changes in arterial blood pressure (BP) are sensed by carotid, aortic, and perhaps coronary baroreceptors.1,2 The signal is integrated in cardiovascular control centers in the brain stem and leads to compensatory adjustments in sympathetic and parasympathetic activity. BP reduction is followed by baroreflex-mediated increases in heart rate (HR) and sympathetic vasomotor tone.1,3,4 An increase in BP attenuates HR and sympathetic vasomotor tone.1,3,4 Thus, the baroreflex serves as a buffer to prevent excessive BP swings.5,6 The buffering function is severely impaired in patients with bilateral damage to baroreceptors or baroreflex afferents (baroreflex failure).7,8 These patients feature extreme volatility of BP. Neuronal degeneration of sympathetic and parasympathetic efferents is tantamount to autonomic failure. Patients with autonomic failure have severe orthostatic hypotension.9,10 These rare diseases illustrate the pivotal role of baroreflexes in short-term cardiovascular regulation in humans. Even subtle changes in baroreflex function may elicit important cardiovascular effects. Adrenergic mechanisms in the brain have an important role in human baroreflex regulation. For example, norepinephrine transporter inhibition causes a selective impairment in baroreflex adjustment of sympathetic vasomotor tone.11 α-2 Adrenoceptors are particularly important in central nervous baroreflex regulation, both in animals and in humans.12–14 We conducted studies with the α-2 adrenoceptor agonist clonidine in freely moving mice and observed a profound increase in the baroreflex HR gain that was explained by parasympathetic activation (unpublished observations). We used the α-2 adrenoceptor agonist clonidine to test the hypothesis that α-2 adrenoceptor stimulation in humans also improves parasympathetic HR control and baroreflex regulation.

**Methods**

We studied 9 healthy control subjects (6 men, 3 women, age 31±2 years, body mass index 24±1 kg/m²). Written informed consent was obtained before study entry. All studies were approved by the institutional review board. Forty-eight hours before study, volunteers received a diet free of substances that could interfere with catecholamine measurements.

**Subjects**

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**Key Words:** antihypertensive, agents ■ blood pressure ■ heart rate ■ baroreflex ■ sympathetic nervous system
Instrumentation
Respiration and ECG were measured continuously (Cardioscreen; Medis GmbH, Ilmenau, Germany). Beat-by-beat BP (Finapres; Ohmeda, Englewood, Cali) and brachial arterial BP (Dinamap; Critikon, Tampa, Fla) were determined. Muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve (Moni- unit; Biomedical Engineering Department, University of Iowa, Iowa City). A unipolar tungsten electrode ( uninsulated tip diameter of 1 to 5 μm, shaft diameter of 200 μm) was inserted into the muscle nerve fascicles of the peroneal nerve at the fibular head for multiunit recordings. Nerve activity was amplified with a total gain of 100 000, band-pass-filtered (0.7 to 2 kHz), and integrated.15

Protocol
All tests were conducted with the subjects in the supine position. The subjects underwent testing on 2 separate days. On 1 day, they were studied after ingestion of a placebo tablet with 50 mL water. On another day, they were tested with intravenous clonidine. After a stable baseline was recorded, clonidine was applied as intravenous loading dose (150 μg over 20 minutes), followed by a continuous infusion at a rate of 20 μg/hour. On both study days, subjects underwent cold-pressor testing to assess the effects of α-2 adrenergic receptor stimulation on the response to external stimuli. Thereafter, incremental infusions of sodium nitroprusside and phenylephrine hydrochloride (0.2, 0.4, 0.8, and 1.6 μg/kg per minute over 5 minutes) were administered. The infusions were stopped after the maximum dose had been administered or after diastolic BP had changed by >15 mm Hg. On the placebo day, the subjects received bolus injections of propranolol every 3 minutes (0.01, 0.02, 0.04, 0.06, 0.08 mg/kg) to achieve near-complete β-adrenoceptor blockade.16 Subjects received a total intravenous 0.21 mg/kg propranolol dose within 15 minutes. β Blockade was used to characterize the effect of complete peripheral sympathoinhibition.

Data Acquisition and Analysis
Data were converted from analog to digital at 500 Hz using the Windaq pro+ software (Dataq Instruments Inc, Akron, Ohio). RR intervals, diastolic BP and systolic BP values, and respiration were defined off-line for the complete records using a program written by one of the authors (A.D.), which is based on PV-wave software (Visual Numerics Inc). MSNA bursts were identified after filtering the integrated signal and defining the baseline.15

Spectral Analysis and Baroreflex Sensitivity
Pharmacological baroreflex sensitivity was determined from the steepest part of the sigmoidal curve obtained during phenylephrine and sodium nitroprusside infusions. HR and BP variability were determined using spectral analysis, cross-spectral analysis, and the transfer function between relative risk intervals and systolic BP. Analysis was performed for 5-minute segments during different baselines before each intervention17,18 and for 128 seconds during each infusion step.

Statistics
All data are expressed as mean±SEM. Intraindividual differences were compared by the paired t test or the Wilcoxon test. ANOVA testing for repeated measures was used for multiple comparisons. A value of P<0.05 was considered significant.

Results
Resting BP, HR, and MSNA
Baseline BP, HR, and MSNA were similar on the placebo day and on the clonidine day. BP decreased to 100±7/55±3 mm Hg with clonidine (P<0.01). The mean RR interval increased from 989±77 ms before to 1073±85 ms during clonidine (P<0.05). MSNA decreased from 18±3 bursts/min before to 4±2 bursts/min during clonidine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (Control Day)</th>
<th>Baseline (Clonidine Day)</th>
<th>Clonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>64±3</td>
<td>62±3</td>
<td>56±3*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>121±4</td>
<td>122±4</td>
<td>100±7†</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>70±2</td>
<td>73±3</td>
<td>55±3†</td>
</tr>
<tr>
<td>MSNA (bursts·min⁻¹)</td>
<td>19±3</td>
<td>18±2</td>
<td>4±1†</td>
</tr>
</tbody>
</table>

Results are presented as mean±SEM. n indicates number of subjects; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MSNA, muscle sympathetic nerve activity; HR, heart rate.

*Significant difference vs baseline, paired t test, P<0.05, †P<0.01.

(P<0.01; Table 1) Breathing frequency was similar before and during clonidine. During the cold pressor test, systolic BP increased 14±3 mm Hg with placebo and 6±2 mm Hg with clonidine (P<0.05).

Responses to Phenylephrine and Nitroprusside
Phenylephrine responsiveness was moderately increased during clonidine infusion (P<0.05 by ANOVA). With phenylephrine at an infusion rate of 0.8 μg/kg per minute and systolic BP increased 13±2 mm Hg with placebo and 18±2 mm Hg with clonidine (Figure 1, top). We were not able to determine nitroprusside dose–response curves on both study days because basal BP was too low on the clonidine day. Furthermore, the increase in phenylephrine infusion rate was limited by marked bradycardia. Therefore, we were able to obtain only a part of the baroreflex curve on clonidine. The maximal steepness of the baroreflex HR curves was not different with placebo or with clonidine (26±3 ms/mm Hg without and 16±2 ms/mm Hg with clonidine) (Figure 1, middle). However, with clonidine, the baroreflex curve was reset to markedly lower BP and HR values. With placebo, the baroreflex curve approached saturation at an RR interval length of approximately 1100 ms. Remarkably, this saturation level was no longer present during clonidine infusion. With both interventions, the operating point was on the steep part of the baroreflex HR curve.

Clonidine infusion caused a resetting of the sympathetic baroreflex curve to lower BP values (Figure 1, bottom). We were not able to determine the maximal gain of the sympathetic baroreflex curve with certainty. Clearly, the operating point was on a rather shallow part of the baroreflex curve during clonidine infusion. With placebo, the operating point was close to the steepest part of the baroreflex curve.

We used the impedance method to assess relative changes in cardiac stroke volume during phenylephrine and nitroprusside infusions. On placebo, we observed a linear relationship between RR interval and cardiac stroke volume. Stroke volume decreased during nitroprusside (−24%±8%, P<0.05) and increased during phenylephrine (14%±6%, P<0.05). Remarkably, stroke volume failed to increase with increasing RR duration during clonidine and during clonidine plus phenylephrine (−1.4±7%).
We determined HR variability at baseline and during baroreflex loading with phenylephrine and baroreflex unloading with nitroprusside.11 Table 2 summarizes the results on HR variability and BP variability. Clonidine increased the relative power in the high-frequency band compared with baseline (39.0±4.7 versus 48.3±3.8, P<0.05) and led to a decrease in the ratio between low- and high-frequency power (1.94±0.41 versus 1.16±0.16, P<0.05). The total power of HR variability, the mean power in the low-frequency band, the mean power in the high-frequency band, and the relative power in the low-frequency band were similar. Baroreceptor loading with phenylephrine during clonidine induced a profound increase in HR variability (Table 2). However, the increase in RR variability with phenylephrine was much more pronounced in the high-frequency range, which further reduced the ratio between low- and high-frequency oscillations (low-frequency/high-frequency ratio). The variability data were plotted against BP (Figure 2). RR variability decreased during baroreflex unloading with nitroprusside and increased during baroreflex loading with phenylephrine. The increase in RR variability with baroreflex loading was profoundly enhanced with clonidine in the high-frequency and low-frequency ranges. For RR variability in the low-frequency range (Figure 2, top left), the slope was 19 ms/mm Hg with placebo and 163 ms/mm Hg with clonidine (P=0.002). For RR variability in the high-frequency range (Figure 2, bottom left), the slope was 58 ms/mm Hg with placebo and 350 ms/mm Hg with clonidine (P<0.0001). Normalized spectral power density in the low-frequency band was reduced during clonidine (LF n.u. in Figure 2, top right). Normalized spectral power density in the high-frequency band (HF n.u. in Figure 2, bottom right) was increased during clonidine (P<0.05). The profound RR interval variability changes with clonidine were associated with a reduction in the low-frequency/high-frequency ratio. Figure 3 illustrates RR interval time series at baseline, during clonidine, and during clonidine plus phentolamine in a representative example.

Overall systolic BP variability did not change during clonidine (SD: 5.2±0.2 mm Hg) compared with baseline (SD: 5.4±0.3 mm Hg). However, the relationship between systolic BP variability in the low-frequency range and systolic BP was shifted to markedly lower pressure levels (Figure 4).

**Response to Systemic β-Adrenoceptor Blockade**

Near-complete β blockade with propranolol did not elicit a depressor response. RR interval length increased from 896±31 ms before to 975±39 ms during propranolol. RR variability in the high-frequency range changed from

### TABLE 2. Heart Rate Variability and Blood Pressure Variability Data at Baseline, During Clonidine, and During Clonidine Plus Phenylephrine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Clonidine</th>
<th>Clonidine Plus Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>TP-RR (msec)</td>
<td>3224±843</td>
<td>3785±976</td>
<td>8943±2329†</td>
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<tr>
<td>LF-RR (msec)</td>
<td>1394±438</td>
<td>1374±369</td>
<td>2857±889</td>
</tr>
<tr>
<td>HF-RR (msec)</td>
<td>851±256</td>
<td>1451±520</td>
<td>6719±2475†</td>
</tr>
<tr>
<td>LF-RR (n.u.)</td>
<td>61.0±4.7</td>
<td>51.7±3.7</td>
<td>39.4±3.3†</td>
</tr>
<tr>
<td>HF-RR (n.u.)</td>
<td>39.0±4.7</td>
<td>48.3±3.8*</td>
<td>60.6±3.2†</td>
</tr>
<tr>
<td>LF/HF-RR</td>
<td>1.94±0.41</td>
<td>1.16±0.16*</td>
<td>0.69±0.10†</td>
</tr>
<tr>
<td>LF-SBP (mm Hg)</td>
<td>12.8±2.6</td>
<td>5.9±1.2</td>
<td>2.6±0.6†</td>
</tr>
</tbody>
</table>

Results are presented as mean±SEM. n indicates number of subjects; TP-RR, total power of heart rate variability; LF-RR, mean spectral power of heart rate variability in the low-frequency band (0.04–0.15 Hz) in msec; LF-RR (n.u.), mean spectral power of heart rate variability in the low-frequency band in normalized units; HF-RR, mean spectral power of heart rate variability in the high-frequency band (0.15–0.4 Hz) in msec; HF-RR (n.u.), mean spectral power of heart rate variability in the high-frequency band in normalized units; LF/HF-RR, low-frequency power-to-high-frequency power ratio; LF-SBP, mean spectral power of systolic blood pressure variability in the low-frequency band.

*Significant difference vs baseline, P<0.05, (1-way ANOVA).
†Significant difference vs clonidine, P<0.05, (1-way ANOVA).
841±253 ms² before to 973±221 ms² during propranolol; RR variability in the low-frequency range was 1710±224 ms² before and 2376±258 ms² during propranolol.

Discussion

We determined the effect of α-2 adrenoreceptor stimulation with clonidine on the regulation of sympathetic vasomotor control and HR. The important finding in our study is that clonidine had a differential effect on baroreflex HR and vasomotor regulation. Clonidine profoundly augmented baroreflex-mediated bradycardia, particularly during baroreceptor loading. This effect is consistent with an increased parasympathetic tone. The results are similar to those we found in mice in which we could test the parasympathetic effects more directly (unpublished observation). In contrast, clonidine reduced resting sympathetic vasomotor tone and shifted the operating point of the sympathetic baroreflex to a flat part of the sympathetic baroreflex curve. The shift decreased the ability of the baroreflex to withdraw sympathetic vasomotor tone during baroreflex loading.

We first determined the effect of α-2 adrenoreceptor stimulation on phenylephrine responsiveness. In healthy subjects, the pressor response to phenylephrine is attenuated by the baroreflex, the so-called baroreflex BP buffering. Hence, near-complete lesions of baroreflex afferents or efferents are associated with a profound increase in phenylephrine responsiveness.19–22 Disruption of one component of the efferent baroreflex arc is sufficient to decrease baroreflex BP buffering. For example, the selective impairment in sympathetic vasomotor regulation with norepinephrine transporter inhibition led to a 2- to 3-fold increase in phenylephrine responsiveness.11 In the present study, phenylephrine responsiveness was moderately increased with clonidine. The observation suggests that the ability of the baroreflex to buffer BP changes may be reduced because of the fact that sympathetic withdrawal during phenylephrine infusion is absent during α-2 adrenoreceptor stimulation. Changes in vascular responsiveness to phenylephrine may also have contributed to our findings.

We observed marked changes in baroreflex-mediated adjustments of HR and sympathetic vasomotor tone during α-2 adrenoreceptor stimulation. As expected, α-2 adrenoreceptor stimulation reduced basal sympathetic vasomotor tone. The reduction in neural activity was associated with reduction in low-frequency systolic BP oscillations (Mayer waves). Mayer waves are related to oscillations in sympathetic vasomotor discharge.23–25 All these findings are consistent with the known central sympatholytic effect of clonidine. Given the marked decrease in BP with clonidine, the decrease in sympathetic vasomotor tone cannot be explained by baroreflex-mediated inhibition. We manipulated BP with nitroprusside and phenylephrine to obtain sympathetic baroreflex curves. With placebo, the operating point was on the steep part of the sympathetic baroreflex curve. Small changes in BP resulted in substantial changes in sympathetic vasomotor tone and low-frequency oscillations in systolic BP. In contrast, the operating point of the sympathetic baroreflex was at a rather shallow part of the curve during clonidine infusion. Thus, α-2 adrenergic activation limits the ability of the baroreflex to withdraw sympathetic activity during baroreflex loading. Similarly, norepinephrine transporter inhibition elicits a selective decrease in baroreflex vasomotor...
control. Animal studies suggest that this phenomenon may also be related to \( \alpha-2 \) adrenoreceptor stimulation.

In addition to the effect of \( \alpha-2 \) adrenoreceptor stimulation on sympathetic vasomotor tone, we observed dramatic changes in HR regulation. Normally, the baroreflex HR curve has a sigmoidal shape that prevents an excessive baroreflex-mediated decrease in HR. \( \alpha-2 \) Adrenoreceptor stimulation caused a resetting of the baroreflex HR curve to much lower BP and HR values. Indeed, we did not see a saturation of the baroreflex HR curve at low HRs. As a consequence of the baroreflex changes, we observed marked bradycardia, particularly during concomitant phenylephrine infusion. However, the steepness of the baroreflex HR curve was similar with placebo and with clonidine. Similar results were obtained in an earlier study using the modified oxford technique for baroreflex assessment. In contrast, Sleight et al found an improvement in baroreflex slope in rabbits and in humans. Interestingly, a dissociation between resting HR and baroreflex HR control was also observed after exercise.

We analyzed HR variability during each nitroprusside and phenylephrine infusion step to further assess changes in cardiac regulation during clonidine. The depressor and bradycardic response to clonidine makes the interpretation of HR variability data difficult. Nevertheless, stimulation of \( \alpha-2 \) adrenoceptors resulted in a profound increase in HR variability in the low- and high-frequency ranges. Moreover, the relative low frequency power decreased and the relative high-frequency power of the RR interval increased during \( \alpha-2 \) adrenoceptor stimulation. Hence, the low-frequency/high-frequency ratio, which is sometimes termed sympato-
vagal balance, decreased. Complete $\beta$-adrenoceptor blockade with propranolol did not lead to a similar response. Therefore, sympathetic deactivation at the level of the heart may not be sufficient to explain the HR and HR variability changes with $\alpha$-2 adrenoceptor stimulation. The HR variability data and our studies in freely moving mice (unpublished observation) are consistent with the idea that clonidine increased parasympathetic outflow to the heart.

Various mechanisms may contribute to parasympathetic activation of the heart with clonidine. The response may result from a sympathovagal interaction at the level of the brain stem. Clonidine decreases sympathetic outflow from the rostral ventrolateral medulla. Decreased activity in this area may lead to a secondary increase in parasympathetic activity. It is also possible that clonidine directly influences afferent areas in the brain stem like vagal nuclei or the caudal ventrolateral medulla. Indeed, $\alpha$-2 adrenoceptors are highly expressed in vagus motor nuclei in animals and in humans. An interaction between parasympathetic cardiac activation may also occur at the level of the heart. Stimulation of presynaptic $\alpha$-2 adrenoceptors may modulate acetylcholine release from cholinergic neurons innervating the heart.

Remarkably, clonidine changed the relationship between systolic BP changes and cardiac stroke volume. With placebo, stroke volume increased as RR interval increased. On clonidine, stroke volume failed to increase as RR interval further increased. Reductions in cardiac stroke volume with clonidine have also been shown in animal studies. Thus, a pressor stimulus elicits an excessive reduction in cardiac output during $\alpha$-2 stimulation. The change in the cardiac response to a pressor stimulus tends to improve baroreflex buffering. The previously unknown mechanism may explain why overall BP buffering is only slightly impaired during $\alpha$-2 stimulation even though baroreflex vasomotor control is worsened. Our study provides a strong impetus to determine cardiac stroke volume changes during baroreflex testing.

Our studies were performed in normal humans and normal mice. We have not tested these responses in hypertensive patients or animal models. Nevertheless, an important role for $\alpha$-2 adrenoceptors in hypertension and various forms of heart failure have been performed that underscore the importance of central sympatholytic effects. Our findings suggest that, in addition, a concomitantly increased parasympathetic tone may also participate, particularly in alleviating hypertensive cardiomyopathy or perhaps diastolic dysfunction.

**Limitations of the Study**
One possible limitation of our study is that we did not use a muscarinic blocker, such as atropine, to confirm parasympathetic activation at the level of the heart. However, we demonstrated that clonidine-induced bradycardia and HR variability changes in mice can be abolished with atropine (unpublished observations). Another potential limitation of our study is that we used 5-minute intervals for phenylephrine and nitroprusside infusions and analyzed the last 128 seconds during each infusion step using fast Fourier transformation. Longer infusion intervals and the use of nonparametric methods of time series analysis might be used in further studies.

**Perspectives**
Our study may have several important clinical implications. $\alpha$-2 Adrenoceptor stimulation disables a safety mechanism that prevents excessive bradycardia. The mechanism may explain some of the side effects of clonidine and related compounds. Moreover, the $\alpha$-2 effect on parasympathetic outflow to the heart may contribute to the pathogenesis of neurocardiogenic (vasovagal) syncope, a condition characterized by episodic bradycardia and hypotension. Blockade of $\alpha$-2 adrenoceptors with yohimbine improves orthostatic tolerance in a subset of these patients. Stimulation of $\alpha$-2 adrenoceptors with clonidine worsens the symptoms. Decreased HR variability and impaired baroreflex control of the heart is associated with increased cardiovascular risk. Pharmacological augmentation of HR variability and baroreflex function may be beneficial in this setting. Our study suggests that medications that augment $\alpha$-2 adrenergic transmission may be particularly useful in this regard. Finally, infusion of phenylephrine during $\alpha$-2 adrenoceptor stimulation may provide a graded increase in parasympathetic activity. The approach may be useful in determining “parasympathetic reserve.” Moreover, the methodology may permit distinguishing functional from structural changes in cardiac parasympathetic regulation.

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**References**


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