2005

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A Potential Mechanism for Long Term Morbidity?

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Abstract—Previous studies have demonstrated that antihypertensive treatment resets baroreflex control of heart rate (HR) and increases cardiac vagal baroreflex sensitivity. However, it is uncertain whether baroreflex control of muscle sympathetic nerve activity (MSNA) also resets after treatment. We tested the hypothesis that chronic antihypertensive therapy alters baroreflex regulation of MSNA in patients with untreated moderate hypertension. Seven newly diagnosed patients with systolic blood pressure (BP) of 159±5 mm Hg (mean±SE) and diastolic BP of 103±4 mm Hg were studied before and after 1 to 2 weeks ´ and 3 months (chronic) of antihypertensive treatment with losartan–hydrochlorothiazide (Hyzaar). MSNA and hemodynamics were measured supine, during a Valsalva maneuver (VM), and at 70° head-up tilt (HUT) for 10 minutes. Data were compared with those obtained in 7 age-matched healthy controls. We found that Hyzaar lowered mean BP acutely and chronically by 20±4 and 23±3 mm Hg (both $P<0.01$) but did not change HR. Supine MSNA increased by 43±11% and 34±11% after acute and chronic treatment (both $P<0.01$). However, MSNA responses to VM and HUT did not differ after treatment compared with before treatment, indicating unchanged reflex control. These data indicate that sympathetic neural activity was augmented substantially by antihypertensive treatment with Hyzaar, consistent with an ongoing baroreflex unloading, and did not return to baseline or "reset" after 3 months of therapy. We speculate that persistent and marked sympathetic activation by the baroreflex may be a potential mechanism for hypertension that is refractory to antihypertensive therapy and may provide a target mechanism for persistent morbidity despite adequate BP control. (***Hypertension*****. 2005;45:513-521.)**

Key Words: baroreceptors ■ autonomic nervous system ■ renin-angiotensin system

Lowering blood pressure (BP) by antihypertensive agents decreases morbidity and mortality in patients with hypertension.[1] However, the risk of stroke, myocardial infarction, or congestive heart failure remains high in such patients, even with adequate BP control.[2] Moreover, many patients have inadequate BP control despite medical therapy often involving multiple drug regimens.[3] One potential mechanism for both of these problems may be persistent or even augmented sympathetic activation by the baroreflex.

Previous studies have demonstrated that antihypertensive treatment resets the vagally mediated baroreflex control of heart rate (HR) to a lower pressure level and increases cardiac vagal baroreflex sensitivity acutely and chronically.[4–6] However, it is uncertain whether baroreflex control of sympathetic neural activity also resets after antihypertensive therapy. Results regarding the effects of acute antihypertensive treatment on sympathetic baroreflex function are few and controversial. Enhanced,[7] attenuated,[8,9] or unchanged[10] baroreflex sensitivity has been reported. There have been fewer studies on baroreflex regulation of muscle sympathetic nerve activity (MSNA) during chronic antihypertensive therapy, with similarly contradictory findings.[9–12] The discrepancy among these studies may be attributable to differences in pharmacological actions of antihypertensive drugs, efficacy and duration of treatment, or methodologies implemented for evaluation of the baroreflex.

Hyzaar is a fixed-dose combination of losartan potassium and hydrochlorothiazide, in which the former is an angiotensin II (Ang II) type 1 (AT_1) receptor antagonist and the latter is a diuretic. A recent study showed that the combination of losartan and hydrochlorothiazide provided greater reductions in clinic and ambulatory BP than hydrochlorothiazide or losartan monotherapy.[13] It was reported recently that losartan decreased MSNA in hypertensive patients with chronic renal failure,[14] whereas administration of hydrochlorothiazide alone for 12 weeks did not affect MSNA in obese hypertensive individuals.[11] We thereby reasoned that administration of Hyzaar may decrease MSNA in hypertensive patients, which

Received December 7, 2004; first decision January 2, 2005; revision accepted January 7, 2005.

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Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000158312.63381.c1**

would suggest resetting of sympathetic baroreflex function associated with control of BP.

The present study was performed specifically to test the hypothesis that chronic antihypertensive therapy with Hyzaar decreases MSNA and resets baroreflex control of MSNA in patients with untreated moderate hypertension. To accomplish this objective, we measured MSNA and systemic hemodynamics in the supine position, during a Valsalva maneuver (VM), and at 70° head-up tilt (HUT) before and after 1 to 2 weeks ´ and 3 months (chronic) of antihypertensive treatment. Data were compared with those obtained from the age-matched healthy controls.

Methods

Study Participants

Seven newly diagnosed male patients with moderate essential hypertension (defined as average awake systolic BP [SBP] between 155 and 179 mm Hg or diastolic BP [DBP] between 95 and 109 mm Hg on 24-hour ambulatory BP measurements), aged 44±3 years (mean±SE), and body mass index (BMI) of 30.7±1.6 kg/m^2 were recruited. All were free of any known cardiovascular diseases, renal diseases, diabetes mellitus, cerebral vascular diseases, or any other chronic severe medical condition on the basis of the medical history and a careful physical examination before the study. We also studied 7 healthy individuals (6 males and 1 female) matched for age (41±2 years of age) and BMI (26.3±1.4 kg/m^2) with the patients. Written informed consent was obtained from all participants. The study was approved by the institutional review boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas.

Study Design

All participants underwent 3 studies: baseline, acute, and chronic. After completion of the baseline study, patients were treated with Hyzaar (losartan/hydrochlorothiazide, 50/12.5 to 100/25 mg once daily) for 1 week. If BP was not controlled to within the normal or high-normal range with the lower dose, the dose was doubled and acute studies performed 1 week later. The antihypertensive drug was taken in the morning and the treatment was maintained for 3 months. Acute and chronic studies were performed 1 to 2 weeks and 3 months after the baseline study. Throughout the entire study, no other antihypertensive drugs or other medications were permitted. Twenty-four hour ambulatory BP measurement (Accutracker II; SunTech) was repeated the day before the acute study, 6 weeks after initiation of antihypertensive therapy, and the day before the chronic study in all patients; however, it was only repeated once the day before the chronic study in healthy individuals. The following protocol was performed during each study.

Experimental Protocol

The experiment was performed in the morning ≥2 hours after a light breakfast and ≥12 hours after the last caffeinated or alcoholic beverage in a quiet, environmentally controlled laboratory with an ambient temperature of ≈25°C. Patients took Hyzaar 2 to 3 hours before the test during the acute and chronic studies. A venous catheter was inserted into an antecubital vein for blood samples.

HR was monitored continuously from the ECG (Hewlett-Packard), and beat-to-beat arterial pressure was derived by finger photoplethysmography (Finapres; Ohmeda). Arm BP was measured intermittently by electrosphygmomanometry (model 4240; SunTech), with a microphone placed over the brachial artery to detect Korotkoff sounds.

Cardiac output (Qc) was measured with modification of the acetylene rebreathing technique (model MGA1100; Marquette).[15] Stroke volume (SV) was calculated from Qc and the HR measured during rebreathing. Total peripheral resistance (TPR) was calculated as the quotient of mean BP and Qc, multiplied by 80. Mean BP was calculated as [(SBP−DBP)/3]+DBP, where SBP and DBP are cuff SBP and DBP measured during rebreathing.

MSNA signals were obtained with the microneurographic technique.[16] Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2 to 3 cm from the recording electrode. The nerve signals were amplified (gain 70 000 to 160 000), band-pass filtered (700 to 2000 Hz), full-wave rectified, and integrated with a resistance-capacitance circuit (time constant 0.1 s). Criteria for adequate MSNA recording included: (1) pulse synchrony; (2) facilitation during the hypotensive phase of the VM, and suppression during the hypertensive overshoot after release; (3) increases in response to breath holding; and (4) insensitivity to emotional stimuli.[16]

After ≥30 minutes of quiet rest in the supine position, baseline data were collected for 6 minutes. All participants were then asked to perform 2 VM at 40 mm Hg for 15 s after a normal inspiration, separated by a 2-minute recovery period. After sufficient recovery, the subject was tilted passively to a 70° upright position for 10 minutes. A belt was placed across the subject's waist to make sure he/she would not fall. The subject supported the body weight by standing on a plate at the end of the tilt bed on one leg, allowing the other leg to be relaxed for microneurography. HR, BP, and MSNA were recorded continuously. Qc was measured and blood samples were taken in the supine position and at the end of tilting. Plasma catecholamines were measured by high-performance liquid chromatography,[17] and plasma renin activity (PRA) and aldosterone were measured by radioimmunoassay.[18] Hematocrit (Hct) was determined with a microcentrifuge.

Data Analysis

Sympathetic bursts were identified by a computer program using a 3:1 signal-to-noise ratio threshold within a 0.5-s search window and an expected burst reflex latency from the preceding R-waves of 1.3 s,[19] and then were confirmed by an experienced microneurographer. The number of bursts per minute (burst frequency), the number of bursts per 100 heart beats (burst incidence), and the sum of the integrated burst area per minute (total activity) were used as quantitative indexes. Because the amplitude of bursts of sympathetic nerve activity depends critically on electrode position, whereas determinations of burst frequency are stable between recording sessions,[20] the total activity was normalized to the resting supine value to allow comparisons of test responses among different sessions. Therefore, the supine baseline recording served as a reference (value of 1.0) and subsequent measures were related to this value.

HR, BP, and MSNA were averaged for 6 minutes of supine baseline. During HUT, data were collected from the third to eighth minute and were averaged for 6 minutes. To analyze the MSNA responses to the VM, the areas under all bursts during 1-minute baseline were integrated, divided by 4 (total activity of 15 s), and then were assigned a score of 1. Using this method, subsequent changes of integrated MSNA during a 15-s straining period of the VM were compared with the baseline value and expressed as either >1 or <1 in this study.

Assessment of Baroreflex Function

Sympathetic baroreflex function was assessed by relating all sympathetic bursts occurring during the 15-s straining period of the VM to the maximum fall of DBP as measured from the highest level just after the beginning of straining (phase I) to its nadir.[21] Additionally, we used the SV/MSNA relationship to test sympathetic baroreflex function during changes in posture under different treatment conditions because DBP and MSNA are elevated during upright tilt, whereas MSNA has been demonstrated to be directly and inversely related to the changes in SV during orthostatic stress.[22,23]

Cardiac vagal baroreflex sensitivity was assessed during early phase II of the VM, beginning at the highest SBP value recorded at the onset of straining and ending at the nadir of SBP during the 15-s straining period. The slope of the linear relationship between the reductions in SBP and the corresponding decreases in R-R interval

TABLE 1. Effects of Antihypertensive Therapy With Hyzaar on Supine Resting Values

	Patients (n=7)			Controls (n=7)		
Variables	Baseline	Acute	Chronic	Baseline	Acute	Chronic
24-hour ABPM (awake)						
SBP (mm Hg)	159±5†	134±4*	131±4*	120±3	Not measured	126±3
DBP (mm Hg)	103±4†	85±3*	83±2*	74±3	Not measured	78±2
HR (bpm)	80±3	80±5	81±4	71±4	Not measured	71±3
SV (mL)	94±7	88±5	90±5	121±14	I114±16	115±13
Qc (L/min)	7.11±0.32	6.71±0.35	6.68±0.52	8.18±0.63	7.51±0.68	8.04±0.61
TPR (dyne.s.cm^{-5})	1262±83†	1205±80	1193±114	876±93	1029±130	950±130
PRA (ng/mL per hour)	0.75±0.17	11.67±4.55*†	6.84±2.36	0.83±0.14	0.60±0.13	0.70±0.17
Aldosterone (ng/dL)	7.96±1.23	7.87±1.16	10.51±2.96	5.33±0.74	4.00±0.37	4.70±1.14
Hct (%)	44.9±0.7†	45.2±0.9	42.6±1.1	42.0±0.8	41.9±1.1	41.9±1.0

Values are mean±SE.

*$P<0.05$ compared with the baseline study within the group; †$P<0.05$ compared with the control group during the same study in the same position.

was estimated to evaluate cardiac vagal baroreflex sensitivity. This index was also assessed during phase IV of the VM, identified as a time interval from the first increase in SBP after the release of VM straining to the first noticeable pressure drop after overshot.

Statistical Analysis

Data are expressed as mean±SE. Subject characteristics between the groups were compared by unpaired *t* tests. The effects of acute and chronic antihypertensive therapy on 24-hour ambulatory BP, supine MSNA, and resting supine hemodynamics in patients were determined using 1-way repeated-measures ANOVA. The effects of acute and chronic treatment on MSNA and cardiovascular responses to the VM and HUT in patients were determined by 2-way repeated-measures ANOVA. The Bonferroni corrected *t* test was used post hoc for multiple comparisons. The effects of acute and chronic antihypertensive therapy on the sensitivity of baroreflex control of MSNA assessed by the VM were determined by 1-way repeated-measures ANOVA. The relationship between mean values of MSNA and SV in the supine and upright positions in all patients was determined by least-squares linear regression analysis. The relationship between R-R interval and the corresponding SBP during early phase II and phase IV of the VM was determined by least-squares linear regression analysis in each subject, and the slope was compared using 1-way repeated-measures ANOVA within group and 2-way repeated-measures ANOVA between groups during all studies. All statistical analyses were performed with a personal computer-based analysis program (SigmaStat; SPSS). A P value of <0.05 was considered statistically significant.

Results

Twenty-Four–Hour Ambulatory BP and Supine Resting Values

Before treatment, BP was significantly higher ($P<0.01$), whereas HR did not differ ($P=0.104$) in patients compared with controls (Table 1). Although resting SV and Qc were not different between the groups ($P=0.318$ and 0.138), TPR was greater in patients than in controls (Table 1; $P=0.009$). Resting PRA and aldosterone were similar in both groups ($P=0.791$ and 0.200). Figure 1 shows original tracings of integrated MSNA of 1 representative patient and healthy control in the supine position during baseline, acute, and chronic study. Resting MSNA burst frequency was significantly higher (Figure 2; $P=0.032$), whereas burst incidence tended to be higher (Table 2; $P=0.072$) in patients than in healthy controls. Supine resting plasma norepinephrine did not differ in patients and controls before treatment (Table 2; $P=0.572$).

Hyzaar lowered mean BP acutely and chronically by 20±4 and 23±3 mm Hg (both $P<0.01$) but did not change HR in patients, indicating a resetting of baroreflex control of HR (Table 1). Resting SV and Qc remained unchanged during either acute or chronic antihypertensive therapy (Table 1; treatment effect $P=0.646$ and 0.706). However, resting TPR was lowered by Hyzaar (Table 1). PRA increased acutely, then began to drop during chronic therapy (Table 1; $P=0.024$ and 0.073). Aldosterone and Hct did not change during acute and chronic antihypertensive therapy (Table 1).

Supine resting MSNA increased dramatically during acute therapy (43±11%), and remained persistently elevated after 3 additional months of treatment (Figures 1 and 2; Table 2; both $P<0.01$). The typical error of measurement (SD of the differences between acute and chronic study/square root 2) for repeat determinations of MSNA expressed as coefficient of variation was 18.4%, which included day-to-day variability of MSNA in the population of moderate hypertensives, plus variation in the persistence of the increase in MSNA

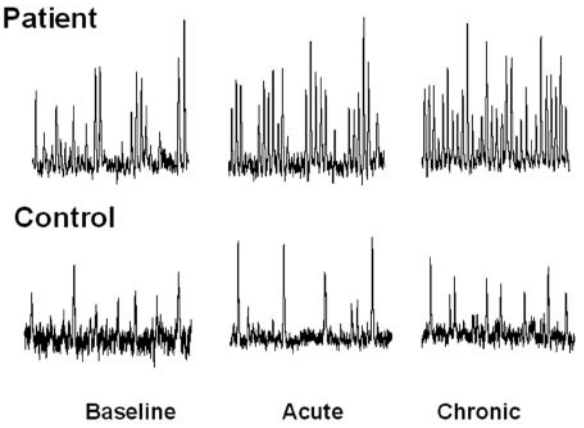

Figure 1. Original tracings of MSNA of 1 representative patient and healthy control in the supine position during the baseline, acute, and chronic study.

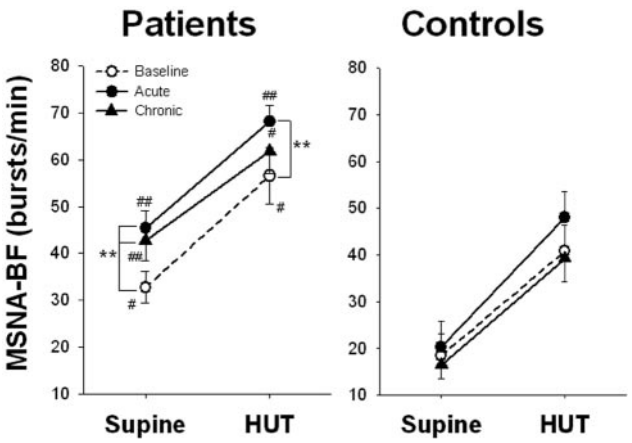

Figure 2. MSNA burst frequency (MSNA-BF) responses to 70° HUT in the patient group and the control group during the baseline, acute, and chronic study. Values are mean±SE. MSNA-BF was increased significantly at 70° HUT in both groups during 3 studies. $^{**}P<0.01$ compared with the baseline study. $\#P<0.05$ and $\#\#P<0.01$ compared with the control group within the same study.

during antihypertensive therapy. This level of precision compares favorably with the typical error of repeated MSNA measurements in healthy controls of 14.2%. The mean difference of supine MSNA between baseline and acute study in the patient group (43%) was more than twice the typical error of measurement, providing strong evidence that this difference was a true effect, rather than chance variation. Supine plasma norepinephrine increased significantly in patients during acute and chronic antihypertensive therapy (Table 2; $P=0.014$ and 0.009).

MSNA and Cardiovascular Responses to Upright Posture

Before treatment, MSNA increased significantly during tilting in patients and controls (Figure 2; Table 2; both $P<0.01$). MSNA burst frequency tended to be higher in patients (Figure 2; $P=0.079$), but burst incidence was similar between the groups during tilting (Table 2; $P=0.259$). In the upright position, plasma norepinephrine and epinephrine concentrations were not different between the groups during the baseline study (Table 2; $P=0.713$ and 0.121). SBP tended to be higher ($P=0.097$), whereas DBP was significantly higher in patients than in controls during tilting (Table 2; $P<0.01$). HR was not different between the groups in the upright position (Tables 1 and 2; $P=0.269$).

The increase in MSNA with upright tilt did not change during acute and chronic antihypertensive treatment in patients (Figure 2; Table 2), suggesting no change in reflex control of MSNA. Plasma norepinephrine concentration in

TABLE 2. Effects of Antihypertensive Therapy With Hyzaar on Cardiovascular and Sympathetic Responses to 70° HUT

	Patients			Controls		
Variables	Baseline	Acute	Chronic	Baseline	Acute	Chronic
SBP (mm Hg)						
Supine	148±6†	134±5*	130±5*	120±4	126±5	128±6
HUT	154±9†	140±10	141±7	130±4	137±6	127±4
DBP (mm Hg)						
Supine	93±5†	82±2*	79±5*	67±3	73±4	71±4
HUT	110±5†	95±7*	95±4*	81±4	91±1	81±3
HR (bpm)						
Supine	77±3	77±5	75±3	69±3	69±4	72±4
HUT	95±5	102±7	94±5	86±6	83±1	82±5
MSNA-BI (bursts/100 heart beats)						
Supine	45±4	63±5*†	63±7*†	29±7	30±8	24±4
HUT	61±7	70±5	67±6	51±8	58±7	51±8
Total activity (ratio)						
Supine	1±0	1±0	1±0	1±0	1±0	1±0
HUT	2.8±0.4	2.6±0.2	2.4±0.3	4.1±1.1	4.7±1.1	3.7±0.7
Plasma norepinephrine (pg/mL)						
Supine	264±44	367±56†	363±66†	213±25	221±37	190±26
HUT	558±74	784±116*	578±52	558±101	530±95	503±108
Plasma epinephrine (pg/mL)						
Supine	14±2	15±1	21±10	19±3	16±2	19±3
HUT	29±5	24±8	26±5	49±9	44±12	45±9

Values are mean±SE.

MSNA-BI indicates MSNA burst incidence.

*$P<0.05$ compared with the baseline study within the group; †$P<0.05$ compared with the control group during the same study in the same position.

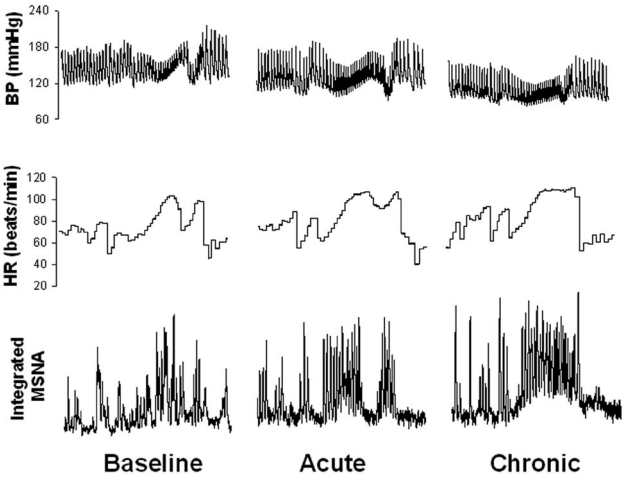

Figure 3. Original tracings of BP, HR, and MSNA responses to the VM of 1 representative patient before (baseline) and after acute and chronic antihypertensive therapy with Hyzaar. Although BP was decreased significantly, HR and MSNA responses remained unchanged during the VM.

the upright position was higher during acute ($P=0.038$, before versus acute treatment) but not chronic ($P=0.823$; before versus chronic treatment) antihypertensive therapy, which may be attributable to an upregulated norepinephrine clearance, leading to an apparent normalization of norepinephrine levels despite persistently elevated sympathetic nerve activity. Plasma epinephrine was not affected by the treatment (Table 2). BP was decreased significantly ($P<0.01$), whereas HR did not change in the upright position in patients during acute and chronic treatment with Hyzaar (Table 2), indicating resetting of the cardiac vagal baroreflex.

Baroreflex Function

Figure 3 shows original tracings of BP, HR, and MSNA responses to the VM of 1 representative patient before and after antihypertensive therapy. Normalized MSNA response was smaller in patients than in controls before treatment ($P=0.002$); however, MSNA responses to the VM did not change during acute and chronic antihypertensive therapy in patients (Figure 4; treatment effect $P=0.225$).

The sensitivity of baroreflex control of MSNA, assessed by relating all bursts occurring during the 15-s straining period to the maximum fall of DBP during the VM, was not affected by either acute or chronic antihypertensive therapy (-1.83 ± 0.57 bursts/min per mm Hg before treatment; -1.51 ± 0.46 during acute treatment; and -1.40 ± 0.25 during chronic treatment; $P=0.104$ and 0.485 for acute and chronic treatment compared with before treatment). A very strong linear correlation ($r=-0.961$) between mean values of MSNA and SV in the supine and upright positions was found in all patients before and after treatment (Figure 5; $P=0.002$), indicating that baroreflex control of MSNA in response to the hemodynamics of upright posture was not reset during acute and chronic antihypertensive therapy.

The sensitivity of baroreflex control of HR during decreasing BP (early phase II) was not different between the groups (Figure 6A; $P=0.920$). Interestingly, the sensitivity tended to be lower in patients than in controls during increasing BP

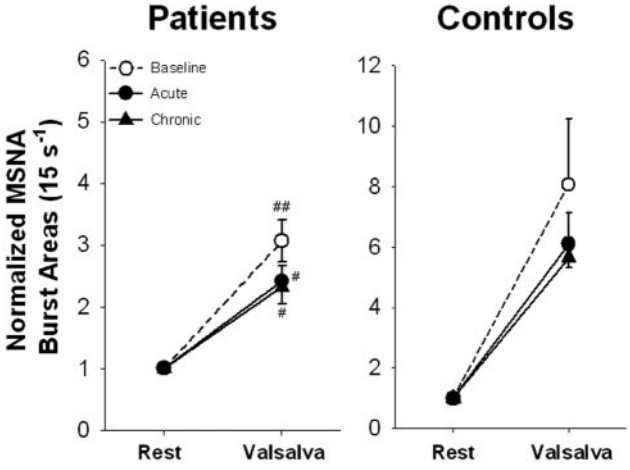

Figure 4. Normalized MSNA responses to the VM in the patient group and the control group during the baseline, acute, and chronic study. Values are mean±SE. #$P<0.05$ and ##$P<0.01$ compared with the control group within the same study.

(phase IV) during baseline study (Figure 6B; $P=0.05$). Although baroreflex control of HR was reset in patients during acute and chronic antihypertensive therapy, the sensitivity did not change compared with before treatment (Figure 6A and 6B; treatment effect $P=0.249$ and 0.140). Cardiac vagal baroreflex sensitivity was not different between the groups during the acute and chronic studies (Figure 6A, $P=0.884$ and 0.475; Figure 6B, $P=0.308$ and 0.137).

Discussion

Main Findings

The major findings from this study are that: (1) supine sympathetic nerve activity was increased significantly during chronic antihypertensive therapy with Hyzaar in patients with moderate hypertension, suggesting that the antihypertensive effect of pharmacological therapy was chronically and vigorously opposed by the baroreflex; and (2) the changes in MSNA during the VM and upright tilt were not affected by the treatment, arguing against any alteration in reflex control

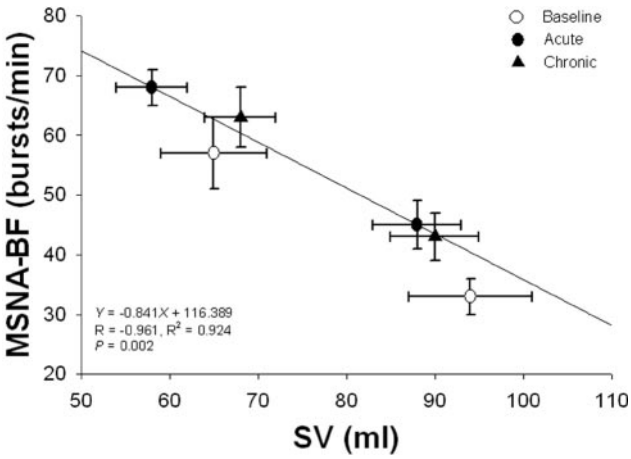

Figure 5. The relationship between the SV and the corresponding MSNA burst frequency (MSNA-BF) in the supine and upright positions in the patient group before and after acute and chronic antihypertensive therapy with Hyzaar.

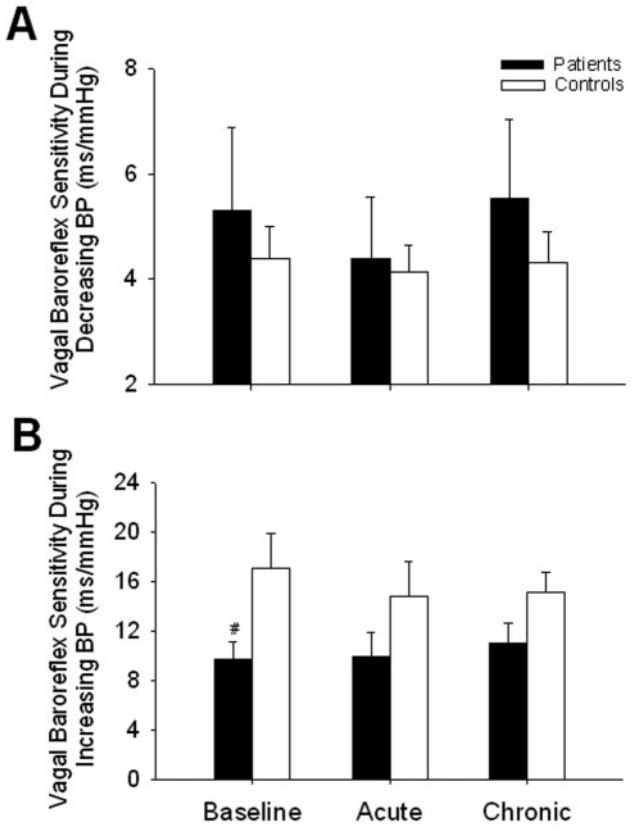

Figure 6. The sensitivity of baroreflex control of HR in the patient group and the control group assessed during early phase II (A) and phase IV (B) of the VM during the baseline, acute, and chronic study. Values are mean±SE. #$P \leq 0.05$ compared with the control group.

of MSNA despite 3 months of antihypertensive therapy. Thus, these results do not support our hypothesis that chronic antihypertensive therapy with Hyzaar decreases MSNA and resets baroreflex control of MSNA in patients with moderate hypertension. These findings may provide a potential mechanism for treatment failure in patients with resistant hypertension, and may suggest a mechanism for persistent morbidity (ie, persistent sympathetic activation) even in patients with adequate BP control.

Baroreflex Function and Antihypertensive Therapy

The evidence that human hypertension is associated with impairment of baroreflex control of cardiac vagal outflow is strong.[24] Moreover, there is nearly universal agreement that antihypertensive treatment resets the baroreflex control of HR to a lower pressure level.[4–6,25–27] On the other hand, it is still uncertain whether human hypertension is associated with a corresponding enhancement of sympathetic outflow, or whether baroreflex control of MSNA also resets after antihypertensive therapy.

In the present study, we found that before treatment, although BP was much higher, resting HR was relatively normal in patients than in healthy controls, suggesting that cardiac vagal baroreflex had been reset to a higher arterial pressure range in patients with untreated moderate hypertension. The increase in BP was caused primarily by an increase in TPR, which was likely mediated by sympathetic activation under resting conditions, because Qc was normal in these patients before treatment. These results are consistent with previous findings by Yamada et al[28] and Anderson et al[29] but different from those of Wallin et al[30–32] and Lake et al.[33]

Because antihypertensive therapy resets the cardiac vagal baroreflex in hypertensive patients, we expected that the sympathetic baroreflex would also have been reset to a lower pressure level during treatment. However, our data showed that resting MSNA was increased dramatically, MSNA responses to upright tilt and the VM were intact, and baroreflex regulation of MSNA remained unchanged during antihypertensive therapy. The increase in resting MSNA was consistent with an ongoing arterial baroreceptor unloading, evoked by reductions in BP during acute treatment. Surprisingly, resting MSNA did not return to baseline, and the sympathetic baroreflex did not reset even after 3 months of antihypertensive therapy with Hyzaar.

Hyzaar and Absence of Sympathetic Baroreflex Resetting

Previous studies by Sundlöf et al[34] and Wallin et al[35] demonstrated that in patients with untreated essential hypertension, a marked acute reduction in arterial pressure resulted in an increase in MSNA during administration of a cardioselective β-adrenoceptor antagonist metoprolol or a low dose of a central sympathoinhibitory agent clonidine. Recently, Grassi et al[36] found that acute administration of an antihypertensive drug with peripheral vasodilator agent prazosin or a combination of central and peripheral modes of action urapidil activated the sympathetic nervous system to a similar extent in untreated hypertensive patients, and they thereby concluded that adrenergic activation was generalized to any drug-induced acute BP fall, presumably because of a lack of a sympathetic baroreflex resetting during acute antihypertensive treatment. This notion was further supported by the recent finding that administration of AT_1 receptor blockade eprosartan for 1 week in men with normal to mild hypertensive BP values decreased arterial pressure and increased resting MSNA but did not blunt sympathetic activation caused by lower body negative pressure or mental stress.[37] Our results obtained during acute antihypertensive treatment with Hyzaar are consistent with these previous reports. It is likely that the increase in resting MSNA and the absence of sympathetic baroreflex resetting can be seen during acute treatment with various antihypertensive agents.

The present study extends these previous studies by making similar measurements after 3 months of chronic therapy. We found that resting MSNA remained high, whereas the sympathetic baroreflex still did not reset despite 3 additional months of antihypertensive therapy with Hyzaar. This observation appears to be in conflict with earlier studies by Mitchell et al[38] and Weinberger,[39] showing that chronic administration of the angiotensin-converting enzyme (ACE) inhibitor captopril decreased plasma norepinephrine concentration in severely hypertensive patients. However, the sensitivity of plasma norepinephrine concentration as a marker of sympathetic activity is narrow because of its limited

reproducibility[40] in addition to its dependence on tissue clearance and secretion from the sympathetic nerve terminals.[41,42] On the other hand, Grassi et al[10] measured MSNA in untreated mild to moderate essential hypertensive patients and found that sympathetic nerve traffic was not affected by chronic ACE inhibitor lisinopril treatment. The baroreflex ability to modulate central sympathetic outflow was also unaffected, whereas the sympathetic baroreflex was reset to the lower BP values during 2 months of treatment. They considered that the sympathetic baroreflex resetting was, to a large extent, attributable to a nonspecific effect of the BP reduction itself, although a specific influence of ACE inhibition on the afferent, central, or efferent portion of the reflex arc could not be excluded. However, our results gained during chronic treatment with Hyzaar do not support this notion. The specific reasons why our findings are different from theirs are unclear. We cannot exclude the possibility that during chronic antihypertensive therapy, different pharmacokinetic or pharmacodynamic properties of the drugs may have different effects on sympathetic baroreflex function.

Hyzaar is a combination of losartan and hydrochlorothiazide. It seems likely that losartan acts at vascular AT_1 receptors but not presynaptic AT_1 receptors located on the sympathetic nerve terminal, causing a reduction in Ang II–mediated vasoconstriction with no influence on sympathetic activation and baroreflex function. Additionally, losartan may increase central sympathetic outflow through an increase in circulating Ang II. It has been demonstrated that a high concentration of circulating Ang II is able to pass the blood–brain barrier and increase sympathetic outflow centrally.[43–45] However, losartan may also pass the blood–brain barrier and then bind to AT_1 receptors in the brain.[46,47] The central effects of Ang II on the sympathetic nervous system may be attenuated during chronic administration of losartan. Hydrochlorothiazide is a diuretic and can result in a reduction in plasma volume after high-dose administration.[48] The reduction in plasma volume can cause an increase in resting MSNA with no influence on sympathetic baroreflex function. However, this possibility seems to be small because hydrochlorothiazide was used at a relatively low dose; in addition, our preliminary data showed that plasma volume measured by the Evans blue dye method remained unchanged after 3 months of low-dose administration of hydrochlorothiazide in hypertensive patients. We also did not see any significant changes in Hct concentration, resting SV, or Qc in the patient group throughout the entire study, providing further evidence against a chronic reduction in plasma volume by hydrochlorothiazide in this study. The specific mechanisms underlying the absence of sympathetic baroreflex resetting during chronic antihypertensive therapy with Hyzaar cannot be elucidated in the present study. Further investigations with more subjects, including sufficient numbers of women and diverse ethnic groups taking multiple different drugs, are needed to address these questions.

Clinical Implications

Our findings have potential significance to medical practice. The persistent and marked sympathetic activation by unloading of the baroreflex without resetting during chronic antihypertensive therapy (such as with Hyzaar) may be an important mechanism underlying the persistent increased morbidity in patients with hypertension despite adequate BP control.[2] For example, chronic sympathoexcitation may promote cardiac hypertrophy and apoptosis[49] and may also predispose to dysrhythmias in these patients.[50] It is possible that for optimal reduction in clinical events, hypertensive patients (who may already have left ventricular hypertrophy), treated with AT_1 receptor antagonists (and a diuretic) should also take centrally acting sympathoinhibitory agents to prevent the secondary chronic sympathetic activation and adrenergic stimulation by the baroreflex, even if BP is adequately controlled. Whether this combination should be considered in very high-risk patients treated with other antihypertensive medications remains to be determined. For some patients in whom BP control is difficult despite multiple medications, it is possible that chronic sympathoexcitation, possibly manifested by sustained elevation in plasma catecholamines in the supine position, may overwhelm the direct vasodilatory effects of the drugs. Under such circumstances, centrally acting sympatholytic drugs may be particularly beneficial. These speculations will require direct confirmation in clinical trials involving larger numbers of patients.

Study Limitations

There are 2 limitations in this study. First of all, the number of subjects was small. We studied 7 patients with moderate hypertension and 7 healthy individuals as controls. It was extremely difficult to recruit subjects, especially healthy subjects in the present study, because very few people were willing to have 3 direct intraneural microneurographic recordings within several months. Hence, we present the exact *P* values as much as possible in our report. Moreover, numerous studies have demonstrated that for each healthy individual, MSNA burst frequency and burst incidence responses are remarkably constant, not only during a whole experiment, but also when recordings are repeated with intervals of weeks or months. Thus, studying more subjects may not have significant effects on the results of this study.

Second, we did not study placebo-treated hypertensive patients or use a paired, crossover design because of ethical issues involving the withholding of treatment for >3 months in patients with moderate hypertension. Although our study design may not have been perfect, it is unlikely that placebo-treated hypertensive patients would have had such dramatic changes in their sympathetic nerve activity without the BP-lowering effects of antihypertensive therapy. The increase in MSNA with antihypertensive therapy in this study was marked, very consistent among individual patients, and virtually identical between the acute and chronic study, associated with low typical error of measurement.

In summary, Hyzaar lowered BP acutely and chronically but did not change HR in patients with moderate hypertension. Supine MSNA was increased significantly, whereas MSNA responses to upright tilt and the VM remained unchanged during acute and chronic antihypertensive treatment. These data indicate that sympathetic neural activity was augmented substantially by antihypertensive treatment with Hyzaar, consistent with ongoing baroreflex unloading, and

did not return to baseline or "reset" after 3 months of therapy. We speculate that persistent and marked sympathetic activation by the baroreflex may be a potential mechanism for hypertension that is refractory to antihypertensive therapy and may provide a target mechanism for persistent morbidity despite adequate BP control.

Acknowledgments

This study was supported by the American Heart Association Texas Affiliate beginning grant-in-aid (0060024Y), NIH (HL075283), and the Wallace, Barbara, and Kelly King Foundation trust. The time and effort put forth by the subjects is greatly appreciated. The authors thank Emily R. Martini and M. Dean Palmer for expert technical assistance, Diane Bedenkop for recruiting healthy volunteers and patients, Kimberly Williams and Marta Newby for skillful nursing help. We also thank Drs Shawna Nesbitt and Ronald Victor from the Division of Hypertension at the University of Texas Southwestern Medical Center at Dallas for their encouragement, advice, and support.

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