Relationship between size and latency of action potentials in human muscle sympathetic nerve activity

Aryan Salmanpour,1,2 Lyndon J. Brown,1 Craig D. Steinback,2 Charlotte W. Usselman,2 Ruma Goswami,2 and J. Kevin Shoemaker2,3
1Department of Electrical and Computer Engineering; 2Neurovascular Research Laboratory, School of Kinesiology, Faculty of Health Sciences; and 3Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

Submitted 23 September 2010; accepted in final form 22 March 2011

Sympathetic burst both at baseline (P < 0.001) and LBNP (r = 0.61 ± 0.12; P < 0.001). In addition, the amplitude of detected action potentials within sympathetic bursts was directly related to the increased burst size at both baseline (r = 0.59 ± 0.16; P < 0.001) and LBNP (r = 0.61 ± 0.12; P < 0.001). Also, the amplitude of detected action potentials within sympathetic bursts was directly related to the increased burst size at both baseline (r = 0.59 ± 0.16; P < 0.001) and LBNP (r = 0.61 ± 0.12; P < 0.001). In addition, the number of detected action potentials and the number of distinct action potential clusters within a given sympathetic burst were correlated at baseline (r = 0.7 ± 0.1; P < 0.001) and during LBNP (r = 0.74 ± 0.03; P < 0.001). Furthermore, action potential latency (i.e., an inverse index of neural conduction velocity) was decreased as a function of action potential size at baseline and LBNP. LBNP did not change the number of detected action potentials and unique clusters per sympathetic burst. It was concluded that there exists a hierarchical pattern of recruitment of additional faster conducting neurons of larger amplitude as the sympathetic bursts become stronger (i.e., larger amplitude bursts). This fundamental pattern was evident at rest and was not altered by the level of baroreceptor unloading applied in this study.

postganglionic sympathetic nerve activity; microneurography; action potential detection; size principle

THE FIRST RECORDINGS OF HUMAN postganglionic sympathetic nerve recordings were reported by Hagbarth and Vallbo (1968), initiating an exciting era in research regarding autonomic outflow and the neural control of the cardiovascular system (Delius et al. 1972; Wallin and Charkoudian 2007). Traditionally, due to relatively poor signal-to-noise aspects, analysis of this multifiber recording from human peripheral nerves has, to a large extent, been constrained to the integrated neurogram signal through band-pass filtering, rectification, and integration. These steps reduce background noise and provide a quantitative measure of sympathetic outflow but eliminate all action potential information. For instance, the number of action potentials and their morphologies that contribute to a given sympathetic burst are lost during the integration process.

Nonetheless, observations based on the integrated neurogram have led investigators to hypothesize details about the properties of the single neurons that make up the multifiber response. For example, variations in the size of the integrated bursts are deemed to reflect recruitment patterns from a pool of axons within the recording field of the electrode; however, uncertainty continues regarding the size of the pool of postganglionic sympathetic axons from which variations in recruitment can occur. Also, a provocative observation in human sympathetic recordings is that the conduction of sympathetic traffic, based on the delay of the burst from a representative R wave of the ECG (Wallin et al. 1994), is inversely related to burst size. As burst size is expected to be related to the number of action potentials occurring within the same burst, the shorter reflex latency of larger bursts is hypothesized to be due to 1) variations in synaptic delays between the brain stem and peripheral nerve; that is, more discharges of the same neurons per burst; and/or 2) more than one population of efferent sympathetic neurons with progressive recruitment thresholds and conduction velocities (Wallin et al. 1994). Variations in baroreflex control of burst occurrence vs. burst size have led some investigators to postulate different sites of control over these two features (i.e., the number of units recruited within a sympathetic burst; Kienbaum et al. 2001). Macefield and Wal- lin (1999) have provided considerable advances regarding the discharge properties of single sympathetic neurons in humans. These studies indicate that such neurons discharge in a probabilistic manner, typically firing only once in a sympathetic burst (Macefield et al. 1994; Macefield and Wallin 1999). However, multiple within-burst firings of the same neuron occur with increasing stress (Macefield and Wallin 1999) and with some pathologies (Elam and Macefield 2001; Elam et al. 2002). From these studies on single-fiber recordings, Macefield and Wallin (1999) have proposed that 1) between-individual variations exist in the number of active sympathetic neurons and 2) increases in firing probability of active neurons may be the primary mechanism by which burst intensity (size) is augmented.

By analogy, one may consider that the properties of excit- able neurons are similar across neural systems. For example,
recruitment of additional motor units of increasing size is the strategy employed during muscular contraction of growing intensity (Adrian and Bronk 1929; Henneman 1957). Here the larger neurons have a higher recruitment threshold (they are not recruited at rest) and, compared with smaller axons, exhibit faster conduction velocities (Henneman et al. 1965). Moreover, the firing rates of active neurons increases after recruitment (Westgaard and De Luca 2001). Whether such an ordered pattern is a property of other excitable nerve systems, such as the sympathetic nervous system, remains untested.

To study postganglionic sympathetic discharge patterns, we developed a technique that enables the identification and morphological classification of multiple vasomotor neurons contributing to sympathetic bursts (Salmanpour et al. 2010a,b). This method was applied to human muscle sympathetic nerve activity (MSNA) data obtained at rest and during lower body negative pressure (LBNP) to elicit a wide range of MSNA. Using this approach, we tested the hypothesis that variations in human MSNA are related to recruitment of a new population of efferent sympathetic fibers that differ in terms of action potential size and latency. The alternate outcome is that the increase in MSNA is accomplished primarily by increasing the firing probability of low-threshold axons that are present under baseline conditions. Our aim was to extract action potential content from extracellular sympathetic recordings during baseline and LBNP of –60 mmHg and classify these action potentials based on their peak-to-peak amplitude. LBNP was used to increase the sympathetic activity via baroreflex unloading to test the role of baroreceptor input in the regulation of action potential recruitment. This approach of action potential classification complements the single-unit approach that has been proposed previously (Macefield et al. 1994; Macefield and Wallin 1999) by emphasizing the total number of action potentials per sympathetic burst, and the size of these action potentials, but not how a single axon behaves over time.

METHODS

Subjects

Ten healthy individuals (5 males, 5 females) participated in this study. They were 23–30 yr in age, 47–98 kg in weight, and 160–188 cm in height. All participants were healthy as determined by a medical questionnaire and family history. The participants provided signed consent to the study procedures, and these procedures were approved by the Human Subjects Research Ethics Board at The University of Western Ontario.

Experimental Protocol

Data were obtained from supine individuals during 5 min of baseline and 3 min of LBNP –60 mmHg. In addition, in a second study, MSNA was recorded during a 3-min rest period and 3 min of –80 mmHg LBNP in five individuals from whom adequate nerve signals were sustained at this level of simulated orthostasis. The difficulties of obtaining quality SNA data at high levels of LBNP include movement of subject (leg), syncope, appearance of apnea but not during arousal to a loud noise (Hagbarth and Vallbo 1968), and burst probability was inversely related to diastolic blood pressure (Sundlof and Wallin 1978). Neurograms were measured with a nerve traffic analysis system (662C-3; Bioengineering of University of Iowa, Iowa City, IA). The neural signal was preamplified with a gain of 1,000 (using preamplifier and isolation amplifier, gain = 100 and 10, respectively) and further amplified with a gain of 75 (using a variable gain amplifier gain = 0.1–99). This neuronal activity was then band-pass filtered (bandwidth of 700–2,000 Hz), and the filtered MSNA was rectified and integrated. Integration is accomplished with a leaky integrator set with a 0.1-s time constant. All signals were digitized with a data acquisition system (PowerLab software ADInstruments) at a sampling rate that varied from 100 Hz for the rectified and integrated neurogram to 10 kHz for the amplified and filtered neurogram. Previous work from our laboratory (unpublished data) indicates that the band-pass filter settings applied to the raw data do not impair our ability to detect action potential waveforms. However, the impact of signal conditioning, including amplifier characteristics and band-pass filter settings, on action potential morphology requires further assessment.

Integrated MSNA Burst Analysis

Integrated bursts of MSNA were identified as exhibiting pulse-synchrony, having a signal-to-noise ratio of least 2:1 (i.e., the ratio of the amplitude of the burst and the baseline) with respect to the previous period of neural silence between bursts, having characteristic rising and falling slopes, and increasing in incidence and size during end-expiratory apnea but not startle. Burst occurrence was confirmed by visually inspecting the corresponding raw neurogram.

The level of sympathetic activity was expressed both as number of bursts per 100 heartbeats (burst incidence) and burst per minutes (burst frequency). In addition, the strength, or size, of each burst was quantified based on the height of each burst from its previous minimum baseline level. The impact of LBNP on burst strength (height) was determined using the absolute burst height values at baseline and LBNP.

Reflex latency was determined using the approach introduced by Fagius and Wallin (1980) and used frequently since. This process is simply the time delay between the R wave related to the burst (the previous R wave in which the burst was observed) and the peak of the burst (the highest point of the integrated MSNA burst). Reflex latency was calculated for each burst and averaged for all detected bursts in each condition and volunteer (mean reflex latency). The baroreflex threshold diagram was used to compare the arterial baroreflex control of MSNA at rest and LBNP –60 mmHg. To do so, diastolic pressures of individual heartbeats were grouped in intervals of 1 mmHg and for

Finometer were validated with manual sphygmomanometer blood pressure measurements. Changes in cardiac output were acquired using the Finometer Modelflow algorithm. Heart rate was measured with a standard lead II ECG (Pilot 9200; Colin Medical Instruments, San Antonio, TX). Data were collected on Chart 5 Powerlab data acquisition system (ADInstruments, Colorado Springs, CO).

Sympathetic Neural Recordings

Recordings of MSNA were obtained from the fibular (peroneal) nerve using a 200-μm diameter, 35-mm long tungsten microelectrode tapering to an uninsulated 1- to 5-μm tip. This electrode was inserted percutaneously into the nerve just posterior to the fibular head. A reference electrode was positioned subcutaneously 1–3 cm from the recording site. An MSNA site was obtained by manually manipulating the microelectrode until a characteristic pulse synchronous burst pattern was observed. The recording site was taken to provide MSNA when J) the burst pattern was not associated with skin paresthesias, 2) increased in burst strength in response to voluntary apnea but not during arousal to a loud noise (Hagbarth and Vallbo 1968), and J) burst probability was inversely related to diastolic blood pressure (Sundlof and Wallin 1978). Neurograms were measured with a nerve traffic analysis system (662C-3; Bioengineering of University of Iowa, Iowa City, IA). The neural signal was preamplified with a gain of 1,000 (using preamplifier and isolation amplifier, gain = 100 and 10, respectively) and further amplified with a gain of 75 (using a variable gain amplifier gain = 0.1–99). This neuronal activity was then band-pass filtered (bandwidth of 700–2,000 Hz), and the filtered MSNA was rectified and integrated. Integration is accomplished with a leaky integrator set with a 0.1-s time constant. All signals were digitized with a data acquisition system (PowerLab software ADInstruments) at a sampling rate that varied from 100 Hz for the rectified and integrated neurogram to 10 kHz for the amplified and filtered neurogram. Previous work from our laboratory (unpublished data) indicates that the band-pass filter settings applied to the raw data do not impair our ability to detect action potential waveforms. However, the impact of signal conditioning, including amplifier characteristics and band-pass filter settings, on action potential morphology requires further assessment.

Integrated MSNA Burst Analysis

Integrated bursts of MSNA were identified as exhibiting pulse-synchrony, having a signal-to-noise ratio of least 2:1 (i.e., the ratio of the amplitude of the burst and the baseline) with respect to the previous period of neural silence between bursts, having characteristic rising and falling slopes, and increasing in incidence and size during end-expiratory apnea but not startle. Burst occurrence was confirmed by visually inspecting the corresponding raw neurogram.

The level of sympathetic activity was expressed both as number of bursts per 100 heartbeats (burst incidence) and burst per minutes (burst frequency). In addition, the strength, or size, of each burst was quantified based on the height of each burst from its previous minimum baseline level. The impact of LBNP on burst strength (height) was determined using the absolute burst height values at baseline and LBNP.

Reflex latency was determined using the approach introduced by Fagius and Wallin (1980) and used frequently since. This process is simply the time delay between the R wave related to the burst (the previous R wave in which the burst was observed) and the peak of the burst (the highest point of the integrated MSNA burst). Reflex latency was calculated for each burst and averaged for all detected bursts in each condition and volunteer (mean reflex latency). The baroreflex threshold diagram was used to compare the arterial baroreflex control of MSNA at rest and LBNP –60 mmHg. To do so, diastolic pressures of individual heartbeats were grouped in intervals of 1 mmHg and for
each interval burst incidence was calculated and plotted against the mean of the pressure interval (Sundlof and Wallin 1978).

Action Potential Detection and Analysis

Action potentials were detected and extracted from the filtered raw MSNA signal using the techniques developed previously (Salmanpour et al. 2010a). Briefly, this technique uses a continuous wavelet transform (CWT) for action potential detection. The CWT used a “mother wavelet” that was adapted to an actual average action potential waveform constructed from physiological recordings of postganglionic sympathetic action potentials (i.e., matched mother wavelet had the same morphology as a physiological sympathetic action potential; Salmanpour et al. 2010a). The designing process involves 1) detecting action potentials from the filtered raw MSNA signal and averaging them to build a mean action potential template and 2) matching the amplitude and the phase of the new mother wavelet to that of the mean action potential template in the frequency domain (Salmanpour et al. 2010a).

The CWT with the matched mother wavelet was applied to the filtered MSNA to provide a wavelet coefficient between the signal of interest (i.e., an action potential) and the mother wavelet such that the wavelet coefficient was largest in the presence of the action potentials and negligible when applied to noise. Wavelet coefficients related to action potentials and noise were separated based on thresholding analysis (Salmanpour et al. 2010a). The exact location of the negative peak for each action potential was then detected by isolating the largest suprathreshold wavelet coefficient. With the use of this location information, the action potential waveforms were obtained from the original signal by putting the estimated location of action potentials in the center of a predefined window (3.2 ms). In this way, the amplitude and morphology of each extracted action potential remained unaltered. Extracted action potentials were then ordered based on peak-to-peak amplitude, and histogram analysis was performed to separate action potentials into amplitude-based clusters based on Scott’s rule (Scott 1979). Scott’s rule proposes the optimal histogram bin width based on the sample size and an estimate of the SD of the data. As such, the number of total clusters varied by subject.

The signal-to-noise ratio for a period of data was determined as the amplitude of the negative peak of the mean action potential over the SD of the background noise (i.e., during sympathetic silence). Action potential latency was determined as the time delay between the R wave of the ECG related to the burst of action potentials (i.e., the previous R wave in which the burst of action potentials was observed) and the negative peak of the action potential waveform, similar to that described previously (Macefield et al. 1994).

Action Potential Summation Simulation

A limitation to the study of total action potential content in a burst is the potential problem of action potential summation if two (or more) axons fire simultaneously such that their electrical discharges summate in the neurogram. We have quantified the potential risk of this problem [for detailed explanation, see Salmanpour et al. (2010a)]. With the use of published data from recordings of single postganglionic sympathetic axons (Macefield and Wallin 1999), a simulation analysis was performed to determine the probability of action potential overlap. From these previous data, it was assumed that each axon was active only once within a burst of activity, had a mean firing frequency of 0.33 Hz, and had a firing probability (percentage of heartbeats during which a neuron was active) of 35%. The shape and amplitude of each action potential were the same between “neurons” and the duration was fixed at 3.2 ms. A population of 30 simulated “neurons” was used for this simulation. Each “neuron” was active independently from the others. With the use of these criteria, 10,000,000 simulated bursts of sympathetic activity (containing $1.2 \times 10^6$ simulated action potentials) were generated. These data were then assessed for overlapping action potentials. The overlap was calculated based on the distance between the negative peaks of two action potentials where the complete overlap occurred if the distance was $\leq 0.3$ ms and partial overlaps were counted if the distance was $>0.3$ ms and $\leq 2$ ms. Two possible patterns of summation were expected. First, complete superposition would produce a larger than expected action potential that would occupy a bin that was not contiguous in the binning designation. Second, incomplete summation would produce an aberrant waveform whose shape and amplitude would be misleading but, nonetheless, composed of two action potentials that could be counted. Thus action potential clusters were eliminated if they were larger than all others and in a bin that was not contiguous with the remaining data. Further, action potential waveforms that were not triphasic, and/or of small amplitude, were eliminated from the analysis.

For practical reasons, this simulation model was designed on the assumption that each sympathetic neuron fired once per sympathetic bursts, although it is known that sympathetic neurons can fire multiple times within a single burst on some occasions (Macefield et al. 1994; Macefield and Wallin 1999). Regardless, how a single neuron fires within a single burst of activity is irrelevant in the context of superposition and summation of action potentials because a single neuron cannot superimpose upon itself. Rather, the important aspect is the temporal distance between two adjacent action potentials regardless of their origin from one or two axons.

Statistical Analysis

Values are given as means ± SD. The effect of condition (baseline vs LBNP) was assessed using a two-tailed, paired, t-test. Probability values $<0.05$ were considered statistically significant. Linear regression analysis was used to quantify the relationship between the following variables: burst amplitude vs. burst latency, burst size vs. number of action potentials within a burst, burst size vs. number of unique action potential clusters within a burst, and number of action potentials within a burst vs. number of unique action potential clusters within a burst.

RESULTS

This section summarizes the results of integrated burst and action potential analyses in sympathetic neural recordings. We divided our data into two different groups. Group A contains and compares sympathetic burst parameters and action potential content in MSNA data at baseline and LBNP = 60 mmHg for nine subjects (4 males, 5 females; subjects 1 to 9). Subject 10 was excluded from this group because a high level of motor unit activity was present only during LBNP = 60 mmHg.

Group B compares the same parameters in MSNA data at baseline and LBNP = 80 mmHg for five subjects (3 males, 2 females) in whom successful studies were performed. Although subject 10 showed a high level of motor unit activity at LBNP = 60 mmHg, clean sympathetic nerve signals (i.e., without motor unit activity) were recorded at baseline and LBNP = 80 mmHg. One of the additional recordings in group B at baseline and −80 mmHg was from a participant (subject 2) in the original sample who was studied a second time. We refer to this subject as subject 2–2.

Baseline vs. LBNP = 60 mmHg

Integrated bursts analysis. Filtered raw and integrated data were obtained for 300 s at baseline and 180 s of steady-state LBNP for burst and action potential analysis. An example of the raw MSNA data at base-line and LBNP = 60 mmHg is shown in Fig. 1.
Compared with baseline, burst frequency (13 ± 3 vs. 36 ± 7 bursts/min; P < 0.001) and burst incidence (23 ± 7 vs. 47 ± 12 burst/100 heartbeats; P < 0.001) were increased at LBNP −60 mmHg. Compared with baseline (0.22 ± 0.07 vs. 0.28 ± 0.09 V; P < 0.05), mean burst amplitude was increased slightly at LBNP −60 mmHg. In each participant, the burst incidence was linearly related to diastolic blood pressure in a manner that was reset to higher sympathetic outflow with LBNP, indicating that the sympathetic bursts reflected baroreflex-mediated sympathetic activity (see Fig. 2).

There was a linear relationship between the burst amplitude and corresponding burst reflex latency both at baseline (slope = −0.00079 ± 0.0002; r = −0.5 ± 0.09; P < 0.001) and LBNP −60 mmHg (slope = −0.00099 ± 0.00026; r = −0.56 ± 0.12; P < 0.001). Figure 3 illustrates this linear relationship for one representative subject at baseline and LBNP −60 mmHg.

**Action potential analysis.** For sympathetic action potential analyses, 77 ± 21 integrated sympathetic bursts consisting of a total of 815 ± 499 detected action potentials at baseline and 102 ± 36 bursts consisting of a total of 1,265 ± 831 detected action potentials at LBNP −60 mmHg were analyzed per subject. Compared with baseline, action potentials per minute (145 ± 84 vs. 461 ± 227 spikes/min; P < 0.05) and action potentials per 100 heartbeats (254 ± 164 vs. 606 ± 327 spikes/100 heartbeats; P < 0.05) were increased during LBNP −60 mmHg. This increase in total action potential content over time was due to the increase in burst frequency and burst incidence because the number of action potentials per integrated burst at baseline was not changed by LBNP −60 mmHg (see Table 1).

The number of distinct “clusters” of action potentials was not different when detected action potentials were binned based on peak-to-peak amplitude at LBNP −60 mmHg and the same bin size was used for clustering at baseline (see Table 1).

A representative example of detected sympathetic action potential clusters is shown in Fig. 4. The extracellular action potential in human MSNA is triphasic and templates similar to those produced in the current study have been shown in (Inglis et al. 1996; Macefield and Wallin 1999).

With the use of linear regression analysis, there were strong relationships between relative integrated burst size and the number of action potentials per sympathetic burst both at...
The number of active action potential clusters at baseline [r = 0.75 ± 0.13 (range 0.61 to 0.9); P < 0.001 in all subjects] and LBNP −60 mmHg [r = 0.75 ± 0.12 (range 0.61 to 0.87); P < 0.001 in all subjects]. There were moderate to strong relationships between relative integrated burst size and the number of active action potential clusters at baseline [r = 0.59 ± 0.16 (range 0.47 to 0.77); P < 0.001 in all subjects] as well as LBNP −60 mmHg [r = 0.61 ± 0.12 (range 0.44 to 0.83); P < 0.001 in all subjects]. In addition, the number of detected action potentials and the number of distinct clusters within a given sympathetic burst were strongly correlated at baseline [r = 0.7 ± 0.1; P < 0.001 (range 0.55 to 0.81)] and at LBNP −60 mmHg [r = 0.74 ± 0.03; P < 0.001 (range 0.69 to 0.8)]. Figure 5 represents these linear relationships between 1) relative integrated burst size and the number of action potentials per burst, 2) the relative integrated burst size and the number of unique action potential clusters per burst, and 3) the number of action potentials vs. the number of unique action potential clusters per bursts for one subject. Similar patterns in these relationships were observed for the remaining subjects (see Fig. 5, A3, B3, and C3, for mean responses).

The relationship between the occurrence of individual action potential clusters as a function of burst amplitude in one subject is shown in Fig. 6A during baseline and in Fig. 6B at LBNP −60 mmHg. In general, action potential clusters present at rest were also present during LBNP. Also, the larger action potential clusters tended to fire within larger bursts but not smaller bursts.

At both baseline and LBNP, −60 mmHg action potential cluster latency decreased in each subject as an inverse function of peak-to-peak amplitude (see Fig. 7). The mean response was fitted using an exponential function (R² = 0.9857; P = 0.0012) at baseline and (R² = 0.9935; P = 0.0016) at LBNP −60 mmHg. It was observed that the whole latency-size profile shifted upward ∼8 ms during LBNP as the integrated mean reflex latency increased ∼10 ms. The increase in mean reflex latency during LBNP was also reported in (Fagius et al. 1987).

As mentioned above, the effect of any action potential summation (i.e., 2 action potentials firing at exactly the same time to produce a summated action potential waveform) within larger bursts of activity was tested using a simulation (Salmanpour et al. 2010a). The simulation results indicated that the probability of complete overlap and summation to produce a single, larger amplitude, waveform (i.e., the time difference between the arrival times of the 2 spikes >0.3 ms) was ∼0.28%. However, partial overlap occurred when two action potentials fired within >0.3 and ≤2 ms with a probability of 3%. Within this range, action potentials with a separation between >0.7 ms could be detected as distinct waveforms and only action potentials with a separation >1.4 ms.

Table 1. Postganglionic sympathetic action potentials and action potential clusters within sympathetic bursts detected at baseline and LBNP −60 mmHg for each participant

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline</th>
<th>LBNP −60 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP/bursts</td>
<td>Clusters/bursts</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>2–1</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>11 ± 6</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

AP, action potentials; LBNP, lower body negative pressure.
were classified further based on peak-to-peak amplitude. For a complete performance evaluation of our spike detection algorithm on overlapping spikes, see Salmanpour et al. (2010a). Notice that this cutoff of 1.4 ms will also capture multiple firings of single units that appear to display maximal instantaneous firing rates of up to 230 Hz in single-unit sympathetic recordings (Macefield et al. 1994; Macefield and Wallin 1999).

**Baseline vs. LBNP −80 mmHg**

Figure 8 shows an example of the raw MSNA data at baseline and LBNP −80 mmHg. Notice the increase of the amplitude of integrated sympathetic bursts during LBNP −80 mmHg for this individual.

Compared with baseline, burst frequency (15 ± 8 vs. 48 ± 16 bursts/min; \( P < 0.05 \)) and burst incidence (26 ± 13 vs. 55 ± 15 burst/100 heartbeats; \( P < 0.05 \)) were increased at LBNP −80 mmHg. Compared with baseline (0.23 ± 0.07 vs. 0.38 ± 0.14 V; \( P < 0.05 \)), mean burst amplitude was increased at LBNP −80 mmHg. Figure 9, A and B, illustrates the distribution of absolute burst amplitudes at (baseline vs. LBNP −60) and (baseline vs. LBNP −80 mmHg). Notice that larger bursts are showing up at stronger levels of suction.

Similar to group A, there was a linear relationship between the burst amplitude and corresponding burst reflex latency both at baseline (slope = −0.00089 ± 0.00024; \( r = −0.55 \pm 0.1; \ P < 0.001 \)) and LBNP −80 mmHg (slope = −0.00087 ± 0.00028; \( r = −0.55 \pm 0.16; \ P < 0.001 \)). Figure 10 illustrates this linear relationship for one representative subject at baseline and LBNP −80 mmHg.

The total number of action potentials per sympathetic bursts and the number of active action potential clusters per sympathetic bursts were increased for three subjects but not for the remaining two recordings. (see Table 2.)

Figure 11, A and B, illustrates the relationship between the occurrence of individual action potential clusters as a function of burst amplitude in subject 8 during baseline and LBNP −80 mmHg, respectively, and shows the increased firing of action potentials during LBNP −80 mmHg.

**DISCUSSION**

There were three important new findings of the current study. First, clusters of larger axons exhibited shorter latency and, from a probabilistic perspective, were more likely to be present in larger bursts, which also exhibited a shorter reflex latency. Second, this range of action potential size and latency was observed at rest and persisted unaltered during −60 mmHg of LBNP. Thus there exists a fundamental pattern of recruitment of additional larger, faster conducting neurons as the sympathetic bursts become stronger (i.e., larger amplitude bursts) and that moderate levels of baroreceptor unloading do little to modify this fundamental pattern. Although LBNP of −60 mmHg increased burst frequency and burst incidence, it did not change the number of detected action potentials or the number of distinct clusters within a sympathetic burst. This latter observation implies that the same population of neurons at baseline fires more often during LBNP −60 mmHg. Yet, within each condition, there appears to be a recruitment strategy whereby the larger action potentials are recruited with a lower probability and, once recruited, contribute to the larger integrated MSNA burst. Third, during more severe orthostasis, as produced by −80 mmHg LBNP, the introduction of new clusters with larger amplitude was observed in three of the five individuals. Thus there is evidence supporting the hypothesis that a subpopulation of large postganglionic axons exists and is recruited under conditions of intense baroreceptor unloading requiring high sympathetic outflow, at least in some individuals.

**Integrated Burst and Action Potential Analysis**

In the current study, the integrated bursts were explained by a baroreflex mechanism, as their occurrence was linearly
related to diastolic blood pressure. However, the strength of each integrated burst was not explained by blood pressure, consistent with the understanding that burst occurrence, but not strength, is under baroreflex modulation (Kienbaum et al. 2001), at least when assessed under conditions of supine rest when baroreflex inputs are strong. The current study extends this understanding to high (but not extreme) levels of baroreceptor unloading.

Recently, we (Steinback et al. 2010) reported observations that severe chemoreflex activation produced important increases in the strength of integrated MSNA bursts in humans. This earlier study revealed that the pattern of action potential recruitment during a prolonged breath hold displayed an ordered pattern whereby larger bursts were more likely to contain larger action potentials with faster conduction velocity. This earlier study also indicated that progressive and severe chemoreflex stress can elicit very large bursts of MSNA with the interpretation that large action potentials are reserved for high stress circumstances.

However, this earlier study did not emphasize action potential discharge patterns at baseline. The current data suggest that this ordered pattern of sympathetic action potential recruitment is present even under baseline conditions and may, therefore, not necessarily be reflex specific but rather be evidence for a generalized principle of postganglionic sympathetic neural recruitment.

However, the conclusion of a generalized sympathetic discharge pattern that is extended by severe chemoreflex stress to include very large action potentials raises the question of why baroreceptor unloading would not lead to a greater probability of larger axons being recruited, if they are supposedly reserved for conditions of high sympathetic need? LBNP of ~60 mmHg is analogous to upright posture, which can elicit ~300% increase in sympathetic outflow. Nonetheless, it remains possible that this level of baroreceptor unloading is not severe enough to elicit the recruitment of latent postganglionic neurons. To test this idea, we examined the MSNA outflow in five individuals in whom the MSNA recording site was maintained at ~80 mmHg of LBNP. These data indicate that more severe baroreceptor unloading can lead to the appearance of new and larger amplitude action potential clusters indicative of a latent but recruitable population of neurons just like the chemoreflex responses reported previously (Steinback et al. 2010). However, this new popula-
tion of action potentials was elicited in only three of five individuals, suggesting that individual variations exist in terms of postganglionic recruitment of MSNA. We do not know if this reflects the severity of the baroreflex unloading, which at −80 mmHg may be closer to maximal tolerance in three subject but not the other two subjects. None of these participants showed any sign of presyncope.

Overall, the larger bursts had a shorter reflex latency. Also, action potential latency continued to be related to cluster size in an exponential manner. This exponential pattern was consistent with the well-defined relationship between action potential amplitude (proportional to the square of axon diameter) and conduction velocity (Clamann and Henneman 1976) (see Fig. 7). Therefore, an important observation is that new clus-
ters of larger, faster conducting neurons were present in the larger bursts during LBNP – 80 mmHg.

The mechanisms mediating varying levels of action potentials of different recruitment thresholds are likely to include the proportion and activity of strong vs. weak preganglionic inputs, which are known to impact postganglionic recruitment (Janig and McLachlan 1992; McAllen and Trevaks 2003). It remains to be understood how this anatomical basis produces functional differences in multifiber recruitment of larger vs. smaller axons, and burst composition. Nonetheless, our findings are in agreement with the previous study by Wallin et al. (1994). These authors, in the absence of action potential analysis, measured integrated burst reflex latencies during apnea and LBNP and concluded that the recruitment of faster conducting sympathetic neurons might be one mechanism explaining the variation of reflex latencies between large and small sympathetic bursts. In addition, the inverse relationship between action potential amplitude and its latency follows the pattern of recruitment based on the “size principle” originally described for motor unit neurons (Henneman 1957), which indicates that neurons with high threshold of activation

![Fig. 7. Mean action potential latency for each cluster as a function of normalized cluster amplitude (cluster 1 = 100%) for each participant during baseline (A) and at LBNP –60 mmHg (B). Mean cluster latency, binned across participants, as a function of normalized cluster amplitude during baseline and LBNP –60 mmHg (C). Numbers indicate the number of subjects per bin. Decrease in cluster latency as a function of amplitude was fit with a modified exponential decay (see text) (baseline, solid line; LBNP dashed-line). *P < 0.05, latency significantly different from first bin, in both baseline and LBNP –60 mmHg.](image)

![Fig. 8. A representative example of filtered and integrated muscle sympathetic nerve activity at rest (A) and LBNP –80 mmHg (B).](image)
have larger diameters (i.e., higher amplitude action potential) and higher conduction velocities (i.e., shorter latency) than neurons with low thresholds of activations. If this rule was valid for the human MSNA signal, it can be expected that small sympathetic bursts contain neurons that exhibit a low activation threshold and a low conduction velocity (i.e., low amplitude action potentials with longer latencies), whereas large, integrated bursts would contain these smaller action potentials as well additional neurons with higher threshold, larger amplitudes, and faster conduction velocities. Our results would support this expectation.

The shorter latency of larger action potential clusters is the basis of an assumption that these action potentials are generated by larger neurons. It remains possible, however, that the larger amplitude action potentials could be the result of summation of

---

**Fig. 9.** Distribution of burst amplitudes at baseline vs. LB NP −60 (A) and baseline vs. LB NP −80 mmHg (B).

**Fig. 10.** Relationship between relative integrated burst size and corresponding reflex latencies at baseline (A) and LB NP −80 mmHg (B). In A and B, 100% represents mean burst size at baseline and LB NP −80 mmHg. C: mean response relation (i.e., reflex latency vs. burst size) during baseline (○, solid line) and LB NP −80 (●, dashed line) (n = 5). Mean values are from each subject’s lowest and highest burst amplitude at baseline (○) and LB NP (●). Error bars indicate SD. Baseline regression: $y = -0.00089x + 1.38$; LB NP regression: $y = -0.00087x + 1.38$. 
any two medium size action potential that fire at the same time (Spickler and Kezdi 1969; Andresen and Yang 1989). We believe this is possible but rare. First, the same axon will not superimpose upon itself even though it can fire at the theoretical maximum of 500 Hz. Even if such high rates were observed for single units, our analysis excludes classification of any spikes with interspike distances of < 2 msec. Second, our simulation analysis argues against substantial superimposition of axons (Salmanpour et al. 2010a). It is also possible that the larger amplitude clusters simply reflect action potentials from neurons that are geographically close to the recording electrode. However, this would not explain their shorter latency.

Baroreflex Control?

A common observation in integrated MSNA reports is that the baroreflex affects the probability of burst occurrence but not its size. In an analogous manner, the current data suggest that the level of baroreceptor loading affected the number of action potentials per minute but had little impact on the recruitment of larger action potentials (i.e., the number of spikes per bursts was not different across levels of LBNP). Thus it may be that these larger action potentials with faster conduction velocities reflect a level of control that is minimally affected by baroreflex pathways, at least, perhaps, until severe baroreceptor unloading occurs.

Although LBNP −60 mmHg produced modest increases in the amplitude of integrated bursts, it seems that this amount of increase was not the result of a systematic recruitment of additional neurons. That is, the action potential clusters present during LBNP were those already present at baseline. This could be explained using a hypothetical model for arterial baroreceptor influence on MSNA presented by Kienbaum et al. (2001). Based on the observation that the baroreflex regulated the occurrence of sympathetic bursts but not their strength, these authors suggested that two different sites of control were involved in the modulation of these two independent features of sympathetic outflow. These earlier conclusions were based on observations made in resting humans. The current data, assessed at moderate-to-high levels of lower body suction, support and extend this hypothesis to a wider range of baroreceptor loading. Nonetheless, one may argue that severe baroreflex stress may be required to expose baroreflex control over burst size. Certainly, removal of some baroreceptor input in the current study did result in a small shift towards increased burst size and probability of larger action potentials. Furthermore, our data from −80 mmHg LBNP support that idea that a more severe orthostatic challenge may expose a greater inhibitory role of baroreceptor inputs on MSNA burst strength with a low-pressure threshold at least in some individuals. In support of this conjecture, the recent work by (Cooke et al. 2009) showed that the severe levels of LBNP (e.g., suction ≥80 mmHg) may affect both the amplitude and reflex latency of integrated bursts. Thus one would expect that the higher levels of LBNP might affect the action potential content differently than that of lower/medium levels of LBNP.

Limitations

The current approach for action potential detection in MSNA bursts provides information on the size and latency of action potentials within MSNA bursts. A limitation of the approach is that it cannot be determined whether separate action potentials with the same morphology (i.e., size) are from the same or different neurons. Generally, these clusters active during baseline continued to be active during LBNP. However, whether or not these are the same neurons is not known. All that can be said here is that action potentials of similar size are present both at rest and during LBNP.

Also, the correct detection of action potentials by this method is a function of the signal-to-noise ratio. We have tested the accuracy of the current action potential detection method using simulated MSNA data with varying sets of burst rate, action potential rate, and signal-to-noise ratios (Salmanpour et al. 2010a). In the present study, the average signal-to-noise ratio for the data was 3.7 ± 0.3. Based on a previous performance analysis (Salmanpour et al. 2010a), this level of signal-to-noise would produce a correct detection rate of 91 ± 11% and a false positive detection rate of 3%. In addition, the observed exponential relationship between size and latency of action potentials suggests that large action potentials fire in the first part of the burst; moreover, any complete overlapping action potential causing a larger action potential could occur anywhere within a sympathetic burst, which is not necessarily fitted with the observed size-latency relationship. Therefore, the potential for the larger action potentials to be due to signal summation, or to be a function of the analytical approach, is small.

Summary and Conclusions

Earlier, Wallin et al. (1994) pondered the mechanism(s) producing the shorter latency of larger integrated bursts and suggested that altered synaptic delays and/or latent populations of neurons with higher recruitment thresholds and faster conduction velocities could account for such burst-by-burst variations in latency. When combined with information derived from the behavior of single sympathetic units (Macefield et al. 1994; Macefield and Wallin 1999), the current data lend support to both options of additional sympathetic outflow. This outflow can change spontaneously, and such variations occur at baseline and during reflex activation. At rest, burst strength predicts not only the number of active sympathetic units but also the size and latency of these units. Thus the current results suggest that various populations of sympathetic postganglionic units exist with varying recruitment thresholds and conduction velocities. Baroreceptor unloading enhances burst incidence due to the increased probability of recruiting the same action potential population that was present at baseline. The shorter latency of larger bursts, both at rest and during LBNP, is, at least partly, the result of more and faster conducting larger action potentials in the larger bursts.

Table 2. Postganglionic sympathetic action potentials and action potential clusters within sympathetic bursts detected at baseline and LBNP −80 mmHg for group B

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline AP/bursts</th>
<th>Baseline Clusters/bursts</th>
<th>LBNP −80 mmHg AP/bursts</th>
<th>LBNP −80 mmHg Clusters/bursts</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–2</td>
<td>7</td>
<td>4</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>8</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>3</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>5</td>
<td>33</td>
<td>9</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>10 ± 6</td>
<td>5 ± 0</td>
<td>18 ± 9</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>
In conclusion, this study has provided new evidence regarding postganglionic action potential discharge patterns and provides detailed information about size and latency of action potentials in human MSNA recordings. The findings indicate that a hierarchical pattern of recruitment of a new population of efferent sympathetic fibers can explain variations in MSNA burst size at rest and baroreflex unloading. Moreover, the moderate levels of baroreceptor unloading do not change the pattern of neural sympathetic recruitment in human MSNA.

Fig. 11. Relationship between the occurrence of individual action potential clusters as a function of burst amplitude in 1 subject during baseline (A) and LBNP – 80 mmHg (B). In each section A or B, integrated sympathetic bursts are ordered along the top by burst size where 100% represents mean burst size at baseline (A) and LBNP – 80 mmHg (B) separately. Below, occurrences of postganglionic sympathetic action potentials as a function of integrated burst size are indicated for each action potential cluster (numbers at left show action potential clusters). Notice that large action potential clusters are predominately present within larger bursts. Notice the increase of the activity of preexisting action potential clusters (clusters 1–17) and firing of the new additional clusters (clusters 18–22) during LBNP – 80 mmHg.
GRANTS
This work was supported by research grants from the Natural Sciences and Research Council of Canada (to K. Shoemaker).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES