Firing Properties of Single Muscle Vasoconstrictor Neurons in the Sympathoexcitation Associated With Congestive Heart Failure

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Background—Congestive heart failure (CHF) in humans is associated with a marked sympathoexcitation, including an augmented muscle sympathetic nerve activity (MSNA) in intraneural multiunit recordings. In the present study, single-unit recording was used to evaluate whether the firing properties of individual muscle vasoconstrictor neurons can reveal underlying mechanisms for this increase in MSNA.

Methods and Results—Eight patients with CHF (NYHA class II to IV; left ventricular ejection fraction, 29±5%, mean±SEM) were studied. In standard multiunit recordings, MSNA burst incidence (bursts/100 heartbeats) ranged from 65% to 100% (88±5%). Using selective tungsten microelectrodes, we made recordings from 16 single muscle vasoconstrictor axons. Mean unit firing probability (ie, the percentage of cardiac intervals in which a single axon fired) was 54.5±5.2% (range, 21 to 89%), and mean firing frequency was 0.98±0.22 Hz (0.14 to 3.86 Hz), both of which were higher than seen previously in healthy subjects (P<0.001). Although single neurons occasionally generated multiple spikes per sympathetic burst, such multiple firing was rare and was not different from that seen in healthy subjects.

Conclusions—An increased firing frequency of individual vasoconstrictor neurons is one mechanism for the increased number of multiunit MSNA bursts at rest in CHF. The neurons discharge in more diastoles than in healthy subjects (ie, firing probability is increased), but the likelihood of discharging >1 impulse per sympathetic burst is not increased. Despite the intense multiunit activity at rest, the firing characteristics of individual vasoconstrictor axons indicate a remaining capacity for transient increases of MSNA in CHF. (Circulation. 1999;100:1708-1713.)

Key Words: nervous system, autonomic ■ heart failure

The neurohumoral activation associated with congestive heart failure (CHF) includes a marked sympathoexcitation, which is considered to be an important pathophysiological and prognostic factor. Using direct intraneural recording techniques, several studies have shown a marked augmentation of sympathetic nerve activity to the muscle vascular bed (MSNA) in patients with moderate to severe CHF and significantly altered MSNA even in mild CHF, whereas sympathetic activity in cutaneous nerves appears to be unaltered. Studies using isotope dilution techniques have demonstrated that the augmented MSNA is paralleled by increased cardiac, renal, and cerebral norepinephrine spillover.

Most microneurographic studies of MSNA in awake humans have relied on the analysis of multiunit recordings, which reflect the summed activity of many vasoconstrictor neurons. In multiunit recordings, impulses from different neurons occur in synchronized bursts, which are generated during the diastoles, when arterial baroreflex inhibition is at its weakest. Because the absolute strength of the bursts depends on the proximity of the electrode to the active fibers (a variable that cannot be adequately controlled), interindividual or intergroup comparisons of the strength of multiunit MSNA can only be based on the frequency of sympathetic bursts (bursts/100 heartbeats or bursts/min). Recently, a technique for recording action potentials from individual vasoconstrictor nerve fibers was described. This approach extends the possibility of making more detailed quantitative comparisons of MSNA between groups of subjects. Thus, for each vasoconstrictor nerve fiber, data can be obtained on (1) the firing probability (ie, the relative proportion of diastoles in which the neuron fires), (2) the degree of multiple within-burst firing (ie, how often the neuron fires >1 spike per burst), and (3) the mean firing frequency.

In the present study, we used this single-unit approach to assess whether the firing properties of individual vasoconstrictor fibers in CHF patients, compared with previous data from healthy subjects, can reveal mechanisms underlying the augmentation of MSNA in CHF. Our hypothesis was that if single-unit firing properties were unaltered in CHF, this would suggest that the increase in multiunit MSNA that occurs during the development of the disease was due to the...
recruitment of previously silent vasoconstrictor fibers. Conversely, if there were increases in unit firing probability and/or multiple within-burst firing, this would indicate that increased mean firing frequencies contributed to the sympathoexcitation, either alone or in combination with recruitment of previously silent fibers.

Methods

Subjects
Data were obtained from 1 female and 7 male CHF patients (mean age, 52 years; range, 42 to 58 years; NYHA functional class II to IV) recruited from the Department of Cardiology. Five patients had idiopathic dilated cardiomyopathy, and 3 had ischemic heart disease. Left ventricular ejection fractions (LVEFs) ranged between 13% and 40% (29±5%). All patients remained on optimal pharmacological treatment while being assessed for cardiac transplantation. Drugs included diuretics, ACE inhibitors, and digitalis; 6 patients were also on β-adrenergic receptor antagonists. One subject had a pacemaker. Three patients subsequently underwent heart transplantation after the present experiments. Each patient provided informed written consent to the procedures, which were conducted under the approval of the human ethics committee of the University of Göteborg.

General Procedures
ECG activity was recorded with standard Ag-AgCl chest electrodes, respiratory movements with a strain-gauge transducer attached to a strap around the chest, and continuous finger blood pressure by pulse plethysmography (Finapres, Ohmeda). With the patient in a comfortable supine position, the thigh was supported by a vacuum cast, and the common peroneal nerve was located behind the fibular head by palpation and electrical stimulation via a surface probe. A laboratory-produced tungsten microelectrode of relatively low impedance was inserted percutaneously into a motor fascicle of the nerve, and a site was located in which spontaneous pulse-synchronous sympathetic activity could be recorded. A nearby subdermal electrode with a larger uninsulated tip served as the reference electrode. Resting multiunit bursts and heart rate were recorded during 5 minutes of quiet breathing, so as to allow measurement of burst incidence (bursts/100 heartbeats). After removal of this microelectrode, a second, high-impedance microelectrode (type 25–10-1, Frederick Haer Co, or type TM33B20, World Precision Instruments) was inserted into the same or an adjacent motor fascicle, and the microelectrode was manipulated until large unitary discharges appeared out of the multiunit sympathetic bursts.

Data Acquisition and Analysis
Neural activity was amplified (5×105), filtered (0.3 to 5.0 kHz), digitized at 12.8 kHz (12 bits), and stored on disk via the SC/Zoom data acquisition and analysis system (Department of Physiology, University of Umeå, Sweden). The amplified and filtered nerve signal was also led to an audiometer and through a resistance-capacitance circuit (time constant, 100 ms). The latter output, the “integrated nerve signal,” was digitized at 800 Hz and stored as 8 bits.

Results

With standard tungsten microelectrodes of relatively low impedance, the multiunit burst incidence in the CHF patients was 88.1±4.7 bursts/100 heartbeats (range, 65 to 100 bursts/100 heartbeats).

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With high-impedance microelectrodes, stable unitary recordings were made from 16 intrafascicular sites in the 8 patients. All units generated triphasic, negative-going spikes, consistent with an extra-axonal recording from a C fiber. However, spike configuration varied enough between different units to allow identification of single nerve fibers on the basis of uniform morphologies of the superimposed spikes (Figure 1).

The firing probability of single vasoconstrictor fibers was 55.1±5.1% (range, 21% to 93%). As illustrated in Figure 1, firing probability could be low (this unit fired in 35% of cardiac intervals) despite a high multiunit MSNA burst incidence (82% in this patient with severe CHF). The average firing rate, calculated as the inverse of all interspike intervals, was 0.98±0.22 Hz (range, 0.14 to 3.86 Hz). As illustrated in Figure 2, the distribution of instantaneous frequencies (calculated from each interspike interval) was very wide but strongly skewed toward low firing rates. The number of spikes a unit contributed to a sympathetic burst was generally low. The majority of units (14 of 16) fired at most 2 to 5 spikes per cardiac interval (median, 3), but 2
units discharged up to 7 and 9 spikes, respectively. The percentages of cardiac intervals in which units were quiescent, fired a single spike, or generated multiple spikes are shown for 2 units in Figure 3. Whereas the unit in Figure 3A had the low firing probability shown by most units, the one in Figure 3B—the same unit that generated the highest average (3.86 Hz) and instantaneous (400 Hz) firing rates as well as the highest number of spikes/burst (9)—had both a high firing probability and a high incidence of multiple firing. Pooled data calculated from the mean values for each unit are shown in Figure 4A. Considering only those cardiac intervals in which the units fired, units in CHF patients generated 1 spike in 70.6% of intervals, 2 spikes in 18.2%, 3 spikes in 7.3%, and 4 spikes in only 3.0% of cardiac intervals (Table and Figure 4C).

Fourteen (88%) of 16 units occasionally generated 2 spikes separated by very short interspike intervals (<20 ms), as previously described in healthy subjects. Figure 5 shows the 2 “doublets” with the highest instantaneous frequencies (336 and 400 Hz, respectively), which occurred in the unit depicted in Figure 3B. In this subject, such doublets occurred in 9.6% of recorded cardiac intervals. The average amount of doublets across all units was 4.4%, but it was only 2.2% if the exceptional unit in Figures 3B and 5 was excluded.

Comparison With Firing Properties in Normal Healthy Subjects

The Table summarizes the average firing properties of all 16 units recorded in CHF patients and compares them with 33 units recorded from healthy subjects (n=14; mean age, 31 years; range, 21 to 61 years) and reported in 2 previous studies. Heart rate differed significantly between the 2 groups (P<0.02): in healthy control subjects, the mean cardiac interval was 1.19±0.09 seconds, corresponding to a mean heart rate of 50.4 beats/min, and in the CHF patients, the mean cardiac interval was 0.87±0.06 seconds (69.0 beats/min). The CHF patients had more multiunit MSNA bursts, and their individual vasoconstrictor fibers had significantly higher firing probabilities (Figure 4, A and B) and mean firing frequencies. In contrast, the relative proportion of single versus multiple firing within sympathetic bursts did not differ between CHF patients and healthy subjects (Figure 4, C and D).
In the present study, we have made the first single-unit recordings from muscle vasoconstrictor neurons in patients with CHF, in an attempt to elucidate mechanisms for the sympathoexcitation prevailing in this condition. The results show that qualitatively, the discharge characteristics of single fibers in the patients were similar to those found in healthy subjects. Quantitatively, however, the fibers in CHF patients had a significantly higher average firing probability, an unchanged probability of multiple within-burst firing, and a significantly higher mean firing frequency.

**Firing Properties of Single Muscle Vasoconstrictor Neurons in Heart Failure**

Compared with healthy subjects, the vasoconstrictor units in CHF patients had increased firing probability. This means that activated muscle vasoconstrictor neurons discharged in a larger proportion of cardiac intervals in the patients. This will contribute to the increased mean firing frequency. Another factor contributing to the increased firing rate is that the patients had a higher heart rate than the healthy subjects, i.e., more cardiac intervals were available in which the units could fire. It is noteworthy that a higher heart rate would have this effect even with an unchanged firing probability. Thus, because firing is initiated only during diastoles, heart rate per se may influence the number of sympathetic impulses that reach the blood vessels. This provides an interesting functional coupling between the heart and the peripheral vascular beds, which will tend to amplify baroreflex-induced changes of vascular resistance at constant firing probabilities.

Although recruitment of previously silent vasoconstrictor neurons cannot be directly illustrated in a short-term experiment, the increased average unit firing probability may imply such a mechanism. It is tempting to speculate that units with low firing probabilities (units firing in only 20% to 30% of cardiac intervals) in CHF patients may have been silent before the development of cardiac failure.

In contrast to firing probability, the probability for multiple within-burst firing was not increased in the CHF patients, i.e., in both healthy subjects and patients, ≈2 spikes occurred in only 30% of those cardiac intervals in which the units fired. Thus, multiple within-burst firing is not an important mechanism for the chronically elevated vasoconstrictor drive in CHF. This is in contrast to the findings during acute sympathoexcitation evoked by voluntary inspiratory apneas in healthy subjects, in which increased multiple within-burst firing does occur.

A possible explanation for the low degree of multiple within-burst firing at rest, in CHF patients as well as in healthy subjects, is that the discharge of an individual postganglionic neuron is governed primarily by the firing of a single preganglionic neuron with a strong synaptic connection. Multiple firing and the generation of short interspike intervals would occur only rarely, when an additional preganglionic neuron with a sufficiently strong synapse fires almost at the same time as the first neuron, thereby increasing the synaptic current and hence the likelihood that the postganglionic neuron generates multiple action potentials. This would agree with the firing characteristics of superior cervical sympathetic ganglion cells in the rat.

One exceptional unit, recorded in a patient with severe ventricular dysfunction (LVEF 16%), showed a much higher degree of multiple within-burst firing than all other units. Whether the behavior of this one unit was artifactual (see Methodological Considerations below), originated from an unusual type of normal fiber, or was truly pathological and related to disease severity is unclear. However, the normal firing characteristics of 3 fibers recorded from 2 patients with even more compromised left ventricular function (LVEF 13% and 15%, respectively) make the last alternative less likely.

**Residual Capacity for Sympathoexcitation**

Despite the high MSNA burst incidence (mean, 88 bursts/100 heartbeats) in multunit records from our CHF patients, individual muscle vasoconstrictor neurons fired on average in only 55% (range, 21% to 93%) of cardiac intervals. Thus,

<table>
<thead>
<tr>
<th>Units, n</th>
<th>Burst Inc, %</th>
<th>Firing Prob, %</th>
<th>Mean Freq, Hz</th>
<th>1 Spike, %</th>
<th>2 Spikes, %</th>
<th>3 Spikes, %</th>
<th>4 Spikes, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF patients</td>
<td>16</td>
<td>88.1±4.7</td>
<td>55.1±5.1</td>
<td>0.98±0.22</td>
<td>70.6±5.8</td>
<td>18.2±2.4</td>
<td>7.3±2.6</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>33</td>
<td>44.6±7.3</td>
<td>30.8±2.5</td>
<td>0.40±0.04</td>
<td>72.7±3.3</td>
<td>18.4±2.0</td>
<td>5.1±1.1</td>
</tr>
</tbody>
</table>

Group difference † † † NS NS NS NS

† $P<0.001$.

Mean data on multiunit burst incidence (Burst Inc), single-unit firing probability (Firing Prob), mean firing rate (Mean Freq), and the percentage of heartbeats in which units generated 1, 2, 3, or 4 spikes, as measured only from those cardiac intervals in which the units fired.

* See References 16 and 17.
many vasoconstrictor neurons remained inactive in a majority of cardiac intervals, and no unit was activated in all cardiac intervals, in a recording at rest. Together with the preserved propensity for firing only 1 spike per cardiac interval (ie, per multiunit sympathetic burst), these findings may have important pathophysiological and clinical implications. Multiunit MSNA responses to a hypotensive challenge have been found to be blunted in CHF patients as well as in healthy subjects with high MSNA, suggesting that the capacity for further increases in sympathetic activity is reduced in such subjects. However, single-unit recordings in normal subjects with high multiunit MSNA (75±5 bursts/100 heartbeats) have shown that unit firing probability and the degree of multiple firing both increase significantly when the sympathetic drive is elevated acutely, suggesting that the blunted multiunit MSNA responses to, for example, a hypotensive challenge are not a result of the firing of individual neurons having reached an upper limit. Thus, there should be ample opportunity for the vasoconstrictor fibers recorded in our CHF patients to increase their firing in response to acute stimuli, which could allow the system to maintain a role in beat-to-beat blood pressure homeostasis in severe CHF as well.

Methodological Considerations
As discussed in previous studies on the firing properties of single sympathetic neurons in healthy human subjects, an important caveat is whether the recordings can be accepted as originating from single sympathetic nerve fibers. To accept a unit, our requirements were that it should fire in association with multiunit discharges, that the spike morphology agree with that of an extracellular recording from a C fiber, and that the shape be reproducible between successive spikes. Despite these precautions, it is difficult to exclude with certainty that 2 units of identical shape and size may be active in the same recording, which is of particular importance when multiple spikes with short interspike intervals are generated within a sympathetic burst. Thus, the highest instantaneous frequencies observed in the present study (336 and 400 Hz, doublets shown in Figure 5) may be an overestimate. However, we could not exclude any of the relevant spikes on the basis of shape. And although the frequency is high, it may not be impossible. The theoretical limit at which an individual C fiber is capable of firing is determined by its absolute refractory period. For sympathetic postganglionic axons in the cat, this value is 2 ms, and it is the same for sensory C fibers in the hippocampus of the guinea pig. Doublets (interspike interval <20 ms) do occur in healthy subjects as well and may be due to a single postganglionic neuron being driven by 2 or more strong synaptic inputs—a consequence of chance occurrences in which the preganglionic neurons discharge essentially simultaneously. However, several observations point to the possibility that the exceptional unit depicted in Figures 3B and 5 may in fact include 2 different units. The mean firing rate of this unit was twice as high as that of any other unit recorded in CHF patients or healthy volunteers, and its ability to fire 9 spikes per sympathetic burst contrasts with the finding that no other unit fired >7 spikes per burst. Furthermore, this unit fired doublets 4 times more frequently (9.6% of cardiac intervals) than those of normal subjects with low resting MSNA (2.4%). normal subjects with high MSNA (2.4%, calculated from original data in Reference 17), or the remaining 15 units in our present CHF group (2.2%). Thus, with the exception of this one unit, the incidence of randomly occurring doublets is remarkably similar in all recorded muscle vasoconstrictor units, be it in CHF patients or in healthy volunteers. These exceptional characteristics may cast doubt on the unitary integrity of this particular recording. Still, our inclusion requirements do not allow us to exclude the unit. It should be underlined, however, that exclusion of the unit would further strengthen the findings and conclusions of the study.

The age of our CHF patients (mean, 52 years; range, 42 to 58 years) was significantly higher than that of the control subjects (mean, 32 years; range, 21 to 61 years), and the question arises whether the increased firing probabilities and mean firing frequencies in the patients could be related to the difference in age. This is unlikely. Two studies of single muscle vasoconstrictor fibers have been made previously, in healthy subjects 22 years old (range, 21 to 32 years) (with an average multiunit MSNA of 21 bursts/100 heartbeats) and 36 years old (range, 24 to 61 years) (with an average multiunit MSNA of 75 bursts/100 heartbeats), respectively. When the firing characteristics between these groups were compared, there were no significant differences in firing probability or multiple within-burst firing, but the older subjects with more multiunit bursts had significantly lower mean firing frequencies, which could be explained by a lower heart rate. Although these studies did not aim specifically at comparing age effects, the results provide no indication that higher ages should be associated with a higher single-unit firing frequency, as found here in our CHF patients.

Conclusions
Although it has been known for more than a decade that multiunit muscle sympathetic outflow is greatly augmented in patients with CHF, the nature of this hyperactivity has not been clarified. The present results demonstrate that an increased firing frequency of individual muscle vasoconstrictor neurons contributes to the increased multiunit activity. The increased frequency occurs because, compared with normal subjects, the neurons discharge in more diastoles, whereas the probability for >1 impulse per sympathetic multiunit burst is not increased. The facts that all vasoconstrictor neurons were silent in some cardiac intervals and that they preferentially fired only 1 spike per cardiac interval indicate a remaining capacity to transiently increase their firing in CHF patients, despite an intense hyperactivity in the resting state.

Acknowledgments
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