Sympathetic Neural Burst Amplitude Distribution
A More Specific Indicator of Sympathoexcitation in Human Heart Failure

Yrsa Bergmann Sverrisdóttir, PhD; Bengt Rundqvist, MD, PhD;
Gudmundur Johannsson, MD, PhD; Mikael Elam, MD, PhD

Background—Human muscle sympathetic nerve activity (MSNA) is usually measured as the number of pulse-synchronous bursts in multiunit mean voltage recordings. We recently suggested burst amplitude distribution as a more sensitive indicator of altered MSNA in congestive heart failure (CHF). Here, we test whether this distribution can discriminate between different conditions with increased MSNA burst frequency and whether it reflects single vasoconstrictor fiber firing intensity.

Methods and Results—We analyzed resting multiunit MSNA in 36 CHF patients (24 with mild to moderate CHF, 12 with severe CHF investigated before and after heart transplantation), 14 patients with pituitary deficiency, 25 matched healthy control subjects, and an additional 56 healthy men with a wider age range (21 to 71 years). Pituitary deficiency was associated with increased MSNA burst frequency (60 versus 37 bursts/min in control subjects), equivalent to that in mild to moderate CHF (61 bursts/min). However, burst amplitude distribution in hypopituitary patients (median burst amplitude, 37%) did not deviate from matched control subjects (36%), whereas amplitudes increased with disease severity in CHF (43% in mild to moderate, 52% in severe) and normalized after transplantation (36%). In the larger healthy group, MSNA burst frequency increased with age, and burst amplitude distribution remained unaffected. In 8 CHF patients, single-unit firing frequency showed a close positive relationship to multiunit burst amplitude distribution ($r=0.82$, $P<0.01$) but none to burst frequency ($r=0.39$, $P=0.3$).

Conclusions—Muscle vasoconstrictor fiber activity is better reflected by multiunit MSNA burst amplitude distribution than by burst frequency, at least in CHF. This distribution can discriminate between conditions with increased burst frequency. (Circulation. 2000;102:2076-2081.)

Key Words: nervous system, autonomic ■ heart failure ■ hormones ■ aging

Human muscle sympathetic nerve activity (MSNA) arises from vasoconstrictor fibers directed to the muscle vascular bed and is involved mainly in dynamic blood pressure regulation. An inhibitory influence from arterial baroreceptors provides a dominating control mechanism for MSNA, resulting in cardiac rhythmicity and an inverse relationship to diastolic blood pressure variations. Quantification of MSNA in intraneural recordings is usually based on counting the neural bursts identified by inspection of a mean voltage neurogram. Burst area or amplitude can be measured to evaluate relative changes in MSNA within a recording session, but absolute measures of burst size cannot be used for interindividual comparisons for technical reasons. Thus, interindividual or group comparisons of resting MSNA are usually based on burst counts over time (burst frequency) or, when differences in heart rate are accounted for, on bursts per 100 heartbeats (burst incidence). Because of the large interindividual variability of these 2 quantitative measures in healthy subjects, moderate differences in MSNA between groups of subjects in cross-sectional studies are difficult to identify. However, a recent study from our laboratory of patients with mild to moderate congestive heart failure (CHF) indicated that the relative burst amplitude distribution is shifted toward larger bursts before the occurrence of a significantly augmented burst frequency, suggesting that analysis of MSNA burst amplitude distribution may provide a more sensitive indicator of altered sympathetic discharge in early stages of CHF.

In the present study, we tested the hypothesis that MSNA burst amplitude distribution may also discriminate between conditions with increased MSNA burst frequency by comparing amplitude distributions in multiunit MSNA recordings from 3 conditions known to be associated with a high burst frequency. The first condition was CHF, in which increased MSNA burst frequency is a well-established finding indicating that the relative burst amplitude distribution is shifted toward larger bursts before the occurrence of a significantly augmented burst frequency, suggesting that analysis of MSNA burst amplitude distribution may provide a more sensitive indicator of altered sympathetic discharge in early stages of CHF.

Received March 30, 2000; revision received May 19, 2000; accepted June 8, 2000.
From the Departments of Clinical Neurophysiology (Y.B.S., M.E.) and Cardiology (B.R.) and the Research Center for Endocrinology and Metabolism (G.J.), Sahlgren University Hospital, Göteborg, Sweden.
Correspondence to Dr Yrsa Bergmann Sverrisdóttir, Institute for Clinical Neuroscience, Department of Clinical Neurophysiology, Sahlgren University Hospital, S-41345 Göteborg, Sweden. E-mail yrsa.sverrisdottir@neuro.gu.se
© 2000 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org
ized after orthotopic heart transplantation.\textsuperscript{11–15} we also analyzed the burst amplitude distribution before and after transplantation. The second group consisted of patients with pituitary deficiency and untreated growth hormone deficiency (GHD), a neuroendocrine disease recently shown to be associated with increased MSNA burst frequency, in all probability of central origin.\textsuperscript{16} The patient categories were

\begin{table}[h]
\centering
\caption{Basic Characteristics of the Primary Study Group}
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Variables & Control & GHD & CHF (II-IIIA) & CHF (IIIB-IV) & HTx \\
\hline
Number/Sex & 25 (22 M/3 F) & 14 (11 M/3 F) & 24 (19 M/5 F) & 12 M & 12 M \\
Age, y & 52 (38–68) & 56 (46–69) & 56 (40–75) & 51 (36–61) & 51 (36–61) \\
BMI, kg/m\textsuperscript{2} & 25 (22–35) & 26 (19–31) & 26 (19–33) & 26 (20–32) & 26 (21–34) \\
Heart rate, bpm & 61 (47–88) & 61 (48–86) & 78 (58–98)\textsuperscript{*} & 86 (68–112)\textsuperscript{*} & 87 (40–107)* \\
MAP, mm Hg & 94 (79–115) & 101 (85–123)* & 91 (76–119)\textsuperscript{†} & 77 (67–87)\textsuperscript{*} & 109 (97–127)\textsuperscript{†} \\
MSNA, bursts/min & 37 (17–58) & 60 (41–76)* & 61 (44–92)* & 78 (64–96)* & 38 (25–53)|| \\
MSNA, bursts/100 heartbeats & 59 (30–93) & 83 (63–99)* & 78 (56–98)* & 89 (71–98)* & 45 (28–64)§ \\
MSNA, median burst amplitude, % & 36 (24–50) & 37 (25–49) & 43 (28–59)* & 52 (44–61)* & 36 (23–45)§ \\
\hline
\multicolumn{6}{|l|}{Data are presented as means (ranges).} \\
\multicolumn{6}{|l|}{\textsuperscript{*}Statistically significant difference (P<0.001) between control subjects and patient groups.} \\
\multicolumn{6}{|l|}{\textsuperscript{†}Statistically significant difference (P<0.05) between GHD and CHF groups; \textsuperscript{‡}P<0.001.} \\
\multicolumn{6}{|l|}{\textsuperscript{§}Statistically significant difference (P<0.01) between CHF (IIIB-IV) and HTx group; \textsuperscript{||}P<0.001.} \\
\end{tabular}
\end{table}

Cardiac Transplant Recipients
A separate study group consisting of 12 severe cardiac failure patients (left ventricular ejection fraction, 0.18±0.08), previously investigated before and after cardiac transplantation (HTx), was reanalyzed to evaluate putative changes in burst amplitude distribution related to the reduction in MSNA burst frequency that occurs after HTx.\textsuperscript{15} The patients in the severe CHF group were classified as being in NYHA functional class IIIB to IV, 5 with coronary artery disease and 7 with idiopathic dilated cardiomyopathy.

Aging
In addition to the main study group, we also reanalyzed a larger group (n=56) of previously investigated normotensive (mean arterial pressure, 91±10.7 mm Hg), normal-weight (BMI, 24.4±2.5 kg/m\textsuperscript{2}), healthy men (unpublished data) with an age range of 21 to 71 years, (43±18 years) to evaluate the effect of age on the different measures of MSNA.

Comparison With Single-Unit Discharge
Of the above CHF patients, 8 were previously used in a study of the firing characteristics of single muscle vasoconstrictor fibers.\textsuperscript{18} These data are in the present study compared with the burst amplitude distribution and burst frequency of a multunit MSNA recording performed in the same experimental session as the previously reported single-unit data. In subjects in whom >1 individual vasoconstrictor nerve fiber was recorded from (2 fibers in 2 patients, 3 fibers in 3), data for ≥2 fibers were averaged before comparison with multunit data from that subject.

The Human Ethics Committee at the University of Göteborg approved the experimental procedures, and all subjects gave their informed consent to the procedure.

Methods

Subjects
The main study group consisted of 2 patient categories with marked augmentation of MSNA burst frequency and a group of healthy matched control subjects.

Cardiac Failure Patients
Twenty-four patients with mild to moderate cardiac failure (left ventricular ejection fraction, 0.28±0.09) in NYHA II to IIIA were investigated, 6 on the basis of coronary heart disease and 18 with idiopathic dilated cardiomyopathy. All the CHF patients and transplant recipients (see below) were investigated without withdrawal of ongoing medication (ACE inhibitors, digoxin, diuretics, nitrates, standard triple immunosuppression, and calcium channel blockers).

Pituitary-Deficient Patients
This group consisted of 14 patients with hypopituitarism caused primarily by nonsecreting pituitary adenomas and its treatment. All patients in this group had untreated GHD as verified by an insulin tolerance test. When appropriate, the patients had received stable replacement therapy with glucocorticoids (n=6), thyroxine (n=8), and gonadal steroids (n=8) ≥6 months before the study.

Control Subjects
Twenty-five healthy subjects matched for age, sex, and body mass index (BMI) were recruited as MSNA control subjects for the mild to moderate CHF and GHD patient groups (Table 1).

Nerve Recording
Multunit recordings of effenter postganglionic sympathetic nerve activity were obtained with a tungsten microelectrode with a tip diameter of a few microns inserted into a muscle fascicle of the peroneal nerve posterior to the fibular head. A low-impedance reference electrode was inserted subcutaneously a few centimeters away. When a muscle nerve fascicle had been identified, small electrode adjustments were made until a site was found in which
spontaneous, pulse-synchronous bursts of neural activity that increased during voluntary apnea but did not respond to arousal stimuli (such as noise or pinching) could be recorded. Details of the nerve recording technique and criteria for MSNA have been reported previously. The original nerve signal was amplified with a gain of 50,000 and fed through a band-pass filter with a band width of 700 to 2000 Hz and then through an integrating network with a time constant of 0.1 second to obtain a mean voltage display of nerve activity. Both the filtered and mean voltage neurograms were stored on analog tape (Racal V-Store, Racal Recorders Ltd) and on a computer (sampling frequency, 200 Hz), together with an ECG (via standard chest leads) and respiratory movements (via a strain gauge attached to a rubber strap around the chest). During the experiments, recorded variables were also monitored on a storage oscilloscope (Tektronix 549, Tektronix Beaverton) and an ink-jet recorder (modified Mingograph 800, Siemens-Elema Ltd).

**Statistical Analysis**

After a stable recording site was acquired, resting MSNA was recorded for 15 to 20 minutes. Data from the last 5 minutes were used for analysis. Bursts were identified by inspection of the mean voltage neurogram, aided by computer software developed in the laboratory, and MSNA was expressed as burst frequency (bursts per minute) and burst incidence (bursts per 100 heartbeats). To obtain a relative burst amplitude distribution, the amplitude of the largest burst that occurred during the analyzed period was set to 100%, and other burst amplitudes were expressed as a percentage of the maximal burst. From the burst amplitude distribution, a median burst amplitude (the value at which 50% of the burst amplitudes were larger and 50% were smaller) was extracted and used for statistical analysis.

The results are presented as the mean ± SD (range). Comparisons between the study groups were performed with the Kruskal-Wallis ANOVA median test followed by the Mann-Whitney U test when appropriate. Correlations were examined by calculating the Pearson linear correlation coefficient. P < 0.05 was considered significant.

**Results**

The subjects in the main study group did not differ in terms of age, sex, or BMI (Table 1). The pituitary-deficient patients did not deviate from the control subjects in heart rate but had significantly higher mean arterial pressure (P < 0.05). In CHF patients, heart rate increased with disease severity and was high in the denervated heart after HTx. Both CHF groups and the HTx group had significantly higher heart rate than the control subjects and the pituitary-deficient patient group. The mean arterial pressure ranged from lower than that of control subjects in the severe CHF (P < 0.001), equal to that of control subjects in mild to moderate CHF (P = 0.2), to higher than that of control subjects in the HTx group (P < 0.001; Table 1).

**Differences in MSNA Between CHF and Hypopituitary Patients**

Whereas MSNA burst frequency and incidence were increased to a similar degree in both the mild to moderate CHF and hypopituitary patient groups compared with control subjects (Table 1 and Figure 1), the MSNA median burst amplitude was increased only in the CHF patients (P < 0.001), and burst amplitude distribution in the hypopituitary patients did not differ from the control subjects (P = 0.97). In the CHF group (mild, moderate, and severe), all MSNA parameters increased with disease severity and were significantly reduced after HTx. MSNA burst frequency and median burst amplitude in the HTx group did not differ from the control subjects, whereas MSNA burst incidence was significantly lower (P < 0.01).

**Age-Related Changes in MSNA**

In the larger group of healthy subjects with an age range of 21 to 71 years, MSNA burst frequency and incidence showed a positive relationship with age (r = 0.65 and 0.64, respectively; P < 0.0005 for both). In contrast, MSNA median burst amplitude remained unchanged throughout the age range (Table 2).

**Relationship Between Multiunit and Single-Unit MSNA**

In 8 CHF patients in whom both single-unit and multiunit MSNA was analyzed, multiunit burst amplitude distribution was closely correlated to the firing frequency of individual muscle vasoconstrictor nerve fibers (r = 0.84, P < 0.01; Figure 2), whereas no significant correlation was found between single-unit activity and multiunit burst frequency (r = 0.39, P = 0.3).

**Discussion**

The main findings of the present study are that (1) the distribution of multiunit MSNA burst amplitudes can discriminate between different conditions with a similar MSNA burst frequency augmentation and (2) the firing frequency of individual vasoconstrictor fibers is related more closely to MSNA burst amplitude distribution than to MSNA burst frequency. This indicates that burst amplitude distribution...
may be a more sensitive indicator of MSNA intensity, which can be used to distinguish between conditions with sympathetic nerve hyperactivity of different origin.

**Burst Amplitude Distribution Illustrates Sympathoexcitation in CHF**

The sympathetic activation in CHF is well established,\(^1\) with increased MSNA being paralleled by increased total body, renal, cardiac, and central nervous system norepinephrine spillover.\(^1\) Studies focusing on milder degrees of CHF have found an increased number of MSNA bursts early in the disease. In contrast to the previous study from our laboratory,\(^2\) the present study also finds a significant augmentation of MSNA burst number in mild to moderate CHF. This difference between 2 studies from the same laboratory highlights the previously recognized problem with a large interindividual variability of MSNA burst number in group comparisons, making subject selection crucial. In fact, the discrepancy between studies of mild CHF recently prompted our first attempt to use MSNA burst amplitude distribution for group comparisons. In agreement with the findings in our present mild to moderate CHF group, the patients in the earlier study from our laboratory were subsequently shown to have a greater proportion of large bursts than healthy control subjects, despite not differing in burst number, and we suggested that burst amplitude distribution may be a more sensitive indicator of altered discharge intensity than traditional burst counts. This notion is strongly underlined by the present finding that the firing frequency of individual vasoconstrictor fibers is closer related to multiunit burst amplitude distribution than to burst frequency (see also below) in a group of patients with CHF ranging from mild to severe.

The underlying mechanisms for sympathetic hyperactivity in CHF are not fully elucidated, but impaired baroreflex control has been shown in severe and mild to moderate CHF.\(^2\) This impairment of baroreflex restraint of sympathetic neuronal outflow, whether caused by peripheral or central alterations,\(^3\) has therefore been proposed as a plausible mechanism for the sympathoexcitation characterizing this condition. However, sympathoexcitation as a compensatory response, elicited by still-functioning baroreceptors sensing an increasingly lowered blood pressure, may contribute. The improved hemodynamic situation and the normalization of baroreflex function within weeks after HTx\(^4\) could both be responsible for the rapid postoperative reduction in MSNA\(^5\) and the normalization of MSNA burst amplitude distribution shown in this study.

Ignoring the present findings concerning the relationship between single-unit firing frequency and multiunit burst amplitude distribution, we could argue that alterations in MSNA burst amplitude distribution are simply a consequence of the decreased heart rate per se being responsible for the change in MSNA burst amplitude distribution. The diastolic blood pressure variability (determined as the within-person SD) was significantly reduced in the severe compared with the mild to moderate CHF group (\(P<0.001\)) but did not differ between mild to moderate CHF and hypopituitary patients, making diminished blood pressure variations a less likely cause for the change in MSNA burst amplitude distribution. In fact, the increased proportion of large MSNA bursts in our mild to moderate CHF patients, despite a blood pressure variability similar to that in hypopituitary patients, may indicate that this change in distribution is reflecting the impairment of baroreflex control over sympathetic outflow.

**Burst Amplitude Distribution Is Normal in Other Conditions With High Burst Frequency**

Both MSNA burst frequency and burst incidence are known to increase with age,\(^1\) a development recently found to be linked to the decrease in secretion of insulin-like growth factor-I levels in somatopause (unpublished observations). Adults with GHD and aging healthy subjects thus share a decline in insulin-like growth factor-I and a rise in MSNA burst frequency.\(^6\) Our present finding that MSNA burst amplitude distribution remains unaffected during aging and in hypopituitary patients contrasts the findings in CHF, clearly illustrating that this MSNA variable can be used to discriminate between different conditions characterized by increased MSNA burst frequency. This discrepancy between CHF and aging/hypopituitarism may support the view of McAllen and Malpas\(^7\) that sympathetic burst probability and burst amplitude are controlled independently.

The notion that burst amplitude distribution could be maintained by baroreflex modulation agrees well with the fact that baroreflex control of MSNA has been shown to remain unchanged during aging.\(^8\) Hypopituitarism and untreated GHD are linked to a higher incidence of hypertension,\(^9\) but although our patients had a higher blood pressure than the matched control subjects, they were not hypertensive. Given that impairment of baroreflex function has also been found in hypertension, one could argue that hypopituitary patients (and those with other conditions) who develop...
significantly hypertension eventually should alter their MSNA burst amplitude distribution, if this measure indeed reflects baroreflex modulation. However, in contrast to the impaired baroreflex control of heart rate, the baroreflex control of MSNA has been shown to remain intact in both primary and secondary hypertension. Thus, the difference in MSNA burst amplitude distribution between CHF and hypopituitary patients would probably persist even if the latter patient group developed hypertension.

**Study Limitations**

Given the possibility that baroreflex modulation governs MSNA burst amplitude distribution (see above), the lack of a baroreflex test is a limitation of the present study. However, reciprocal changes in MSNA median burst amplitude and blood pressure during pharmacological baroreflex provocation have been demonstrated in normal subjects with intact baroreflex function. The present data include reanalysis of several previous studies from our laboratory, precluding the addition of a baroreflex test, and we base our suggestion of baroreflex involvement on the previously published evidence for decreased baroreflex control of sympathetic outflow in CHF that is normalized after HTx and on the evidence for normal sympathetic baroreflex control in aging. Baroreflex control of MSNA is usually investigated with short-term pharmacological interventions, raising blood pressure with phenylephrine and/or lowering blood pressure with nitroprusside. If future studies can establish that the burst amplitude variation at rest is governed by baroreflex modulation, analysis of MSNA burst amplitude distribution may provide a less invasive, and thus safer, test of baroreflex control of sympathetic outflow.

Our conclusion that multunit MSNA burst amplitude distribution reflects the firing frequency of individual vasoconstrictor neurons, whereas multunit burst frequency does not, is limited to CHF because no such comparisons were made in hypopituitary or aging subjects. However, in 2 previous reports by Macefield and coworkers on healthy subjects with low and high multunit MSNA burst frequency, the average firing frequency of individual vasoconstrictor neurons was lower in the study group with higher multunit burst frequency. Thus, the poor ability of multunit MSNA burst frequency to predict the discharge intensity of individual vasoconstrictor fibers seems to be a general phenomenon.

Finally, the fact that ongoing pharmacological treatment was maintained in our CHF patients may be considered a limitation of the study. However, we chose this strategy to avoid rebound cardiovascular responses and associated baroreceptor-mediated effects on sympathetic nerve traffic.

In summary, muscle vasoconstrictor fiber activity is better reflected by multunit MSNA burst amplitude distribution than by burst frequency. The amplitude distribution can discriminate between conditions with increased MSNA burst frequency of different origin.

**Acknowledgments**

This study was supported by the Swedish Medical Research Foundation (grant 12170), the Swedish Heart and Lung Foundation, and the Medical Faculty of Göteborg. We thank Tomas Karlsson and Gun Bodehed-Berg for excellent technical assistance.

**References**


Sympathetic Neural Burst Amplitude Distribution: A More Specific Indicator of Sympathoexcitation in Human Heart Failure
Yrsa Bergmann Sverrisdóttir, Bengt Rundqvist, Gudmundur Johannsson and Mikael Elam

_Circulation_. 2000;102:2076-2081
doi: 10.1161/01.CIR.102.17.2076

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/17/2076

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/