for 3 min. The subjects were instructed to remain relaxed, breathe normally, and avoid Valsalva-like maneuvers during hand immersion. One minute after the onset of hand immersion, bolus infusions of sodium nitroprusside and phenylephrine HCl were once again administered using the same time course and doses as were used in the control trial (Fig. 1). Data analysis. Data were sampled at 200 Hz via a commercial data acquisition system (Biopac System; Santa Barbara, CA) and analyzed using LabView software (National Instruments; Austin, TX). Beat-by-beat heart rate was calculated from the R-R interval of the electrocardiogram. Beat-by-beat systolic and diastolic blood pressures were obtained from the arterial blood pressure waveform.

The integrated neurogram was normalized by assigning the largest amplitude of a sympathetic burst during the first minute before the administration of drugs or the onset of hand immersion in ice water to a value of 100. All bursts for that trial were then normalized against that value (8). Taking into account burst latency from the R-wave of the electrocardiogram, MSNA bursts were identified by manual inspection of the neurogram. Burst area of the integrated neurogram and systolic and diastolic blood pressures were measured simultaneously on a beat-by-beat basis. Total MSNA activity of the burst was defined as the burst area of the rectified and integrated neurogram.

The sensitivity of baroreflex control of MSNA was identified from the linear relationship between MSNA and diastolic pressure during pharmacologically induced changes in blood pressure (3, 8, 19). Diastolic pressure was used because MSNA correlates closely with diastolic pressure but not with systolic pressure (24). To perform a linear regression between nerve activity and blood pressure, values for MSNA were averaged over 3-mmHg diastolic blood pressure ranges. Because MSNA was often completely suppressed when blood pressure exceeded a particular threshold, the relationship

![Fig. 1. Representative tracings obtained from 1 subject during the cold pressor test. Integrated muscle sympathetic nerve activity (MSNA), blood pressure (BP; via Finapres), and beat-by-beat heart rate (HR) are shown. One hand of the subject was immersed to the wrist in ice water (hand immersion). A bolus of sodium nitroprusside (100 μg) was administered 1 min after the onset of hand immersion, which decreased BP 20–30 s after administration. Phenylephrine HCl (150 μg) was then administered ~60 s after the administration of sodium nitroprusside. Phenylephrine HCl caused an increase in BP 20–30 s after its administration. The cold pressor test ended ~60 s after the administration of phenylephrine HCl. Arrows indicate the aforementioned events for this subject.](http://ajpheart.physiology.org/)

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Table 1. Hemodynamic and MSNA responses during the control trial

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Nitroprusside</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>125.3 ± 3.7</td>
<td>111.2 ± 5.6*</td>
<td>136.1 ± 4.9*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>67.5 ± 1.9</td>
<td>55.3 ± 2.1*</td>
<td>72.2 ± 2.7*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86.0 ± 2.4</td>
<td>74.0 ± 3.0*</td>
<td>93.4 ± 3.3*</td>
</tr>
<tr>
<td>Heart rate,</td>
<td>53.2 ± 1.6</td>
<td>72.9 ± 2.8*</td>
<td>47.4 ± 1.3*†</td>
</tr>
<tr>
<td>MSNA, x 10^3</td>
<td>42.6 ± 6.7</td>
<td>161.3 ± 18.2*</td>
<td>18.8 ± 3.7*†</td>
</tr>
</tbody>
</table>

The data for baseline are mean values of ~1 min before the infusion of sodium nitroprusside, the data for nitroprusside are mean values of ~15 s during the lowest blood pressure induced by sodium nitroprusside, and the data for phenylephrine are mean values of ~15 s during the highest blood pressure induced by phenylephrine HCl. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity. \*P < 0.05 compared with baseline; †P < 0.05 compared with nitroprusside.

Table 2. Hemodynamic and MSNA responses during the cold pressor test

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Cold Pressor</th>
<th>Nitroprusside</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>127.4 ± 3.0</td>
<td>159.3 ± 9.1*</td>
<td>152.3 ± 8.2*</td>
<td>174.3 ± 5.6*‡</td>
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<td>DBP, mmHg</td>
<td>66.5 ± 1.5</td>
<td>92.7 ± 4.1*</td>
<td>83.1 ± 3.3*‡</td>
<td>94.0 ± 3.0*‡</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86.6 ± 1.9</td>
<td>114.9 ± 5.5*</td>
<td>106.1 ± 4.7*</td>
<td>120.7 ± 3.5*</td>
</tr>
<tr>
<td>Heart rate,</td>
<td>55.0 ± 1.1</td>
<td>60.5 ± 1.9</td>
<td>74.8 ± 2.5*†</td>
<td>47.1 ± 2.1*††</td>
</tr>
<tr>
<td>MSNA, x 10^3</td>
<td>37.7 ± 7.7</td>
<td>190.4 ± 35.9*</td>
<td>284.2 ± 26.9*††</td>
<td>121.2 ± 24.1*††</td>
</tr>
</tbody>
</table>

Data for baseline are mean values of ~1 min before the onset of the cold pressor test, data for the cold pressor test are mean values of ~15 s during the cold pressor test and just before the administration of sodium nitroprusside (~1 min after onset of the cold pressor test), data for nitroprusside are mean values of ~15 s during the lowest blood pressure induced by sodium nitroprusside during the cold pressor test, and data for phenylephrine are mean values of ~15 s during the highest blood pressure induced by phenylephrine HCl during the cold pressor test. \*P < 0.05 compared with baseline; †P < 0.05 compared with cold pressor test; ‡P < 0.05 compared with nitroprusside.

RESULTS

During control trials, blood pressure decreased significantly by sodium nitroprusside infusion and then increased significantly by phenylephrine HCl infusion (Table 1). These changes in blood pressure resulted in baroreflex-mediated changes in heart rate and MSNA. There were no significant differences (P > 0.05) between hemodynamic parameters before the onset of hand immersion (Table 2) relative to the period before drug administration for the control trials (Table 1).

Recordings of integrated MSNA, blood pressure, and beat-by-beat heart rate during the cold pressor test for a representative subject are shown in Fig. 1. MSNA and blood pressure began to increase ~30 s after the onset of hand immersion and continued to increase until the onset of sodium nitroprusside administration. Vasoactive drugs caused significant changes in blood pressure, which resulted in baroreflex-mediated changes in heart rate and MSNA during the second and third minutes of the cold pressor test (Table 2). Breath holding was not observed in any subjects during the procedures.

An example of the linear regression between MSNA and diastolic blood pressure for a representative subject is shown in Fig. 2. A strong relationship between MSNA and diastolic blood pressure was seen for each subject for the control trial (mean r^2 = 0.82 ± 0.03) and the cold pressor test trial (mean r^2 = 0.78 ± 0.02). The curve was shifted upward and to the right to reflect the increases in MSNA and diastolic blood pressure that occurs with the cold pressor test. The slope of the relationship between MSNA and diastolic blood pressure was more negative when baroreflexes were perturbed in combination with cold pressor stimulation relative to the control trial (cold pressor: −244.9 ± 26.3 units·beat^−1·mmHg^−1; control: −138.8 ± 18.6 units·beat^−1·mmHg^−1, P < 0.005; Fig. 3). These results indicated that the cold pressor test increased the gain of baroreflex modulation of MSNA.

There was also a strong relationship between heart rate and systolic blood pressure for each subject during the control (mean r^2 = 0.88 ± 0.02) and cold pressor test trials (mean r^2 = 0.78 ± 0.07). The relationship between the change in heart rate relative to the change in systolic blood pressure was shifted to the right to reflect the higher blood pressures during the cold pressor test (Tables 1 and 2). However, the slope of the relationship between heart rate and systolic blood pressure was similar (P = 0.41) between the cold pressor (−0.86 ± 0.09 beats·min^−1·mmHg^−1) and control trials (−0.84 ± 0.10 beats·min^−1·mmHg^−1, Fig. 4). These results suggest that sympathetic stimulation via the cold pressor test does not alter the sensitivity of baroreflex regulation of heart rate but shifts the...
DISCUSSION

The main finding of the present study is that baroreceptors remain capable of modulating MSNA and heart rate during sympathetic activation induced by the cold pressor test. Furthermore, the slope of the relationship between MSNA and diastolic blood pressure during the cold pressor test was more negative relative to the control condition. Finally, the cold pressor test resets the baroreflex curve expressing the relationship between diastolic blood pressure and MSNA upward and to the right. These finding suggest that the cold pressor test increases the sensitivity of baroreflex control of MSNA in humans and resets the baroreflex curve to accommodate the changes in MSNA and blood pressure that occur with the cold pressor test.

It is recognized that the cold pressor test increases MSNA and blood pressure (1, 11, 15, 21, 26). The increase in MSNA likely contributes to the increase in blood pressure. Thus it may be that baroreceptor inhibition of MSNA is overridden during the cold pressor test (26). However, Fagius et al. (5) previously speculated that baroreflexes remain functional during a cold pressor test because MSNA still showed cardiac-rhyth-
micity during the test. However, in that study, blood pressure during the cold pressor test was not altered so the investigators were unable to confirm their hypothesis. To specifically address this question, we acutely changed blood pressure via bolus injection of sodium nitroprusside and phenylephrine HCl after MSNA and blood pressure were elevated during a cold pressor test. During the cold pressor test, nitroprusside-induced decreases in blood pressure resulted in increases in MSNA, whereas phenylephrine-induced increases in blood pressure resulted in decreases in MSNA. Therefore, the present data clearly show that baroreceptors remain capable of modulating MSNA during the cold pressor test and confirm the prior speculation by Fang et al. (5). However, during the cold pressor test, phenylephrine-induced increases in blood pressure did not return MSNA to levels similar to, or less than, MSNA before the cold pressor test (Table 2). This observation suggests that baroreceptor-mediated suppression of MSNA was insufficient to completely overcome activation of MSNA induced by the cold pressor test.

In the present study, baroreflex modulation of MSNA was assessed by calculating the slope of total activity of MSNA to diastolic blood pressure on a beat-to-beat basis. This relationship has been used extensively to probe the role of baroreflexes in humans (4, 8). We found that the slope of the change in total activity of MSNA relative to the change in diastolic blood pressure was more negative during the cold pressor test. This observation indicates that the sensitivity or gain of arterial baroreflex control of MSNA was significantly elevated by the cold pressor test. Moreover, the baroreflex curve expressing the relationship between diastolic blood pressure and MSNA was reset upward and to the right to accommodate the change in blood pressure and MSNA that occurred during the cold pressor test.

The mechanism for an increase in baroreflex sensitivity of MSNA during the cold pressor test is not clear. Sympathetic excitation during hand immersion in cold water occurs only when skin temperature falls to levels that produce a sensation of intense pain (12). Fagius et al. (5) reported a weak but statistically significant correlation between the rating of perceived pain and the increase in MSNA during 1-min immersion of a hand in 2°C water. Pain induced with several methods is capable of elevating MSNA (16, 20), and the increase in MSNA during the cold pressor test may be driven by painful sensation induced with ice water (12). Therefore, pain or noxious stimuli may play a role in resetting the baroreflex during the cold pressor test. However, this suggestion is purely speculative and warrants further investigation to verify.

The benefits of an elevated gain expressing baroreflex control of MSNA during the cold pressor test can only be speculated upon at this time. The main purpose of baroreflex modulation of MSNA is to buffer changes in vascular tone. Thus a heightened sensitivity of baroreflex control of MSNA may serve to better maintain blood pressure during, for example, a hypotensive challenge under conditions of stress or painful situation similar to that caused by the cold pressor test.

The effects of the cold pressor test on baroreflex control of MSNA are similar to what we previously reported during posthandgrip exercise ischemia (3). In that study, the gain of baroreflex control of MSNA was similarly elevated during posthandgrip ischemia. We concluded that metaboreceptor stimulation was responsible for the change in the sensitivity of baroreflex control of MSNA. However, given the findings of the present study, coupled with the painful sensation during postexercise ischemia, we cannot exclude a possible role of increased perception of pain during muscle ischemia in mediating the previously observed response. Nevertheless, metaboreceptor stimulation may still be the main factor for the elevation in baroreflex sensitivity during posthandgrip ischemia, because pressor responses to muscle ischemia are independent of pain associated with the ischemia (7, 18). Moreover, no significant relationship was observed between MSNA responses and the perception of pain during posthandgrip muscle ischemia (17). Because the cold pressor test and posthandgrip muscle ischemia have similar effects on baroreflex control of MSNA, an alternative hypothesis may be both painful stimuli and metaboreceptor stimulation alter baroreflex responsiveness via common neural pathways.

Consistent with prior observations (26), heart rate at the beginning of the second minute of the cold pressor test in the present study was not significantly different with heart rate before immersion of the hand in cold water. The dissociation between responses of heart rate and MSNA during cold pressor test may imply that they are governed by different mechanisms (26). In the present study, pharmacologically induced decreases and increases in blood pressure during the cold pressor test caused appropriate baroreflex-mediated increases and decreases in heart rate, respectively (Fig. 1). This finding confirms that baroreflexes remain capable of modulating heart rate during a cold pressor test despite the observation that blood pressure increases without changes in heart rate during the cold pressor test without pharmacological interventions (26). Compared with the control condition, the curve expressing baroreflex control of heart rate was shifted to higher blood pressures but the sensitivity of this reflex, as indicated by the slope of the response between heart rate and systolic blood pressure, was unaffected by the cold pressor test. Thus a dissociation was observed between the effects of the cold pressor test on the sensitivity (i.e., slope) of baroreflex control of MSNA and baroreflex control of heart rate. It is interesting to note that we saw a similar dissociation during assessment of baroreflex function during post-handgrip ischemia (3). A possible explanation for the dissociation between these baroreflex-mediated responses may due to factors associated with parasympathetic innervation of the heart.

Study limitations. In the present study, the sensitivity of baroreflex control of MSNA was estimated from the linear slope of the relationship between MSNA and...
diastolic blood pressure. The relationship of MSNA and diastolic blood pressure is likely sigmoidal across a wide range of blood pressures. In the present study, relatively small changes in diastolic blood pressure occurred during the pharmacological intervention. Thus we do not know whether the cold pressor test alters the maximal gain of baroreflex control of MSNA. We can, however, conclude that factors associated with the cold pressor test increase baroreflex control of MSNA within the tested diastolic blood pressure range around the operating point.

The basic premise of the current study was that without pharmacologically induced changes in blood pressure, MSNA would have remained constant during the second and third minute of the cold pressor test. Previous studies show that MSNA increases significantly after 30 s (26) and 1 min (12, 13) after the onset of the cold pressor test. However, these studies also showed that there were no significant differences in MSNA during the period from the end of minute 1 through the end of the 3-min cold pressor test (12, 13). It is in this period of time that the baroreflexes were pharmacologically perturbed in the present study. Therefore, increased baroreflex modulation of MSNA during the second and third minute of the cold pressor test is not likely caused by time-dependent changes of MSNA during the period when the drugs were administered.

Significant variation in baroreflex control of heart rate was observed between subjects (Fig. 4). For example, the slope of the relationship between heart rate and blood pressure increased in five subjects and decreased in five subjects during the cold pressor test. Similar variability was observed when identifying the effects of metaboreceptor stimulation (3) or heat stress (2) on baroreflex control of heart rate. We recognize that the relatively large degree of variability between subjects increases the likelihood of committing a beta error. However, given the present variability, an inordinate number of subjects would be required to confirm that the cold pressor test does not alter baroreflex regulation and heart rate. Nevertheless, we believe our interpretation of the data represents the overall effects of cold pressor test on baroreflex regulation of heart rate.

In conclusion, the results from this study suggest that the cold pressor test resets baroreflex control of MSNA and heart rate to accommodate the elevation in blood pressure and MSNA that occurs during the cold pressor test. Moreover, the sensitivity of baroreflex modulation of MSNA is elevated during the cold pressor test without affecting the sensitivity of baroreflex modulation of heart rate.

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REFERENCES


