Cardiac sympathetic afferent sensitivity is enhanced in heart failure

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Wang, Wei, Harold D. Schultz, and Rong Ma. Cardiac sympathetic afferent sensitivity is enhanced in heart failure. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H812–H817, 1999.—A previous study from this laboratory has shown that cardiac sympathetic afferent stimulation by epicardial application of bradykinin (BK) and capsaicin was significantly enhanced in the dog with experimental heart failure (HF). The present study determined whether activity from cardiac sympathetic chemosensitive afferent endings is enhanced in HF. Rapid ventricular pacing was induced in six dogs. Five sham dogs served as controls. At the time of the acute experiment, the dogs were anesthetized with pentobarbital sodium (30 mg/kg iv). A thoracotomy was performed in the second intercostal space, and single afferent fiber discharge from the left cardiac sympathetic nerve was recorded. Baseline cardiac sympathetic afferent discharge rate (spikes/s) and its responses to intra-atrial injection of BK were compared between sham and HF groups. Baseline cardiac sympathetic afferent discharge rate in the HF group was significantly elevated compared with the sham group (4.3 ± 0.5 vs. 2.2 ± 0.6 spikes/s, P < 0.05). In addition, cardiac sympathetic afferent responses to left intra-atrial injection of bradykinin (2 and 5 µg/kg) and capsaicin (5 and 10 µg/kg) were also significantly augmented. The sensitized cardiac sympathetic afferent responses to BK (2 and 5 µg/kg, left intra-atrial injection) in the HF group were significantly reduced by the cyclooxygenase inhibitor indomethacin (5 mg/kg iv). The sensitized cardiac sympathetic afferent response to capsaicin (5 and 10 µg/kg, left intra-atrial injection) in the HF group was preserved. It is suggested that the cardiac sympathetic chemosensitive afferent sensitivity is significantly enhanced in dogs with HF even though the baseline cardiac sympathetic afferent discharge is elevated.

cardiovascular reflex; rapid pacing; sympathetic nerves; bradykinin; capsaicin; indomethacin

IT HAS BEEN SHOWN that the congestive heart failure state is characterized by elevated sympathetic tone and depressed cardiac vagal tone (3, 7). The mechanism(s) responsible for the neurohumoral activation in heart failure are not well understood. In the last several years, it has been reported that the sustained increase in neurohumoral drive in chronic congestive heart failure is mediated by blunted arterial baroreceptor and cardiopulmonary receptor reflexes (13). In addition to cardiopulmonary vagal reflexes, it is well known that cardiac “sympathetic afferent” reflexes contribute to increases in sympathetic outflow and may be involved in signaling cardiac pain during acute ischemia (12).

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Cardiac sympathetic afferent reflex in heart failure

Model of chronic heart failure. The model of low cardiac output heart failure used in the present study was that of chronic ventricular pacing in the dog. In brief, after control measurements were made in the conscious state, the dogs were paced (right ventricular) at 250 beats/min using a Medtronic 8340 pacemaker (Medtronic, Minneapolis, MN), which was modified to pace at this rate. Approximately 1 wk was allowed for the dogs to recover from surgery before pacing was begun.

Acute experiments. When dogs were paced for 3–4 wk and their left atrial and left ventricular end-diastolic pressures (LVEDP) were significantly elevated (>20 mmHg), acute experiments were carried out. Each dog was anesthetized with pentobarbital sodium (30 mg/kg iv) and intubated. A femoral artery was catheterized for measurement of systemic, diastolic, pulse, and mean arterial pressure (MAP). A femoral vein was cannulated for administration of supplemental doses of anesthesia (1/10 of initial dose of pentobarbital per hour) and drugs. Arterial blood gases were measured throughout the experiment and kept within normal limits (pH 7.35–7.45; PaCO₂, 30–40 mmHg; PaO₂, 85–95 mmHg).

Cardiac sympathetic afferent recording. The third and fourth ribs on the left side were removed. The cardiac sympathetic trunk from the stellate, which is known to contribute to the innervation of the heart (2, 16), was dissected into fine filaments using a dissecting microscope. Nerve filaments were desheathed and continuously split until single units were recorded. Cardiac sympathetic afferent discharge was amplified (Grass P18 Micro Electrode DC Amplifier), displayed on an oscilloscope (model 7313, Tektronix), and recorded on a strip-chart recorder (Gould ES 1000B). The impulses were fed into a loud speaker for monitoring and into a window discriminator (Frederick Haer) to exclude extraneous spikes. Pulses from the discriminator were fed into a rate meter, which can be set as required to count impulses per second. It has been shown that cardiac sympathetic afferents have Aδ- and C-fibers. Aδ-fibers have faster conduction velocities (CVs) (>2 m/s), and C-fibers have slower CVs (<2 m/s) (12). CVs of the afferent fiber were obtained to determine fiber type. To determine CVs, we used the spike-triggered averaging technique (19). Spike-triggered averaging uses multifiber activity recorded from the whole nerve or a slip of the nerve and uses single fiber activity recorded simultaneously from a distal filament dissected from the nerve. The single fiber or its window discriminator pulse was used to trigger a MacLab Scope system. The averaged single fiber and averaged whole nerve activities were displayed, and after sufficient averaging (32 cycles) a distinct potential was observed in the whole nerve activity originating from the single fiber. By dividing the time between the peak of the two potentials by the distance between the two pairs of recording electrodes, the CV was calculated. Mechanosensitive endings were identified by their cardiac pattern of discharge and by their immediate and vigorous response when the receptor site was gently stroked with a fine probe or bristle; endings in the left heart were stimulated when the descending thoracic aorta was occluded briefly by a snare to increase blood pressure upstream. Chemosensitive endings have a sparse and irregular discharge without cardiac modulation, and there is little change in their impulse activity when vascular pressures are increased and an increase in their discharge after myocardial ischemia by occlusion of the main left coronary artery for 1 min. Although they could be stimulated by probing or pinching the heart, the evoked discharge usually lags behind the stimulus and has a pronounced afterdischarge. Finally, all chemosensitive fibers responded to left atrial injection of capsaicin.

Experimental protocols. In sham and heart failure animals, baseline cardiac sympathetic afferent discharge rate was recorded and averaged for 5 min. Bradykinin (2 and 5 μg/kg, left intra-atrial bolus injection) was administered while single cardiac sympathetic afferent fiber activity was recorded for 30 s. The discharge activity during the last 10 s was averaged. Successive injections of bradykinin were separated by at least 15 min to avoid tachyphylaxis (5). In addition to bradykinin, capsaicin (5 and 10 μg/kg) was also injected into the left atrium while single cardiac sympathetic afferent fiber activity was recorded. Bradykinin and capsaicin were randomly injected. These procedures were repeated 20 min after administration of the cyclooxygenase inhibitor indomethacin (5 mg/kg iv).

Statistical analysis. Cardiac sympathetic afferent discharge rates and hemodynamics were compared between sham and heart failure dogs using a Student’s t-test. Two-way ANOVA followed by post hoc analysis using Duncan’s test was used to determine the level of significance of mean data in response to bradykinin and capsaicin at each dose in the two groups of animals. A paired t-test was used when a control response was compared with the response after an intervention in the same animal. All statistical analyses were done using computer software (SigmaStat, Jandel). All data are expressed as means ± SE. A P value of <0.05 was considered statistically significant.

RESULTS

Hemodynamics. LVEDP, left ventricular systolic pressure (LVSP), MAP, and heart rate were measured in anesthetized sham and heart failure groups. As seen in the Table 1, the LVSP was significantly decreased (102.8 ± 3.0 vs. 139.6 ± 5.1 mmHg, P < 0.05), the LVEDP was significantly elevated (22.5 ± 2.1 vs. 2.1 ± 0.5 mmHg, P < 0.001), and MAP was significantly decreased (82.8 ± 3.2 vs. 118.8 ± 6.8 mmHg, P < 0.05) in dogs with heart failure. Heart rate was not different most likely because of the influence of pentobarbital anesthesia.

Characteristics of cardiac sympathetic afferent discharge. Five single cardiac sympathetic afferent units from sham dogs and six units from heart failure dogs were recorded. Most of these afferents responded to coronary ischemia and none responded to mechanical distension of the left ventricle by occlusion of the descending thoracic aorta. All afferent endings were located in the left anterior ventricle (Fig. 1). CVs of the afferents in both sham and heart failure groups were <2 m/s (1.06 ± 0.09 m/s in the sham vs. 1.07 ± 0.12 m/s in the heart failure dogs), classifying them as C-fibers.

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<th>Table 1. Hemodynamics of anesthetized sham and heart failure dogs</th>
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Values are means ± SE; n, number of dogs. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; HR, heart rate; NS, not significant.
A representative recording of a cardiac sympathetic afferent is shown in Fig. 2. Averaged discharge rate in the heart failure group was significantly elevated (4.36 ± 0.47 spikes/s) compared with that in the sham group (2.30 ± 0.57 spikes/s, P < 0.05). The responses to bradykinin and capsaicin were examined in five sham and six heart failure dogs. Figure 2 shows a representative recording of the response to bradykinin in a sham and a heart failure dog. As can be seen, the response to bradykinin (5 µg/kg left intra-atrial injection) was greater in the heart failure dog compared with the sham dog. In fact, in this dog this dose was just at threshold. Arterial pressure fell in both dogs. The mean data are shown in Fig. 3A. Changes in the discharge rate of the cardiac sympathetic afferents to bradykinin were significantly greater in the heart failure dogs at both doses of bradykinin [2.73 ± 0.51 vs. 1.26 ± 0.25 spikes/s, P < 0.05 (2 µg/kg) and 4.30 ± 0.78 vs. 2.48 ± 0.71 spikes/s, P < 0.05 (5 µg/kg)]. The mean response of the discharge rates of the cardiac sympathetic afferents to capsaicin are shown in Fig. 3B. Changes in the discharge rates of the cardiac sympathetic afferents to capsaicin were markedly higher in the heart failure dogs [4.20 ± 1.30 vs. 1.49 ± 0.60 spikes/s, P < 0.05 (5 µg/kg) and 7.40 ± 1.35 vs. 2.40 ± 0.35 spikes/s, P < 0.05 (10 µg/kg)].

Effect of bradykinin and capsaicin on cardiac sympathetic afferent discharge after cyclooxygenase blockade. In five sham and six heart failure dogs, cardiac sympathetic afferent responses to epicardial bradykinin and capsaicin were determined after the cyclooxygenase blocker indomethacin (5 mg/kg iv) was administrated. Indomethacin did not alter baseline cardiac sympathetic afferent discharge in either sham or heart failure groups (2.61 ± 0.54 vs. 2.23 ± 0.56 spikes/s in the sham dogs and 4.53 ± 0.77 vs. 4.75 ± 0.43 spikes/s in the heart failure dogs). However, changes in the response of the cardiac sympathetic afferents to bradykinin were significantly blunted in both sham and heart failure

![Fig. 1. Location of cardiac sympathetic afferent endings in sham (open circles) and heart failure (filled circles) dogs.](image1)

![Fig. 2. Representative recordings from a sham (left) and heart failure (right) dog. Cardiac sympathetic afferent fiber discharge before and after left intra-atrial injection of bradykinin (5 µg/kg). Event marks indicate when injection was made. Traces from top to bottom are arterial blood pressure (ABP), mean arterial blood pressure (MAP), discharge rate (rate meter output), and neurogram (raw nerve activity).](image2)
groups (Fig. 4A) 0.68 ± 0.47 spikes/s in the sham vs. 0.80 ± 0.39 spikes/s in the heart failure for bradykinin (2 µg/kg) and 0.48 ± 0.20 in the sham vs. 1.55 ± 0.21 spikes/s in the heart failure for bradykinin (5 µg/kg)).

After indomethacin administration there were no longer any significant differences between sham and heart failure groups.

Indomethacin did not alter changes in the discharge rates of the cardiac sympathetic afferent to capsaicin (Fig. 4B). The responses to capsaicin were significantly enhanced in the heart failure group compared with the sham groups after indomethacin [3.45 ± 0.91 vs. 1.19 ± 0.13 spikes/s, P < 0.05 (5 µg/kg) and 5.94 ± 1.42 vs. 1.86 ± 0.72 spikes/s, P < 0.05 (10 µg/kg)].

DISCUSSION

Stimulation of cardiac sympathetic afferents elicits a sympathosympathetic reflex, resulting in an increase in blood pressure, heart rate, and sympathetic outflow (8, 12, 34). Consistent with this stimulation, it has been shown that at least one stimulus to sympathetic afferents is myocardial ischemia (23). Cardiac sympathetic afferents can be stimulated by a variety of substances that are released by the myocardium during ischemia. These include potassium, hydrogen ion, bradykinin, and prostaglandins (14, 23, 26, 27). It has been shown that coronary flow is decreased (20), and oxygen consumption is increased in experimental heart failure, which is most likely induced by increased left ventricular wall tension (24). We have previously shown that the renal sympathetic nerve activity responses to epicardial application of bradykinin and capsaicin are significantly enhanced in dogs with pacing-induced congestive heart failure (31). This enhancement was confirmed in our recent study (29). Whether other components in addition to sympathetic afferent endings play a role in the enhanced cardiac sympathetic afferent reflex in heart failure is unclear. In our recent study, the enhanced cardiac sympathetic afferent reflex in the heart failure state was due, in part, to an increase in central sensitivity (11). In that study it was shown that electrical stimulation of a cardiac sympathetic afferent nerve resulted in an enhanced reflex response in dogs with heart failure. It is still possible that an increase in afferent sensitivity may contribute to the enhanced reflex sensitivity. In our previous study it was shown that blockade of cardiac sympathetic afferents with epicardial application of lidocaine significantly reduced baseline renal sympathetic nerve activity in the heart failure dogs, whereas having little effect in sham dogs (31). These data suggest that cardiac sympathetic afferents in heart failure provide a tonic input to the
central nervous system. In the present study baseline discharge rates of the cardiac sympathetic afferents in the heart failure group were significantly higher than in the sham dogs. This finding substantiates the fact that indeed an augmented and sustained input from cardiac sympathetic afferents occurs in the heart failure state. All cardiac sympathetic afferents recorded in this study were located in the left anterior descending coronary region of the left ventricle, and most were primarily responsive to chemosensitive stimulation by occlusion of the coronary artery rather than mechano-sensitive stimulation elicited by an increase in left ventricular pressure by occlusion of the descending aorta. CVs of the afferents in both the sham and heart failure groups were <2 m/s, consistent with the fact that the cardiac sympathetic afferents that we recorded were C-fibers. Because C-fiber afferents respond primarily to chemical stimuli (1, 17, 21, 25, 28), it is likely that the interstitial myocardial environment is altered in such a way as to promote an enhanced sensitivity in response to bradykinin and capsaicin. This is further suggested by a recent study from this laboratory in which vagal C-fiber responsiveness was also enhanced in dogs with pacing-induced heart failure (18). In addition to the enhanced tonic discharge of the cardiac sympathetic afferents in the heart failure group, an increased responsiveness to left atrial injection of bradykinin and capsaicin was observed. These data strongly suggest that the enhanced sensitivity of the cardiac sympathetic afferent reflex has both a central (11) and an afferent component.

The heart failure state is characterized by neurohumoral activation. Among those substances that are elevated in heart failure is bradykinin. Cheng et al. (4) have recently shown that plasma bradykinin is increased almost fivefold in dogs with pacing-induced heart failure. Furthermore, these investigators show that bradykinin contributes to maintenance of coronary blood flow in the heart failure state. It has been shown that several effects of bradykinin are mediated by prostaglandins (6, 15, 22, 33). Our previous studies (29, 31) have shown that excitation of the cardiac sympathetic afferent reflex induced by epicardial application of bradykinin can be partially prevented by the cyclooxygenase inhibitor indomethacin. In the present study, indomethacin did not change baseline cardiac sympathetic afferent discharge in either sham and heart failure groups; however, changes in the response to bradykinin were significantly blunted in both sham or heart failure groups. The enhanced cardiac sympathetic afferent fiber discharge response to capsaicin in the heart failure group was preserved after indomethacin. These data suggest that the cardiac sympathetic afferent responses to bradykinin are mediated by prostaglandins. These findings are supported by several studies in which the response of ischemically sensitive abdominal visceral afferents to bradykinin were also shown to be dependent, in part, on prostaglandin synthesis (9, 10). Because those visceral afferents are electrophysiologically similar to cardiac sympathetic afferents, it is likely that a similar pathway exists for the mechanism of bradykinin stimulation.

Limitation of study. Indomethacin may decrease cardiac sympathetic afferent sensitivity to bradykinin in the heart failure state; however, this does not appear to affect basal cardiac sympathetic afferent activity. It is still unclear why a higher basal cardiac sympathetic afferent activity was seen in the heart failure group. This may be related to a state of relative ischemia due, in part, to increased ventricular wall tension as a result of chronic cardiac dilation and an increase in heart rate. In addition, only five single fibers from the sham animals and six fibers from the heart failure group were recorded. Whereas there is clearly a normal spectrum of discharge rates and sensitivities for these afferents, the single fibers were all from the left ventricle and were all chemosensitive. Finally, the standard error for the discharge frequency was relative low. This indicates that we recorded from a group of fibers with very similar discharge and chemosensitive in both groups. Therefore, it is unlikely that the variability of these fibers played a major role in this study.

In summary, an enhanced response of the cardiac sympathetic afferents to stimulation with bradykinin and capsaicin was observed in dogs with pacing-induced heart failure. The enhanced cardiac sympathetic afferent fiber discharge response to bradykinin in the heart failure group was related to augmented prostaglandin synthesis, because the cyclooxygenase inhibitor indomethacin prevented this enhanced sensitivity. This phenomenon may contribute to the sympathoexcitative state in chronic heart failure.

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