Sympathetic Neural Activation in Nondiabetic Metabolic Syndrome and Its Further Augmentation by Hypertension

Robert J. Huggett, Joanna Burns, Alan F. Mackintosh, David A.S.G. Mary

Abstract—Hypertension is a major cardiovascular risk factor in the metabolic syndrome (MS) in which the presence of insulin resistance, glucose intolerance, abnormal lipoprotein metabolism, and central obesity all confer an increased risk. Because essential hypertension (EHT), insulinemia, and visceral fat are associated with sympathetic hyperactivity, which is itself known to increase cardiovascular risk, the aim of this study was to see if MS is a state of sympathetic nerve hyperactivity and if the additional presence of EHT intensifies this hyperactivity. In 69 closely matched subjects, comprising hypertensive MS (MS+EHT, 18), normotensive MS (MS-EHT, 17), hypertensives without MS (EHT, 16), and normotensive controls without MS (NC, 18), we measured resting muscle sympathetic nerve activity (MSNA) as assessed from multiunit discharges and from single units with defined vasoconstrictor properties (s-MSNA). The s-MSNA in MS+EHT (76±3.1 impulses/100 beats) was greater (at least \( P<0.01 \)) than in MS-EHT (62±3.2 impulses/100 beats) and in EHT (60±2.3 impulses/100 beats), and all these were significantly greater (at least \( P<0.01 \)) than in NC (46±2.7 impulse/100 beats). The multi-unit MSNA followed a similar trend. These findings suggest that MS is a state of sympathetic nerve hyperactivity and that the additional presence of hypertension further intensifies this hyperactivity. The degree of sympathetic hyperactivity seen in this study could be argued at least partly to contribute to the higher cardiovascular risk and metabolic abnormalities seen in MS+EHT patients. (Hypertension. 2004;44:847-852.)

Key Words: sympathetic nervous system ■ hypertension ■ metabolism

little information exists on the level of sympathetic nerve activity in the metabolic syndrome (MS) and whether the presence of essential hypertension (EHT), which is itself a state of sympathetic nerve hyperactivity,1–4 augments this activity. Sympathetic activation has already been associated with many of the individual components of the MS, such as visceral obesity,5 insulinemia,6 EHT,1–4 and type 2 diabetes.7 The majority of treated hypertensives have in addition at least one other MS component,8 also, it is now widely recognized that MS constitutes a cluster of major cardiovascular risk factors,9 represented mainly by insulin resistance and obesity in which hypertension as an individual component overlaps the least with the other components.10 Hypertension is known to be one of the highest predictors of cardiovascular morbidity and mortality associated with the MS.11,12 Furthermore, sympathetic activation in EHT is believed to contribute to cardiovascular risk.13,14

We therefore tested the hypothesis that the level of sympathetic nerve activity would be increased in normotensive MS and that the additional presence of EHT would further amplify this sympathetic activation.

Methods

Subjects

A total of 72 white subjects were examined, comprising 18 subjects with hypertensive MS (MS+EHT), 18 normotensive MS (MS-EHT), 18 hypertensives without MS (EHT), and 18 normotensive controls without MS (NC) subjects. All had similar sedentary occupational status and dietary habits, including a sodium intake of \( \sim 150 \) mmol/d, as assessed during the initial screening in the dietetics department of St. James’s University Hospital. In addition, all patients were screened by history, physical, and laboratory examination. They were excluded if there was evidence of secondary hypertension, familial dyslipidemia, diabetes, cardiac dysrhythmias, peripheral vascular disease, and chronic disease that may influence the autonomic nervous system. Stable microneurographic data to obtain single unit activity could not be obtained in 3 subjects, and complete data were therefore obtained from 69 subjects. These comprised 18 subjects with MS+EHT, 17 with MS-EHT, 16 with EHT, and 18 NC subjects.

The diagnosis of MS was confirmed using the criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)9 as the MS criteria for entry into the trial. Thus, the 2 MS groups had fasting venous glucose level between 6.1 mmol/L (110 mg/dL) and 7.0 mmol/L (126 mg/dL) (with a 2-hour plasma glucose of <11.1 mmol/L (200 mg/dL) during a 75-g oral glucose tolerance test to exclude diabetes mellitus), abdominal obesity (waist circumference >102 cm in men and >88 cm in women), triglyceridemia of >1.695 mmol/L (150 mg/dL), and a low high-density lipoprotein (HDL) <1.036 mmol/L (40 mg/dL) for men and <1.295 mmol/L (50 mg/dL) for women. The hypertensive groups had established hypertension for no longer than 48 months, with pretreatment arterial pressure level of ≥141/90 mm Hg that has been controlled by therapy for at least 6 months. Arterial pressure was documented using the average of at least 3 seated
recordings taken on separate occasions. The type and number of patients taking different antihypertensive therapy in MS+EH and EHT groups, respectively, were angiotensin-converting enzyme (ACE) inhibitors (8 and 6), angiotensin-II receptor blockers (ARB) (3 and 2), thiazide diuretic (3 and 6), and β-blockers (8 and 6). Also, 2 and 1 patients, respectively, were on no therapy at the time of the study. The investigation was conducted according to the principles of the Declaration of Helsinki (2000) of the World Medical Association and performed with the approval of St. James’s University Hospital Ethics Committee, with all subjects providing informed written consent.

Anthropometry
Regional body fat was assessed by the mean of 3 repeated skinfold-thickness measurements obtained using skinfold calipers (British Indicators Ltd, UK) at biceps, triceps, subscapular, and suprailiac regions. Sum and log10 sum of the 4 skinfold measurements were obtained and percentage fat mass was calculated using the Siri equation for age and sex.15 Waist circumference was measured as the minimal circumference measured at the navel, and the hip circumference as the widest circumference measured at the hips and buttocks. Waist-to-hip ratio was used as an index of regional adipose tissue distribution.

Assays
Venous fasting plasma insulin, glucose, and lipid profiles were obtained. Insulin was measured by solid phase 2-site immunoassay (ADVIA Centaur; Bayer Diagnostics, Newbury, UK). Blood glucose was measured by an automated analyser (ADVIA 1650; Bayer Diagnostics). Insulin resistance was assessed by the previously validated16,17 homeostasis model assessment (HOMA), whereby the HOMA index of insulin resistance (HOMA-IR)=fasting glucose (mmol/L)×[fasting insulin (µU/mL)/22.5]. The lipid profile, total cholesterol, HDL, and triglycerides levels were measured using a direct enzymatic method (ADVIA 1650). Low-density lipoprotein cholesterol was calculated using the Friedewald formula.18

General Protocol
In all subjects, the measured variables were obtained in an identical manner as has been published previously.4,5 Resting arterial pressure was measured from the upper arm using a standard mercury sphygmomanometer. Changes in heart rate and arterial pressure were monitored and recorded using a standard ECG and a Finometer device (FMS; Arnhem, the Netherlands). Calf blood flow (CBF) to the muscle of the left calf was obtained using standard strain-gauge plethysmography (Hokanson, Bellevue, Wash). CBF was obtained as the average of 12 recordings, and calf vascular resistance (CVR) was obtained as the product of mean arterial pressure over CBF and was expressed in arbitrary units. The intraobserver reproducibility of CBF measurement in this laboratory, obtained as twice 95% confidence interval of differences between repeated within-session plethysmography, amounted to 2.4% of the value of the measurement.

Microneurography
During each session microneurography was performed with subjects studied in the semisupine position. Postganglionic muscle sympathetic nerve activity49 was recorded from the right peroneal nerve, alongside the other data for 5 minutes after attaining steady state for at least 30 minutes.4,5 The neural signal was amplified (×50 000), filtered (bandwidth of 700 to 2000 Hz), and integrated (time constant 0.1 second) to output multunit muscle sympathetic nerve activity (MSNA). Single units (s-MSNA) in the raw action potentials were obtained by adjusting the electrode probe while using fast monitor sweep to screen for the presence of consistent action potential morphology, as previously described.4,20 Single unit activity was confirmed during subsequent analysis by superimposing the action potentials electronically (Figure 1). The output of action potentials and bursts from this assembly was passed to a PC-based data acquisition system (LabVIEW; National Instruments Corp), which digitized the acquired data at 12 000 samples/second (16 bits).

Only vasoconstrictor discharge was accepted after showing appropriate responses to spontaneous arterial pressure changes, the Valsalva maneuver, isometric handgrip exercise, and cold pressor tests.5,7,19,20 This was confirmed by simultaneous measurement of CBF and CVR. Baroreceptor reflex sensitivity controlling the heart interval through vagal effects was obtained from stage IV of the Valsalva maneuver21 as the slope of best linear relationship between systolic arterial pressure and its heart interval (phase 0) or the succeeding one (phase 1).

Analysis was independently performed off-line, using dedicated software and the LabVIEW system (National Instruments Corp). MSNA bursts were identified by inspection when the signal-to-noise ratio was >3. An electronic discriminator window was used objectively to count MSNA bursts and s-MSNA units, which were then quantified as mean frequency per minute and per 100 cardiac beats. We measured both s-MSNA and MSNA frequencies because s-MSNA reflects the mean resting sympathetic nerve frequency supplying the peripheral vessels. However, the MSNA bursts contain different efferent units, including those recruited through the operation of reflex mechanisms,4 and their data make our findings comparable with other publications in terms of potential confounding factors. The variability of measuring both s-MSNA and MSNA within sessions and between sessions in the same subject in this laboratory did not exceed 10%.4

Statistics
One-way ANOVA with Newman-Keuls post-tests was used to compare data between groups. The least-square technique was used...
TABLE 1. Clinical and Metabolic Characteristics in the Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NC (n=18)</th>
<th>EHT (n=16)</th>
<th>MS-EHT (n=17)</th>
<th>MS+EHT (n=18)</th>
<th>Comparisons Between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>8/10</td>
<td>7/9</td>
<td>8/9</td>
<td>8/10</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>51±2.0</td>
<td>54±2.3</td>
<td>50±1.2</td>
<td>51±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32±1.1</td>
<td>30±0.9</td>
<td>33±1.0</td>
<td>32±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91±0.02</td>
<td>0.94±0.01</td>
<td>0.94±0.03</td>
<td>0.98±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>98±2.2</td>
<td>99±2.0</td>
<td>101±2.1</td>
<td>100±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2±0.1</td>
<td>5.4±0.1</td>
<td>6.4±0.08</td>
<td>6.5±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>120 minute glucose, mmol/L</td>
<td>5.7±0.2</td>
<td>6.1±0.2</td>
<td>7.4±0.5</td>
<td>7.7±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL</td>
<td>8.8±1.0</td>
<td>9.4±2.2</td>
<td>20.8±2.8</td>
<td>27.8±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA², units</td>
<td>2.0±0.2</td>
<td>2.3±0.6</td>
<td>6.0±0.8</td>
<td>7.6±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.2±0.2</td>
<td>5.1±0.1</td>
<td>5.7±0.2</td>
<td>5.6±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.0±0.1</td>
<td>3.1±0.1</td>
<td>3.6±0.1</td>
<td>3.5±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.04</td>
<td>1.3±0.03</td>
<td>1.0±0.05</td>
<td>0.9±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4±0.08</td>
<td>1.5±0.05</td>
<td>2.3±0.2</td>
<td>3.2±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total body fat, kg</td>
<td>32±2.0</td>
<td>32±1.3</td>
<td>34±1.8</td>
<td>32±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>60±1.7</td>
<td>59±1.5</td>
<td>57±1.9</td>
<td>60±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>34±1.3</td>
<td>35±1.0</td>
<td>37±1.4</td>
<td>34±1.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data as mean±SEM for normotensive controls (NC), hypertensive controls (EHT), and normotensive (MS-EHT) and hypertensive metabolic syndrome (MS+EHT) groups.
Comparisons were made using ANOVA post-tests (Newman-Keuls) and are shown in the right 4 columns.
NS indicates insignificant differences.

Results

The details of the 4 groups are shown in Tables 1 and 2. They were well matched for age, body weight, body mass index, waist/hip ratio, waist circumference, and gender ($\chi^2=0.81; P>0.90$). Also, there were no differences in arterial pressure indices. As previously shown in hypertensive and normotensive populations, there was no significant correlation within any of the groups between sympathetic nerve activity and arterial pressure (at least $P>0.06$). Sympathetic activity indices showed a significant correlation to age in each of the 4 groups ($r$ between 0.5 and 0.70; at least $P<0.02$) as has previously been reported. The EHT groups had lower baroreceptor reflex sensitivity than the other 2 groups as previously reported. Thus, baroreceptor reflex sensitivity in

TABLE 2. Hemodynamic and Sympathetic Measures in the Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>NC (n=18)</th>
<th>EHT (n=16)</th>
<th>MS-EHT (n=17)</th>
<th>MS+EHT (n=18)</th>
<th>Comparisons Between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>65±1.4</td>
<td>67±1.4</td>
<td>68±1.8</td>
<td>69±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial pressure, mm Hg</td>
<td>95±1.3</td>
<td>94±1.4</td>
<td>95±1.5</td>
<td>97±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean</td>
<td>129±1.7</td>
<td>130±2.1</td>
<td>132±2.2</td>
<td>134±1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic</td>
<td>78±1.6</td>
<td>75±1.6</td>
<td>77±1.5</td>
<td>78±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>46±2.7</td>
<td>60±2.3</td>
<td>62±3.2</td>
<td>76±3.1</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>s-MSNA, impulses/100b</td>
<td>30±1.6</td>
<td>40±1.7</td>
<td>42±2.2</td>
<td>52±2.3</td>
<td>&lt;0.001 NS</td>
</tr>
<tr>
<td>s-MSNA, impulses/min</td>
<td>40±2.3</td>
<td>51±1.7</td>
<td>52±2.3</td>
<td>63±2.6</td>
<td>&lt;0.01 NS</td>
</tr>
<tr>
<td>MSNA, bursts/100b</td>
<td>26±1.4</td>
<td>34±1.2</td>
<td>35±1.6</td>
<td>43±1.8</td>
<td>&lt;0.001 NS</td>
</tr>
<tr>
<td>Calf vascular resistance, units</td>
<td>33±2.3</td>
<td>41±4.4</td>
<td>48±3.5</td>
<td>56±3.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data as mean±SEM for normotensive controls (NC), hypertensive controls (EHT), and normotensive (MS-EHT) and hypertensive metabolic syndrome (MS+EHT) groups.
MSNA (multunit bursts) and s-MSNA (single unit activity) are expressed per 100 cardiac beats (100b) and per minute (min).
Comparisons were made using ANOVA post-tests (Newman-Keuls) and are shown in the right 4 columns.
NS indicates insignificant differences.
Discussion

There are 2 important new findings in this study. First, the sympathetic neural discharge to the periphery was increased in MS+EHT. Second, activation of vasoconstrictor sympathetic activity in MS+EHT was greater than that seen in either EHT or MS-EHT alone. These findings suggest that the metabolic abnormalities found in MS are associated with an enhanced sympathetic drive that is further intensified by the presence of hypertension.

We defined the presence of MS in our patients according to the ATP III criteria, which include central obesity, dyslipidemia, impaired fasting glucose, and EHT. Matching between groups was achieved while accepting differences between the hypertensive and normotensive groups, specifically in terms of antihypertensive therapy. This comprised ACE inhibitors, ARBs, thiazide diuretics, and β-blocking agents. Although we cannot entirely exclude some influence of these agents, it is known in hypertensive patients that chronic ACE inhibitor therapy does not affect MSNA levels. ARBs may decrease it, and β-blockers may have no effect or decrease MSNA. In obese hypertensive subjects, chronic hydrochlorothiazide therapy that lowers arterial pressure did not significantly affect MSNA levels. We did not stop antihypertensive therapy in order to obtain comparable arterial pressure levels. Analyzing the data in patients not taking ARB or β-blocking agents did not affect our findings. Also, there was no correlation between sympathetic nerve activity and arterial pressure as has previously been documented. Therefore, although we cannot completely exclude an effect of medications in our 2 hypertensive groups, they could not have entirely caused the excessive sympathetic hyperactivity. In addition, the present study was designed to avoid traditional confounding factors that are known to affect sympathetic activity. Other data in our laboratory indicate that the overweight condition without metabolic or hormonal abnormalities is not associated with sympathetic nerve hyperactivity. Furthermore, the 4 groups had similar heart rate levels, thus minimizing its effect on sympathetic nerve activity.

There may be differences in the sympathetic output to regions other than the leg in our patients. However, it is noteworthy to mention that our results are consistent with other reported findings in conditions related to MS despite involving different populations, regions, and techniques. For instance, indices such as heart rate variability and urinary normetanephrine have suggested an increased sympathetic.
drive and its effects in MS patients. Also, obesity in combination with EHT has been found to either increase MSNA\textsuperscript{29} or have no effect.\textsuperscript{30} These considerations are consistent with our findings of MS being a state of sympathetic nerve hyperactivity, particularly in the presence of hypertension. Furthermore, our results on increased CVR despite similarities in arterial pressure level support our findings of increased vasoconstrictor peripheral sympathetic activity. In this respect, and as we observed, it has been known that there is no significant correlation between vasoconstrictor MSNA and arterial pressure levels.\textsuperscript{4,7} However, the increased CVR is consistent with the reported impairment of basal and insulin-induced stimulation of blood flow in skeletal muscle tissue in obesity\textsuperscript{11} and insulin resistance.\textsuperscript{32}

Based on our results, it is possible to suggest factors affecting the association between MS and sympathetic hyperactivity. First, MS components such as visceral obesity, peripheral insulin resistance, and reactive hyperinsulinemia have been found to result in sympathetic hyperactivity.\textsuperscript{5-7} In addition, the coexistence of EHT and type 2 diabetes results in an even greater sympathetic neural activation than that seen in EHT or type 2 diabetes alone.\textsuperscript{7} Increased sympathetic activity has been associated with increased plasma insulin levels regardless of the presence or absence of additional hypertension.\textsuperscript{30,33} However, hyperinsulinemia resulting from insulinoma has not been associated with increased arterial pressure\textsuperscript{34} or MSNA,\textsuperscript{35} perhaps reflecting the vasodilatory effect of insulin or downregulation of central insulin receptors.\textsuperscript{34,35} Second, it is possible that the sympathetic activation may be a primary event in the MS, eventually resulting in insulin resistance and increased sympathetically mediated lipolysis.\textsuperscript{36-38} Although accepting such an association between MS and sympathetic activity, our findings do not establish a causal relationship between them. Finally, because insulinemia and sympathetic nerve activity can differ in levels and effects according to ethnicity,\textsuperscript{39,40} we cannot extrapolate our findings beyond those found in our white subjects.

**Perspectives**

Although we did not test the risk of, or differences in, regional sympathetic activation in our patients, the finding of increased peripheral sympathetic nerve activity in MS and its further augmentation by the presence of EHT could be argued to lead to increased susceptibility to vascular complications such as atherosclerosis in patients with MS and EHT. It is already known that MS or EHT patients have a high cardiovascular risk, but what is more important regarding our results is that the combination of MS and hypertension has been reported to result in an even higher morbidity and mortality.\textsuperscript{11} It is possible, at least at the level of conjecture, that sympatholytic agents may be of use, as it has already been shown that sympatholytic agents can have a beneficial effect not only on blood pressure but also on lipid parameters and measures of glucose tolerance.\textsuperscript{41}

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References

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