

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

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**Cui, Jian, Thad E. Wilson, and Craig G. Crandall.** Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *Am J Physiol Heart Circ Physiol* 282: H1717–H1723, 2002. First published January 3, 2002; 10.1152/ajpheart.00899.2001.—The purpose of this project was to test the hypothesis that baroreceptor modulation of muscle sympathetic nerve activity (MSNA) and heart rate is altered during the cold pressor test. Ten subjects were exposed to a cold pressor test by immersing a hand in ice water for 3 min while arterial blood pressure, heart rate, and MSNA were recorded. During the second and third minute of the cold pressor test, blood pressure was lowered and then raised by intravenous bolus infusions of sodium nitroprusside and phenylephrine HCl, respectively. The slope of the relationship between MSNA and diastolic blood pressure was more negative ( $P < 0.005$ ) during the cold pressor test ( $-244.9 \pm 26.3$  units·beat<sup>-1</sup>·mmHg<sup>-1</sup>) when compared with control conditions ( $-138.8 \pm 18.6$  units·beat<sup>-1</sup>·mmHg<sup>-1</sup>), whereas no significant change in the slope of the relationship between heart rate and systolic blood pressure was observed. These data suggest that baroreceptors remain capable of modulating MSNA and heart rate during a cold pressor test; however, the sensitivity of baroreflex modulation of MSNA is elevated without altering the sensitivity of baroreflex control of heart rate.

baroreflex sensitivity; heart rate; nitroprusside; phenylephrine

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 ve-

nous 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.

MSNA is normally under negative feedback control from the arterial and cardiopulmonary baroreflexes. Under resting conditions, there is a close inverse relationship between the occurrence of MSNA bursts and diastolic blood pressure (24). Besides arterial and cardiopulmonary baroreflexes, the sympathetic nervous system is influenced by other reflex mechanisms. For example, increases in MSNA induced by the cold pressor test occur independent of baroreflex-mediated sympathetic activation. Fagius et al. (5) demonstrated that the typically observed inverse relationship between MSNA and spontaneous changes in blood pressure was abolished during the cold pressor test. A positive correlation between the increase in blood pressure and the increase in MSNA by a cold pressor test might imply that baroreceptor inhibition of MSNA was "overridden" by the cold pressor test (26). However, Faguis et al. (5) also reported that cardiac rhythmicity of MSNA was preserved during the cold pressor test. Given this observation, Faguis et al. (5) suggested that the cold pressor test resets baroreflex control of MSNA while preserving the capacity of baroreflex buffering of MSNA; however, this speculation has not been tested.

Therefore, it is unknown whether baroreflexes still modulate MSNA and heart rate during the cold pressor test when blood pressure is elevated and, if so, whether the cold pressor test alters the sensitivity of baroreflex modulation of these variables. Thus the present study was undertaken to test the hypothesis that baroreceptor modulation of MSNA and heart rate are preserved during the cold pressor test. Second, we tested the hypothesis that the cold pressor test alters the sensitivity of arterial baroreflex modulation of MSNA and heart rate. To test these hypotheses, baroreflex control of MSNA and heart rate were assessed on a beat-by-beat basis during rapid pharmacologically induced changes in arterial pressure during control conditions and during exposure to the cold pressor test.

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## METHODS

**Subjects.** Ten subjects (7 men and 3 women) participated in this study. The subjects' average age was  $34 \pm 2$  (SE) yr, and all were of normal height ( $173 \pm 3$  cm) and weight ( $74 \pm 3$  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 cardi tachometer (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 piezoelectric 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  $\mu$ g sodium

nitroprusside was administered, followed  $\sim 60$  s later by 150  $\mu$ g 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  $\mu$ g) was administered 1 min after the onset of hand immersion, which decreased BP 20–30 s after administration. Phenylephrine HCl (150  $\mu$ g) was then administered  $\sim 60$  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  $\sim 60$  s after the administration of phenylephrine HCl. Arrows indicate the aforementioned events for this subject.

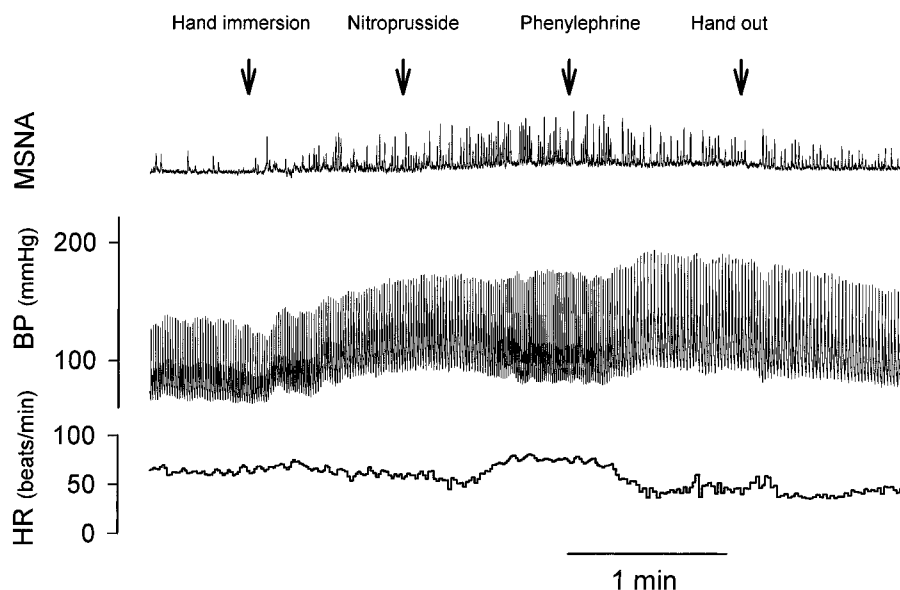


Table 1. Hemodynamic and MSNA responses during the control trial

	Baseline	Nitroprusside	Phenylephrine
SBP, mmHg	125.3 ± 3.7	111.2 ± 5.6*	136.1 ± 4.9†
DBP, mmHg	67.5 ± 1.9	55.3 ± 2.1*	72.2 ± 2.7†
MAP, mmHg	86.8 ± 2.4	74.0 ± 3.0*	93.4 ± 3.3†
Heart rate, beats/min	53.2 ± 1.6	72.9 ± 2.8*	47.4 ± 1.3*†
MSNA, × 10 <sup>3</sup> units/min	42.6 ± 6.7	161.3 ± 18.2*	18.8 ± 3.7†

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.

between MSNA and blood pressure was frequently nonlinear at higher blood pressures. In the present analysis, only the linear portion of the data was used to estimate the slope of relationship between MSNA and diastolic pressure. Baroreflex modulation of heart rate was identified from the relationship between beat-by-beat heart rate and systolic blood pressure during pharmacologically induced changes in blood pressure. Beat-by-beat heart rates were also pooled over 3-mmHg systolic blood pressure ranges, followed by linear regression analysis between heart rate and systolic blood pressure.

Statistical analyses were performed using commercially available software (StatView 5.0). Differences in hemodynamic responses within control and cold pressor trials were evaluated using a repeated-measures one-way ANOVA. Baseline values from the two trials were compared via paired  $t$ -tests. The effects of the cold pressor tests on baroreflex gains were compared with the control trials via paired  $t$ -tests. All values are reported as means ± SE.  $P$  values < 0.05 were considered statistically significant.

## 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  $r^2 = 0.82 \pm 0.03$ ) and the cold pressor test trial (mean  $r^2 = 0.78 \pm 0.02$ ). 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:  $-244.9 \pm 26.3$  units·beat<sup>-1</sup>·mmHg<sup>-1</sup>; control:  $-138.8 \pm 18.6$  units·beat<sup>-1</sup>·mmHg<sup>-1</sup>,  $P < 0.005$ ; 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  $r^2 = 0.88 \pm 0.02$ ) and cold pressor test trials (mean  $r^2 = 0.78 \pm 0.07$ ). 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 ( $-0.86 \pm 0.09$  beats·min<sup>-1</sup>·mmHg<sup>-1</sup>) and control trials ( $-0.84 \pm 0.10$  beats·min<sup>-1</sup>·mmHg<sup>-1</sup>; 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

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

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

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.

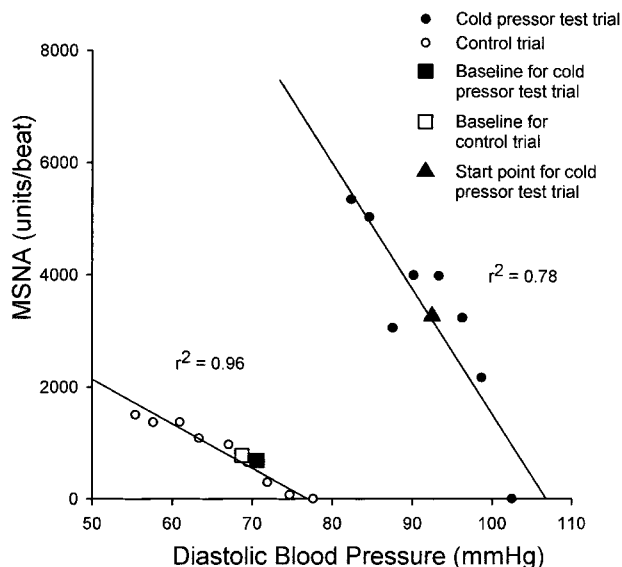


Fig. 2. An example of the linear regression between MSNA and diastolic BP (DBP) for a representative subject. Only the linear portion of the data was used for the regression analysis during both control and cold pressor trials. Baseline MSNA and DBP were similar before drug infusion for the control trial relative to before the cold pressor test. The cold pressor test increased both MSNA and DBP, as indicated by a shift in the operating point immediately before infusion of the first drug (starting point for cold pressor test trial) relative to the period before the onset of the cold pressor test. Finally, it is clear that the slope relating the change in MSNA relative to the change in DBP is more negative during the cold pressor test.

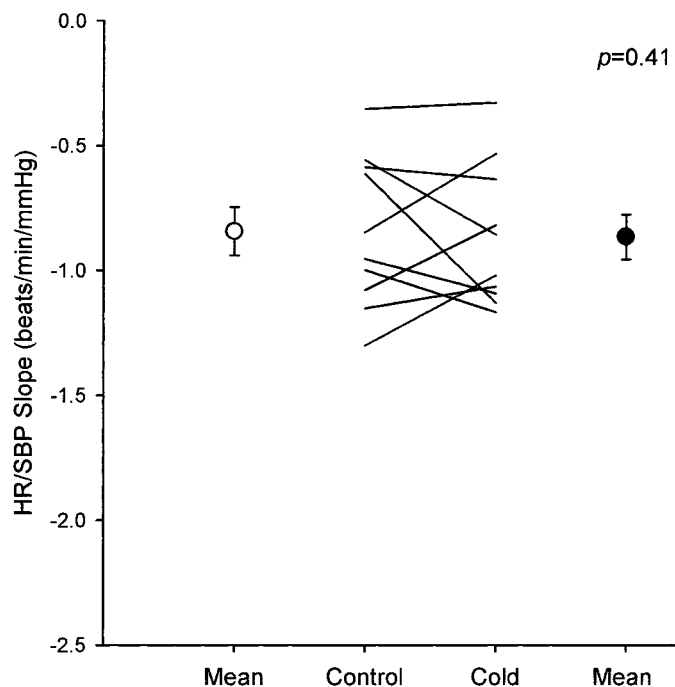


Fig. 4. The slopes from the linear regression between HR and systolic BP (SBP) for each subject (lines) as well as mean slopes (circles) from control and cold pressor test periods. The cold pressor test did not significantly alter the average slope of the relationship between the change in HR relative to the change in SBP. Symbols are as described in Fig. 3.

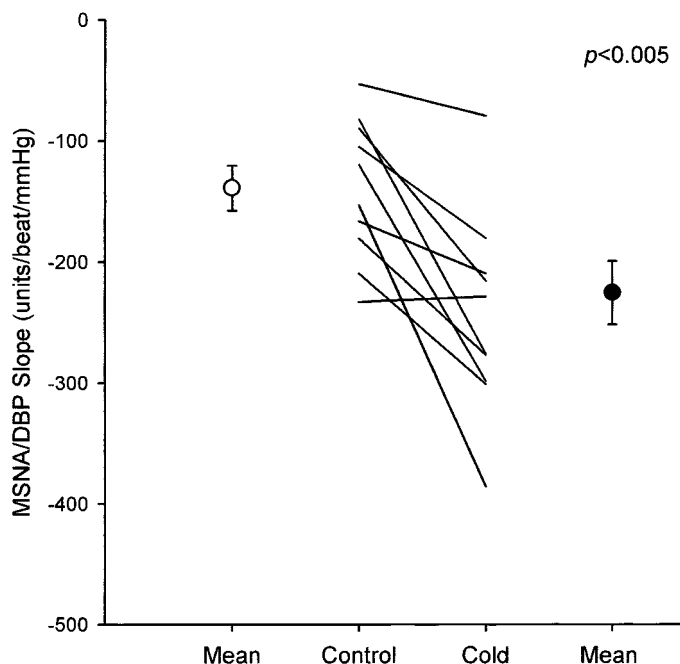


Fig. 3. Change in slopes from the linear regression between MSNA and DBP for each subject (lines) as well as mean slopes (circles) from control and cold pressor test periods (cold). The cold pressor test significantly altered (i.e., more negative) the average slope (●) between the change in MSNA relative to the change in DBP compared with the control condition (○).

baroreflex curves to accommodate the elevation in blood pressures that occurs with the cold pressor test.

**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 findings 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. In contrast, this same perturbation shifts the baroreflex curve expressing the relationship between heart rate and systolic blood pressure to high blood pressures but does not alter the sensitivity of this reflex.

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 Fagius 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-by-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 posthandgrip ischemia (3). A possible explanation for the dissociation between these baroreflex-mediated responses may be 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

1. Calhoun DA, Mutinga ML, Collins AS, Wyss JM, and Oparil S. Normotensive blacks have heightened sympathetic response to cold pressor test. *Hypertension* 22: 801–805, 1993.
2. Cui J, Wilson TE, and Crandall CG. Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans. *Am J Physiol Regul Integr Comp Physiol*. 282: R252–R258, 2002.
3. Cui J, Wilson TE, Shibasaki M, Hodges NA, and Crandall CG. Baroreflex modulation of muscle sympathetic nerve activity during posthandgrip muscle ischemia in humans. *J Appl Physiol* 91: 1679–1686, 2001.
4. Ebert TJ and Cowley AW Jr. Baroreflex modulation of sympathetic outflow during physiological increases of vasopressin in humans. *Am J Physiol Heart Circ Physiol* 262: H1372–H1378, 1992.
5. Fagius J, Karhuvaara S, and Sundlof G. The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta Physiol Scand* 137: 325–334, 1989.
6. Floras JS. Inhibitory effect of atrial natriuretic factor on sympathetic ganglionic neurotransmission in humans. *Am J Physiol Regulatory Integrative Comp Physiol* 269: R406–R412, 1995.
7. Freund PR, Rowell LB, Murphy TM, Hobbs SF, and Butler SH. Blockade of the pressor response to muscle ischemia by sensory nerve block in man. *Am J Physiol Heart Circ Physiol* 237: H433–H439, 1979.
8. Halliwill JR. Segregated signal averaging of sympathetic baroreflex responses in humans. *J Appl Physiol* 88: 767–773, 2000.
9. Halter JB, Stratton JR, and Pfeifer MA. Plasma catecholamines and hemodynamic responses to stress states in man. *Acta Physiol Scand Suppl* 527: 31–38, 1984.
10. Hines EA and Brown GE. The cold pressor test for measuring the reactivity of the blood pressure: data concerning 571 normal and hypertensive subjects. *Am Heart J* 11: 1–9, 1936.
11. Jones PP, Spraul M, Matt KS, Seals DR, Skinner JS, and Ravussin E. Gender does not influence sympathetic neural reactivity to stress in healthy humans. *Am J Physiol Heart Circ Physiol* 270: H350–H357, 1996.
12. Kregel KC, Seals DR, and Callister R. Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. *J Physiol (Lond)* 454: 359–371, 1992.
13. Mizushima T, Tajima F, Nakamura T, Yamamoto M, Lee KH, and Ogata H. Muscle sympathetic nerve activity during cold pressor test in patients with cerebrovascular accidents. *Stroke* 29: 607–612, 1998.
14. Muzi M, Goff DR, Kampine JP, Roerig DL, and Ebert TJ. Clonidine reduces sympathetic activity but maintains baroreflex responses in normotensive humans. *Anesthesiology* 77: 864–871, 1992.
15. Ng AV, Callister R, Johnson DG, and Seals DR. Sympathetic neural reactivity to stress does not increase with age in healthy humans. *Am J Physiol Heart Circ Physiol* 267: H344–H353, 1994.
16. Nordin M and Fagius J. Effect of noxious stimulation on sympathetic vasoconstrictor outflow to human muscles. *J Physiol (Lond)* 489: 885–894, 1995.
17. Ray CA, Dahl NP, Osevala N, and Ertl AC. Endogenous opioids fail to alter pain perception and sympathetic nerve activity to ischemic muscle and cold-induced pain (Abstract). *FASEB J* 15: A1144, 2001.
18. Rowell LB, Hermansen L, and Blackmon JR. Human cardiovascular and respiratory responses to graded muscle ischemia. *J Appl Physiol* 41: 693–701, 1976.
19. Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, and Eckberg DL. Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol Heart Circ Physiol* 276: H1691–H1698, 1999.

20. **Schobel HP, Ringkamp M, Behrmann A, Forster C, Schmieder RE, and Handwerker HO.** Hemodynamic and sympathetic nerve responses to painful stimuli in normotensive and borderline hypertensive subjects. *Pain* 66: 117–124, 1996.
21. **Seals DR.** Sympathetic activation during the cold pressor test: influence of stimulus area. *Clin Physiol* 10: 123–129, 1990.
22. **Somers VK, Mark AL, and Abboud FM.** Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. *J Clin Invest* 87: 1953–1957, 1991.
23. **Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI.** Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034–2039, 1991.
24. **Sundlof G and Wallin BG.** Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol (Lond)* 274: 621–637, 1978.
25. **Vallbo AB, Hagbarth KE, Torebjork HE, and Wallin BG.** Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 59: 919–957, 1979.
26. **Victor RG, Leimbach WN Jr, Seals DR, Wallin BG, and Mark AL.** Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* 9: 429–436, 1987.
27. **Ward MM, Mefford IN, Parker SD, Chesney MA, Taylor CB, Keegan DL, and Barchas JD.** Epinephrine and norepinephrine responses in continuously collected human plasma to a series of stressors. *Psychosom Med* 45: 471–486, 1983.

