Single-Unit Sympathetic Discharge: Quantitative Assessment in Human Hypertensive Disease

John P. Greenwood, MB ChB; John B. Stoker, BSc, MB ChB; David A.S.G. Mary, PhD, MB ChB

Background—Reports demonstrating sympathetic hyperactivity in hypertension with the use of microneurography have been inconsistent. One possible reason is that previous studies have assessed muscle sympathetic nerve activity (MSNA) from integrated voltage waves (“bursts”) recorded from multiunit discharges. We studied single units with defined vasoconstrictor properties (s-MSNA) to further characterize sympathetic output in hypertensive disease.

Methods and Results—We examined 74 subjects with a wide range of arterial blood pressure that were considered to be either normal (NT), high normal (HN), or stages 1 to 3 essential hypertension (EHT-1, EHT-2/3). All had their peripheral sympathetic activity measured from both multiunit bursts and single-unit vasoconstrictor impulses. There was a significant correlation between s-MSNA and MSNA, and results of variability studies were similar. The EHT-1 and EHT-2/3 groups had greater s-MSNA and MSNA than did the matched NT group (always P<0.01). The HN group also had greater s-MSNA and MSNA than did the NT group (mean±SEM: 43±5 vs 29±2 impulses/100 beats, P<0.05; 36±4 vs 24±2 bursts/100 beats, P<0.05). In addition, the EHT-1 group had significantly greater s-MSNA than did the EHT-2/3 group (63±6 vs 51±3 impulses/100 beats, P<0.05), which could not be demonstrated with MSNA bursts.

Conclusions—Quantification from single vasoconstrictor units has provided additional evidence in established essential hypertension of increased central sympathetic output. Furthermore, in the mild or early stages of hypertension, this technique has provided new evidence of augmented sympathetic output compared with more severe hypertension. (Circulation. 1999;100:1305-1310.)

Key Words: sympathetic nervous system ■ hypertension ■ blood pressure ■ action potentials

Since its introduction 3 decades ago, the technique of microneurography has been used extensively to examine peripheral sympathetic activity and its effect on vascular resistance.1,2 In the pathogenesis of essential hypertension there now exists a wealth of data to suggest a state of sympathetic hyperactivity, but this has been far from consistent, even from studies of direct nerve recordings.3–11 Furthermore, in what has been considered to be “borderline” hypertension, the situation is more complicated, in part because of its ambiguous definition. Here the evidence for a basal increase in sympathetic discharge is more limited and inconsistent,12–16 with additional data suggesting only augmented reflex responses.12,14,16

Accounting for these inconsistencies are the fundamental difficulties in quantifying human autonomic activity and the absence of matching of age, weight, or disease characteristics in some earlier studies.8–10,17 In addition, all previous reports conducted with microneurography to investigate hypertension have assessed muscle sympathetic nerve activity (MSNA) from recordings of multiunit bursts. However, multiunit recordings contain a variable number of firing units that may have different firing frequencies and function. Recently, for the first time, this potential inconsistency has been avoided by characterizing the behavior of single-unit muscular sympathetic nerve activity (s-MSNA) with demonstrable vasoconstrictor properties.18 Although much more technically demanding, by only studying these single units, it is postulated that one could obtain a more specific assessment of sympathetic activity responsible for the control of vascular resistance.

The present investigation was designed to see whether recording from single vasoconstrictor units in conjunction with multiunit bursts could further characterize peripheral sympathetic discharge in matched subject groups with a range of hypertensive disease compared with normotension.

Methods

Subjects

The study was conducted between September 1995 and February 1999 in 74 consecutive white subjects in whom it was possible to identify and record single-unit activity. This represented ~60% of the total number of subjects studied; in the other 40% it was not possible to obtain a stable recording from a single vasoconstrictor unit. All patients were screened by history and physical and...
laboratory examinations. Subjects were excluded if there was evidence of secondary hypertension, arrhythmia, or chronic disease that may influence the autonomic nervous system. Of the 74 subjects, 38 were women and 36 were men, ranging in age between 22 and 75 years. The subjects were grouped according to their resting blood pressure (average of 3 seated recordings on separate occasions) by use of the JNC-VI classification:16 Normal (NT) resting blood pressure was considered to be systolic pressure <130 and diastolic <85 mm Hg. High normal (HN) resting blood pressure was considered to be systolic pressure of 130 to 139 mm Hg or diastolic of 85 to 89 mm Hg. Essential hypertension was divided into 3 stages: Stage 1 (EHT-1) was described as systolic pressure of 140 to 159 mm Hg or diastolic of 90 to 99 mm Hg; stage 2 (EHT-2), systolic pressure of 160 to 179 mm Hg or diastolic of 100 to 109 mm Hg; and stage 3 (EHT-3), systolic pressure of ≥180 mm Hg or diastolic of ≥110 mm Hg.

All patients with essential hypertension were studied before and after antihypertensive treatment had been discontinued and had no evidence of target organ damage. The 13 patients with stage 2 hypertension and the 8 with stage 3 disease were considered as a single group with established hypertension.

General Protocol

Under the approval of St James's University Hospital Ethics Committee, subjects provided informed written consent to the investigation. All subjects were studied between the hours of 9 AM and noon and were asked to avoid nicotine and caffeine products for 12 hours and alcohol and strenuous exercise for 24 hours before the investigation. Subjects maintained a normal dietary intake of sodium, and alcohol and strenuous exercise for 24 hours before the investigation. All patients with essential hypertension were studied before and after antihypertensive therapy was commenced and had no evidence of secondary hypertension, arrhythmia, or chronic disease that may influence the autonomic nervous system. Of the 74 subjects, 38 with stage 1 disease were considered as a single group with established hypertension.

Microneurography

Postganglionic MSNA was recorded from the right peroneal nerve as previously described.2 The neural signal was amplified (×50,000), and for the purpose of generating bursts representing multunit discharge, the signal was filtered (bandwidth of 700 to 2000 Hz) and integrated (time constant 0.1 second). The output of action potentials and bursts from this assembly were passed to a data acquisition system (FASTDAQ, Lectromed UK Ltd) for on-line monitoring and storage with the use of a minicomputer (Elonex UK Ltd). The FASTDAQ system digitized the action potentials at 12 000 samples/s and all other data channels at 2000 samples/s (8 bits). Long-term storage was achieved with the use of a high-capacity drive (Iomega zip drive, Iomega Europe GmbH).

With the exploring electrode in the nerve, electrical stimulation (0.1 to 1.0 V, 1 Hz, 0.2 ms) caused muscular twitches without paraesthesia. MSNA was differentiated from skin sympathetic activity by previously accepted criteria.2 Single units were sought by repeatedly making tiny adjustments to the exploring electrode position. When a unit was identified in the raw action potential morphology (Figure 1), as previously described.18

Other Procedures

Only vasoconstrictor units were accepted and examined, the criteria of acceptance being appropriate responses to spontaneous changes in arterial blood pressure, the Valsalva maneuver, and isometric hand-grip exercise. During Valsalva, sympathetic activity increased during the latter part of phase II (blood pressure compensation) and/or phase III (release of strain and fall in blood pressure) and decreased during phase IV (increase and overshoot of blood pressure). Isometric handgrip exercise, performed with the use of a dynamometer (MIE Medical Research Ltd), produced a late increase in arterial blood pressure and sympathetic neural activity. Further confirmation that the sympathetic activity obtained in this manner was destined to supply the muscle vascular bed was confirmed in some subjects by obtaining a direct relation between the frequency of sympathetic discharge and calf vascular resistance (unpublished observation).

Data Analysis

Without knowledge of the patient diagnosis, data analysis was performed off-line by a single experienced operator using signal processing software (FASTDAQ, Lectromed UK Ltd). An electronic discriminator was used to count the spikes of s-MSNA and the R wave of the ECG. The former was quantified as mean frequency of impulses per minute and impulses/100 cardiac beats; this avoided any interference by the length of the cardiac cycle.20 The bursts of MSNA on the mean voltage neurogram were identified by inspection when the signal-to-noise ratio was >3 and were quantified as mean number of bursts per minute and bursts/100 beats.

In each subject, the resting mean frequencies of s-MSNA impulses and MSNA bursts were obtained simultaneously. For variability of measurements, these frequencies were obtained for a minimum of 2 minutes during the steady state, twice within 30 minutes during the same impairment of the peroneal nerve. A similar method of analysis was used to estimate the variability of the technique by obtaining the frequencies from 2 separate impalpations of the peroneal nerve (ie, different units), within a 60-minute period.

Statistics

The directional relation between s-MSNA and MSNA was examined by the use of Pearson correlation coefficients (r). The variability of obtaining mean frequencies of either s-MSNA impulses or MSNA bursts was estimated as the 95% CIs of the individual differences relative to the mean of the repeated measurements. One-way ANOVA with Newman-Keuls multiple post-test comparisons were used to compare data between the different clinical conditions. Values of P<0.05 were considered statistically significant. Data are presented as mean±SEM.

Results

Four groups were examined, all free from cardiovascular therapy and closely matched for age, body weight, and body mass index (Table 1). With regard to sex, there were no significant differences between the ratio of men to women in the 4 groups (χ²=2.37; P>0.4).

As expected, the average indexes of arterial blood pressure were significantly different between all 4 groups (Table 1). Although overall the heart rate tended to increase with the severity of hypertension, there was no significant difference between the group pairs.

During the resting state there was no systematic difference in the mean frequency of s-MSNA and MSNA from both the same and different units. Considering measurements from the same unit recorded twice within a 30-minute period, the variability of repeated measurements amounted to <10% for both s-MSNA and MSNA (Table 2). When measurements were made from 2 different units recorded twice within a 60-minute period, the variability again amounted to <10% for both s-MSNA and MSNA.

Within the 4 groups of subjects there were significant correlations between measurements of s-MSNA and MSNA.
in the same individual subjects; the lowest correlation ($r$) was 0.76 ($P<0.0007$) for the mean frequency per minute in the EHT-1 group. As expected, there was a significant positive correlation for both s-MSNA and MSNA with the subject’s age (0.42<$r$<0.59; $P<0.0002$). However, there was no significant correlation within any group (consistently $r$<0.38, $P$>0.09), between resting sympathetic discharge (s-MSNA or MSNA) and the levels of resting arterial blood pressure (systolic, diastolic, or mean pressure).

**TABLE 1. Characteristics of the 4 Subject Groups**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>NT</th>
<th>HN</th>
<th>EHT-1</th>
<th>EHT-2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (men)</td>
<td>17 (7)</td>
<td>17 (8)</td>
<td>19 (11)</td>
<td>21 (10)</td>
</tr>
<tr>
<td>Age, y</td>
<td>37±2.0</td>
<td>44±2.5</td>
<td>43±2.8</td>
<td>45±2.3*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72±2.7</td>
<td>78±3.7</td>
<td>80±3.8</td>
<td>80±4.0*</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>26±0.7</td>
<td>27±1.0</td>
<td>27±1.0</td>
<td>28±1.0*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66±2.0</td>
<td>67±2.6</td>
<td>72±2.2</td>
<td>73±1.8†</td>
</tr>
<tr>
<td>Arterial pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>89±1.2</td>
<td>102±0.7</td>
<td>111±0.8</td>
<td>129±2.6‡</td>
</tr>
<tr>
<td>Systolic</td>
<td>117±1.9</td>
<td>134±1.1</td>
<td>148±1.5</td>
<td>171±4.6‡</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75±1.3</td>
<td>86±1.0</td>
<td>92±1.2</td>
<td>108±2.6‡</td>
</tr>
</tbody>
</table>

Data are mean±SEM. One-way ANOVA, *$P$<0.10, †$P$<0.05, ‡$P$<0.0001.

The main objective of this study was to determine whether or not the examination of single-unit sympathetic discharge could assist in the characterization of subjects with hypertension compared with normotensive individuals (Table 3). First, the EHT-1 and EHT-2/3 groups had significantly greater s-MSNA and MSNA than in NT subjects (Figures 2 and 3). This remained true whether the mean rate of discharge was expressed as frequency per minute or per 100 cardiac beats. Second, these values were significantly greater in the EHT-1 group compared with the HN group. Third, the HN group had significantly greater s-MSNA and MSNA (irrespective of how activity was expressed) than did the NT group. Finally, although s-MSNA was significantly greater in the EHT-1 group than the EHT-2/3 group (Figure 2), this could not be demonstrated from the study of multiunit bursts of MSNA (Figure 3).

**Discussion**

This is the first study in which sympathetic vasoconstrictor discharge has been studied directly from single units in human hypertensive disease. Although more technically demanding, by using this technique we have shown that the resting mean frequency of s-MSNA was greater in hypertensive subjects than in closely matched normotensive control.
subjects. In addition, the group with stage 1 hypertension had higher sympathetic activity than the HN group, who in turn had higher activity than the NT group. Finally, we have shown that the mean frequency of s-MSNA was greater in mild rather than more severe established essential hypertension, a fact not apparent from multiunit recordings. These findings provide evidence that the elevated sympathetic drive in essential hypertension does not simply arise from the recruitment of additional firing units but also involves an increase in the actual impulse frequency for any particular unit. In addition, they add further weight to the argument that elevated sympathetic drive is important during the development of essential hypertension.

Microneurography is a well-established technique that has been used successfully in the assessment of peripheral sympathetic activity. Previous studies have examined the frequency and incidence of MSNA bursts derived from multiunit recordings but have not been specific to any of the individual actions potentials that constitute the burst. This limitation has recently been addressed after the first recordings of single-unit muscle sympathetic discharge in normal subjects. Although only a small number of subjects were examined, perhaps reflecting the difficulty of obtaining these single units, their mean resting frequency (0.47 Hz) was similar to that seen in this investigation (0.38 Hz). These values are considered comparable to those obtained from animal studies. The technique of directly measuring the mean frequency of single-unit discharge may be considered to reflect the true resting central sympathetic output to the periphery. This is because it allows quantification from specific units with demonstrable vasoconstrictor function without interference from other uncharacterized units. In addition, it can avoid the inclusion of recruited units that could otherwise be counted during recording of MSNA bursts. However, the latter technique may be more useful during reflex maneuvers, which induce large amounts of recruitment. For this reason, single-unit and multiunit recording techniques should be considered complementary.

### TABLE 2. Reproducibility of s-MSNA and MSNA Recordings From the Same Unit Within 30 Minutes and 2 Different Units Within 60 Minutes

<table>
<thead>
<tr>
<th></th>
<th>Recording 1 (Mean±SEM)</th>
<th>Recording 2 (Mean±SEM)</th>
<th>Mean of Difference ±95% CI</th>
<th>Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same unit (n=25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-MSNA, imp/min</td>
<td>33.8±6.4</td>
<td>34.1±6.3</td>
<td>−0.2±1.2</td>
<td>7.1%</td>
</tr>
<tr>
<td>s-MSNA, imp/100 beats</td>
<td>48.1±8.7</td>
<td>48.9±8.7</td>
<td>−0.8±1.8</td>
<td>7.4%</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>26.6±3.6</td>
<td>26.8±3.6</td>
<td>−0.2±0.9</td>
<td>6.7%</td>
</tr>
<tr>
<td>MSNA, bursts/100 beats</td>
<td>39.1±5.5</td>
<td>40.4±5.5</td>
<td>−1.6±1.4</td>
<td>7.0%</td>
</tr>
<tr>
<td>Different units (n=44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-MSNA, imp/min</td>
<td>55.1±6.4</td>
<td>55.6±6.5</td>
<td>−0.5±1.7</td>
<td>6.1%</td>
</tr>
<tr>
<td>s-MSNA, imp/100 beats</td>
<td>78.1±9.1</td>
<td>77.9±9.0</td>
<td>0.1±3.0</td>
<td>7.7%</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>31.1±2.5</td>
<td>30.7±2.6</td>
<td>0.2±1.0</td>
<td>6.5%</td>
</tr>
<tr>
<td>MSNA, bursts/100 beats</td>
<td>44.1±3.9</td>
<td>44.0±4.2</td>
<td>0.3±1.7</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

*Imp indicates impulses. Activity is expressed per minute and per 100 cardiac beats.*

### TABLE 3. Sympathetic Neural Activity From Single-Unit and Multiunit Recordings Expressed as Activity per Minute and per 100 Cardiac Beats in the 4 Groups of Subjects

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>HN</th>
<th>EHT-1</th>
<th>EHT-2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-MSNA, imp/min</td>
<td>19±1.6</td>
<td>27±3.0</td>
<td>45±4.2</td>
<td>37±2.2</td>
</tr>
<tr>
<td>s-MSNA, imp/100 beats</td>
<td>29±2.4</td>
<td>43±5.0</td>
<td>63±5.6</td>
<td>51±3.2</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>16±1.4</td>
<td>23±2.1</td>
<td>35±1.6</td>
<td>35±2.2</td>
</tr>
<tr>
<td>MSNA, bursts/100 beats</td>
<td>24±2.2</td>
<td>36±4.0</td>
<td>49±2.8</td>
<td>48±3.4</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. Imp indicates impulses.*

**Figure 2.** s-MSNA, quantified per minute and per 100 cardiac beats for the 4 groups of subjects: normal (NT), high normal blood pressure (HN), stage 1 hypertension (EHT-1), and stage 2 or 3 hypertension (EHT-2/3). Column height represents mean; bars indicate 95% CIs. Compared with NT, *P<0.05, †P<0.001, ‡P<0.01. Compared with HN, §P<0.001, ¶P<0.01, #P<0.05, #P<0.01. Compared with EHT-1, **P<0.05.
In the current study, both methods of assessment were used to investigate peripheral sympathetic activity in hypertensive disease. The values of frequency obtained from MSNA recordings in this study were similar to those previously published in age-comparable normotensive and hypertensive subjects.\(^8\)\textsuperscript{–}10\textsuperscript{,}21\ There was also a significant correlation between the resting mean frequency of s-MSNA and that of MSNA bursts that were simultaneously assessed. Over a period of time, there were fewer MSNA bursts than s-MSNA impulses, which could be anticipated because a burst may consist of a variable number of impulses (between 1 and 7) from 1 or more units.\(^18\)\textsuperscript{ Previous reports have also shown that the intraindividual variability of repeated measures of MSNA is small.\textsuperscript{\textsuperscript{20\textsuperscript{,}}22} In agreement, we found that the variability of repeated measures of resting s-MSNA from 2 different units was low and remarkably similar to that relating to the same unit. Not only do these findings confirm the existence of a steady state during which the recordings were made, but they also support the validity of the new technique of single-unit assessment relative to that which has been established from the use of multunit bursts.

Although many reports have assessed the resting mean frequency of MSNA bursts in patients with essential and “borderline” hypertension, the results have been inconsistent. In essential hypertension, the mean resting frequency of MSNA was found to be either increased\(^8\)\textsuperscript{,}9\textsuperscript{,}21\textsuperscript{,}23\ or similar\textsuperscript{3\textsuperscript{–}}6\textsuperscript{,}24\ to that seen in normotensive subjects. Likewise, in “borderline” hypertension, resting MSNA was also either increased\textsuperscript{13\textsuperscript{–}15} or similar\textsuperscript{12\textsuperscript{,}16} to that seen in normotension. Furthermore, it has been reported that the mean frequencies of MSNA bursts were not consistently greater in “borderline” hypertension than in essential hypertension.\textsuperscript{8\textsuperscript{–}12\textsuperscript{,}15\textsuperscript{,}23} With improvement in understanding of the influences on peripheral sympathetic output, some of the inconsistency in earlier studies could be explained by the presence of confounding factors. These include the subject characteristics of age, sex, and obesity\textsuperscript{8\textsuperscript{,}17\textsuperscript{,}25} or the presence of gastric and bladder distention.\textsuperscript{26\textsuperscript{,}27} All these confounding factors were avoided in the current study. Also, because a low-sodium diet may increase sympathetic activity,\textsuperscript{13} all groups were examined after receiving a sodium intake of \(\approx400\) mmol/d.

In the JNC-VI classification of blood pressure used in this study, the term “borderline hypertension” has been replaced by “high-normal blood pressure.”\textsuperscript{19} Although this may appear to make it difficult to draw comparisons, earlier studies have in fact used varying definitions of both normotension and in particular borderline hypertension. We have shown that hypertensive patients have higher levels of s-MSNA and MSNA than the normotensive group. Of particular interest was the fact that levels of s-MSNA were higher in the EHT-1 group than in the EHT-2/3 group despite the lower arterial blood pressure in the former group. This was not true for MSNA. In contrast, the HN group had greater levels of s-MSNA and MSNA than did the NT group despite higher blood pressure levels. Insight into this change in sympathetic drive in hypertensive disease has been made possible for the first time by the use of the single-unit technique. In particular, the measurement of s-MSNA has allowed greater discrimination of high-frequency central sympathetic drive in hypertensive subjects, the potential of which may have been suspected by the fact that individual bursts can contain a variable number of action potentials.\textsuperscript{18}

The current findings therefore confirm that hypertension is associated with a state of increased central sympathetic drive, but it is likely that numerous other non-neural mechanisms are important. This could be suspected from the wide variance and overlap between the ranges of sympathetic activity in the normal and hypertensive groups and the lack of correlation to resting arterial blood pressure. It has been reported that in addition to the mean discharge frequency, the irregularity of firing could contribute to the vasoconstrictor effect.\textsuperscript{18} Furthermore, central sympathetic drive and the degree of peripheral vasoconstriction are both believed to be modulated by a multitude of humoral factors such as nitric oxide, endothelin-1, insulin, and so on. Finally, differences in plasma renin activity might contribute to the variance in neural activity in this study. Although the sympathetic nervous system is known for its heterogeneous regional output, there is a correlation between resting sympathetic discharge to the periphery and the kidney.\textsuperscript{28}

More importantly, in terms of central vasoconstrictor sympathetic drive, the current study has shown a clear difference between normotensive and hypertensive subject groups. In the former groups (NT, HN), higher blood pressure was associated with higher sympathetic drive; in the latter groups (EHT-1, EHT-2/3), an increase in the severity of hypertension was associated with a lower sympathetic drive.
This is in keeping with reports that the responses of central sympathetic output are augmented in borderline hypertension\textsuperscript{12,14,16} but are affected to a lesser degree in established hypertension\textsuperscript{9,21}. Also, baroreceptor reflex control of the heart rate and sympathetic output are more consistently impaired or reset in established hypertension\textsuperscript{9,21} than in borderline hypertension. Overall, these findings support the theory that the actual origins of raised arterial pressure involve the sympathetic nervous system and that with time, neural and hemodynamic responses may be modulated by various central, reflex, hormonal, and structural changes.\textsuperscript{29–31}

In conclusion, for the first time single-unit sympathetic vasoconstrictor activity has been quantified in human hypertension and has shown that greater sympathetic drive occurs in milder stages of the disease process. In nonhypertensive subjects, greater sympathetic drive occurred in those with a high-normal blood pressure level. Thus the current investigation has provided strong support to the hypothesis that the central sympathetic drive is a major factor in the development of hypertension.

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