Multiple firing of single muscle vasoconstrictor neurons during cardiac dysrhythmias in human heart failure

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Elam, Mikael, and Vaughan Macefield. Multiple firing of single muscle vasoconstrictor neurons during cardiac dysrhythmias in human heart failure. J Appl Physiol 91: 717–724, 2001.—Single vasoconstrictor nerve fibers in humans normally fire only once but have the capacity to fire as many as eight times, per cardiac interval. Our laboratory recently demonstrated that the mean firing frequency of individual vasoconstrictor fibers is more than doubled in the sympathoexcitation associated with congestive heart failure. Six patients with congestive heart failure (New York Heart Association functional class II–IV; left ventricular ejection range 13–37%, average 22%) were studied at rest and during premature ectopic heartbeats. Analyzed for a total of 60 premature beats, the average firing probability of 10 vasoconstrictor fibers increased from 61 to 80% in the prolonged cardiac interval (i.e., reduced diastolic pressure) after premature beats. The incidence of multiple within-burst firing increased markedly, with two spikes being more common than one. Our results illustrate two different mechanisms (increases in firing probability and multiple within-burst firing), and indirectly indicate a third mechanism (recruitment of previously silent fibers), for acute sympathoexcitatory responses.

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The loss of pulse rhythmicity in muscle sympathetic nerve activity (MSNA) after local anesthesia of the glossopharyngeal and vagus nerves (7) illustrates that this firing pattern depends on the integrity of the baroreceptor reflex arc. Leimbach and co-workers (12), the first investigators demonstrating that MSNA is increased but that pulse rhythmicity is preserved in congestive heart failure (CHF), consequently interpreted their finding as indicating a retained arterial baroreceptor function in this cardiac disorder. This notion was recently underlined by Ando and co-workers (3), who used a severe CHF patient with pulsus alternans and resulting alternans in both blood pressure and MSNA to highlight the fact that beat-to-beat baroreflex control of MSNA remains highly sensitive and rapidly responsive in CHF. However, slower blood pressure changes (such as those elicited in standard baroreflex tests using intra-arterial infusions of phenylephrine and/or nitroprusside) result in smaller MSNA changes in CHF patients compared with healthy controls (8, 10), leading several investigators to the seemingly contrasting interpretation that baroreflex inhibition of MSNA is impaired in CHF. Although the exact nature of this baroreflex impairment remains unclear, it has been suggested as one underlying mechanism for the sympathoexcitation prevailing in CHF (17, 18).

In a recent study of the firing characteristics of single muscle sympathetic neurons in moderate to severe CHF (14), our laboratory found that individual vasoconstrictor neurons in patients were recruited in more cardiac intervals compared with healthy subjects, whereas they retained a propensity to fire only once per cardiac interval (e.g., once per burst of multi-unit activity). Because the neurons had the ability to occasionally fire up to eight spikes per burst, our group concluded that the low degree of multiple within-burst firing offered ample opportunity for further increases in firing of vasoconstrictor neurons during excitatory stimuli (such as falls in blood pressure), indicating a remaining homeostatic capacity despite the chronic sympathoexcitation in CHF.

The primary aim of the present study was to investigate whether an increase in multiple within-burst firing is indeed used by individual vasoconstrictor fibers to increase their output in response to acute sympathoexcitatory stimuli. To this end, we searched through all available single-unit recordings from CHF patients from our laboratory’s previous study (14), looking for cardiac dysrhythmias followed by transient falls in blood pressure. In our laboratory’s previous single-unit study of MSNA in CHF, as in most multi-unit MSNA studies published, such events were ex-
METHODS

Subjects. Single-unit recordings from eight CHF patients (14) were reexamined. Data from cardiac dyshyriaismas were obtained from one female and five male CHF patients (mean age 53 yr, range 42–58 yr; New York Heart Association functional class II-IV). The patients were recruited from the Department of Cardiology at Sahlgren University Hospital. Four patients had idiopathic dilated cardiomyopathy, and two had ischemic heart disease. Left ventricular ejection fractions (LVEF) averaged 22% (range 13–37%). All patients remained on optimal pharmacological treatment while being assessed for cardiac transplantation. Drugs included diuretics, angiotensin-converting enzyme inhibitors, and digitalis; four patients were also on β-adrenoceptor antagonists. One subject had a pacemaker. Three patients subsequently underwent heart transplantation after the present experiment. 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. Electrocardiographic 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, Louisville, CO). With the patient in a comfortable supine position, the thigh was supported by a vacuum cast and the common peroneal nerve 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 min of quiet breathing so as to allow measurement of burst incidence (bursts per 100 heartbeats). After removal of this microelectrode, a second, high-impedance microelectrode (type 25-10-1, Frederick Haer, Brunswick, ME, or type TM33B20, World Precision Instruments, Sarasota, FL) was inserted into the same, or an adjacent, motor fascicle and the microelectrode manipulated until large unitary discharges appeared out of the multiunit sympathetic bursts. High-impedance electrodes have a smaller recording area, allowing the preferential recording of activity from a single or a few fibers. In electrode positions yielding such unitary discharges, 5-min recordings were made under resting conditions.

Data acquisition and analysis. Neural activity was amplified (5 × 10^5), filtered (0.3–5.0 kHz), digitized at 12.8 kHz (12 bits), and stored on disk via the SC/ZOOM data acquisition and analysis system (Dept. of Physiology, University of Umeå, 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. During off-line analysis the morphology of every spike of a candidate unit was carefully checked using the spike recognition facility incorporated in the SC/ZOOM software (14, 16).

After visual inspection of the records for identification of premature ectopic heartbeats, the computer measured the number of spikes each single unit fired during the large multiunit MSNA burst triggered by the transient fall in blood pressure associated with the ectopic beat (Fig. 1). These data were compared with the average firing properties of each unit during sinus rhythm, regarding the probability of firing (= the percentage of bursts/cardiac intervals during which ≥1 spikes occurred) and the degree of multiple within-burst firing (= the number of bursts/cardiac interval with >1 spike in relation to all bursts/cardiac interval with any spike; in %) (14). Ectopic beats often occurred in regular or irregular trains (Fig. 1), and in such trains only the first ectopic beat with its associated neural burst was included in the analysis. For isolated ectopic beats, data on single-unit firing, integrated MSNA burst size, cardiac interval length, and blood pressure level were analyzed for each heartbeat during a period from six beats before to four beats after the ectopic beat. Single-unit firing probability and multiple firing were also analyzed at rest and during voluntary apneas. During

Fig. 1. Recording from a single muscle vasoconstrictor neuron in a patient with severe heart failure, showing a sequence with alternating premature beats. This unit generally fired only once per cardiac interval (indicated by asterisks) at regular rhythm but fired multiple spikes in the hypotensive intervals (i.e., in the large multiunit burst) after premature beats. Superimposed spikes show uniform spike morphologies, indicating that action potentials originated from the same sympathetic axon. ECG, electrocardiograph; BP, blood pressure.

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apneas our CHF patients often exhibited isolated, or trains of, ectopic heartbeats. All ectopic beats, together with four subsequent heartbeats, were excluded from the apnea analysis. For one patient who developed a sustained supraventricular tachycardia at the end of the recording session, neural and cardiovascular parameters were analyzed in the time and frequency (fast Fourier transform) domains over longer periods before and after the start of tachyarrhythmia (see RESULTS).

Statistics. All values are expressed as means ± SE. All statistical evaluation of the data was performed in STATISTICA for Windows v.5.1 (StatSoft, Tulsa, OK), using unpaired t-tests. Differences were considered statistically significant at P < 0.05.

RESULTS

Premature ectopic heartbeats occurred during the recordings from 10 vasoconstrictor fibers in 6 of our 8 CHF patients (3 fibers in 1 patient, 2 in 2 patients, and 1 fiber in 3 patients). A total of 60 ectopic heartbeats were included in the analysis, some of which appeared as the first in a series of ectopic beats (Fig. 1). Only 23 ectopic beats were isolated enough to allow sequence-analysis of regular heartbeats before and after the premature beat.

Premature heartbeats, resulting in a transient fall in systolic and diastolic pressure, were consistently followed by large multiunit MSNA bursts in which single vasoconstrictor units showed an average firing probability of 80%, compared with an average of 61% after regular heartbeats. In those cardiac intervals in which the fibers were active, there was a significant shift toward multiple within-burst firing (P < 0.001): the percentage of solitary spikes decreased from 60% in sinus rhythm to 27% in the burst associated with the premature beat, whereas the incidence of double spikes increased from 23 to 40% (Fig. 2).

On the basis of 23 isolated ectopic beats illustrated in Fig. 3, the number of spikes fired by single units was 215% higher after premature beats compared with preceding regular beats, because of increases in both firing probability and multiple firing. The corresponding increase in multiunit MSNA burst area (taking both increased burst amplitude and duration into account) was 384%. The increases in burst amplitude and duration were both positively related to the prolonged cardiac interval and inversely (and equally) related to systolic and diastolic pressures.

No change in single- or multiunit sympathetic discharge or in hemodynamic variables preceded the arrhythmic events. The fall in both single- and multiunit activity immediately before the large burst is related to the fact that a sympathetic burst corresponding to the

Fig. 2. Histograms with pooled data from 10 single vasoconstrictor fibers, recorded during a total of 60 premature heartbeats, showing the percentage of cardiac intervals in which units were quiescent, fired a single spike or multiple spikes during sinus rhythm (A and C) and after premature beats (B and D). Premature beats elicited an increased firing probability (i.e., reduced number of quiescent intervals) and a marked increase in multiunit within-burst firing, with 2 spikes being more common than 1.
short cardiac interval leading up to the premature heartbeat usually could not be identified. As previously described (6, 27), the large neural burst associated with the premature beat was followed by almost complete inhibition of sympathetic traffic in the subsequent cardiac interval, most likely due to the transient increase in systolic, but not diastolic, pressure.

Sustained tachyarrhythmia occurred during recording in one patient with moderate heart failure (New York Heart Association functional class III, LVEF 29%). At the end of a 30-min recording session during which the patient was relaxing and reported feeling comfortable, the sinus rhythm of 70 beats/min (mean cardiac interval 0.84 s) was broken by the occurrence of two supraventricular extrasystoles, initiating a sustained supraventricular tachycardia of ∼130 beats/min (mean cardiac interval 0.449 s; Figs. 4 and 5). During the tachycardia, the subject reported a slight dyspnea but no chest pain.

As for isolated ectopic heartbeats, the onset of sustained tachycardia was not preceded by any obvious trigger, change in single- or multiunit sympathetic nerve activity, or change in heart rate or blood pressure. On average, systolic and diastolic pressures increased from 138.1 ± 0.4/87.2 ± 0.3 mmHg during sinus rhythm to 144.6 ± 0.2/98.8 ± 0.3 mmHg during tachyarrhythmia. However, the tachycardia was also associated with a moderate degree of blood pressure alternans, resulting in systolic and diastolic pressures of 148.4 ± 0.3/100.2 ± 0.2 mmHg for every second pressure wave and 140.8 ± 0.3/98.1 ± 0.2 mmHg for those in between, when averaged for 626 heartbeats. Despite the rather limited difference between low and high alternating pressure waves, multiunit MSNA became entrained with the alternans rhythm (i.e., one-half the actual heart rate). This is shown in the time domain in Fig. 5A by averaging multiunit MSNA from every second R wave, before and after the start of tachyarrhythmia, and in the frequency domain in Fig. 5B.

Total integrated MSNA (average level of activity per minute; area under curve in arbitrary units) did not change significantly from sinus rhythm to tachycardia (23,200 and 23,000 arbitrary units, respectively). In contrast, the firing frequency of the single vasoconstrictor fiber dropped from 0.62 to 0.35 Hz, and its firing probability fell from 36 to 15%. Furthermore, the degree of multiple within-burst firing was markedly reduced: the percentage of cardiac intervals in which only solitary spikes were generated increased from 76 to 96% during the tachycardia, whereas the percentage of double spikes decreased from 21 to 4%.

DISCUSSION

Comparisons of single-unit and multiunit MSNA at regular heart rate and during dysrhythmias revealed two different mechanisms for acute increases in human sympathetic nerve discharge: 1) increased firing probability of vasoconstrictor fibers that are already active (i.e., firing in a larger proportion of cardiac

Fig. 3. Graphs illustrating neural and cardiovascular variables [spikes per burst (A), burst amplitude (B) and burst duration (C)] averaged for 5 heartbeats before to 4 beats after isolated premature heartbeats (n = 23). The hypotensive interval after premature heartbeats was associated with a large burst of multiunit muscle sympathetic nerve activity, including increased single-unit firing, followed by almost complete inhibition of sympathetic traffic in the subsequent interval, possibly due to the transiently increased arterial pressure resulting from an increased end-diastolic volume. Note that no changes in nerve activity or cardiovascular function preceded the premature beats; the drop in nerve activity immediately before the large burst illustrates that usually no nerve activity corresponding to the short cardiac interval leading up to the ectopic beat could be identified. au, Arbitrary units.

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intervals) and 2) increased multiple within-burst firing (i.e., individual vasoconstrictor fibers firing >1 spike per cardiac interval).

**Sympathetic response to premature heartbeats.** In a recent study of single vasoconstrictor fibers in CHF patients, our laboratory demonstrated that the chronically augmented MSNA at rest in these patients depended on an increased firing probability of individual fibers, whereas the normal propensity for firing only one spike per cardiac interval remained unchanged (14). Because fibers occasionally could fire as many as eight spikes per burst, our group suggested that the low average degree of multiple within-burst firing indicated a remaining capacity to transiently increase firing further. The present data show that, in response to a rapid fall in blood pressure, individual vasoconstrictor fibers in CHF patients 1) increase their firing probability further and 2) increase the number of spikes fired per sympathetic burst. Interestingly, the increased firing probability prevailing at rest in CHF patients may make the multiple within-burst firing mechanism more important in these patients. It has been discussed whether the ability to further increase sympathetic outflow is limited in CHF patients with intense sympathetic drive at rest (9, 28). Given the above firing characteristics of single vasoconstrictor units at rest and after ectopic heartbeats in moderate-to-severe CHF patients, the mechanism of multiple within-burst firing would appear to provide a capacity for further augmentation of sympathetic outflow, even though resting sympathetic drive may have reached a level at which all vasoconstrictor fibers were recruited in all cardiac intervals.

**Is the risk with ectopic heartbeats linked to sympathetic activation?** We found no evidence for stronger volleys of sympathetic traffic before dysrhythmic episodes. Premature beats were consistently followed by large sympathetic bursts, whereas the postextrasystolic beat was followed by virtually complete neural silence, as previously shown in multiunit recordings of MSNA (6, 27). Thus, averaged over several heartbeats, there is no net increase in total MSNA during periods with premature heartbeats in CHF patients. However, as discussed by Welch and co-workers (27), the large volleys of MSNA after premature beats could be paralleled by similar surges of cardiac sympathetic discharge, a notion underlined by the demonstration of a correlation between muscle sympathetic nerve activity and cardiac norepinephrine spillover (26). In this context, it is important to note that, although sympathoexcitatory stimuli have been shown to further increase MSNA in CHF patients with a markedly increased discharge even at rest, the relative increase in response to hypotension (8, 10) or mental stress (19) is lower than in healthy subjects. However, the new information gained from the recording of single vasoconstrictor fibers is that large bursts of sympathetic discharge after premature heartbeats differ from sympathetic neural bursts at sinus rhythm: they include more multiple within-burst firing of individual vasoconstrictor neurons. This firing pattern will augment the transmitter concentration at the neuroeffector junction through temporal summation. In general, the probabilistic nature of the discharge of single sympathetic neurons means that, as far as the neuroeffector junction is concerned, the neural input will be irregular. Multiple firing increases the irregularity of the
input by generating volleys with high within-burst frequencies. Interestingly, sympathetic effectors show more vigorous responses to irregular firing than when the same number of impulses arrive in a regular pattern (2, 11, 22; cf. Ref. 25). Thus, even if only transient, the marked rise in multiple within-burst firing after premature heartbeats in CHF patients may cause exaggerated effector organ responses. This response pattern may be one underlying mechanism for the increased risk of sudden death after periods of frequent ectopic beats (20, 23) and contribute to the arrhythmogenic effect of sympathoexcitation suggested by experimental (5, 13) and clinical (21, 24) studies.

*Sustained tachyarrhythmia.* In healthy volunteers, a stable rise in blood pressure of a few millimeters of Hg induced by steady-state infusions of phenylephrine elicits a stable and almost complete inhibition of MSNA (1, 4). The sudden occurrence of a sustained supraventricular tachycardia in one of our patients led to a rise in diastolic blood pressure level of >10 mmHg, but multiunit MSNA remained at pre-tachycardia strength, although entrained with the alternans rhythm (i.e., one-half the heart rate). This finding could suggest that MSNA primarily responds to acute beat-to-beat changes in blood pressure rather than to longer term changes in average pressure level, resulting in a large burst induced by the beat-to-beat shifts of the alternans pressure despite the overall increase in pressure level. On the other hand, this response pattern may be related to heart failure per se rather than illustrating a general principle, because CHF seems to be characterized by blunted MSNA responses to slow, but not rapid, blood pressure changes (see the Introduction). Regardless of which interpretation is favored, the present findings clearly support the conclusion by Ando and co-workers (3) that arterial baroreceptor control of MSNA remains exquisitely sensitive to rapid blood pressure changes in human heart failure, because multiunit MSNA became entrained with the alternans rhythm despite the limited difference between the alternating pressure waves, being on average only 2 mmHg for diastolic pressure.

Obviously, information gained from the recording of a single neuron is limited. However, it is interesting to note that, in contrast to multiunit MSNA, the firing frequency of a single vasoconstrictor fiber was approximately halved during tachyarrhythmia, because of reductions in both firing probability and multiple with-

Fig. 5. Analysis of the recording in Fig. 4. A: averaged integrated multiunit muscle sympathetic nerve activity, ECG, and BP at sinus rhythm (left) and during tachycardia (right), with arrows indicating the alternating lower pressure waves. B: fast Fourier transform analysis of muscle sympathetic nerve activity, ECG, and BP at sinus rhythm (left) and during tachycardia (right); note 2 peaks in the BP curve, occurring at the heart rate and one-half the heart rate, whereas muscle sympathetic nerve activity only shows a peak at one-half the heart rate during tachycardia.
in-burst firing. This may indicate that the maintained average level of multunit MSNA was achieved via recruitment of previously silent vasoconstrictor neurons and indirectly suggests this as a third possible mechanism for acute changes in sympathetic outflow. That this mechanism is relevant is also underlined by our experience that we often observe additional units, with a different spike morphology, firing transiently during sympathoexcitatory stimuli.

Limitations. Our limited electrocardiograph recording montage does not allow us to specify the type or site of origin of the premature beats, but our objective was simply to use the blood pressure reductions after the premature beats as a short-lasting baroreflex stimulus. A few isolated premature heartbeats occurring during the recording of single-fiber activity of vasoconstrictor muscles in subjects without CHF (1 event in 1 healthy subject, 3 events in 2 patients with obstructive sleep apnea) were all followed by multiple within-burst firing, indicating that this altered firing pattern in response to transient baroreceptor unloading is not specific to CHF patients (Macefield and Elam, unpublished observations). Although the increased firing probability at rest in CHF patients may set the stage for a shift toward multiple within-burst firing in response to acute excitatory stimuli (cf. above), our limited data from ectopic heartbeats in subjects without CHF do not allow us to draw such a conclusion. Needless to say, interpretations based on a single case of sustained tachycardia must be cautious. However, the major technical difficulties involved in achieving recordings from single sympathetic nerve fibers, especially during stimuli affecting sympathetic discharge, lead us to include the observations from this case because we consider it unlikely that additional information from similar events in other patients will be collected. Finally, the ongoing pharmacological treatment of our patients is a limitation of our study. In this, as in several previous studies of MSNA in CHF from our laboratory, we chose this strategy to avoid rebound cardiovascular responses and associated baroreceptor-mediated effects on sympathetic nerve traffic.

In conclusion, recordings from single vasoconstrictor nerve fibers of the muscle vascular bed in CHF patients illustrate two different mechanisms (increases in firing probability and multiple within-burst firing) and indirectly indicate a third mechanism (recruitment of previously silent fibers), for acute sympathoexcitatory responses. The marked shift toward multiple within-burst firing during hypotensive periods after cardiac dysrhythmias may constitute a risk factor per se because it entails neural volleys with high within-burst firing frequencies in a large proportion of sympathetic fibers.

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