

Augmented single-unit muscle sympathetic nerve activity in heart failure with chronic atrial fibrillation

著者	Ikeda Tatsunori, Murai Hisayoshi, Kaneko Shuichi, Usui Soichiro, Kobayashi Daisuke, Nakano Manabu, Ikeda Keiko, Takashima Shin-ichiro, Kato Takeshi, Okajima Masaki, Furusho Hiroshi, Takamura Masayuki
journal or publication title	Journal of Physiology
volume	590
number	3
page range	509-518
year	2012-02-01
URL	http://hdl.handle.net/2297/30371

doi: 10.1113/jphysiol.2011.223842

**Augmented single-unit muscle sympathetic nerve activity
in heart failure with chronic atrial fibrillation**

Tatsunori Ikeda, Hisayoshi Murai, Shuichi Kaneko, Soichiro Usui, Daisuke Kobayashi,
Manabu Nakano, Keiko Ikeda, Shin-ichiro Takashima, Takeshi Kato, Masaki Okajima,
Hiroshi Furusho and Masayuki Takamura

Department of Disease Control and Homeostasis, Graduate School of Medical Science,
Kanazawa University, Kanazawa, Japan

Running head: Sympathetic nerve activity in heart failure with atrial fibrillation

Running head: Sympathetic nerve activity in heart failure with atrial fibrillation

Abstract: 248 words

Total words: 4863 words

Corresponding author:

Shuichi Kaneko

Disease Control and Homeostasis, Graduate School of Medical Science, Kanazawa
University, 13-1 Takara-machi, Kanazawa 920-8641, Japan

Telephone No: 81-76-265-2238

Fax No: 81-76-234-4248

E-mail: skaneko@m-kanazawa.jp

Abstract

Background- Atrial fibrillation (AF) is a common complication in heart failure (HF) patients.

However, it remains unclear whether irregular ventricular response patterns induced by AF increase sympathetic nerve activity.

Methods and Results- We measured resting multi- and single-unit muscle sympathetic nerve activity (MSNA) in 21 age-matched HF patients with chronic AF ($n = 11$) rhythm or sinus rhythm (SR, $n = 10$). The multi-unit MSNA, which was expressed as total activity, was similar between HF + AF patients and HF + SR patients. However, the single-unit MSNA in HF + AF patients was significantly greater than that in HF + SR patients (62 ± 9 spikes/min vs. 42 ± 4 spikes/min, $p < 0.05$). Moreover, the incidence of multiple firing of single-unit MSNA within a given burst was augmented in HF + AF patients as compared with HF + SR patients ($48 \pm 8\%$ vs. $26 \pm 3\%$, $p < 0.01$). A significant negative relationship was observed between the reduced diastolic pressure induced by a prolonged cardiac interval in AF subjects and single-unit MSNA frequency within one cardiac interval in each HF + AF subject.

Conclusions- The firing characteristics of single-unit MSNA were different between HF patients with AF and HF patients with SR; particularly, those with a prolonged long RR interval showed multiple firings of single-unit MSNA. These findings suggest that AF per se leads to the instantaneous augmentation of single-unit MSNA induced by decreased diastolic pressure, which might partially contribute to disease progression in HF patients.

Key words: atrial fibrillation, heart failure, muscle sympathetic nerve activity

Introduction

Atrial fibrillation (AF) is a well known and common complication in chronic heart failure (HF) (Braunwald, 1997). The prevalence of AF in patients with HF also increases in parallel with the severity of the disease, ranging from 5 to 10% in patients with mild HF, 5 to 26% in patients with moderate HF, and up to 50% in patients with severe HF (Kareti et al. 2005). AF has also been assumed to facilitate and worsen the development of chronic HF (CHF). The onset and coexistence of these cardiac disorders produces a vicious cycle, leading to advanced pump failure in HF patients (Ehrlich et al. 2002). However, the pathophysiological relationship between AF and HF has remained unclear.

Sympathetic nerve activity during AF is considered to play an important role in the deterioration of cardiac function. As compared with studies examining the acute state of paroxysmal AF (Wasmund et al. 2003; Grassi et al. 2003), little is known regarding sympathetic nerve activity during the chronic AF state in HF. A previous study demonstrated that cardiac sympathetic nerve activity is not elevated in well-treated CHF patients with AF as compared with those in SR using the cardiac norepinephrine (NE) spillover method (Gould et al. 2003). However, a U.S. carvedilol study demonstrated that beta blockers improve the outcome in HF patients with AF (Ramaswamy, 2003). These results suggest that activation or disturbance of the sympathetic nervous system in AF rhythm may be involved in the progression of disease severity in HF.

With respect to the evaluation of sympathetic nerve activity in humans, direct recording of muscle sympathetic nerve activity (MSNA) has been conducted through integrated multi-unit fiber activity to allow for the examination of sympathetic outflow to the peripheral system. The degree of sympathetic activation can be quantified by counting the number of bursts or the burst amplitude distribution (Sverrisdottir et al. 2000). However, AF rhythm is recognized as one of the exclusion criteria for evaluating sympathetic outflow using multi-unit MSNA analysis in human studies because an irregular ventricular response could produce a large sympathetic burst activity followed by prolonged sympathetic inhibition, which can lead to the underestimation of true sympathetic nerve activity. A newly developed technique has demonstrated that a unitary recording of single-unit MSNA provides more detailed sympathetic properties (Macefield et al. 1994; Murai et al. 2006; Lambert et al. 2008). The greatest advantage of the single-unit approach is the identification of instantaneous firing patterns of the central sympathetic drive during one cardiac interval. We have previously reported a greater increase in firing probability and that multiple firing of muscle vasoconstrictor neurons occurs during the handgrip exercise in CHF (Murai et al. 2009). Additionally, a recent study reported that multiple firing within one cardiac interval was related to cardiac norepinephrine spillover (Lambert et al. 2011). Thus, it is likely that this method will be useful for evaluating the instantaneous alteration of central sympathetic nerve activity in the AF rhythm.

In the present study, we hypothesized that irregular ventricular responses in AF would increase sympathetic augmentation in patients with HF. To elucidate the influence of chronic AF on sympathetic outflow, we compared direct recordings of both single and multi-unit MSNA between HF patients with sinus rhythm (SR) or AF. Additionally, we examined the relationship between the RR interval, blood pressure, and single-unit MSNA to investigate whether single-unit MSNA is regulated by arterial baroreflex in HF patients with AF.

Methods

Subjects

Data were obtained from 11 age-matched HF patients (7 males, 4 females) with AF, aged 63 ± 7 years, and 10 HF patients (7 males, 3 females) with SR, aged 64 ± 2 years. Patients were classified as being in New York Heart Association functional class II (9 patients) or III (2 patients) in the HF + AF group and class II (8 patients) or III (2 patients) in the HF + SR group. The inclusion criteria for the HF patients with and without AF were aged between 18 and 80 years, displaying symptoms of HF, and on stable medical therapy for > 3 months. Chronic AF was defined as being treated with rate control therapy for > 6 months. Patients were excluded if they had suffered acute coronary syndrome, clinical relevant sleep apnea, or acute exacerbation of HF within the preceding 3 months. No patient underwent regimented exercise training. The study protocol and its purpose were explained in detail to each subject, and informed consent was obtained from all of the subjects. The study protocol was approved by the ethical panel of the Graduate School of Medical Science, Kanazawa University.

Study protocol

All experiments were performed in a quiet, electrically shielded room, at the same time in the morning (9:00–12:00 AM), with the subject in the supine position. Following the positioning of the tungsten electrode, MSNA was determined and all subjects were allowed to rest for 15 min.

The patients underwent recordings of MSNA, heart rate (HR), and continuous, non-invasive blood pressure (BP) monitoring during a 5-min period. To minimize any confounding effects of a distended bladder on sympathetic nerve recordings, diuretics were withheld on the morning of the study, but other medications were continued as prescribed.

Measurements

All experiments were conducted in a quiet, electrically shielded room with the subjects in the supine position. The subject's HR was determined from the time between successive R-wave intervals, recorded from an electrocardiogram sampled at 1000 Hz. Arterial pressure was recorded continuously from the radial artery and digitized at 800 Hz using a non-invasive tonometry monitoring system (JENTOW-7700; Nihon Colin, Komaki, Japan). Postganglionic muscle sympathetic nerve activity was recorded from the left peroneal nerve, as described previously (Murai et al. 2006; Murai et al. 2009). Briefly, the common peroneal nerve was located posterior to the head of the fibular bone by palpation and surface electrical stimulation. A high-impedance tungsten microelectrode (type 25-5-1; Frederick Haer, Brunswick, ME, USA) was inserted percutaneously into a motor fascicle, and then adjusted until spontaneous pulse-synchronous multi-fiber bursts of sympathetic activity were observed. To determine the shape of a single-unit sympathetic action potential, further adjustments of the microelectrode were required until a large unitary spike discharge could be observed in the raw nerve recordings. In the current study, the

single- and multi-unit MSNA were recorded simultaneously from the same microelectrodes.

Data analysis

Sympathetic neural activity was amplified ($\times 70,000$), band-pass filtered (0.5–3.0 kHz), sampled at 12 kHz, and stored on a digital audio tape recorder (DT120RT; Sony, Tokyo). To produce a mean voltage neurogram for the analysis of multi-unit MSNA, the amplified and filtered nerve activity was full-wave rectified, passed through a resistance-capacitance integrating circuit with a time constant of 0.1 s, and connected to an audio speaker. Multi-unit integrated nerve activity was digitized at a sampling rate of 1000 Hz. Both the raw nerve signals and the mean voltage neurogram were displayed visually on an oscilloscope (Neuropack 2; Nihon Kohden, Tokyo). During the off-line analysis, the morphology of single-unit MSNA spikes was analyzed using the Sony PC Scan II software. All candidate action potentials were then superimposed in an attempt to determine that the single-unit spikes of MSNA originated from a single fiber, as described previously (Murai et al. 2006). We used the following criteria that were refined by Macefield et al. (1994) to identify single-unit spikes of MSNA: (1) synchronization with multi-unit MSNA bursts, (2) a triphasic spike morphology, with the main phase being negative, and (3) constancy of morphology using the superimposition method (Macefield et al. 1994). When the single-unit MSNA was superimposed, minimal variation was observed in the single-unit spike amplitude or shape. Typical recordings of single- and multi-unit MSNA from patients with HF with and without

AF are shown in Figure 1. The multi-unit MSNA bursts in the AF patients were consistent with similar burst onset latency. The largest spike waveform was isolated from the raw action potential synchronous with multi-unit MSNA bursts. As in healthy subjects, in AF patients, single-unit MSNA spike waveforms were observed in each burst and in subsequently performed recordings.

For each subject, the mean voltage neurogram multi-unit MSNA burst frequency (bursts/min) and burst incidence (bursts per 100 heart beats) were determined. The normalized mean burst amplitude was calculated by assigning the largest absolute burst amplitude recorded at rest as an arbitrary value of 10 and expressing all other burst amplitudes as a percentage of this maximum burst height. Total MSNA activity was then calculated as the product of mean burst frequency and the mean normalized burst amplitude. Single-unit MSNA was expressed as both the total number of spikes per minute (spike frequency) and per 100 heart beats (spike incidence). The percentage of bursts or cardiac intervals showing one or multiple single-unit spikes was calculated from the number of bursts or cardiac intervals in which single-unit spikes were fired divided by all of the bursts and cardiac intervals with at least one spike.

Statistical analyses

Results are expressed as means \pm standard error of the mean (SEM). Statistical analyses were performed using the unpaired t-test and Fisher's exact test for group comparisons. Within group comparisons, the paired t-test was used. All calculations were performed using the statistical

package Stat View (Abacus Concepts, Inc., Berkeley, CA, USA; 1995). To assess the relationship between the RR interval, arterial pressure, and single-unit MSNA, we performed a linear regression analysis between the cardiac parameters and corresponding single-unit MSNA frequency. p -values < 0.05 were deemed to indicate statistical significance.

Results

Baseline comparisons

The baseline characteristics of HF patients with AF or SR are shown in Table 1. We observed no significant difference between the groups regarding age, heart rate, arterial pressure, body mass index, drugs, or etiology of HF. The results of the analysis of multi-unit MSNA are shown in Figure 2. The multi-unit MSNA burst frequency (Fig. 2A) and burst incidence (Fig. 2B) significantly decreased in the HF + AF group as compared with the HF + SR group (30 ± 7 bursts/min vs. 44 ± 2 bursts/min, and 50 ± 8 bursts/100 heart beats vs. 67 ± 5 bursts/100 heart beats, respectively; $p < 0.05$). However, the total multi-unit MSNA (Fig. 2C) did not differ between HF patients with and without AF. In contrast, single-unit MSNA frequency (62 ± 9 spikes/min vs. 42 ± 4 spikes/min, $p < 0.05$, Fig. 3A) and incidence (100 ± 13 spikes/100 heart beats vs. 67 ± 5 spikes/100 heart beats, respectively, $p < 0.05$, Fig. 3B) were significantly greater in the HF+AF group than in the HF+SR group.

Distribution of the incidence of multiple single-unit spikes between HF patients with and without AF

The percentages of multiple single-unit MSNA that fired within a given burst in HF patients with AF or SR are shown in Figure 4. The maximum firing frequency within a given burst was four single-unit spikes in the HF + SR group, while five single-unit spikes were observed in the

HF + AF group. In HF + AF patients, the percentages of multiple single-unit spikes that occurred were significantly decreased in one spike ($56 \pm 5\%$ vs. $74 \pm 3\%$, respectively; $p < 0.05$) and increased in two spikes ($25 \pm 6\%$ vs. $18 \pm 2\%$, respectively; $p < 0.05$), three spikes ($13 \pm 3\%$ vs. $5 \pm 0.8\%$, respectively, $p < 0.05$), four spikes ($6 \pm 3\%$ vs. $1.4 \pm 1.2\%$, respectively, $p < 0.05$), and five spikes ($3 \pm 1.0\%$ vs. $0.0 \pm 0.0\%$, respectively, $p < 0.05$).

Correlation between single-unit MSNA with systolic pressure, diastolic pressure, and the preceding R-R interval

To determine the underlying mechanism of the increase in SNA, we examined the relationship between the number of single-unit MSNA and arterial pressure and the preceding R-R interval in one cardiac interval in all HF + AF patients. A total of 307 ± 42 RR intervals during 5 min in HF + AF patients and 312 ± 46 in HF + SR patients were analyzed. The results are shown in Table 2. No significant correlation was observed between systolic arterial pressure and single-unit MSNA in HF + AF patients, except for one case. However, single-unit MSNA had a significant negative correlation with diastolic arterial pressure in all HF + AF patients. Additionally, a significant positive correlation was observed between the preceding R-R interval and single-unit MSNA in 10 of the 11 HF + AF patients. However, in the HF + SR group, a significant correlation between the diastolic pressure or preceding R-R interval and single-unit MSNA was observed in only 2 of 10 in HF +SR patients ($p < 0.05$, vs HF+AF patients).

Discussion

The current study is the first report to demonstrate the firing characteristics of the central sympathetic outflow to the peripheral system by evaluating single- and multi-unit sympathetic nerve activity in HF patients with AF. The novel and important findings of the present study are that (1) no difference in multi-unit MSNA, which was expressed as total MSNA, was observed between HF patients with and without AF. Additionally, the multi-unit MSNA frequency expressed as both burst frequency and burst incidence was decreased in HF patients with AF; (2) the discharge frequency of single-unit MSNA was significantly augmented and the firing pattern of single-unit MSNA spikes expressed as a percentage of multiple firing within one cardiac interval was significantly increased in HF patients with AF; and (3) a significant negative relationship was observed between diastolic pressure and single-unit MSNA frequency within one cardiac interval. These findings suggest that irregular ventricular responses induced by AF cause the instantaneous augmented multiple firings of single-unit MSNA.

We measured single-unit and multi-unit MSNA to evaluate the accurate activity of one unitary sympathetic firing in AF patients. The analysis of multi-unit MSNA in AF rhythm is not without limitations in that prolonged irregular ventricular responses would cause a large burst followed by prolonged sympathetic inhibition. In our study, multi-unit-burst counting was reduced in HF patients with AF. Previous assessments of SNA in acute paroxysmal AF patients using

multi-unit MSNA are controversial. Grassi and colleagues used multi-unit MSNA to assess SNA during AF and SR in patients with paroxysmal AF and observed a reduction in SNA during AF (Grassi et al. 2003). By contrast, Wasmund and coworkers found a significant augmentation of SNA during AF that was induced by right atrial pacing (Wasmund et al. 2003). As shown previously, single-unit MSNA provides a more detailed examination of neuron firing, particularly during one cardiac interval (Murai et al. 2006). The amplitude and/or size of multi-unit MSNA can be used as a surrogate analysis to quantify the sympathetic nerve outflow to the peripheral system, including cardiac intervals, but is still unable to evaluate the number of sympathetic nerve firings during one cardiac interval. In the present study, although the multi-unit MSNA frequency expressed as the total MSNA activity (the relative amplitude multiplied by burst frequency) was similar between HF patients with and without AF, single-unit MSNA was significantly greater in AF as compared with SR patients. These results indicate that the counting of multi-unit MSNA and total MSNA activity would be insufficient to express the total quantity and quality of sympathetic nerve activity in humans.

The evaluation of sympathetic nerve activity in patients with AF has not been adequately analyzed, because an irregular R-R interval makes it difficult to assess the quantity of sympathetic nerve activity. Sympathetic nerve activity is recognized as one of the therapeutic targets that can be used to decrease the severity of heart failure. Previous studies have indicated that no difference

was evident in the total systemic or cardiac NE spillover between AF and SR patients, both of which show LV dysfunction (Gould et al. 2003). In the current study, we observed no significant SNA differences with respect to the total multi-unit MSNA between HF patients with and without AF.

These results suggest that the total level of sympathetic activity expressed as a time interval should be similar between SR and AF rhythm in HF patients. However, the analysis of single-unit MSNA revealed the instantaneous sympathetic pattern (quality of sympathetic nerve firing pattern) in one cardiac interval. Our data demonstrate that single-unit MSNA is significantly greater in HF patients with AF together with an increased multiple firing incidence. The occurrence of multiple spikes is not specific to heart failure patients. Previously, our study demonstrated that, in healthy subjects, the percentage of multiple firing spikes during one burst was increased during the Valsalva maneuver, although the percentage was low at rest (Murai et al. 2006). These instantaneous multiple spike firings are thought to influence strong effector organ responses by a greater release of norepinephrine. In fact, acute irregular and rapid nerve stimulation has been shown to evoke a greater effector organ response than regular stimulation in anesthetized rats (Dibona et al. 1999). In humans, Lambert et al. observed that the incidence of multiple firing is associated with cardiac norepinephrine spillover in humans (Lambert et al. 2011). Recent research has shown that, at rest, a high firing frequency or increased incidence (percentage) of multiple spikes is related to cardiovascular risk factors such as hypertension (Lambert et al 2008), obstructive sleep apnea (Elam

et al 2002), and congestive heart failure (Macefield et al. 1999; Murai et al 2009). AF and/or ectopic premature ventricular contraction are thought to contribute to reduced left ventricular systolic function, although the mechanisms of reduced left ventricular contraction remain unclear. Our results might aid in our understanding of the mechanisms underlying worsening left ventricular function in AF patients.

A greater correlation was observed between the preceding R-R interval or diastolic pressure and single-unit MSNA in HF + AF patients as compared with HF + SR patients. Our results indicate that afferent sympathetic regulatory mechanisms in HF patients with AF would be different from that in HF patients with SR. Disordered arterial baroreceptor function has been assumed to be a mechanism involved in the augmentation of SNA in heart failure. However, recent evidence has demonstrated that arterial baroreceptor function is preserved, maintaining appropriate blood pressure in heart failure (Floras, 2009). In the present study, the firing frequency of single-unit MSNA within one cardiac interval was significantly associated with diastolic blood pressure and the preceding RR interval. Elam and coworkers demonstrated that instantaneous augmentation of multiple single-unit firing was induced following premature ventricular contraction in HF patients (Elam et al. 2001). These data indicate that arterial baroreflex mechanisms play an important role in the regulation of single-unit MSNA in HF patients with AF. However, because correlation coefficients of diastolic pressure were not high ($r = 0.46 \pm 0.05$), augmented multiple

firing of single-unit MSNA seems to be attributed to not only decreased diastolic arterial pressure, but also other factors. Kaye and coworkers reported that cardiac filling pressures expressed as the pulmonary capillary wedge pressure (PCWP) were related to cardiac sympathetic nerve activity (Kaye et al. 1994). Complicated interactions that exaggerate cardiac sympathetic afferent stretch receptors and/or blunt cardiopulmonary baroreceptors are important factors regulating efferent sympathetic outflow in HF patients. The elevated cardiac filling pressure induced by a prolonged RR interval in HF patients with AF might contribute to an increase in single-unit MSNA. Further studies are needed to elucidate the afferent integrated signals that regulate the altered to increased multiple firing pattern of single-unit MSNA in HF +AF patients.

Recent large-scale trials demonstrated that rate control therapy was not inferior to rhythm control in AF patients (Van Gelder et al. 2002; Wyse et al. 2002), even in HF patients with AF (Roy et al. 2008). One conceivable reason for this discrepancy is that no ideal drug for rhythm control therapy currently exists. Conversely, in the rate control group of the HF study, about 80% of patients were treated with a beta blocker as rate control therapy, which might cause beneficial effects on instantaneous sympathetic firing (Roy et al. 2008). Indeed, pulmonary vein ablation for AF with no arrhythmic drug was reported to improve cardiac function and exercise capacity (Hsu et al. 2004). In the present study, sympathetic nerve activity, which was expressed as single-unit MSNA frequency and the percentage of multiple spike firing, was significantly augmented in HF patients

with AF. An augmentation of sympathetic nerve activity is known to accelerate heart failure, increasing fatal arrhythmias and enhancing coagulation (Cohn et al. 1984; Kaye et al. 1995; Otowa et al. 2008). Thus, our results suggest that, regarding the autonomic nervous system, the restoration of SR would be more effective in reducing the instantaneous augmentation of multiple spike firing of single-unit MSNA in HF patients with AF.

Study limitations

Two main limitations arise from the current study protocol. First, during the study protocol, the heart rate of AF patients was pharmacologically controlled, meaning that sympathetic nerve activity was affected by drugs. However, the baseline drugs did not differ between HF patients with and without AF. Second, the optimal heart rate was not determined in the treatment of AF. The average heart rate in AF patients was 61 ± 4 bpm, although AF patients without rate control therapy were not recruited in this study. These heart rates raise the concern that an exceeded rate control could accelerate sympathetic activity. However, the average range of rate control did not decrease below 50 bpm. Thus, our rate control therapy was considered to be in an acceptable range during clinical treatment.

Conclusions

The present study provides important information regarding the analysis of single-unit MSNA in patients with chronic AF. More intense single-unit MSNA within one cardiac interval

occurred in HF patients with AF than patients without AF, even in AF patients receiving heart rate control therapy. Accordingly, our results suggest that AF per se augments central sympathetic activity and the restoration of sinus rhythm may be more effective in the treatment of HF patients with AF.

Acknowledgments

We would like to thank Dr. Shigeo Takata (Kanazawa Municipal Hospital) and Dr. Toyoshi Yuasa for help with our microneurography techniques.

Disclosures

None.

Figure legends

Figure 1. Typical recording of single- and multi-unit muscle sympathetic nerve activity (MSNA) in a heart failure (HF) patient with sinus rhythm (SR) (A) and an HF patient with atrial fibrillation (AF) (B). The arrows indicate the single-unit MSNA derived from the raw action potentials. Single-fiber action potentials occur with synchronizing multi-unit MSNA discharges in both HF patients. Little variation in spike amplitude or shape was observed in superimposed single-unit spikes, indicating that the action potentials originated from the same sympathetic fiber.

Figure 2. Comparison of multi-unit muscle sympathetic nerve activity (MSNA) between heart failure (HF) patients with sinus rhythm (SR) and HF patients with atrial fibrillation (AF). Burst frequency (A) and burst incidence (B) were significantly decreased in HF patients with SR versus HF patients with AF. However, the total multi-unit MSNA did not differ between the two groups (C). Values are expressed as means \pm SEM. * $P < 0.05$, compared with HF patients with SR.

Figure 3. Comparison of single-unit muscle sympathetic nerve activity (MSNA) between heart failure (HF) patients with sinus rhythm (SR) and HF patients with atrial fibrillation (AF).

Spike frequency (**A**) and spike incidence (**B**) were significantly greater in HF patients with AF versus HF patients with SR. Values are expressed as means \pm SEM. * $P < 0.01$, compared with HF patients with SR.

Figure 4. Percentages of multiple spikes in heart failure (HF) patients with sinus rhythm (SR) and HF patients with atrial fibrillation (AF). In HF patients with AF, the percentage of one to five spikes per cardiac interval was significantly decreased in one spike and increased in two to five spikes. Values are expressed as means \pm SEM. * $P < 0.05$, compared with HF patients with SR.

REFERENCES

- Braunwald E (1997). Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med* **337**, 1360-1369
- Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB & Rector T (1984). Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med* **311**, 819-823
- Dibona GF & Sawin LL (1999). Functional significance of the pattern of renal sympathetic nerve activation. *Am J Physiol* **277**, 346-353
- Ehrlich JR, Nattel S & Hohnloser SH (2002). Atrial fibrillation and congestive heart failure: specific considerations at the intersection of two common and important cardiac disease sets. *J Cardiovasc Electrophysiol* **13**, 399-405
- Elam M & Macefield V (2001). Multiple firing of single muscle vasoconstrictor neurons during cardiac dysrhythmias in human heart failure. *J Appl Physiol* **91**, 717-724
- Elam M, McKenzie D & Macefield V (2002). Mechanisms of sympathoexcitation: single-unit analysis of muscle vasoconstrictor neurons in awake OSAS subjects. *J Appl Physiol* **93**, 297-303
- Floras JS (2009). Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. *J Am Coll Cardiol* **54**, 375-385
- Gould PA, Esler MD & Kaye DM (2003). Chronic atrial fibrillation does not influence the

magnitude of sympathetic overactivity in patients with heart failure. *Eur Heart J* **24**, 1657-1662

Grassi G, Seravalle G, Bertinieri G & Mancia G (2003). Behaviour of the adrenergic cardiovascular

drive in atrial fibrillation and cardiac arrhythmias. *Acta Physiol Scand* **177**, 399-404

Hsu LF, Jais P, Sanders P, Garrigue S, Hocini M, Sacher F, Takahashi Y, Rotter M, Pasquie JL,

Scavee C, Bordachar P, Clementy J & Haissaguerre M (2004). Catheter ablation for atrial

fibrillation in congestive heart failure. *N Engl J Med* **351**, 2373-2383

Kareti KR, Chiong JR, Hsu SS & Miller AB (2005). Congestive heart failure and atrial fibrillation:

rhythm versus rate control. *J Card Fail* **11**, 164-172

Kaye DM, Lambert GW, Lefkovits J, Morris M, Jennings G, & Esler MD (1994). Neurochemical

evidence of cardiac sympathetic activation and increased central nervous system norepinephrine

turnover in severe congestive heart failure. *J Am Coll Cardiol* **23**, 570-578

Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A & Esler MD (1995). Adverse

consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol*

26, 1257-1263

Lambert E, Dawood T, Schlaich M, Straznicky N, Esler M & Lambert G (2008). Single-unit

sympathetic discharge pattern in pathological conditions associated with elevated cardiovascular

risk. *Clin Exp Pharmacol Physiol* **35**, 503-507

Lambert EA, Schlaich MP, Dawood T, Sari C, Chopra R, Barton DA, Kaye DM, Elam M, Esler MD

& Lambert GW (2011). Single-unit muscle sympathetic nervous activity and its relation to cardiac noradrenaline spillover. *J Physiol* **589**, 2597-2605

Macefield VG, Wallin BG & Vallbo AB (1994). The discharge behaviour of single vasoconstrictor motoneurons in human muscle nerves. *J Physiol* **481** (Pt 3):799-809

Murai H, Takata S, Maruyama M, Nakano M, Kobayashi D, Otowa K, Takamura M, Yuasa T, Sakagami S & Kaneko S (2006). The activity of a single muscle sympathetic vasoconstrictor nerve unit is affected by physiological stress in humans. *Am J Physiol Heart Circ Physiol* **290**, H853-H860

Murai H, Takamura M, Maruyama M, Nakano M, Ikeda T, Kobayashi D, Otowa K, Ootsuji H, Okajima M, Furusho H, Takata S & Kaneko S (2009). Altered firing pattern of single-unit muscle sympathetic nerve activity during handgrip exercise in chronic heart failure. *J Physiol* **587**, 2613-2622

Otowa K, Takamura M, Murai H, Maruyama M, Nakano M, Ikeda T, Kobayashi D, Ootsuji H, Okajima M, Furusho H, Yuasa T, Takata S & Kaneko S (2008). Altered interaction between plasminogen activator inhibitor type 1 activity and sympathetic nerve activity with aging. *Circ J* **72**, 458-462

Ramaswamy K (2003). Beta blockers improve outcome in patients with heart failure and atrial fibrillation: U.S. carvedilol study. *Card Electrophysiol Rev* **7**, 229-232

Roy D, Talajic M, Nattel S, Wyse DG, Dorian P, Lee KL, Bourassa MG, Arnold JM, Buxton AE,

Camm AJ, Connolly SJ, Dubuc M, Ducharme A, Guerra PG, Hohnloser SH, Lambert J, Le

Heuzey JY, O'Hara G, Pedersen OD, Rouleau JL, Singh BN, Stevenson LW, Stevenson WG,

Thibault B & Waldo AL (2008). Rhythm control versus rate control for atrial fibrillation and

heart failure. *N Engl J Med* **358**, 2667-2677

Sverrisdottir YB, Rundqvist B, Johannsson G & Elam M (2000). Sympathetic neural burst amplitude

distribution: A more specific indicator of sympathoexcitation in human heart failure. *Circulation*

102, 2076-2081

Van Gelder IC, Hagens VE, Bosker HA, Kingma JH, Kamp O, Kingma T, Said SA, Darmanata JI,

Timmermans AJ, Tijssen JG & Crijns HJ (2002). A comparison of rate control and rhythm

control in patients with recurrent persistent atrial fibrillation. *N Engl J Med* **347**, 1834-1840

Wasmund SL, Li JM, Page RL, Joglar JA, Kowal RC, Smith ML & Hamdan MH (2003). Effect of

atrial fibrillation and an irregular ventricular response on sympathetic nerve activity in human

subjects. *Circulation* **107**, 2011-2015

Wyse DG, Waldo AL, DiMarco JP, Domanski MJ, Rosenberg Y, Schron EB, Kellen JC, Greene HL,

Mickel MC, Dalquist JE & Corley SD (2002). A comparison of rate control and rhythm control

in patients with atrial fibrillation. *N Engl J Med* **347**, 1825-1833

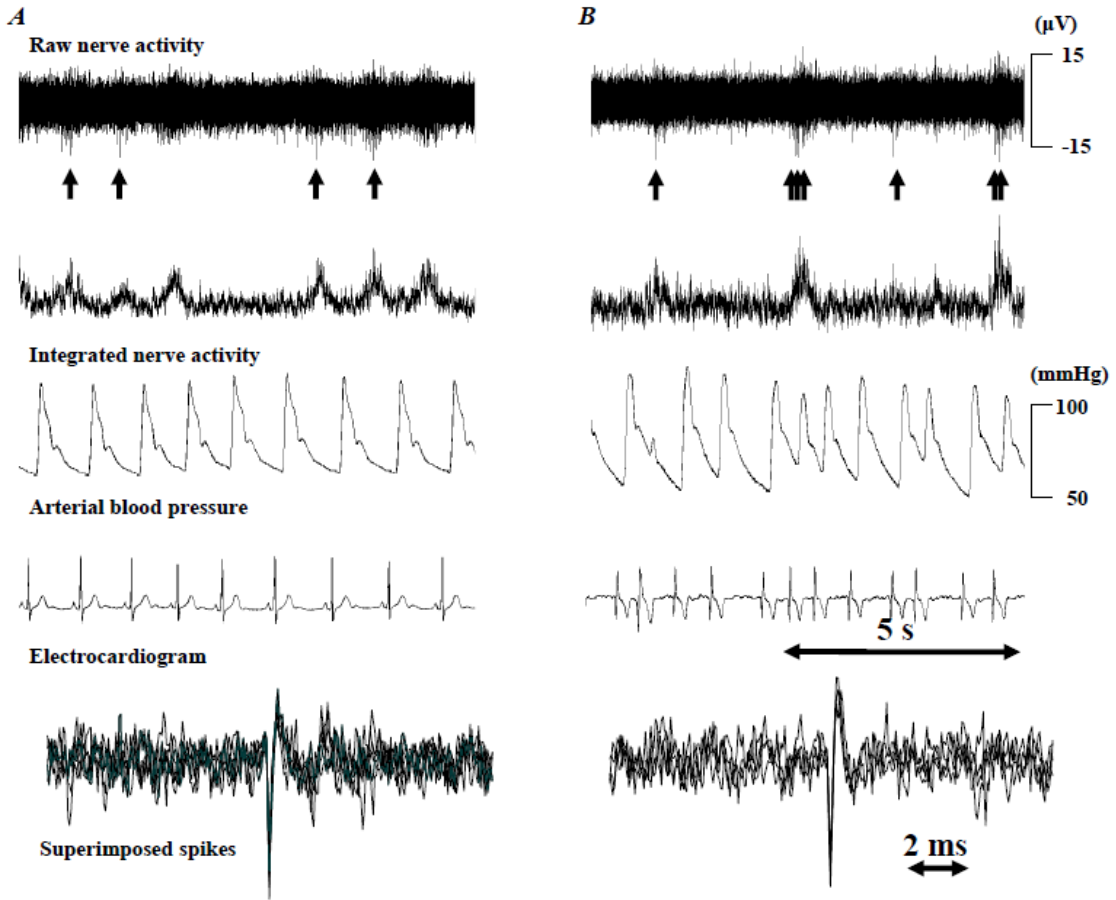


Fig.1

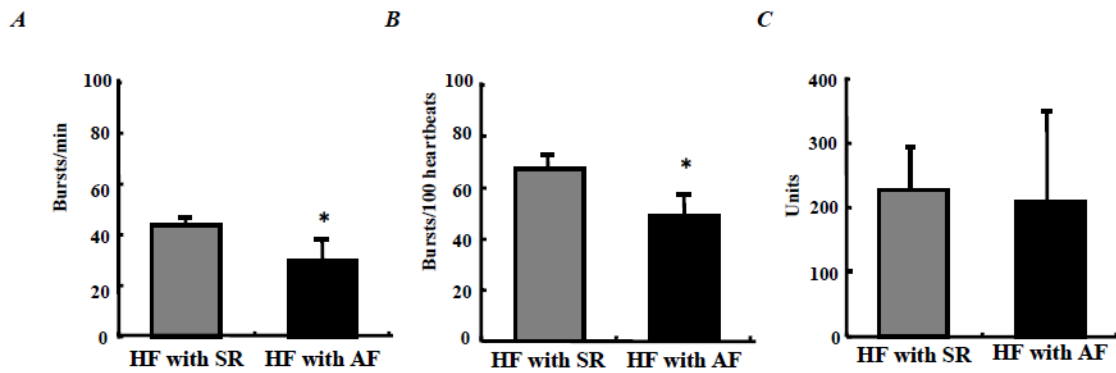


Fig.2

Fig3

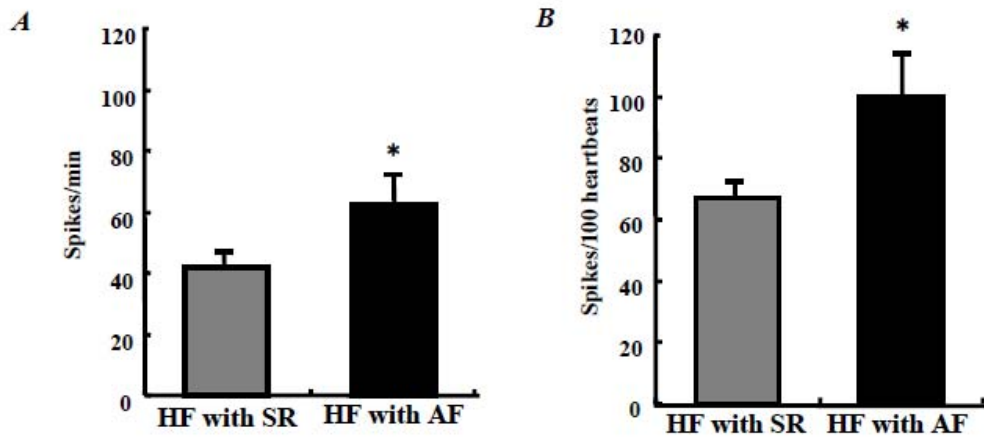


Fig4

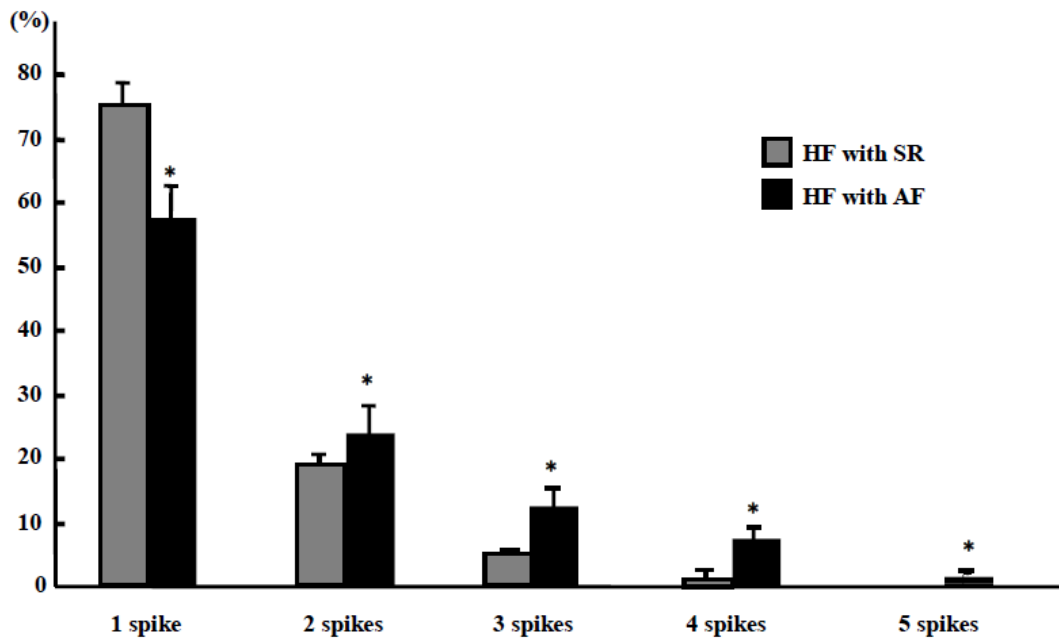


Table.1 Baseline characteristics

	HF with SR (n=10)	HF with AF (n=11)
Age (years)	64 ± 2	63 ± 7
Gender (M/F)	7/3	7/4
Heart rate (beats/min)	63 ± 3	61 ± 4
Height (cm)	163 ± 5	165 ± 6
Body weight (kg)	66 ± 5	66 ± 5
BMI (kg/m²)	25 ± 3	24 ± 3
SAP (mmHg)	111 ± 3	119 ± 13
DAP (mmHg)	63 ± 4	66 ± 8
MAP (mmHg)	72 ± 2	74 ± 8
LVEF (%)	40 ± 3	43 ± 5
LAD (mm)	43 ± 2	47 ± 3
Medication (% (n))		
ACE inhibitor or ARB	100 (10)	100 (11)
β-blocker	80 (8)	82 (9)
Ca channel blocker	50 (5)	46 (5)
Etiology (% (n))		
Coronary artery disease	60 (6)	55 (6)
Idiopathic	40 (4)	36 (5)

Results are expressed as the means ± SEM. BMI=body mass, SAP = systolic arterial pressure, DAP = diastolic arterial pressure, MAP = mean arterial pressure, LVEF = left ventricular ejection fraction, LAD=left atrial diameter, ACE = angiotensin converting enzyme, ARB = angiotensinII receptor blocker

Table 2. Correlation between hemodynamic parameters and single-unit MSNA frequency in HF with AF

Case No.	Systolic arterial pressure		Diastolic arterial pressure		RR interval	
	R	P value	R	P value	R	P value
1	0.005	0.9683	- 0.440	<0.001	0.540	<0.001
2	0.135	0.3349	- 0.332	<0.05	0.460	<0.001
3	0.157	0.2092	- 0.348	<0.01	0.371	<0.01
4	0.117	0.3595	- 0.457	<0.001	0.012	0.9245
5	0.336	<0.05	- 0.381	<0.01	0.715	<0.001
6	0.264	0.0533	- 0.549	<0.001	0.528	<0.001
7	0.046	0.7277	- 0.754	<0.001	0.793	<0.001
8	0.321	0.7942	- 0.771	<0.001	0.876	<0.001
9	0.160	0.2090	- 0.443	<0.001	0.341	<0.01
10	0.089	0.5070	- 0.370	<0.01	0.304	<0.05
11	0.159	0.1257	- 0.289	<0.05	0.275	<0.001

The English in this document has been checked by at least two professional editors,
both native speakers of English. For a certificate, please see:

<http://www.textcheck.com/certificate/cG5o6U>