Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure

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Ramchandra R, Hood SG, Frithiof R, May CN. Discharge properties of cardiac and renal sympathetic nerves and their impaired responses to changes in blood volume in heart failure. Am J Physiol Regul Integr Comp Physiol 297: R665–R674, 2009. First published June 17, 2009; doi:10.1152/ajpregu.00191.2009.—Sympathetic nerve activity (SNA) consists of discharges that vary in amplitude and frequency, reflecting the level of recruitment of nerve fibers and the rhythmic generation and entrainment of activity by the central nervous system. It is unknown whether selective changes in these amplitude and frequency components account for organ-specific changes in SNA in response to alterations in blood volume or for the impaired SNA responses to volume changes in heart failure (HF). To address these questions, we measured cardiac SNA (CSNA) and renal SNA (RSNA) simultaneously in conscious, normal sheep and sheep in HF induced by rapid ventricular pacing. Volume expansion decreased CSNA (−62 ± 10%, P < 0.05) and RSNA (−59 ± 10%, P < 0.05) equally (n = 6). CSNA decreased as a result of a reduction in burst frequency, whereas RSNA fell because of falls in burst frequency and amplitude. Hemorrhage increased CSNA (+74 ± 9%, P < 0.05) more than RSNA (+21 ± 5%, P < 0.09), in both cases because of increased burst frequency, whereas burst amplitude decreased. In HF, burst frequency of CSNA (from 26 ± 3 to 75 ± 3 bursts/min) increased more than that of RSNA (from 63 ± 4 to 79 ± 4 bursts/min). In HF, volume expansion caused no change in CSNA and an attenuated decrease in RSNA, due entirely to decreased burst amplitude. Hemorrhage did not significantly increase SNA in either nerve in HF. These findings support the concept that the number of sympathetic fibers recruited and their firing frequency are controlled independently. Furthermore, afferent stimuli, such as changes in blood volume, cause organ-specific responses in each of these components, which are also selectively altered in HF.

The opposite effect, an increase in total levels of RSNA (3, 27) via a preferential increase in burst amplitude (20). These findings imply that changes in RSNA, induced by alterations in blood volume, are mediated by central pathways that selectively regulate burst amplitude. An important unanswered question is whether burst amplitudes to different organs are regulated independently of each other by these central circuits. In this context, we recently demonstrated inhibition of cardiac SNA (CSNA) during volume expansion and stimulation of CSNA during hemorrhage (31); however, whether this occurs due to selective changes in cardiac sympathetic burst amplitude is unknown.

One of the pathological conditions associated with chronic fluid overload is heart failure (HF), which is associated with increases in CSNA and RSNA. Previous studies indicate that the responses of CSNA (31) and RSNA (5, 41) to volume expansion are impaired during HF. The response of CSNA during hemorrhage is also attenuated in animals in HF (31), and the response of RSNA has not been examined. Determining whether these impaired responses are due to an inability to recruit additional fibers (amplitude) or an inability to generate sympathetic discharges (frequency) will give further insight into the factors leading to sympathetic dysregulation in HF.

The aims of the present study were twofold: 1) to compare the changes in total CSNA and RSNA during volume changes in the normal state and to establish whether these changes were mediated by selective changes in sympathetic burst amplitude and/or frequency in these sympathetic nerves and 2) to determine whether in HF the impaired total SNA responses during volume changes are due to selective changes in either of these parameters. To address these questions, we recorded CSNA and RSNA simultaneously in conscious normal sheep and in sheep with HF during volume expansion and hemorrhage. We examined the extent to which changes in burst amplitude and frequency accounted for the changes in SNA.

METHODS

Adult merino ewes (35–49 kg body wt) were housed in individual metabolism cages in association with other sheep. Experiments were started when sheep were accustomed to laboratory conditions and human contact. Sheep were fed a diet of oaten chaff (800 g/day), and water was offered ad libitum. All experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Surgical procedures. Before the studies, sheep underwent two aseptic surgical procedures, each separated by ≥2 wk of recovery. Anesthesia was induced with intravenous thiopental sodium (15 mg/kg) and, after intubation, maintained with 1.5–2.0% isoflurane-\textsuperscript{O}2. In the first stage, sheep were prepared with a carotid arterial loop. Briefly, a carotid artery was isolated and exteriorized in a fold of skin to form a carotid arterial loop, allowing easy access for arterial cannulation. In a second group of sheep to be induced into HF (n =
8), a pacemaking lead (Medtronic, Minneapolis, MN) was inserted into the right ventricle, as described previously (38). The animals underwent 8–12 wk of rapid ventricular pacing at 200–220 beats/min. Ejection fraction was measured weekly in conscious sheep via echocardiography, and once ejection fraction had decreased to <40%, the sheep underwent the second aseptic surgical procedure. In a separate operation, intrafascicular electrodes were implanted in the cardiothoracic nerves (38), and, in the same session, intravascular electrodes were implanted in the renal nerve (22). Experiments were conducted on conscious sheep standing in their cages. To minimize any effect of surgical stress, experiments were not started until 4 days after implantation of the electrodes.

In all operations, animals were treated with antibiotics [procaine penicillin; Ilium Propen, Troy Laboratories (Smithfield, NSW, Australia) and Mavlab (Qld, Australia)] at the start of surgery and then for 2 days postoperatively. Postsurgical analgesia was maintained with flunixin meglumine (1 mg/kg im; Troy Laboratories or Mavlab) at the start of surgery and then 4 and 16 h after surgery.

On the day before implantation of recording electrodes, arterial and venous cannulas were inserted into the carotid artery and jugular vein, respectively, and the heart was exposed. A pacemaking lead (Medtronic, Minneapolis, MN) was inserted into the right ventricle, as described previously (38). The animals underwent 8–12 wk of rapid ventricular pacing at 200–220 beats/min. Ejection fraction was measured weekly in conscious sheep via echocardiography, and once ejection fraction had decreased to <40%, the sheep underwent the second aseptic surgical procedure. In a separate operation, intrafascicular electrodes were implanted in the cardiothoracic nerves (38), and, in the same session, intravascular electrodes were implanted in the renal nerve (22). Experiments were conducted on conscious sheep standing in their cages. To minimize any effect of surgical stress, experiments were not started until 4 days after implantation of the electrodes.

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Experimental protocols. CSNA and RSNA were recorded differentially between the pair of electrodes with the best signal-to-noise ratio. The signal was amplified (×100,000) and filtered (band pass 300–1,000 Hz), displayed on an oscilloscope, and passed through an audio amplifier and loudspeaker. SNA (5,000 Hz) and arterial and venous blood pressures (100 Hz) were recorded on a computer using a micro 1401 interface and Spike 2 software (Cambridge Electronic Design). The SNA signals were full-wave rectified and integrated using a low-pass time constant of 20 ms.

At 4 days after implantation of sympathetic nerve electrodes, a 5-min recording of resting CSNA, RSNA, arterial pressure, and CVP was made in conscious sheep in the normal state (n = 9) and in HF (n = 8). The animals were then subjected to the volume expansion or hemorrhage protocol on a randomly allocated basis. After ≥24 h of recovery, the second protocol was initiated. Both protocols could not be completed in all sheep because of deterioration of the signal in some sheep. For volume expansion (n = 6 in both groups), Gelofusine (Braun Australia, NSW, Australia) was infused until the total volume infused reached 500 ml. The rate was titrated to 350–500 ml/30 min. For the hemorrhage protocol (n = 6 in both groups), the venous cannula was connected to a blood infusion bag, and blood was removed under gravity at a rate of 20 ml/min, until 500 ml of blood were removed. After the end of the hemorrhage, the blood was reinfused into the animal.

Data analysis. Data were analyzed on a beat-to-beat basis using custom-written routines in the Spike 2 program. For each heartbeat, the program determined diastolic, systolic, and mean arterial blood pressures (MAP), heart period, and the area of the rectified and integrated SNA signals between diastolic pressures. The smallest burst was identified in the entire recording from a spreadsheet of data, and its correct position in the cardiac cycle and absence of artifacts were confirmed visually. The rectified and integrated area between the corresponding diastolic pressures of this burst was noted, and this area was taken as the minimum area for the definition of a burst. When the rectified and integrated area between any heartbeat was greater than the minimum area, this was determined to constitute a burst. For each sheep, the accuracy of burst determination was checked by eye for the data collected over the 5-min control period and at the 1-min periods when successive 50-ml volumes were infused or withdrawn. The burst incidence was calculated as the number of bursts per 100 heartbeats. The burst frequency was calculated as the number of bursts per minute.

For the determination of burst amplitude, the integrated area under the curve for each burst was taken as an index of burst amplitude for the associated heartbeat. The largest burst within the 5-min control period was taken to be 100%, and the sizes of all remaining bursts were calculated as a percentage of this burst. The relative burst amplitudes were calculated by determining the frequency distribution of all the bursts over 5 min during the control period and after infusion of 500 ml of Gelofusine and removal of 500 ml of blood.

Hemodynamic and SNA data were collected from recordings collected over 60-s periods at the end of the 5-min control period, and after each 50 ml of Gelofusin was infused or blood was withdrawn. The total level of SNA was taken as the product of the burst amplitude and the burst incidence. For total levels of SNA, the level of SNA during the 60 s of control was taken as 100%, and changes in total SNA are reported as percent change from control.

Data are expressed as means ± SE. Changes in hemodynamic variables and SNA were analyzed using repeated-measures ANOVA (SigmaStat, Access Softek, version 2.03). Changes in the burst amplitude median after volume expansion were analyzed using a paired t-test. P < 0.05 was considered statistically significant.

RESULTS

Resting hemodynamics and SNA levels in normal and HF animals. Resting levels of CSNA, RSNA, and arterial pressure during control periods in normal (n = 9) and HF (n = 8) sheep are shown in Table 1. In HF, left ventricular ejection fraction and fractional shortening, measured in conscious sheep by echocardiography, were significantly decreased (Table 1). Additional evidence that the sheep were in HF was the finding of significant increases in CSNA, RSNA, and CVP and a tendency for a higher heart rate (HR; Fig. 1, Table 1).

Volume expansion protocol. Volume expansion was associated with a significant increase in CVP and no change in MAP, pulse pressure, or HR in both groups of animals (Fig. 2). These changes were associated with significant decreases in total CSNA and RSNA in the normal animals, whereas, in HF, volume expansion caused no change in CSNA and the decrease in RSNA was greatly attenuated (Fig. 2). Linear regression lines relating the change in SNA for a given change in CVP were drawn for individual sheep in both groups. For a given change in CVP, the change in CSNA was significantly less in

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Values are means ± SE; n, number of sheep. Echocardiographic data for ejection fraction and fractional shortening in the normal group are preparing values from animals in the heart failure (HF) group; other normal variables are from the normal group of animals at the time sympathetic nerve activity (SNA) was recorded. CVP, central venous pressure; MAP, mean arterial pressure; HR, heart rate; CSNA, cardiac SNA; RSNA, renal SNA. *P < 0.05; †P = 0.13.
HF animals than in normal animals. This was reflected in the slopes of the average regression lines, which were significantly lower in the HF (0.36 ± 0.8, \( r^2 = 0.99 ± 0.01 \)) than in the normal (−12.0 ± 5.0, \( r^2 = 0.90 ± 0.02, P < 0.05 \)) animals. The change in RSNA was also significantly less in the HF (−1.8 ± 0.5, \( r^2 = 0.99 ± 0.01 \)) than in the normal (−8.9 ± 1.7, \( r^2 = 0.92 ± 0.02, P < 0.05 \)) animals.

Changes in burst frequency and burst amplitude. There was a significant, progressive decrease in burst incidence and frequency of CSNA and RSNA during infusion of 500 ml of Gelofusine in normal animals (Fig. 3). CSNA burst incidence decreased from 32 ± 5 to 15 ± 5 bursts/100 heartbeats (−59 ± 9%), whereas RSNA burst incidence decreased from 81 ± 6 to 41 ± 13 bursts/100 heartbeats (−53 ± 11%). Inasmuch as there were no changes in HR, the changes in burst frequency were similar to those in burst incidence. In contrast, there was no change in CSNA or RSNA burst incidence or frequency during volume expansion in the HF animals (Fig. 3).

In the normal animals, there was a selective decrease in the burst amplitude of RSNA after volume expansion (median burst amplitude significantly decreased from 62 ± 3% to 54 ± 3%, \( P < 0.05 \)), whereas there was no change in CSNA burst amplitude (60 ± 5% vs. 58 ± 6%; Fig. 4). Similarly, in the HF animals, volume expansion caused a decrease in RSNA burst amplitude (from 58 ± 2% to 50 ± 2%, \( P < 0.05 \)), whereas there was no change in CSNA burst amplitude (57 ± 5% vs. 54 ± 5%).

Hemorrhage protocol. In normal animals, hemorrhage caused a significant decrease in CVP and an increase in HR but no change in MAP (Fig. 5). These changes were associated with a significant increase in total CSNA (\( P < 0.05 \)), whereas the increase in total RSNA did not reach significance (\( P = \)).
Hemorrhage in the HF animals also decreased CVP, with no changes in MAP or HR. The total levels of CSNA and RSNA were not altered during hemorrhage. The slopes of the average regression lines relating the change in CSNA to a given change in CVP were lower in the HF animals than in the normal animals \( r^2 = 0.99 \pm 0.01 \) vs. \( r^2 = 0.95 \pm 0.01 \), \( P < 0.05 \). There was no difference in the slopes of the average regression lines relating the change in RSNA for a given change in CVP between the HF and the normal animals \( r^2 = 0.99 \pm 0.01 \) and \( r^2 = 0.99 \pm 0.01 \), respectively.

**Changes in burst frequency and burst amplitude.** In normal animals, hemorrhage caused an increase in burst incidence [from 31 ± 7 to 53 ± 6 bursts/100 heartbeats (+80 ± 20%) and frequency [from 21 ± 4 to 57 ± 10 bursts/min (+152 ± 28%)] of CSNA, but only an increase in burst frequency [from 66 ± 2 to 99 ± 9 bursts/min (+50 ± 14%)] of RSNA (Fig. 6). In contrast, there was no change in CSNA or RSNA burst incidence during volume expansion in the HF animals (Fig. 6). However, in HF, volume expansion caused significant increases in burst frequency in the cardiac [from 77 ± 6 to 107 ± 9 bursts/min (+41 ± 10%)] and renal [from 80 ± 6 to 107 ± 9 bursts/min (+34 ± 10%)] sympathetic nerves.

In the normal animals, hemorrhage was associated with decreases in the burst amplitudes of CSNA (from 56 ± 6% to 45 ± 6%, \( P < 0.05 \)) and RSNA (from 64 ± 3% to 52 ± 5%, \( P < 0.05 \); Fig. 7). In the HF animals, there was no change in RSNA burst amplitude (from 59 ± 3% to 55 ± 4%), whereas there was a tendency for CSNA burst size to decrease (48 ± 6% vs. 42 ± 6%, \( P = 0.06 \)).

**DISCUSSION**

This is the first study to examine the differential control of SNA to the heart and kidney during changes in volume status in conscious animals, not only in the normal state, but also in HF. The novel findings of this study are as follows. 1) Volume expansion in normal animals was associated with similar percent decreases in CSNA and RSNA. The decrease in CSNA was due to a selective decrease in burst frequency, whereas the decrease in RSNA was due to decreases in burst frequency and amplitude. 2) In HF, volume expansion did not decrease
There was an attenuated decrease in RSNA that resulted from a fall in burst amplitude, not frequency.

In normal animals, hemorrhage increased CSNA and tended to increase RSNA. In both nerves, the increases in activity were due to increases in burst frequency; burst amplitude was reduced.

In HF, the attenuated cardiac sympathoexcitation in response to hemorrhage was due to the smaller increase in burst frequency. Our study also confirms our previous findings that the resting burst frequency of CSNA is significantly lower than that of RSNA and that in HF there is a dramatic increase in CSNA, such that the burst frequencies are similar in both nerves (30). Together, these findings suggest that the amplitude, as well as the frequency, of SNA to the heart and the kidney can be regulated independently.

Volume expansion. We observed similar decreases in CSNA and RSNA during volume expansion in normal conscious sheep. In a previous study of anesthetized, open-chest dogs, an increase in CSNA and a decrease in RSNA were observed with left atrial balloon inflation (10). We did not observe an increase in HR during volume expansion in our study, which, together with the absence of an increase in CSNA, argues against the Bainbridge reflex having a significant effect under the conditions of our study. We cannot rule out, however, that a Bainbridge reflex response would have been initiated if the infusion rate of Gelofusine had been more rapid. Interestingly, during volume expansion, there was no change in HR, despite a decrease in CSNA, suggesting a simultaneous decrease in cardiac vagal nerve activity.

Although the reductions in total SNA in both nerves were similar with volume expansion, the causes of this inhibition were different. The decrease in CSNA during volume expansion was due to a selective decrease in sympathetic burst frequency to the heart, whereas the decrease in RSNA resulted from decreases in burst frequency and amplitude. These findings indicate that there is a selective drive to reduce the recruitment of renal sympathetic fibers during volume expansion but that there is no change in the recruitment of cardiac sympathetic fibers. Previous studies that explored the role of burst frequency and amplitude of RSNA in conscious rats and rabbits found selective decreases in renal burst amplitude accompanied by no changes in burst frequency (6, 15). The reasons for these different findings are unclear, but they may be related to the rates of volume expansion, which were quicker in the previous studies, the time during volume expansion at which measurements were made (within 15 min of the start of volume expansion compared with 30 min in our study), or the species studied.

In HF, the decrease in CSNA during volume expansion in normal animals was abolished, and the inhibition of RSNA was markedly attenuated. In HF, the SNA responses were impaired, because burst frequency did not fall in either nerve during volume expansion. One explanation for the impaired volume-induced
reduction in burst frequency in HF is decreased inhibitory input from atrial vagal afferents, which have been shown to be desensitized in HF (42). In addition, there is evidence that alteration in areas of the brain that play a role in the control of volume, such as the paraventricular nucleus of the hypothalamus (PVN), could contribute to the impaired SNA response to volume expansion during HF (2, 4, 28). Interestingly, the reduction in burst amplitude of RSNA in normal animals during volume expansion was preserved in HF, suggesting that the pathway that modulates burst amplitude is not altered in HF. Although there was no significant change in MAP or pulse pressure in either group during volume expansion, we cannot rule out the possibility that small, nonsignificant changes in MAP may have activated arterial baroreceptor afferents and contributed to the changes.

Hemorrhage. In previous studies in conscious rats (9), rabbits (25), and dogs (26), increases in RSNA and HR were consistently observed during hemorrhage. Studies in human volunteers also indicate increases in directly recorded muscle SNA with lower body negative pressure (33). In a previous study in anesthetized monkeys, increases in CSNA and RSNA were observed during the early phase of hypotensive hemorrhage (34), although these changes were probably largely due to the considerable fall in arterial pressure. None of these studies examined whether the increases in SNA were mediated by selective increases in the amplitude or the frequency of the bursts. In our study in conscious sheep, hemorrhage significantly increased CSNA, but there was only a tendency for RSNA to increase. We found significant increases in burst frequencies of both nerves during hemorrhage, but, in contrast, cardiac and renal SNA burst amplitudes were decreased significantly after 500 ml of blood was removed. This reduction in burst amplitude may be a consequence of the tachycardia that occurred, which will have acted to reduce burst amplitude by reducing the time that blood pressure is below the baroreceptor threshold to inhibit SNA during diastole. The significant increase in CSNA indicates that the increased cardiac burst frequency had a greater effect on total levels than the

Fig. 4. Top: effects of volume expansion on average burst amplitudes of cardiac (●) and renal (○) bursts. *P < 0.05 for variable over time. Middle and bottom: effects of volume expansion on relative burst amplitudes of cardiac and renal sympathetic bursts in normal and HF animals. ●, Amplitudes during 5 min of control; ○, amplitudes after 500 ml of Gelofusine. *P < 0.05 vs. control.
decreased cardiac burst size. In contrast, the effect of the increase in RSNA burst frequency was offset by the decrease in renal burst amplitude, such that the increase in total levels of RSNA did not reach significance.

Previous studies have indicated that the tachycardia in response to hemorrhage is mediated primarily by unloading of arterial baroreceptor afferents (35, 36). In addition, the sympathetic response to lower body negative pressure is reduced in patients with denervated ventricles, but innervated atria and pulmonary veins, indicating an important role for ventricular mechanoreceptors (24). Together, these data suggest that, in normal sheep, the increase in CSNA and the tachycardia during hemorrhage were mediated by changes in cardiopulmonary and baroreceptor afferent input.

The only previous study to examine the contribution of changes in sympathetic burst amplitude and frequency during hemorrhage to total SNA reported a preferential increase in burst amplitude in conscious rabbits (20). It is important to note that, in rabbits, hemorrhage decreased HR, and the period of increased renal burst amplitude coincided with this bradycardia. In addition, previous studies in humans have also documented increases in sympathetic burst amplitude of muscle SNA during lower body negative pressure, which mimics hemorrhage (14, 33). These increases in muscle SNA burst amplitude were observed in the absence of any changes in HR. It is possible that, in our study, the increased HR, and the corresponding decrease in heart period, may have led to the decreased burst amplitude.

In HF, hemorrhage was associated with no significant change in CSNA or RSNA. The change in RSNA was similar to that in the normal sheep, but the increase in CSNA was largely abolished. There was no increase in burst incidence in either nerve during hemorrhage, probably because each was close to its maximum. There was a small increase in the burst frequency of both nerves, which was due to the increase in HR. Despite this increase in burst frequency in both nerves, there was no increase in total SNA levels, probably because of the tendency for burst size to decrease in both nerves. These findings, together with those from our previous study that showed preserved control of CSNA by arterial baroreflexes (30), suggest that the impaired CSNA response to hemorrhage was due to desensitization of cardiopulmonary afferents and/or...
the central mechanisms responding to changes in volume. Arterial pressure in the HF group did not fall during hemorrhage, probably because the tachycardia maintained cardiac output in the face of the decrease in preload, as has been shown in normal sheep (8).

We are unaware of other studies that have recorded CSNA or RSNA during hemorrhage in animals in HF, but human studies of norepinephrine spillover indicate decreases in cardiac norepinephrine spillover during lower body negative pressure (1) or sodium nitroprusside infusion (11) compared with no change in normal subjects. It is difficult to directly compare these results with our data, inasmuch as cardiac norepinephrine spillover is known to be influenced by a reduction in norepinephrine uptake mechanisms in HF (7). In addition, the HF patients were in a more severe state of HF than the sheep in the present experiments, and their medications may have altered CSNA directly or secondary to the hemodynamic improvements they induce.

How might independent control of frequency and amplitude of sympathetic bursts be achieved? After independent control of muscle sympathetic burst amplitude and frequency in humans was observed (13), Wallin (37) proposed that the influence from arterial baroreceptors occurred at two locations. The first is the location where information from the arterial baroreceptors interacts with information from other afferent inputs (e.g., chemosensitive and emotional) and produces a graded input. At another synapse, the afferent input from the arterial baroreceptors acts as a “gating” mechanism and allows presynaptic impulses to pass and produce a burst.

If this theoretical model is taken as a starting point, the data from our study indicate that input from cardiopulmonary afferents have a negative influence on renal burst amplitude at the first synapse but do not affect cardiac burst amplitude. This synapse may be present in the PVN, which has been hypothesized to control the number of recruited fibers for the renal nerve (17). In support of this notion, direct stimulation of the PVN increases RSNA burst amplitude without increasing their frequency of firing (19). As the cardiopulmonary afferent nerves project to the PVN (2, 40), it is feasible that the PVN may play a role in mediating this selective decrease in RSNA amplitude. The reduction in renal burst amplitude during volume expansion in the HF animals suggests that the influence of the cardiopulmonary receptors on renal burst amplitude persists during HF. The differing baseline burst frequencies of CSNA and RSNA indicate that the gating mechanism at the second synapse must also be different for the two nerves. The reduction in renal and cardiac burst frequency during volume expansion in normal animals indicates an inhibitory influence of cardiopulmonary afferents on the gating mechanism at the second synapse. In contrast to the control over renal burst amplitude, this inhibitory influence appears to be abolished in the HF animals.

Fig. 6. Changes in cardiac (●) and renal (○) burst incidence and frequency during hemorrhage in normal and HF animals. *P < 0.05 for variable over time.
Perspectives and Significance

Our results in a large, conscious mammal demonstrate that the amplitude, as well as the frequency, of bursts of SNA to the heart and the kidney can be regulated independently of each other. These findings highlight the importance of measuring both of these components of total SNA. Our data confirm previous findings that different processes regulate the generation of sympathetic discharges and the recruitment of sympathetic fibers and, in addition, that these components of SNA can be differentially modulated to individual organs. Further study is required to establish the functional consequence of changes in burst amplitude vs. burst frequency in health and disease. For example, it is unknown whether larger sympathetic bursts release more norepinephrine and cause greater vasoconstriction than additional bursts of the same size.

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