Naloxone Potentiates Cardiopulmonary Baroreflex Sympathetic Control in Normal Humans

Hans P. Schobel, Ron M. Oren, Allyn L. Mark, and David W. Ferguson

Naloxone, an opioid antagonist, augments baroreflex mechanisms in animals; this occurrence suggests that endogenous opioids blunt baroreflex responses. Limited human studies suggest an inhibitory action of endogenous opioids on baroreflex-mediated vagal responses during arterial baroreceptor deactivation. To evaluate the potential effect of endogenous opioids on cardiopulmonary baroreflex mechanisms in humans, we measured arterial and central venous pressures, heart rate, and efferent muscle sympathetic nerve activity (MSNA, by peroneal microneurography) during unloading of cardiopulmonary baroreceptors with incremental lower body negative pressure (LBNP, from 0 to −15 mm Hg) and during the cold pressor test in 21 normal subjects (aged 24±1 [mean±SEM] years). In 14 subjects, we performed LBNP before and after naloxone (0.15 mg/kg i.v.) and placebo (n=11) on separate days. In six of these 14 subjects and an additional seven subjects (n=13), studies were also performed before and after administration of a lower dose of naloxone (0.075 mg/kg i.v.) on separate days. Neither dose of naloxone significantly altered control arterial or central venous pressures or heart rate. Control MSNA was reduced after the higher but not after the lower dose of naloxone. Comparable reductions in central venous pressure were produced by LBNP in all groups before and after naloxone or placebo, whereas LBNP did not alter arterial pressure. Cardiopulmonary baroreflex sympathetic sensitivity, which was derived as the slope of the linear regression relation between percent change in total MSNA (units) per absolute change in central venous pressure (mm Hg) during incremental LBNP, was significantly augmented after both the high dose (from 18.6±4.7%/mm Hg to 39.3±8.1%/mm Hg, p=0.001) and low dose of naloxone, whereas placebo had no effect. MSNA responses to the cold pressor test were not altered by either dose of naloxone. Thus, naloxone selectively potentiates cardiopulmonary baroreflex regulation of sympathetic neural activity in normal humans. These findings suggest that endogenous opioids exert a tonic inhibitory effect on sympathetic responses to orthostatic stress in normal humans. (Circulation Research 1992;70:172–183)

Endogenous opioids are morphinelike substances produced particularly in the brain to regulate neural function. These compounds contribute importantly to cardiovascular regulation and to pain perception. Compelling evidence that the endogenous opioid system interacts with cardiovascular control mechanisms derives from the results of administration of opioid agonists and antagonists in various physiological and pathological states. The most commonly used opioid antagonist is naloxone, which has high affinity for μ opioid receptors and antagonizes all opioid-mediated effects of morphine.1 Naloxone has been shown to improve blood pressure regulation in experimental endotoxic and hemorrhagic shock, thereby suggesting a role of endogenous opioids in these pathological conditions.2–4

Because high concentrations of opioid-containing nerve cells and receptors are found in regions of the neuroaxis (particularly the nucleus tractus solitarius) where baroreceptor and chemoreceptor afferents terminate, there has been increasing interest in the possibility that endogenous opioids are involved in the physiological regulation of baroreflex mecha-
nisms.5-7 Studies using intravenous or intracerebroventricular injections of naloxone in animals suggest that endogenously activated opioid systems blunt baroreflex responses in a number of species.7-12

In humans, the arterial baroreflex control of heart rate during arterial baroreceptor deactivation with nitroprusside is potentiated by naloxone.13 Deactivation of cardiopulmonary baroreceptors during nonhypotensive hemorrhage has been shown to increase the secretion of adrenocorticotropic hormone (ACTH) in animals.14,15 β-Endorphins, which have high affinity for μ opioid receptors, are known to be released concomitantly and in equimolar concentrations with ACTH from the pituitary.16 We considered the possibility that endogenous opioids exert an inhibitory modulating effect on cardiopulmonary baroreflex responses, as has been suggested for arterial baroreflex mechanisms. If so, antagonism of these opioids by a specific pharmacological antagonist would be expected to potentiate cardiopulmonary baroreflex-mediated responses.

To investigate the potential role and mechanism of action of endogenously activated opioids on the cardiopulmonary baroreflex in humans, we performed studies in normal human subjects to test the hypothesis that acute administration of naloxone would potentiate cardiopulmonary baroreflex control of efferent muscle sympathetic nerve activity (MSNA). We used percutaneous peroneal microneurography as a quantitative measurement of the neuromechanical limb of the cardiopulmonary baroreflex during unloading of cardiopulmonary baroreceptors with nonhypotensive lower body negative pressure (LBNP). To examine the specificity of actions of naloxone, we also examined responses to the cold pressor test, a non–baroreflex-mediated sympathoexcitatory stimulus.

Subjects and Methods

Subjects

Twenty-one subjects (19 men and two women aged 24±1 [mean±SEM] years) were studied in three treatment groups: 0.15 mg/kg naloxone (n=14, subjects 1–14), 0.075 mg/kg naloxone (n=13, subjects 9–21), and placebo (n=11, subjects 4–14). Thus, six subjects (subjects 9–14) were studied with both doses of naloxone, and all but three subjects in the higher dose naloxone group were also studied after placebo. The repeat studies were performed an average of 32±6 (range, 19–76) days after initial study. All subjects were studied without sedation in the supine, postabsorptive state and were free of cardiovascular or other systemic diseases, based on medical history and physical examination. Informed written consent was obtained before study, and the protocol was approved by the Human Subjects Review Committee of the University of Iowa.

Measurements

A direct writing, multichannel physiological recorder was used to simultaneously record phasic and mean arterial and central venous pressures, heart rate, respiratory activity, forearm blood flow, level of LBNP, and MSNA. Arterial pressure was measured directly through a 4F polyethylene arterial catheter inserted percutaneously in the right brachial artery. Mean arterial pressure was obtained by an electrical mean signal. Arterial pulse pressure was determined as systolic minus diastolic pressure. Central venous pressure was measured through an 18.5-gauge polyethylene catheter inserted percutaneously in a right median antecebular vein and advanced to an intrathoracic vein. Heart rate and rhythm were recorded continuously by electrocardiogram, and respiratory activity was recorded by a strain-gauge pneumograph. Zero reference point for all measurements was defined at the phlebotastic axis in the midaxillary position.

Forearm blood flow was measured by venous occlusion plethysmography with a mercury-in-silastic Whitney strain gauge as previously described.17,18 Blood flow was measured every 15 seconds, and the average value per minute was determined. Forearm vascular resistance was derived by dividing mean arterial pressure (mm Hg) by forearm blood flow (ml/min/100 ml forearm volume) and expressed as units.

Microneurographic Recordings of Sympathetic Nerve Activity to Muscle

Multunit recordings of postganglionic MSNA were obtained from a muscle nerve fascicle in the peroneal nerve posterior to the fibular head. This technique has been validated and extensively described in studies from our laboratory and elsewhere.19-27 In brief, recordings were obtained by percutaneous insertion of tungsten microelectrodes into the peroneal nerve. The electrodes were connected to a preamplifier, and the nerve signal was fed through a band-pass filter and routed through an amplitude discriminator to a storage oscilloscope and loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance–capacitance integrating network to obtain a mean voltage display of the neural activity. Standard criteria for acceptance of a recording of MSNA were achieved in all subjects.19-27 Resting nerve activity was measured for up to 10 minutes before the study was begun to ensure that a stable baseline of nerve activity had been obtained. Sympathetic bursts were identified by inspection of the mean voltage neurogram. Individual burst frequency was determined as bursts per minute. Individual burst amplitude was measured, and total integrated MSNA was calculated as the total sum of burst amplitudes per minute and expressed as units per minute. Nerve activity was also corrected for heart rate and expressed as bursts or units per 100 heart beats.26 Thus, sympathetic nerve activity was expressed in four ways: 1) bursts per minute, 2) total integrated nerve activity as units per minute, 3) heart rate–corrected activity as sympathetic bursts per 100 heart beats, and, 4) total integrated nerve activity corrected for heart rate, ex-
pressed as units per 100 heart beats. Prior studies in our laboratory determined an intraobserver variability of 5% and an interobserver variability of <10% in this calculation of sympathetic nerve activity.21

Procedures

Orthostatic stress was simulated by the technique of LBNP using a chamber placed over the subject’s body below the iliac crest.18,23,25,28–30 The negative pressure was applied for consecutive, sequential 2-minute periods at levels of 0 (control), −5, −10, and −15 mm Hg to progressively reduce cardiac filling pressures without altering arterial pressures. The cold pressor test was used as a nonbaroreflex-mediated sympathoexcitatory stimulus.31 Responses to the cold pressor test were assessed by immersion of one of the subject’s hands up to the wrist in ice water for 2 minutes. Subjects were instructed to avoid isometric contraction, performance of Valsalva maneuver, or held expiration during performance of the cold pressor test.31 At the end of each cold pressor stimulus, the subjects were asked to rank the discomfort of hand immersion in ice water from most uncomfortable (grade 10) to least uncomfortable (grade 1). The order of experimental interventions was randomized between subjects, but the same order was performed before and after drug administration.

Protocol

Studies were initiated after insertion of all monitoring devices and after a 20-minute rest period, during which all subjects were familiarized with the experimental techniques. Measurements of hemodynamic parameters and MSNA were obtained over 2-minute periods in control state, during LBNP at −5, −10, and −15 mm Hg, and during recovery. Control, intervention, and recovery periods for the cold pressor test were also of 2-minute duration. There was a 5–10-minute rest period between interventions to permit hemodynamic and MSNA parameters to return to control levels. The average response during each period of control, intervention, and recovery was determined.

After the predrug interventions, subjects received intravenous administration of naloxone hydrochloride (Narcan, Du Pont Pharmaceuticals, Wilmington, Del.) in a dose of either 0.15 mg/kg (n=14) or 0.075 mg/kg (n=13), or placebo (normal saline, n=11). The order of administration of naloxone and placebo was randomized in those subjects who received both, and the subjects were blinded as to what agent they received. Naloxone and placebo were administered over 5 minutes. Beginning 10 minutes after drug administration, the subjects underwent repeat application of LBNP and cold pressor test in the same order as in the predrug trials.

Statistical Analysis

All statistical analysis was performed in consultation with biostatisticians in the Clinical Research Center at the University of Iowa. Control hemodynamics and MSNA parameters before and after drug administration were compared within each treatment group by paired t test. Hemodynamic and MSNA responses to LBNP before and after drug administration were compared by repeated-measures analysis of variance (ANOVA). A cardiopulmonary baroreflex sympathetic sensitivity (%/mm Hg) was derived as the slope of the linear regression relation between the change in total MSNA (expressed as percent change from control) and the absolute change in central venous pressure (mm Hg) during incremental LBNP. Intragroup values of the baroreflex sensitivity before and after drug administration were compared by paired t test. Statistical significance was considered as p<0.05. Intragroup hemodynamic and MSNA responses to the cold pressor test were compared by paired t test with the use of the Bonferroni correction for multiple comparisons. Statistical significance with this measure was considered as p<0.004. Values are presented in the text, figures, and tables as mean±SEM.

Results

Effects of Drug Treatment on Control Hemodynamics and MSNA

Tables 1 and 2 and Figure 1 summarize the effects of both doses of naloxone on control (pre-LBNP) hemodynamics and MSNA. Intravenous administration of 0.15 mg/kg naloxone (Table 1) did not significantly alter arterial pressure parameters, central venous pressure, heart rate, forearm blood flow, or forearm vascular resistance. After this dose of naloxone, MSNA burst frequency decreased from 20.3±2.4 to 17.2±2.2 bursts per minute (p=0.03). This effect persisted when MSNA burst frequency was corrected for heart rate (from 38.1±4.2 to 31.7±3.8 bursts per 100 heart beats, p=0.03). Total integrated MSNA activity (expressed as units or units per 100 heart beats) also tended to decrease after this dose of naloxone, but the results did not achieve statistical significance.

Intravenous administration of the lower (0.075 mg/kg) dose of naloxone (Table 2) did not alter arterial pressure parameters, central venous pressure, heart rate, forearm blood flow, forearm vascular resistance, or MSNA parameters.

Intravenous administration of placebo had no statistically significant effect on resting hemodynamic or MSNA parameters.

Effects of Naloxone on Responses to Cardiopulmonary Baroreceptor Deactivation (LBNP)

The hemodynamic and MSNA responses of subjects during application of incremental LBNP (0, −5, −10, and −15 mm Hg) before and after administration of 0.15 mg/kg naloxone (subjects 1–14) and 0.075 mg/kg naloxone (subjects 9–21) are summarized in Tables 1 and 2 and Figures 1 and 2.

Effects of 0.15 mg/kg i.v. naloxone. During the baseline (predrug) trials of LBNP in the higher dose naloxone group, increasing levels of LBNP resulted
TABLE 1. Effects of 0.15 mg/kg Naloxone on Hemodynamic and Muscle Sympathetic Nerve Activity Responses to Cardiopulmonary Baroreceptor Deactivation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before naloxone (0.15 mg/kg i.v.)</th>
<th>After naloxone (0.15 mg/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>−5 mm Hg</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>130.3±2.8</td>
<td>131.0±2.6</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>71.4±1.9</td>
<td>71.4±1.8</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>91.8±2.5</td>
<td>92.1±2.6</td>
</tr>
<tr>
<td>PPR (mm Hg)</td>
<td>58.9±2.3</td>
<td>59.6±2.3</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>6.3±0.7</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>52.3±1.9</td>
<td>51.8±1.9</td>
</tr>
<tr>
<td>FBF (ml/min/100 ml)</td>
<td>3.3±0.4</td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>FVR (units)</td>
<td>33.3±3.8</td>
<td>38.0±3.7</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>20.3±2.4</td>
<td>20.5±2.4</td>
</tr>
<tr>
<td>MSNA (bursts/100 heart beats)</td>
<td>38.1±4.2</td>
<td>39.1±4.5</td>
</tr>
<tr>
<td>MSNA (units/min)</td>
<td>229.2±29.2</td>
<td>245.2±30.5</td>
</tr>
<tr>
<td>MSNA (units/100 heart beats)</td>
<td>427.5±49.5</td>
<td>464.3±53.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM for subjects 1–14. LBNP, lower body negative pressure; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PPR, pulse pressure; CVP, central venous pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity.

*p<0.05 control before drug vs. control after drug (paired t test).
+31.2±9.6%, and +80.0±22.1% at LBNP of -5, -10, and -15 mm Hg, respectively, whereas after drug, total MSNA increased by +44.4±7.9%, +98.1±20.1%, and +162.8±40.7% at LBNP of -5, -10, and -15 mm Hg, respectively (p=0.0001 predrug versus postdrug, by repeated-measures ANOVA) (Figure 2). These augmented responses were observed as well when MSNA was corrected for heart rate and expressed as units per 100 heart beats (Figure 2). The potentiation of reflex responses after this dose of naloxone was also confirmed when examining the absolute changes in MSNA (Table 1).

The forearm vasoconstrictor responses to LBNP were also significantly augmented after 0.15 mg/kg i.v. naloxone. The increases in forearm vascular resistance before drug were +18.2±7.0%, +16.7±7.8%, and +24.0±8.7% at LBNP of -5, -10, and -15 mm Hg, respectively. After naloxone, forearm vascular resistance increased +22.6±8.9%, +35.8±11.0%, and +36.2±9.6% at LBNP of -5, -10, and -15 mm Hg, respectively (p=0.005 before versus after drug, by repeated-measures ANOVA).

**Effects of 0.075 mg/kg i.v. naloxone.** In those subjects receiving the lower dose of naloxone (0.075 mg/kg, subjects 9–21), incremental LBNP during the predrug trials resulted in proportionately decreasing levels of central venous pressure (Figure 1). There were no significant changes in arterial systolic, diastolic, mean, or pulse pressure or heart rate during predrug LBNP in these subjects. After 0.075 mg/kg naloxone, incremental levels of LBNP produced nearly identical reductions in central venous pressure compared with the predrug trials, and there were no significant changes in arterial pressure parameters or heart rate during LBNP performed after this dose of naloxone.

Again, as was observed with the higher dose of naloxone, the overall MSNA response to LBNP was significantly greater after the drug as compared with before. The increases in total MSNA before 0.075 mg/kg naloxone were +21.2±6.1%, +42.6±10.5%, and +66.6±15.9% at LBNP of -5, -10, and -15 mm Hg, respectively. After 0.075 mg/kg i.v. naloxone, total MSNA increased +37.2±6.2%, +68.9±14.8%, and +130.2±30.6% at LBNP of -5, -10, and -15 mm Hg, respectively (p=0.0004 before versus after drug, by repeated-measures ANOVA) (Figure 2). These augmented responses were observed as well when MSNA was corrected for heart rate and expressed as units per 100 heart beats (Figure 2). The potentiation of reflex responses after this lower dose of
The overall forearm vasoconstrictor response to LBNP, however, was not significantly different before versus after 0.075 mg/kg i.v. naloxone (Table 2, \( p=0.58 \), before versus after drug, by repeated-measures ANOVA).

Figures 3 and 4 present portions of experimental recordings of hemodynamics and MSNA from two different normal subjects during LBNP performed before (top panels) and after (bottom panels) administration of the two doses of naloxone. During baseline studies, LBNP produced a dose-dependent decrease in cardiac filling pressures with a resultant increase in MSNA. After administration of both doses of naloxone, identical levels of LBNP produced similar decreases in cardiac filling pressures. However, the sympathetic nerve responses to cardiopulmonary baroreceptor deactivation were markedly potentiated after naloxone.

**Effects of Placebo on Responses to Cardiopulmonary Baroreceptor Deactivation (LBNP)**

Subjects 4–14 were also studied before and after administration of placebo on a separate day to assess the potential effects of time, repeated intervention, and placebo on hemodynamic and MSNA responses during unloading of cardiopulmonary baroreceptors with LBNP. No differences in responses were observed before and after administration of placebo (Figures 1 and 2). Graded application of LBNP resulted in similar decreases in central venous pressure before and after administration of placebo. There were no significant changes in arterial pressure parameters or heart rate during incremental levels of LBNP in the preplacebo and postplacebo trials. The overall MSNA response to LBNP was not significantly altered by administration of placebo (\( p=0.30 \) before versus placebo, by repeated-measures ANOVA) (Figure 2). When MSNA was corrected for heart rate and expressed as units per 100 heart beats, there were again no differences in responses before versus after placebo (Figure 2).

**Sensitivity of the Cardiopulmonary Baroreceptor: MSNA Reflex**

Figure 5 demonstrates the cardiopulmonary baroreflex sympathetic sensitivity of subjects before and after administration of the two doses of naloxone. Administration of 0.15 mg/kg i.v. naloxone increased the sensitivity from 18.6±4.7%/mm Hg to 39.3±8.1%/mm Hg (\( p=0.001 \)), whereas administration of 0.075 mg/kg naloxone increased the sensitivity from 21.4±7.1%/mm Hg to 41.8±13.6%/mm Hg (\( p=0.02 \)). The mean percent increase in sensitivity after the higher dose of naloxone (+211.6±80.6%) did not significantly differ from the increase after the lower dose (+161.2±41.1%, \( p=0.54 \) high versus low dose) of naloxone. Placebo administration (subjects 4–14) did not alter the cardiopulmonary baroreflex sensitivity (from 23.0±5.0%/mm Hg before to 20.2±5.9%/mm Hg after placebo, \( p=0.50 \)) (Figure 5).

Figure 6 presents the data for the cardiopulmonary baroreflex sensitivity before and after treatment in
Examine the arterial pressure parameters, heart rate, and central venous pressure to the cold pressor stimulus were not altered by either dose of naloxone. The MSNA responses to the cold pressor test (expressed as percent increase in total integrated MSNA) were not significantly altered after either the higher dose of naloxone (+142.9 ± 49.4% increase in MSNA before versus +140.8 ± 40.9% increase after this dose, p = 0.95) or after the lower dose of naloxone (+80.6 ± 21.5% increase in MSNA before versus +76.7 ± 22.3% increase after this dose, p = 0.75) (Table 3). The forearm vascular resistance responses to the cold pressor test were also not significantly altered after the higher dose of naloxone (+48.0 ± 9.0% increase in forearm vascular resistance before versus +53.5 ± 8.3% increase after this dose, p = 0.08) or after the lower dose of naloxone (+33.5 ± 4.0% increase in forearm vascular resistance before versus +34.1 ± 10.1% increase after this dose, p = 0.96) (Table 3).

The mean discomfort rating for the cold pressor test before naloxone was 6.5 ± 0.7 before versus 7.4 ± 0.8 after administration of 0.15 mg/kg naloxone (p = 0.07) and was 6.7 ± 0.7 before versus 7.2 ± 0.6 after administration of 0.075 mg/kg naloxone (p = 0.10 for each comparison).
Figure 4. Portions of experimental recordings of hemodynamics and muscle sympathetic nerve activity (MSNA) from a normal subject during lower body negative pressure (LBNP, from 0 to −15 mm Hg) performed before (top panels) and after (bottom panels) administration of naloxone (0.075 mg/kg i.v.). MAP, mean arterial pressure (mm Hg); EKG, electrocardiogram; HR, heart rate (beats per minute); CVP, central venous pressure. During baseline studies, LBNP produced a dose-dependent decrease in CVP with a resultant increase in MSNA. After administration of naloxone, identical levels of LBNP produced very similar decreases in CVP, but the sympathetic response to this cardiopulmonary baroreceptor deactivation was markedly potentiated.

Discussion

Using direct recordings of efferent MSNA, the present studies demonstrate that acute administration of the opioid receptor antagonist naloxone markedly potentiates cardiopulmonary baroreflex regulation of sympathetic nerve responses in normal awake human subjects. These results are consistent with the concept that a tonic release of endogenous opioids in the resting state and/or a stimulus-dependent (e.g., LBNP) release of opioids produces inhibitory actions on sympathetic reflex responses during unloading of cardiopulmonary baroreceptors. Acute withdrawal of this opioid-mediated inhibition after administration of naloxone produces an augmentation of these cardiopulmonary baroreflex-modulated sympathetic responses.

Important Features of the Present Studies

The results of the current studies demonstrate that naloxone potentiates efferent sympathetically mediated neural responses to unloading of cardiopulmonary baroreceptors with simulated orthostatic stress induced by LBNP. In these studies, both direct

Figure 5. Bar graphs comparing effects of intravenous administration of 0.15 mg/kg naloxone (left panel, subjects 1–14), 0.075 mg/kg naloxone (middle panel, subjects 9–21), and placebo (right panel, subjects 4–14) on cardiopulmonary baroreflex (CPBR) sensitivity, derived as the slope of the linear relation between the change in total muscle sympathetic nerve activity (expressed as the percent change from control) and the absolute change in central venous pressure (mm Hg) during incremental lower body negative pressure. Naloxone potentiated the sensitivity; placebo had no effect.
(sympathetic nerve activity) and indirect (forearm blood flow and vascular resistance) parameters of efferent sympathetically mediated responses were measured. Levels of LBNP (from 0 to −15 mm Hg) that produced a graded reduction in cardiac filling pressures without significantly altering arterial pressure were used. Efferent sympathetic responses were therefore determined in response to perturbations of cardiac filling pressure known to unload the cardiopulmonary baroreceptors. As indicated in Tables 1 and 2 and Figure 1, the systemic hemodynamic effects with LBNP were nearly identical before and after drug administration. These levels of LBNP produced a stimulus-dependent unloading of cardiopulmonary receptors (decrease in cardiac filling pressure) that was accompanied by a stimulus-dependent increase in efferent MSNA. Thus, the observed actions of naloxone on responses to LBNP are probably due to actions of the agent on cardiopulmonary baroreflex mechanisms as opposed to actions on arterial baroreflex pathways.

Whereas the MSNA responses to LBNP were clearly potentiated after both doses of naloxone, an augmentation of the forearm vasconstrictor responses was seen only after the higher dose of naloxone. This augmentation of forearm vascular response appeared to be of a lesser degree than the corresponding potentiation of MSNA responses. This finding and the lack of a significant augmentation of the forearm vascular response after the lower dose of naloxone is most likely explained by the fact that forearm vascular resistance is an indirect and less sensitive quantitative parameter of sympathoexcitatory responses during nonhypotensive LBNP than is MSNA. Another possible explanation for variances between MSNA measurements and plethysmographic measurements of forearm blood flow might be related to the fact that forearm blood flow measurements include both skin and muscle blood flow, whereas MSNA measures neural outflow only to muscle vascular beds.

Finally, as naloxone did not alter MSNA responses to the cold pressor stimulus, there was no evidence for a nonspecific influence of the agent on generalized reflex responsiveness. Thus, the results of the present study are consistent with naloxone inducing an augmentation of cardiopulmonary baroreflex mechanisms, presumably through an afferent or centrally mediated mechanism.

Comparison With Prior Studies of Autonomic Actions of Naloxone in Humans

Only a limited number of prior studies have investigated the actions of naloxone on regulation of autonomic mechanisms in humans. Under resting conditions, administration of naloxone in doses up to 20 mg in normal adults has resulted in only minimal alterations of blood pressure, heart rate, respiration, or temperature. However, under conditions of physiological stress when blood flow is compromised, a number of studies have suggested that naloxone administration can result in significant increases in blood pressure and heart rate, presumably through enhanced sympathetic nervous system activity and reduced vagal or reflex-mediated cardiovascular control. Other studies have found that naloxone can attenuate the hypotensive, tachycardic, and bradycardic responses to various stressors and central nervous system stimulants.

**TABLE 3.** Effects of 0.15 and 0.075 mg/kg Naloxone on Hemodynamic and Muscle Sympathetic Nerve Activity Responses to the Cold Pressor Test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.15 mg/kg i.v. (n=13)</th>
<th>0.075 mg/kg i.v. (n=11)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before drug</td>
<td>After drug</td>
</tr>
<tr>
<td></td>
<td>Control CPT</td>
<td>Control CPT</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>90.2±2.4</td>
<td>107.4±3.5*</td>
</tr>
<tr>
<td></td>
<td>87.3±2.8</td>
<td>99.8±3.0*</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>6.1±0.7</td>
<td>6.6±0.7</td>
</tr>
<tr>
<td></td>
<td>6.3±0.8</td>
<td>6.5±0.8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>54.1±2.5</td>
<td>59.6±2.4</td>
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<td>58.0±3.4</td>
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<td>FVR (units)</td>
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<tr>
<td></td>
<td>38.9±5.1</td>
<td>51.9±6.8*</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>15.6±2.0</td>
<td>25.3±2.7*</td>
</tr>
<tr>
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<td>21.7±3.2</td>
<td>28.5±3.1*</td>
</tr>
<tr>
<td>MSNA (units/min)</td>
<td>183.7±26.4</td>
<td>351.5±54.0*</td>
</tr>
<tr>
<td></td>
<td>229.9±41.8</td>
<td>366.1±55.6*</td>
</tr>
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</table>

Values are mean±SEM. CPT, cold pressor test; MAP, mean arterial pressure; CVP, central venous pressure; HR, heart rate; bpm, beats per minute; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity. *p<0.004 and †p<0.05 control vs. CPT.
pressure is perturbed outside its normal range, naloxone appears to significantly influence cardiovascular regulation. Rubin et al.\textsuperscript{13} observed that in humans the arterial baroreflex control of heart rate during arterial baroreceptor deactivation with nitroprusside was significantly blunted by DAMME (a \(\mu\)-receptor agonist) and potentiated by naloxone. Heart rate, blood pressure, and plasma norepinephrine responses during a 5-minute, 70° head-up tilt, however, were not altered by naloxone. Additional studies from the same group reported that the fall in systolic blood pressure observed during initial sleep in normal humans can be prevented by high parenteral doses of naloxone (0.20 mg/kg) administered immediately before onset of sleep.\textsuperscript{35}

Fohlman and Bonde-Petersen\textsuperscript{36} examined the effects of naloxone (0.1 mg/kg) on hemodynamic parameters during simulated hypovolemia with LBNP at levels of -20 and -40 mm Hg in five healthy subjects. They reported a significant effect of naloxone only during hypotensive levels of LBNP at -40 mm Hg, suggesting that naloxone might alter cardiovascular mechanisms only when arterial baroreceptors are involved.

The findings of the present study confirm the former observations in humans that naloxone in doses of <0.15 mg/kg does not alter hemodynamic parameters under resting conditions. In addition, the present studies extend the prior observations by noting a decrease in resting MSNA (burst frequency) after administration of 0.15 mg/kg i.v. naloxone and by demonstrating a potentiation of sympathetic neural responses to unloading of cardiopulmonary baroreceptors with LBNP after administration of this and the lower (0.075 mg/kg i.v.) dose of the drug. Thus, the present studies are the first to clearly suggest that naloxone alters cardiopulmonary baroreflex-mediated mechanisms in normal humans.

The findings in the present study, using measurement of direct (MSNA) indexes of efferent sympathetic mechanisms, appear to contrast with those of Rubin et al.,\textsuperscript{13} which used changes in plasma norepinephrine concentrations as an index of sympathetic responses. We believe that this may be due to the different methods used to measure sympathetic responses. Plasma norepinephrine levels are determined by efferent sympathetic neural activity, regional release and reuptake mechanisms, and peripheral metabolism.\textsuperscript{37,38} In contrast, the microangiographic method is a highly sensitive and quantitative method that provides direct measurement of efferent sympathetic neural activity.

**Potential Mechanisms of Action of Naloxone**

Performance of the current studies in awake and intact human subjects prevents us from defining exactly the mechanism of action of naloxone. However, the potentiation of a physiological process after administration of naloxone suggests that endogenous opioids are involved.

Studies using the injection of opiate antagonists in various physiological and pathophysiological states provide compelling evidence for an interaction of the endogenous opioid system with cardiovascular control systems. Holaday and Faden\textsuperscript{2-5} demonstrated that naloxone improved blood pressure regulation in endotoxic and hypovolemic shock in animals, thereby suggesting that these disease states are associated with endogenously activated opioids that are involved with the development and/or maintenance of the hypotensive state. Intravenous naloxone increases blood pressure during acute hemorrhage in various species, whereas no effect is observed in normal animals.\textsuperscript{39-43} Prophylactic administration of naloxone appears to abolish the sympathoinhibitory phase of acute hemorrhage in rabbits.\textsuperscript{12,44}

The effect of naloxone on the arterial baroreceptor-mediated heart rate reflex remains controversial in the literature. Whereas some investigators observed an increase in the baroreceptor–heart rate reflex after injection of naloxone,\textsuperscript{9,45,46} the much more powerful study by Burke and Dorward\textsuperscript{12} did not reveal an effect of naloxone on the baroreceptor–heart rate reflex, but there was a potentiation of the baroreceptor-mediated renal sympathetic nerve activity reflex. In the complicated study by Weinstock et al.,\textsuperscript{47} if anything, naloxone attenuated the baroreceptor–heart rate reflex. In the previously noted human study by Rubin et al.,\textsuperscript{13} naloxone increased the baroreceptor–heart rate reflex but did not appear to alter reflex sympathetic responses to head-up tilt, leading the authors to conclude that endogenous opioids are involved in blood pressure regulation only when arterial baroreceptors are involved.

Previous studies, however, have demonstrated that the baroreceptors located in the right atrium are the dominant mediators of ACTH secretion in response to hemodynamic stimuli\textsuperscript{14,15} and that \(\beta\)-endorphins (\(\mu\) opioid receptor agonists) are released concomitantly and in equimolar concentrations with ACTH in response to different stresses.\textsuperscript{16} Both the higher (0.15 mg/kg) and the lower (0.075 mg/kg) doses of naloxone used in the present studies were observed to clearly augment cardiopulmonary baroreflex-mediated sympathetic responses to simulated orthostatic stress. If naloxone’s effects are due to blockade of endogenous opioids, the present studies would suggest that there is either a tonic release of such agents in the resting state and/or a release stimulated by unloading of cardiopulmonary baroreceptors. In animals, deactivation of atrial baroreceptors produces concomitant release of ACTH and \(\beta\)-endorphins.\textsuperscript{16}

The findings in the present study that the increase in baroreflex sensitivity in the six subjects who were studied after both doses of naloxone tended to be less with the lower dose as compared with the higher dose are also consistent with a receptor-blocking action of naloxone.

Thus, we believe that the current observations allow us to speculate that the potentiation of cardiopulmonary baroreflex sensitivity induced by naloxone...
is due to opioid receptor blockade and a lessening of the inhibitory action of these endogenous agents on sympathetic responses to simulated orthostatic stress.

**Potential Limitations of Studies**

We recognize several potential limitations in the design and interpretation of the present studies. First, these studies were acute in nature and do not necessarily predict long-term effects of endogenous opioids in normal humans. Second, the present studies were performed in normal human subjects, and one must exercise caution in applying these observations to patients with clinical disorders in whom naloxone might be used therapeutically. Third, we have attempted to examine the cardiopulmonary baroreflex during maneuvers that deactivated these mechanoreceptors (LBNP). It is possible that other effects of naloxone would be observed under conditions of activation (loading) of such receptors. However, by examining efferent sympathetic responses over an extended and physiologically relevant range of cardiac filling pressures, we believe that the present studies are functionally important. A fourth concern is that the techniques used in the present studies required observations to be made with the subjects in a supine state. It is possible that other effects would be observed in subjects studied in the upright position. Finally, in the performance of these studies, we have used recordings of efferent sympathetic nerve activity from only one site, the peroneal nerve. It is known that there are important differences in the control of sympathetic nerve activity to various tissues and vascular beds.20,24 In these studies, all MSNA responses were compared with a stable control recording of sympathetic nerve activity, and each subject served as his/her own control for both effects of interventions and drug. Thus, although we cannot generalize to other organ-specific sympathetic neural responses, the intrasubject comparisons of peroneal MSNA responses remain valid.

In conclusion, the present study indicates that acute administration of naloxone potentiates MSNA responses to LBNP in normal humans without potentiating sympathetic nerve responses to the cold pressor test. These observations suggest that endogenous opioids modulate cardiopulmonary baroreflex regulation of MSNA in humans.

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