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Ganglionic Mechanisms Contribute to Diminished Vagal Control in Heart Failure

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Background—Previous work has shown that spontaneous and stimulated vagal activity is diminished in heart failure (HF) despite upregulation of functional postsynaptic cholinergic mechanisms. We therefore examined function of the postganglionic neuron in the paced canine model of HF as a possible site for diminished control.

Methods and Results—We measured sinus cycle length changes in response to electrical stimulation of preganglionic and postganglionic parasympathetic neurons innervating the sinoatrial node in control and HF dogs (both, n=8). Cervical vagus stimulation (preganglionic) demonstrated attenuated responses in the HF group at all levels of stimulation (P<0.05). Stimulation of the right atrial fat pad, containing both postganglionic nerves and terminals of preganglionic neurons, showed no such difference between control and HF (200±25 versus 192±18 ms). To ensure that preganglionic input and different levels of baseline sympathetic activity did not contribute to the group difference, similar stimulations were done in the presence of ganglionic and β-adrenergic blockade. Under these conditions, postganglionic stimulation showed smaller changes in sinus cycle length, but the HF group response remained significantly higher than in controls (76±10 versus 20±2 ms; P<0.01), indicating that the difference was independent of preganglionic input and sympathetic activity.

Conclusions—A component of attenuated parasympathetic control in HF is located within the peripheral efferent limb. This defect is located within the parasympathetic ganglion. Future work should be focused on determining mechanisms of attenuated ganglionic transmission so that means targeted at restoring vagal activity can be developed. (Circulation. 1999;99:2958-2963.)

Key Words: heart failure ■ vagus nerve ■ nervous system, autonomic ■ physiology

Heart failure (HF) is characterized by significant changes in activity of the autonomic nervous system that are important in the pathophysiology of the disease. The sympathetic division is highly activated and is believed to contribute to the progression of the disease through direct effects on the myocardium as well as through activation of other neurohormonal systems such as the renin-angiotensin system.1-3 This pathophysiological role is highlighted by the use of pharmacological blockade of sympathetic nervous system–driven mechanisms (ACE inhibitors and β-blockers) as the main approach to current therapy.4,5 Treatment with such pharmacological means is not ideal, however, because the responsible neurohumoral agents (norepinephrine and angiotensin II) have many and varied effects that are incompletely blocked by the actions of these agents.

In normal humans and animals, the parasympathetic nervous system has an important antagonistic effect on sympathetic activity and is essential for maintaining hemodynamic homeostasis. In addition to antagonizing sympathetic end-organ responses, parasympathetic activity modulates sympathetic drive at central and peripheral levels. The parasympathetic nervous system also may provide protection from arrhythmia and sudden death independently of sympathetic antagonism.6 However, control of the heart by the parasympathetic nervous system is attenuated in HF in both humans and animal models7-11 and is therefore unavailable to modulate increased sympathetic activity. The anatomic site(s) at which vagal control is reduced is unknown. Identification of the site(s) responsible for attenuated vagal control is important because it will provide insight into means of restoring activity in patients with HF and other conditions.

Previous work by this laboratory12 and others13 has shown that postsynaptic mechanisms in the heart are actually up-regulated in an “intrinsic denervation supersensitivity”–type manner in HF.14,15 M2 receptors in the sinus node are increased in density, Gα2 (the G-protein responsible for cholinergic transduction in the heart) is increased,16-18 as is the mRNA for this protein,19 and these changes have been confirmed to be functional in physiological preparations.13 This has led us to investigate a defective mechanism more proximal in the vagal cascade as a possible site for diminished vagal control. Preliminary data from this laboratory in
isolated canine atria showed that 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, a nicotinic agonist) produces a diminished response in sinus cycle length (SCL) in HF dogs.20 To determine whether this is due to mechanisms at the nicotinic receptor or mechanisms distal to the ganglion, we conducted the present series of experiments. The aim of the present report was to determine whether a defect within the postganglionic neuron contributes to diminished vagal control in HF.

**Methods**

**Induction of HF**

We have previously discussed the technique used for induction of HF.21 All procedures were carried out according to institutional guidelines for the care and use of laboratory animals. Young adult (9- to 12-month-old) male Beagle dogs were anesthetized with sodium pentothal and maintained with fluorothane gas. After the left external jugular vein had been exposed under sterile conditions, a Medtronic pacing wire was passed through the atrium and lodged in the apex of the right ventricle under fluoroscopic guidance. PAC thresholds and resistances were determined to ensure adequate capture. The lead was then tunneled subcutaneously over the scapula and attached to a Medtronic pacemaker that was placed in a subcutaneous pocket behind the left shoulder. Dogs were kept on 250 mg cephradine BID for 3 days after surgery and allowed to recover for 7 to 10 days before the pacemaker was programmed at 250 bpm. The animals were checked daily by auscultation to ensure continued pacing. ECG strips were taken on a weekly basis or otherwise if there was suspicion of intermittent capture. Pacing was continued until the animal showed clinical signs of HF in 4 to 6 weeks. These signs included ascites, tachypnea, rales, decreased appetite, pallor with slowed capillary refill time in the gums, and dilatation of the ventricles on echocardiographic assessment.

**Experimental Protocol**

The experimental protocol was performed within a few days of development of HF. The dogs were anesthetized with a—chloralose via an intravenous drip until toe pinch reflex was absent. Supplemental chloralose was given the same way every 45 minutes. After endotracheal intubation, the dogs were placed on a respirator (Harvard Apparatus) and were ventilated with room air. A heating blanket maintained temperature in the physiological range. The femoral artery and vein were cannulated for continuous BP monitoring and drug administration. A Swan-Ganz catheter was advanced to the pulmonary artery via the right external jugular vein and connected to a cardiac output computer (Edwards Laboratories) for determination of cardiac output.

The vagus nerves were dissected and isolated at the cervical level through a single midline incision. Each nerve trunk was ligated with heavy silk and then sectioned to prevent proximal conduction. Teflon-coated stainless steel wires were inserted into the proximal end of the caudal remnant of the right vagus nerve for stimulation. A bipolar electrode was then placed near the atrial appendage for recording of an electrogram. Two Teflon-coated wires were inserted into the pulmonary vein complex fat pad, a site that has been well documented as containing the ganglia of fibers innervating the sinus node area.22-24 This provided the means for direct electrical stimulation of postganglionic fibers.

**Protocol**

Vagus stimulations were performed at 3, 5, and 10 Hz at 8 V and a pulse width of 1 ms. Atrial electrogram and ECG signals were recorded for 15 seconds of baseline, 30 seconds of stimulation, and 15 seconds of recovery. Sufficient time was given between stimulations to allow heart rate to return to prestimulation levels. To assess the functional status of the postganglionic neuron, the fat pad was stimulated by use of 2 different parameters. Subthreshold stimulations were made at 3, 5, and 10 Hz at 8 V and a pulse width of 0.05 ms. These parameters were sufficient to activate neurons within the tissue but not the myocardium itself. Because postganglionic neurons are unmyelinated and therefore more difficult to activate electrically, subthreshold stimulations were used to activate a combination of preganglionic fibers as well as some postganglionic fibers innervating the sinoatrial node. To activate postganglionic neurons in a more selective manner, suprathermal stimulations were performed at 8 V, 1 ms, and 200 Hz. To avoid capturing the myocardium and affecting spontaneous atrial electrical activity at these parameters, the stimulator (Grass SD9) was configured to be triggered by the atrial electrocardiogram. This was achieved by passing the electrogram signal through a conditioning stimulator (Grass S44), which created a pulse that could be varied in duration and delivered during the atrial refractory period. By altering the duration of the window, we stimulated the fat pad with 1 to 5 pulses per atrial burst. Our experience showed that 5 pulses provided the maximal response in most of the dogs. Because these stimulations were at higher intensities, they activated the unmyelinated postganglionic fibers directly as well as activating preganglionic neurons.

To specifically isolate the postganglionic response to electrical stimulation, we induced ganglionic blockade with hexamethonium bromide 1 mg/kg IV and directly into the right atrial fat pad (2 to 5 mg). Blocking of ganglionic transmission would eliminate the effect of activating preganglionic neurons. This ensured that slowing of SCL was in response to stimulation of postganglionic neurons alone. Once blockade was achieved, the stimulation parameters were repeated as described above. Atropine (1 mg IV bolus) was given in some experiments to show that the SCL response to stimulation of the fat pad was abolished, confirming that the response was totally mediated by cholinergic mechanisms.

Because previous evidence has shown that direct electrical stimulation of the myocardium can activate intrinsic sympathetic neurons and sympathetic nerve endings,25 which could modify the vagal response, we sought to eliminate any β-adrenergic effects. This was done after baseline stimulations with an infusion of esmolol HCl 200 μg/min, a short-acting β-adrenergic blocker. The order in which each of these steps was implemented (hexamethonium or β-blockade) was alternated in half of the experiments to evaluate the influence of β-adrenergic signaling in postganglionic activation.

**Data Capture and Analysis**

ECG and electrogram signals were captured at 500 Hz with an analog-to-digital converter (DATAQ Instruments) and stored on a 486 DX PC. The signals were peak-detected (CODAS software, DATAQ Instruments) and inspected to ensure appropriate electrogram detection. Point data files were generated and the SCLs plotted graphically with Lotus 123. Quantitative analysis of SCL was made with the specific data points averaged over 10 seconds of baseline and during 15 seconds of a 30-second stimulation.

**Results**

**Hemodynamic Parameters**

After an average of 5 weeks, paced dogs showed clear signs of HF. The Table shows hemodynamic parameters for each
group (mean±SD). Heart rate was generally higher in control dogs before and after β-blockade after bilateral vagotomy. Mean BP was lower in the HF group, and mean capillary wedge pressure was doubled in HF dogs, as expected. Cardiac output was markedly lower in HF dogs, and this correlated well with ventricular dilatation calculated from echocardiograms that were taken when clinical signs were evident. Group differences for each parameter were significant at P<0.01 except for heart rate (P=0.05).

Vagal Stimulation (Preganglionic)

Figure 1A shows an anatomic scheme of vagal innervation and the points of stimulation (arrows) used in our protocols. Figure 1B shows an individual SCL response to vagal stimulation in both a control and an HF animal. Stimulation of the cervical right vagus resulted in a prompt increase of SCL, and there was no observable consistent difference in the rate of onset or recovery from stimulation between 2 dogs, which was confirmed by regression analysis (data not shown).

Subthreshold Stimulation

Stimulation at subthreshold levels, which stimulated predominantly preganglionic neurons along with some postganglionic neurons (see Methods), showed a pattern similar to vagal stimulation, that is, the response was immediate and faded during stimulation. Figure 2B shows group mean data for both control and HF subthreshold stimulation at 3, 5, and 10 Hz. The degree to which SCL was increased was much less than cervical vagal stimulation, and this did not change significantly between different dogs in either group. Although no statistical difference was found between control and HF animals (P>0.05), control animals responded slightly more than HF animals (97±35, 197±65, and 244±66 ms compared with 73±10, 113±20, and 219±34 ms at 3, 5, and 10 Hz, respectively).

Suprathreshold Stimulation

Figure 3A shows group mean data for control and HF dogs when suprathreshold stimulation of the fat pad was used. Stimulation at all levels (1 to 5 pulses per atrial burst) shows no significant difference in SCL response between the 2 groups: 119±17, 128±17, 163±18, 195±20, and 205±20 ms in controls compared with 135±20, 151±18, 166±20, 179±20, and 192±10 ms in HF, P>0.05. To completely
isolate the postganglionic neuron, we administered hexamethonium and repeated the highest level of stimulation. Under ganglionic and adrenergic blockade (Figure 3B), the postganglionic response in the control animals (n = 4) was small (20 ± 2 ms) compared with that in HF animals (n = 6) (76 ± 10 ms; P < 0.01), confirming that stimulation of the fat pad previously was activating preganglionic neurons to a significant degree. It also confirmed that the postganglionic mechanisms are functional and more responsive in HF than in control animals.

**Stimulations Under β-Blockade**

In 2 of the control dogs, we performed ganglionic blockade before β-adrenergic blockade. Stimulation of the fat pad under these conditions produced tachycardia. Figure 4A shows an example of this response. The response to stimulation was converted to bradycardia after β-adrenergic blockade, confirming that this was due to sympathetic activation (4B). To ensure that this effect did not influence the change in SCL induced by fat-pad stimulation, we conducted all 3 stimulation types under β-blockade. Figure 5A shows the group mean data for vagal stimulation before and after blockade. The ability to slow the heart through vagal stimulation was greatly attenuated under β-adrenergic blockade (n = 5, 409 ± 63, 572 ± 93, and 1896 ± 493 ms compared with 131 ± 36, 242 ± 70, and 541 ± 14 ms at 3, 5, and 10 Hz, respectively, P < 0.01). A similar effect was seen with suprathreshold postganglionic stimulation (Figure 5B), although to a lesser degree.

**Discussion**

Previous work in humans and animals has indicated that vagal control of the heart is diminished in HF. Eckberg et al. published one of the earliest reports showing that parasympathetic control was reduced in patients with left ventricular dysfunction. They showed depressed chronotropic responses to atropine and impaired baroreflex control of heart rate in response to acute hypertension, suggesting attenuated vagal mechanisms. Porter et al. confirmed that low (vagomimetic) doses of atropine, which induces vagal activity, did not elicit a change in heart rate in HF patients as it did in controls. They too concluded that vagal control was diminished in HF. A decrease of vagal control in HF also has been shown in the pacing-induced canine model of HF through spectral analysis. We have also shown previously in the canine paced model that vagal mechanisms are attenuated early in HF. Despite this information, the precise location(s) and mechanism(s) for abnormal vagal control are unknown.

**Preganglionic Stimulation**

In this study, we tested the functional capacity of the vagal efferent limb by directly stimulating both preganglionic and postganglionic neurons and monitoring end-organ (sinus node) responses. Our data reveal that despite functional upregulation of cardiac M2 receptors, preganglionic vagal
stimulation produces attenuated responses in HF dogs. This finding therefore supports the notion that at least part of the vagal defect seen in HF lies within the peripheral efferent limb. Anatomically, this could be in preganglionic release of acetylcholine, acetylcholine degradation dynamics, binding to the nicotinic receptor on the postganglionic neuron, transmission and release of acetylcholine from the postganglionic neuron, or acetylcholinesterase dynamics at the neurocardiac synapse. Previous work by our laboratory has shown that acetylcholinesterase is in fact downregulated in HF. This would theoretically increase the amount of acetylcholine in the myocardium and thus potentiate the effects of the vagal system. With present evidence indicating the anatomic localization responsible for attenuated vagal activity to be somewhere between the preganglionic neuron and the synapse at the end organ, we investigated the postganglionic neuron as a potential bottleneck in the vagal cascade.

**Postganglionic Stimulation**

To test whether postganglionic mechanisms are functional in HF, we stimulated postganglionic neurons innervating the sinoatrial node directly and monitored changes in SCL. We found that stimulation of the postganglionic neuron in HF elicits a functional response and has no apparent functional abnormalities compared with controls. Thus, despite an attenuation of vagal response during stimulation at the preganglionic level, direct stimulation of the postganglionic neuron produces responses equal to or greater than those seen in controls. This clearly implies a role of the parasympathetic ganglion as a source of diminished vagal activity seen in HF.

When the ganglion is stimulated directly (fat-pad stimulation), both preganglionic and postganglionic neurons are activated. Under normal conditions, postganglionic neurons contribute to a larger component of the excitation. The lack of statistical significance in our data before hexamethionium is reconciled by the fact that augmented postsynaptic mechanisms in the HF group are offset by greater ganglionic transmission in the control group. This concept is seen most strikingly in the prehexamethionium and posthexamethionium data for suprathreshold stimulation of the fat pad. Before ganglionic blockade, there was no significant difference between control and HF. After ganglionic blockade, however, the more effective ganglionic transmission in controls is removed, and there was a larger response in the HF group than in controls.

**Stimulations After β-Blockade**

To examine whether stimulation of the fat pad also activated sympathetic nerve terminals to release epinephrine and whether this modified the response to vagal stimulation, we performed the stimulations with and without β-adrenergic blockade. Figure 4A shows the SCL response to fat pad stimulation with hexamethionium. After a rapid, transient bradycardia (most likely initiated by rapid activation of cholinergic mechanisms), there was a progressive tachycardia in line with the slower dynamics of β-adrenergic signaling mechanisms. This response was transformed into only bradycardia after β-adrenergic blockade (Figure 4B), indicating that the secondary tachycardia was mediated by norepinephrine acting on β-receptors. This supported the notion that sympathetic stimulation could be attenuating the magnitude of bradycardia in response to vagal postganglionic stimulation through direct antagonism of signal transduction mechanisms (see below). As shown in Figure 5A and 5B, with both preganglionic and postganglionic stimulation, the response to vagal stimulation was decreased in the presence of β-blockade in control animals. The most likely explanation for this is that a large component of vagal activity on heart rate is dependent on a sympathetic substrate. This has been described previously in detail by Levy, who coined the term “accentuated antagonism.” In this regard, although vagal stimulation has direct effects on ion channels through binding of acetylcholine and G_{α2}, much of its activity is mediated via antagonizing the β-adrenergic signaling pathway, primarily through G_{α2}, which inhibits adenyl cyclase and the subsequent cAMP signaling effects. Alternatively, it is possible that β-adrenergic receptors may somehow augment the release of acetylcholine; however, such a role has only been reported previously for α-receptors.

These findings are important for understanding changes in vagal control so that we can target means to modulate its activity. Our report shows that in HF, the postganglionic neuron can release acetylcholine in sufficient quantities to create a response equal to or greater than that in controls. Although no interpretation regarding absolute release of acetylcholine can be made, the report shows that regardless of the amount of acetylcholine released, it can produce a normal response at the level of the sinus node in conjunction with upregulated postsynaptic mechanisms. This may be attributed to an adaptation of the postganglionic neuron itself, the downregulation of acetylcholinesterase, the previously reported upregulation in M_{2} receptors on the myocardium, or a combination of these factors.

Irrespective of the mechanisms that serve to normalize responses to postganglionic stimulation, the present data indicate that the parasympathetic ganglion is a primary site of defective transduction of vagal impulses in HF. Future work needs to be focused on determining the specific ganglionic mechanisms responsible, which may include release of acetylcholine from the preganglionic neuron into the ganglionic synapse or a change in ganglionic receptors. Because nicotinic receptors on ganglionic cells are ligand-gated ion channels, the diminished sensitivity would most likely involve changes in subtype or density of receptor rather than signal transduction mechanisms.

**Limitations**

As with all animal models that attempt to mimic human disease, there is a possibility that this defect is unique to dogs. This seems unlikely, because the neural and hormonal changes in the pacing model very closely parallel those seen in humans with HF at both structural and functional levels. In addition, we did not examine the possibility that preganglionic mechanisms of the efferent limb are also defective. Such an additional abnormality may add to the magnitude and ramifications of a defective ganglion.

The implications of a defective ganglion in HF are significant. The magnitude of the defect is likely to be larger than...
that recorded in view of upregulated postsynaptic mechanisms and decreased degradation of acetylcholine at the synapse. It is possible that this defect may contribute significantly to the autonomic imbalance and high susceptibility to arrhythmia seen in HF patients. Furthermore, this finding may provide a new avenue for development of specific pharmacological intervention in the treatment of HF in the future.

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