Elevated Sympathetic Nerve Activity in Borderline Hypertensive Humans

Evidence From Direct Intraneural Recordings

Erling A. Anderson, Christine A. Sinkey, William J. Lawton, and Allyn L. Mark

Reports of elevated plasma catecholamine levels and augmented responses to autonomic blockade suggest increased sympathetic tone in borderline hypertension. It is not known if this reflects greater sympathetic neural outflow. We directly recorded muscle sympathetic nerve activity (microneurography) in 15 normotensive and 12 borderline hypertensive age-matched men to determine whether borderline hypertensive individuals have elevated sympathetic nerve activity. Supine heart rate, blood pressure, plasma norepinephrine, and efferent muscle sympathetic nerve activity (peroneal nerve) were measured after 6 days of both low and high dietary sodium intake (10 and 400 meq sodium/24 hr). Sympathetic nerve activity was elevated significantly in borderline hypertensive individuals on both low (37±1 bursts/min in borderline hypertensive individuals vs. 29±1 bursts/min in normotensive individuals; p<0.01) and high (25±1 bursts/min in normotensive individuals; p<0.01) sodium diets. The borderline hypertensive group had higher systolic (p<0.01) and diastolic (p<0.05) blood pressures independent of sodium intake. Across both groups, high sodium intake reduced muscle sympathetic nerve activity (p<0.001), plasma norepinephrine (p<0.001), diastolic blood pressure (p<0.02), heart rate (p<0.002), and increased weight (p<0.005). A significant (p<0.05) group-by-diet interaction was observed for plasma norepinephrine levels. Specifically, compared with the normotensive group, plasma norepinephrine levels in the borderline hypertensive group tended to be higher on low sodium diet (p=0.08) and lower on high sodium diet (p=0.23). High sodium intake increased diastolic pressure by over 5 mm Hg in six of 27 subjects (four borderline hypertensive and two normotensive). Sympathetic activity in sodium-sensitive subjects was not elevated compared with sodium-resistant subjects and also declined during high sodium intake. This study supports the hypothesis of elevated central sympathetic neural outflow in borderline hypertension. (Hypertension 1989;14:177-183)

Increasing evidence suggests that mild or borderline hypertension is characterized by augmented sympathetic activity both at rest and in response to physical and psychological stressors.\textsuperscript{1-5} For example, Esler et al\textsuperscript{6} found elevated plasma norepinephrine levels (compared with normotensive persons) in younger, but not older, hypertensive humans. Goldstein\textsuperscript{7} reviewed studies of plasma catecholamine levels in hypertension and noted that significant elevations were reported in 11 of 16 studies comparing young (less than 40 years) hypertensive individuals with normotensive individuals but in only eight of 34 studies comparing older (more than 40 years) normotensive and hypertensive individuals. These findings suggest that sympathetic activity is elevated primarily in the young mild hypertensive human.

Several studies where pharmacological probes were used also reported evidence of greater sympathetic drive in young borderline hypertensive humans. Julius and Esler\textsuperscript{8} found that the elevated heart rates and stroke volumes of young borderline hypertensive individuals returned to normal after autonomic blockade. Egan et al\textsuperscript{9} assessed vascular \(\alpha\)-adrenergic tone in 24 mildly hypertensive and 18 normotensive individuals. The intra-arterial norepinephrine concentration that increased forearm vas-
circular resistance by 30% (i.e., α-receptor sensitivity) was similar in both groups. However, mildly hypertensive subjects had elevated plasma norepinephrine levels and increased vascular α-adrenergic tone (assessed by phentolamine-induced reductions in forearm vascular resistance). They concluded that mild hypertension is associated with increased sympathetic drive.

Without directly recording sympathetic nerve activity, it is difficult to determine if elevated norepinephrine levels reflect increased neural outflow or other mechanisms such as augmented transmitter release or impaired reuptake. To date, only Wallin and colleagues have directly recorded muscle sympathetic nerve activity in hypertensive and normotensive individuals. They reported no difference between hypertensive and normotensive individuals after accounting for age (sympathetic nerve activity increases with age11). However, these studies involved patients with moderate-to-severe hypertension who were not age matched nor on controlled sodium intake.

There has been no systematic comparison of directly recorded muscle sympathetic nerve activity in young borderline hypertensive and normotensive individuals. The present study tested the hypothesis that resting sympathetic nerve activity is elevated in young borderline hypertensive individuals. Borderline hypertensive and normotensive subjects were age matched, and sympathetic nerve activity was recorded on two levels of controlled sodium intake.

Subjects and Methods

Subjects

Subjects were 12 borderline hypertensive and 15 normotensive men classified according to four seated blood pressures taken at least 1 week apart by mercury sphygmomanometer. Borderline hypertension was defined as diastolic pressure intermittently above 90 mm Hg. Normotension was defined as diastolic pressure consistently less than 85 mm Hg. Diastolic pressure was recorded using fifth phase Korotkoff sounds.

Subjects were comparable in age (24.6±0.9 years, range 21–31 in borderline hypertensive vs. 24.5±0.5 years, range 22–29 in normotensive individuals). Borderline hypertensive subjects were significantly heavier than normotensive subjects at the initial screening (87±3 vs. 77±2 kg, respectively, p<0.005).

All subjects had normal electrocardiograms, chest x-rays, urinalysis, blood counts, electrolytes, and renal and liver function. The study was approved by the Institutional Review Committee on Human Investigation, and written informed consent was obtained.

Procedure

Both diets were maintained for 6 days. Subjects continued normal activity but were asked to refrain from strenuous exercise. They reported daily to a Clinical Research Center (CRC) to receive meals, be weighed, deliver urine specimens, and have blood pressure recorded. Subjects were admitted to the CRC on dietary day 5.

On the morning of day 6, blood for plasma norepinephrine measurement was drawn from an indwelling cannula in a forearm vein 30 minutes after awakening but before subjects arose from bed. Norepinephrine levels for half the subjects in each group were determined by high-performance liquid chromatography (HPLC) (SmithKline BioScience Labs., Van Nuys, California) and by radioenzymatic assay (Cat-A-Kit, Amersham Corp., Arlington Heights, Illinois) for the other half. The latter assay was performed in the University of Iowa Cardiovascular Center Care Laboratory. Both assays were sensitive to 10 pg/ml with coefficients of variation of 10% and 6%, respectively.

On the afternoon of day 6, resting muscle sympathetic nerve activity, blood pressure, and heart rate were recorded for six consecutive 1-minute periods after 30 minutes of supine rest. Heart rate was recorded by electrocardiograph, blood pressure by an automatic sphygmomanometer (Life Stat 200, Physio Control Corp., Redmond, Washington), and muscle sympathetic nerve activity by microneurography (peroneal nerve).

Microneurography

Multifiber recordings of muscle sympathetic nerve activity were obtained from the peroneal nerve posterior to the fibular head with tungsten microelectrodes (200 μm diameter shaft; 1–5 μm uninsulated tip). A reference electrode was inserted subcutaneously 1–3 cm from the recording electrode. Electrodes were connected to a preamplifier (gain, 1,000) and amplifier (variable gain, 30–80). Neural activity was fed through a band pass filter (bandwidth, 700–2,000 Hz) and then a resistance-capacitance integrating network (time constant, 0.1 second) to obtain a mean voltage neurogram.

Three criteria indicated for an acceptable recording of muscle sympathetic nerve activity. First, electrical stimulation (2–3 V, 0.2 msec, 1 Hz) through the electrode in the peroneal nerve elicited involuntary muscle contractions but not paresthesias. Second, tapping or stretching innervated muscle elicited afferent mechanoreceptor discharges, whereas stroking the skin did not. Third, the neurogram revealed spontaneous, intermittent, pulse-synchronous bursts that increased during held expiration and phases two and three of the Valsalva maneuver. Evidence that this activity represents efferent sympathetic nerve activity was derived from earlier studies12–14 and includes 1) interruption of the activity by local nerve block proximal, but not distal, to the recording site; 2) elimination of activity by ganglionic blockade; and 3) a conduction velocity approximating 1 m/sec.

Neurograms with cutaneous sympathetic activity were not accepted. This was assessed by the response to arousal stimuli that elicited single reflex
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Sympathetic Nerve Activity in Borderline Hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low Na diet</th>
<th>High Na diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
<td>BHT</td>
</tr>
<tr>
<td>Na (meq/24 hr)</td>
<td>11±1</td>
<td>13±3</td>
</tr>
<tr>
<td>K (meq/24 hr)</td>
<td>74±4</td>
<td>70±2</td>
</tr>
<tr>
<td>Ca (mg/24 hr)</td>
<td>161±18</td>
<td>192±14</td>
</tr>
<tr>
<td>24-Hour Urine</td>
<td>343±21</td>
<td>342±21</td>
</tr>
<tr>
<td>Electrolyte</td>
<td>68±3</td>
<td>70±6</td>
</tr>
<tr>
<td>Excretion</td>
<td>228±23</td>
<td>263±20</td>
</tr>
</tbody>
</table>

Values are mean±SEM. NT, normotensives (n=15); BHT, borderline hypertensives (n=12).
*There were no significant differences between NT and BHT groups in these variables.

bursts of cutaneous, but not muscle, sympathetic activity.

Neurograms were recorded at a 5 mm/sec paper speed on a physiological recorder (model 2800S, Gould Inc., Cleveland, Ohio). Sympathetic bursts were identified by inspection and expressed as bursts per minute. Intraobserver variability in identifying bursts is low (mean 4.3%; range 0–24%). In a systematic blind scoring of 96 records from this study, interobserver variability (E.A.A. and C.A.S.) averaged 5.4±0.5% (range 0–20%).

Dietary compliance and sodium balance were assured by analysis of sodium and potassium in daily 24-hour urine collections. All subjects achieved sodium balance by day 5 (Table 1).

### Statistical Analyses

A two-factor (hypertension class and diet), repeated-measures analysis of variance was used to assess hypertension class and diet main effects and the interaction between these factors. A 0.05 level of significance was used for statistical tests. Data are presented as mean±SEM.

#### Results

Comparison of Normotensive and Borderline Hypertensive Subjects

Independent of diet, significant differences between the borderline hypertensive and normotensive groups (i.e., group main effects) were found for muscle sympathetic nerve activity, systolic and diastolic blood pressure, and weight. Independent of diet, muscle sympathetic nerve activity was significantly (*p*<0.03) greater in borderline hypertensive compared with normotensive subjects (31±1 in borderline hypertensive vs. 23±1 bursts/min in normotensive group). Follow-up tests showed that the borderline hypertensive group had significantly higher muscle sympathetic nerve activity on both low (37±1 in borderline hypertensive vs. 29±1 bursts/min in normotensive group; *p*<0.01) and high sodium diets (25±1 in borderline hypertensive vs. 16±1 bursts/min in normotensive group; *p*<0.01; Figures 1 and 2).

Systolic and diastolic blood pressures were significantly higher in borderline hypertensive versus normotensive subjects independent of diet (systolic blood pressure, 133±1 vs. 121±1 mm Hg, respectively, *p*<0.01; diastolic blood pressure, 81±1 vs. 70±6 mm Hg, *p*<0.01).

![Figure 1](http://hyper.ahajournals.org/)

*Figure 1. Neurogram of muscle sympathetic nerve activity in a normotensive subject and in a borderline hypertensive subject on both high and low sodium diets. Spikes indicate bursts of muscle sympathetic nerve activity. Muscle sympathetic nerve activity, which declined from low to high sodium diets, was consistently higher in the borderline hypertensive subject.*
TABLE 2. Blood Pressure and Heart Rate of Borderline Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=15)</th>
<th>Borderline hypertensive (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Na</td>
<td>High Na</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td>122±1</td>
<td>121±1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)†</td>
<td>74±1</td>
<td>71±1</td>
</tr>
<tr>
<td>Heart rate (beats/min)‡</td>
<td>70±1</td>
<td>65±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*Difference between blood pressure groups independent of diet (p<0.006).
†Difference between blood pressure groups independent of diet (p<0.05) and difference between diets independent of group (p<0.02).
‡Difference between diets independent of blood pressure groups (p<0.002).

73±1 mm Hg, respectively; p<0.05). Follow-up tests indicated that the borderline hypertensive group had significantly higher systolic blood pressures on both low (135±2 vs. 122±1 mm Hg, respectively, p<0.001) and high sodium diets (132±2 vs. 121±1 mm Hg, respectively, p<0.001; Table 2). Diastolic pressures (Table 2) were significantly higher in the borderline hypertensive versus normotensive group on both low (83±2 vs. 74±1 mm Hg, respectively, p<0.001) and high sodium diets (78±1 vs. 71±1 mm Hg, respectively, p<0.01).

Borderline hypertensive subjects were significantly heavier than normotensive subjects on both low (85±3 vs. 73±1 kg, p<0.001) and high sodium diets (86±3 vs. 74±1 kg, p<0.001).

The relation between weight and muscle sympathetic nerve activity was determined by correlating weight and nerve activity on both high and low sodium diets. The correlations across all subjects were nonsignificant for both high (r=0.14, p=0.47) and low (r=0.25; p=0.22) sodium diets.

Comparison of High Versus Low Sodium Diets

Significant differences between high and low sodium diets independent of hypertension class (diet main effects) were found for muscle sympathetic nerve activity, plasma norepinephrine levels, heart rate, diastolic blood pressure, and weight. Averaged across both groups, muscle sympathetic nerve activity declined from 33±1 bursts/min on low sodium diet to 20±1 bursts/min on high sodium diet (p<0.001; Figure 2). Plasma norepinephrine levels declined from 305±20 pg/ml on low sodium diet to 178±15 pg/ml on high sodium diet (p<0.001). Across both groups, heart rate declined 5 beats/min from low to high salt diets (from 72±1 to 67±1 beats/min; p<0.002). Across both groups, diastolic pressure declined by 5 mm Hg from low to high salt diets (from 79±1 to 74±1 mm Hg; p<0.02). Weight increased from 78±2 kg on low sodium to 79±2 kg on high sodium diet (p<0.005).

Hypertension Group-by-Diet Interaction

The only variable for which there was a significant group-by-diet interaction was plasma norepinephrine level (p<0.05). Plasma norepinephrine levels of the borderline hypertensive group (Table 3) tended to be higher on low sodium diet (p=0.08) and lower on high sodium diet (p=0.23).

Relation of Plasma Norepinephrine and Sympathetic Nerve Activity

The relation between plasma norepinephrine and nerve activity was determined by correlating nerve activity...
activity and norepinephrine levels measured during both diets. The correlation across all subjects was significant during low (r=0.60, p<0.002) but not high sodium diets (r=0.13, p=0.52).

Sodium-Sensitive Versus Sodium-Resistant Subjects

To further examine the effects of high sodium, subjects were classified as sodium sensitive or sodium resistant as defined by a 5 mm Hg or more increase in diastolic blood pressure from low to high sodium diets. Six sodium-sensitive subjects were identified (two normotensive and four borderline hypertensive). Sodium-sensitive and sodium-resistant subjects differed only in diastolic blood pressure response to the two sodium diets. Diastolic pressure in sodium-sensitive subjects increased from 77±1 on low sodium to 83±1 mm Hg on high sodium diet and declined in resistant subjects from 80±1 on low sodium to 73±1 mm Hg on high sodium diet (group-by-diet interaction, p<0.0001). Muscle sympathetic nerve activity in sodium-sensitive subjects was 36±1 bursts/min on low sodium diet and 23±1 bursts/min on high sodium diet. Muscle sympathetic nerve activity in resistant subjects was 32±1 bursts/min on low sodium diet and 19±1 bursts/min on high sodium diet (group-by-diet interaction, p=0.86). Plasma norepinephrine levels of sodium-sensitive and sodium-resistant subjects were 326±40 and 299±23 pg/ml, respectively, on low sodium and 177±24 and 179±18 pg/ml, respectively, on high sodium diet.

Critique of Methods

The strengths of the study include the comparison of directly recorded sympathetic nerve activity in age-matched borderline hypertensive and normotensive subjects. Further, sympathetic nerve activity was recorded during two levels of rigorously controlled dietary sodium intake.

A possible limitation of the study is analysis of muscle sympathetic nerve activity as bursts per minute (i.e., frequency). Microneurographically measured muscle sympathetic nerve activity can be quantified as burst frequency and as integrated activity (i.e., burst frequency times mean burst amplitude). Whereas integrated activity should most accurately reflect muscle sympathetic nerve activity, burst amplitude is determined by amplifier gain and proximity of the electrode to a nerve fascicle. It is, therefore, not possible to compare integrated activity across recording sessions or between subjects. Rather, burst frequency provides the best index of muscle sympathetic nerve activity when making such comparisons.

A theoretical limitation to analysis of muscle sympathetic nerve activity as burst frequency is the equal weighting of small and large bursts. However, analysis of burst frequency and integrated activity usually yields similar conclusions. In addition, resting sympathetic nerve activity expressed as frequency is quite reproducible across experimental sessions spanning several months. Moreover, nerve activity recorded simultaneously from different nerves (e.g., radial and peroneal) reveal marked similarity in pattern and burst frequency. Finally, muscle sympathetic nerve activity expressed as frequency correlates with forearm venous norepinephrine when measured simultaneously. Thus, despite some limitations, analysis of muscle sympathetic nerve activity as frequency is assumed to accurately reflect sympathetic activity.

Physiological Significance

Our finding of elevated sympathetic nerve activity in borderline hypertensive individuals parallels the reports of increased sympathetic drive by Egan et al and Esler et al. Further, the reduction in muscle sympathetic nerve activity and plasma norepinephrine with high sodium intake is consistent with reports of reduced plasma norepinephrine levels during high sodium diets in normal subjects.

Interestingly, compared with values in normotensive subjects, plasma norepinephrine levels in bor-
derline hypertensive subjects were higher on low sodium and lower on high sodium diets. However, muscle sympathetic nerve activity was higher in borderline hypertensive than in normotensive subjects on both diets. Although muscle sympathetic nerve activity correlates with simultaneously measured plasma norepinephrine levels, each reflects a different aspect of the sympathetic system. Muscle sympathetic nerve activity reflects central sympathetic outflow, whereas plasma norepinephrine levels are influenced by central sympathetic outflow as well as by peripheral mechanisms such as altered release or reuptake into nerve terminals and spillover from different vascular beds. The differential effect of sodium on norepinephrine levels versus muscle sympathetic nerve activity in borderline hypertensive subjects suggests an alteration of peripheral release/reuptake mechanisms by sodium that is independent of changes in central sympathetic outflow.

Although our results demonstrate that central sympathetic outflow is elevated in borderline hypertensive subjects independent of sodium intake, the data also suggest that plasma norepinephrine levels can vary with level of dietary sodium independent of changes in nerve activity. Specifically, plasma norepinephrine levels and sympathetic nerve activity were correlated significantly on low sodium diet ($r=0.60$) but not on high sodium diet ($r=0.13$). Thus, sympathetic nerve activity may reflect norepinephrine levels on low but not high sodium intake. However, these results should be interpreted cautiously since plasma norepinephrine and muscle sympathetic nerve activity were not measured simultaneously.

The current findings contrast with reports by Wallin and colleagues who found no significant difference in muscle sympathetic nerve activity between normotensive and hypertensive subjects after controlling for age differences. However, the differences between the current study and those by Wallin and colleagues were not unexpected. They studied established hypertensive subjects who were considerably older (mean ages 43, 39, and 42 years) than the subjects in the current study (mean age 24 years). Studies of plasma norepinephrine levels suggest that sympathetic nerve activity is more likely to be elevated in young borderline hypertensive persons rather than in older individuals with established hypertension.

Obesity is a risk factor for hypertension. Our borderline hypertensive subjects were approximately 11 kg heavier than the normotensive subjects. However, the correlation between weight and sympathetic nerve activity was quite small. Izzo et al have also reported no correlation between weight and plasma norepinephrine levels. Thus, it seems unlikely that the elevated sympathetic nerve activity in borderline hypertensive subjects can be attributed to weight differences.

High sodium intake can elevate blood pressure in some individuals. Gavras has hypothesized that high sodium intake increases central sympathetic outflow by decreasing central $\alpha_2$-adrenergic receptor affinity for agonist neurotransmitters. However, Zimlichman et al found high dietary sodium intake did not alter plasma norepinephrine decreases in response to clonidine (a centrally acting $\alpha_2$-agonist) in hypertensive or normotensive individuals. In the current study, although high sodium intake reduced diastolic blood pressure by an average of 4 mm Hg across all subjects, six subjects (four borderline hypertensive and two normotensive) had diastolic blood pressure increases of over 5 mm Hg while on the high sodium diet. Sympathetic nerve activity and plasma norepinephrine levels in these sodium-sensitive subjects were comparable with those in sodium-resistant subjects on both high and low sodium diets. This suggests the increase in blood pressure was not related to augmented central sympathetic outflow. However, high sodium intake has been shown to alter peripheral sympathetic mechanisms (e.g., vascular reactivity to infused norepinephrine).

The reduction in plasma norepinephrine contrasts with Campese et al who reported that sodium-sensitive individuals fail to suppress norepinephrine during sodium loading. The differing results may reflect the fact that Campese et al studied essential hypertensive individuals, whereas we studied young borderline hypertensive individuals.

In this study, sympathetic nerve activity was measured during supine rest. Therefore, we can not address the question of whether sympathetic nerve responses to reflex stimuli (e.g., the cold pressor test or lower body negative pressure) or other stimuli known to increase sympathetic outflow (e.g., mental stress) may be greater in borderline hypertensive individuals or exaggerated during sodium loading.

In summary, this study provides direct evidence for increased central sympathetic outflow in borderline hypertensive individuals independent of dietary sodium intake. In addition, high sodium intake reduced sympathetic activity to a similar extent in normotensive and borderline hypertensive individuals as well as in the relatively small number of sodium-sensitive subjects.

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