Vagal Cardiopulmonary Reflexes After Total Cardiac Deafferentation

Anthony J. Minisi, MD

Background—There are conflicting data regarding whether the primary source of afferent input for the vagal cardiopulmonary reflex emanates from receptors located in the ventricles, atria, and/or lungs. This study evaluated the effects of total cardiac deafferentation on the reflex control of efferent renal sympathetic nerve activity (RSNA) in response to a stimulus that affected all vagal receptors in the cardiopulmonary region.

Methods and Results—Experiments were performed in 14 chloralose-anesthetized dogs with sinoaortic denervation. Reflex control of RSNA in response to blood volume expansion was measured before and after interruption of cardiac vagal afferents by pericardial lidocaine (PL). Reflex sensitivity (% change in RSNA/mm Hg change in left atrial pressure) was markedly attenuated after PL (pre, −10.9±2.2; post, −1.6±0.6; \( P=0.002 \)). RSNA responses to intracoronary nicotine and left atrial balloon inflation were abolished after PL, confirming that cardiac afferents were interrupted. RSNA responses to lung inflation were not affected by PL, indicating that pulmonary afferents remained intact. In 8 experiments, reflex sensitivity values returned to baseline levels after the effects of PL had worn off.

Conclusions—These results indicate that the heart provides the primary source of afferent input for the control of sympathetic outflow by the vagal cardiopulmonary reflex during changes in thoracic blood volumes and pressures. (Circulation. 1998;98:2615-2620.)

Key Words: nervous system, autonomic | nervous system, sympathetic | lidocaine

Cardiopulmonary receptors with vagal afferent fibers contribute to the reflex control of sympathetic outflow in response to changes in thoracic blood volumes and pressures.1,2 Cardiopulmonary receptors with vagal afferents are located in both lungs, both atria, and both ventricles.3,4 Receptors from each of these regions are capable of modulating central sympathetic outflow.3 The relative importance of afferent input from each group of receptors is assumed to be equivalent. However, this assumption has been challenged by clinical and experimental observations that suggest indirectly that receptors either in the left ventricle or in the atria are the primary source of afferent input for the vagal cardiopulmonary reflex. A previous study from our laboratory was performed to further define the relative importance of left ventricular vagal afferents. The results of this study indicated that the loss of afferent input from this group of receptors had only mild effects on the control of sympathetic outflow by the vagal cardiopulmonary reflex.8 These results suggested that there is considerable “redundancy” in the afferent limb of the vagal cardiopulmonary reflex and that receptors in the right ventricle, the atria, and/or the lungs were able to compensate for the loss of afferent input from the left ventricle. In an effort to study this concept further, we investigated the effects of total cardiac deafferentation on the control of sympathetic outflow by the vagal cardiopulmonary reflex. On the basis of our previous observations, we hypothesized that the widespread distribution of receptors throughout the thoracic region would “protect” the vagal cardiopulmonary reflex. As a result, normal reflex responses would be preserved after elimination of vagal afferent input from the entire heart.

Methods

Experiments were performed in 14 anesthetized, mechanically ventilated dogs with sinoaortic denervation. The dogs were anesthetized with sodium thiopental (25 to 30 mg/kg IV) followed by α-chloralose (80 mg/kg IV). Supplemental doses of chloralose (10 mg/kg IV) were given hourly. The dogs were ventilated with a mixture of oxygen and room air. Arterial blood gases were monitored at intervals, and the respirator settings were adjusted to maintain pH between 7.35 and 7.45. The femoral artery and vein were cannulated for continuous measurement of arterial pressure and for infusion of drugs and manipulation of blood volume. The left atrium was cannulated for measurement of left atrial pressure (LAP).

Body temperature was maintained by external warming. During the recording of nerve activity, muscular movement was eliminated with pancuronium bromide (2 mg IV). All procedures and anesthetic techniques were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.
Volume Removed, Volume Infused, and Associated Change in LAP During Each Stage of the Experiment

<table>
<thead>
<tr>
<th></th>
<th>Before Lidocaine</th>
<th>After Lidocaine</th>
<th>1 Hour Later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume removed, mL</td>
<td>240±30</td>
<td>279±64</td>
<td>190±74</td>
</tr>
<tr>
<td>Volume infused, mL</td>
<td>518±47</td>
<td>638±48</td>
<td>679±95</td>
</tr>
<tr>
<td>Change in LAP, mm Hg</td>
<td>10.5±1.1</td>
<td>13.3±0.6*</td>
<td>12.3±1.2</td>
</tr>
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Values are mean ± SEM. *P < 0.05 vs prelidocaine value.

Surgical Preparation

Sinoaortic denervation was performed in all animals to facilitate the study of vagal cardiopulmonary reflexes in isolation from reflexes mediated by the arterial baroreceptors. A midline cervical incision was made to expose the carotid arteries and cervical vagi bilaterally. The carotid baroreceptors were denervated by isolation, ligation, and sectioning of all structures that course between the internal and external carotid arteries. Adequate carotid sinus denervation was confirmed by the lack of renal nerve traffic and arterial pressure changes during bilateral carotid occlusion. The aortic arch baroreceptors were denervated by sectioning of the cervical aortic depressor nerves bilaterally. With an operating microscope, these nerves were dissected free in the vagosympathetic trunk just caudal to the nodose ganglion near the junction of the vagus and the superior laryngeal nerves. The aortic nerves were identified by recording of typical pulse synchronous baroreceptor activity. Although this method may not interrupt all aortic baroreceptor fibers, it has been shown to result in immediate, functional aortic denervation.1,2

After sinoaortic denervation, an incision was made in the left fifth intercostal space, and a rib was removed to expose the heart. Three small incisions were made in the pericardium for placement of 3 cannulas. A small cannula (PE-50) was placed into a branch of the left circumflex coronary artery for injection of nicotine. A balloon occlusion catheter (8F; Meditech) was placed into the left atrium through the atrial appendage for mechanical activation of left atrial receptors and for measurement of LAPs. A final cannula (PE-90) was placed into the pericardial space for injection of lidocaine. All pericardial incisions were subsequently closed with purse-string sutures.

Renal Nerve Recordings

An incision was made in the left flank to expose the left renal artery. A small branch of the renal sympathetic nerves was dissected free from the renal artery and the surrounding connective tissue. The nerve was sectioned distally, and the nerve sheath was removed. The nerve was immersed in mineral oil and placed on bipolar platinum-iridium electrodes for the recording of action potentials as described in detail previously.1,3 Briefly, the signal was amplified by a bandpass amplifier (model P511, Grass Instruments Co) with high-frequency cutoff set at 1000 to 3000 Hz and low-frequency filter at 30 to 100 Hz. The output of this amplifier was fed into an audio amplifier and a spike counter that counted and integrated all nerve spike activity whose amplitude exceeded a preselected voltage level (just above noise).

Experimental Protocol

Arterial pressure, LAP, and raw and integrated RSNAs were recorded continuously during serial blood volume expansions. To obtain a larger portion of the stimulus-response curve between LAP and RSNA, blood volume was reduced by hemorrhage before blood volume expansion. Over a period of ≈4 minutes, blood was removed until systolic arterial pressure reached a level of ≈90 mm Hg and RSNA appeared grossly to reach a maximal level. Blood volume was then expanded by reinflation of the shed blood and by infusion of 10% dextran in normal saline. Blood volume expansion was performed over 8 to 10 minutes, and the amount infused was determined by the magnitude of changes that were observed in LAP and RSNA. After volume expansion, infused dextran volume was removed to return the animal to the baseline state. For subsequent blood volume manipulations, sufficient time (15 to 30 minutes) was allowed for stabilization of pressures and nerve activity, and attempts were made to elicit similar changes in LAP.

The responses to blood volume expansion were assessed before and after total cardiac deafferentation in all animals. Total cardiac deafferentation was achieved by instillation of 4 to 6 mL of 4% lidocaine into the pericardial space. The adequacy of deafferentation was assessed in several ways. The effects of pericardial lidocaine on left atrial receptors was assessed by measurement of RSNA responses to inflation of the left atrial balloon (n = 12). The effects of pericardial lidocaine on left ventricular receptors was assessed by measurement of RSNA responses to intracoronary nicotine (50 to 150 µg; n = 12). To assess the effects of pericardial lidocaine on pulmonary afferents, RSNA responses to transient lung inflation were recorded (n = 8). The lungs were inflated by temporary occlusion of the exhalation port of the respirator circuit.

In 8 experiments, lidocaine was flushed out of the pericardial space, and sufficient time was allowed for RSNA responses to left atrial balloon inflation and intracoronary nicotine concentration to return to baseline levels. The responses of LAP and RSNA to blood volume manipulation were then repeated for a third time.

Data Analysis

Arterial pressure, LAP, and raw and integrated RSNAs were recorded continuously on an electrostatic recorder (model ES1000, Gould Electronics). Baseline measurements were made immediately after hemorrhage when parameters had stabilized. Repeat measurements were made for each 0.5 to 1.0 mm Hg rise in LAP that occurred during blood volume expansion. Because recordings were made from multunit nerves in which the number of active fibers varied for each animal, nerve traffic changes were expressed in terms of percent change from baseline values.

The responses to volume expansion were analyzed in 2 ways. First, the responses for each individual animal were analyzed. For each experiment, measurements of LAP and the associated percent change in RSNA were plotted to derive stimulus-response curves. The slope of the linear portion of these curves was computed by linear regression analysis and used as an estimate of the “sensitivity” of the vagal cardiopulmonary reflex (ie, percent change in RSNA per mm Hg change in LAP). Reflex sensitivity values measured in each experiment (n = 14) were averaged, and means ± SEM were computed. A paired t test was used to assess the differences in mean reflex sensitivity values measured before and after total cardiac deafferentation with pericardial lidocaine. In the 8 experiments in which reflex sensitivity was measured a third time after the effects of pericardial lidocaine had worn off, a Bonferroni correction was used to compensate for multiple comparisons.

In addition, the percent changes in RSNA elicited by volume expansion in all of the animals were averaged, and means ± SEM were calculated. These mean percent changes in RSNA for the entire group were plotted against the changes in LAP, and linear regression
was performed. A t test was used to compare the differences in the slopes of these relationships before and after pericardial lidocaine.

The maximal percent changes in RSNA that were elicited by intracoronary nicotine, left atrial balloon inflation, and lung inflation were measured in each experiment, and means ± SEM were calculated. Paired t tests were used to determine whether there were significant differences in these responses before and after pericardial lidocaine. In the 8 experiments in which responses were measured a third time after the effects of pericardial lidocaine had worn off, a Bonferroni correction was used to compensate for multiple comparisons. In all cases, a value of $P < 0.05$ was considered to be statistically significant.

**Results**

The amount of blood removed and infused during each blood volume manipulation and the increase in LAPs associated with volume expansion in all experiments are shown in the Table. There were no significant differences in the amount of blood removed or infused at each stage of the experiment. The increase in LAP associated with volume expansion was significantly greater after pericardial lidocaine than before, indicating that volume expansion after lidocaine should have provided an adequate stimulus to the cardiopulmonary receptors.

**Effect of Pericardial Lidocaine on Vagal Cardiopulmonary Afferents**

Figure 1 shows the RSNA responses to left atrial balloon inflation, intracoronary nicotine administration, and lung inflation. Before pericardial lidocaine, activation of left atrial, left ventricular, and pulmonary receptors elicited reflex decreases in RSNA. After pericardial lidocaine, inhibitory responses to left atrial balloon inflation and intracoronary nicotine were abolished, indicating interruption of cardiac vagal afferents. The inhibitory response to lung inflation was unchanged, indicating intact function of vagal pulmonary receptors and thus selective chemical deafferentation of the atria and ventricles.

Figure 2 shows the RSNA responses to left atrial balloon inflation and intracoronary nicotine in the 8 experiments in which lidocaine was flushed from the pericardial space. After a period of $\approx 1$ hour, activation of left atrial and left ventricular vagal afferents again elicited inhibition of RSNA. This inhibition was similar to the changes in RSNA that were observed before pericardial lidocaine, indicating that the effects of lidocaine on cardiac afferent fibers were reversible.

**Responses to Blood Volume Expansion**

Individual and mean (± SEM) reflex sensitivity values (% change in RSNA/mm Hg change in LAP) measured during blood volume expansion are shown in Figure 3. Before pericardial lidocaine, elevation of LAP was associated with the expected decrease in RSNA in all experiments. After total cardiac deafferentation with pericardial lidocaine, reflex sensitivity values were significantly attenuated. In fact, in 7 of the 14 experiments, the correlation between LAP and RSNA was no longer significant, indicating that pericardial lidocaine essentially abolished the reflex response.

The relationship between the mean percent changes in RSNA and the changes in LAP are shown in Figure 4. Before pericardial lidocaine, increases in LAP elicited by blood volume expansion resulted in reflex inhibition of RSNA. After pericardial lidocaine, this reflex sympathoinhibition was significantly attenuated.

Mean (± SEM) reflex sensitivity values for the 8 dogs in whom blood volume expansion was repeated after lidocaine was flushed from the pericardial space are shown in Figure 5.
Significant attenuation of reflex sensitivity values was observed after pericardial lidocaine. One hour later, when responses to left atrial balloon inflation and intracoronary nicotine had returned (Figure 2), reflex sensitivity values also were restored to baseline levels.

Discussion
Sensory receptors with vagal afferent fibers are located throughout the cardiopulmonary region. A wealth of clinical observations and experimental data indicate that these receptors contribute to the reflex control of the circulation by the autonomic nervous system. However, much less is known about whether afferent input from different areas of the cardiopulmonary region has equivalent impact on this reflex control. This is particularly true for stimuli, such as blood volume manipulation, that globally affect receptors in the ventricles, atria, and lungs. This study attempted to investigate this issue further by examining the effects of selective cardiac deafferentation on vagal cardiopulmonary reflex function.

The major new finding of this study is that the control of sympathetic outflow by the vagal cardiopulmonary reflex is markedly attenuated after interruption of afferent input from the heart. In fact, the reflex inhibition of renal sympathetic nerve activity elicited by increases in cardiopulmonary pressures was frequently abolished after pericardial lidocaine. Because reflex responses to lung inflation were not affected by pericardial lidocaine, it appears that residual functional receptors located in the lungs are unable to compensate for the loss of vagal afferent input from the heart and do not contribute to the sympathoinhibitory response to blood volume expansion. These results suggest that cardiac receptors provide the principal source of afferent input for the vagal cardiopulmonary reflex during changes in blood volume. To the best of my knowledge, this is the first and only study that has examined the effects of selective cardiac deafferentation on vagal cardiopulmonary reflex control of sympathetic outflow during changes in thoracic blood volumes and pressures.

Experimental Model
Our experiments were performed in animals with sinoaortic denervation. This experimental preparation was used to facilitate the investigation of the vagal cardiopulmonary reflex. Blood volume manipulations are usually associated with changes in arterial pressure. Elimination of the confounding effects of the arterial baroreflex by sinoaortic denervation ensured that the changes in sympathetic outflow elicited by blood volume expansion were mediated by the vagal cardiopulmonary reflex.

Blood volume manipulation was used to alter the stimulus to the cardiopulmonary receptors with vagal afferent fibers. As opposed to other stimuli, such as myocardial ischemia, this maneuver equally affects receptors in all areas of the cardiopulmonary region. We have observed a close correlation between changes in pulmonary artery pressures and changes in cardiac filling pressures during blood volume manipulation. Thus, this technique is well suited for evaluating the relative importance of cardiopulmonary receptors in the atria, ventricles, and lungs. Furthermore, the changes in LAPs observed with blood volume manipulation are well within the range that may occur in physiological conditions such as orthostatic stress.

The use of local anesthetic agents placed in the pericardial space to block neural transmission has been described and validated previously. Although lidocaine was used to block neural transmission in this study, similar effects have been demonstrated for procaine and bupivacaine. These agents have been shown to block electrical activity in both afferent and efferent cardiac nerves. In a pilot study reported previously, we demonstrated that pericardial lidocaine abolished reflex responses to activation of left ventricular, left atrial, and right ventricular vagal afferents. The effects of pericardial lidocaine on right atrial afferents could not be evaluated in this pilot study, because selective activation of right atrial receptors did not produce a measurable reflex response. Nevertheless, it is likely that right atrial afferents were also blocked by pericardial lidocaine. In this study, we confirmed the efficacy of pericardial lidocaine in eliminating reflex responses to activation of cardiac vagal afferents. Furthermore, these data confirm previous observations that pericardial local anesthetics do not affect pulmonary receptors with vagal afferents and that the effects of these agents are reversible. Thus, these results support the view that pericardial lidocaine can be used to create an experimental model of total cardiac deafferentation that is reversible.

Physiological Relevance
Cardiopulmonary receptors with vagal afferent fibers exert tonic inhibition of sympathetic outflow from the central nervous system. Early observations by Mancia and Donald indicated that receptors in the atria, ventricles, and lungs each had the potential to inhibit central sympathetic outflow on a tonic basis. This observation suggested that receptors from all parts of the cardiopulmonary region could participate in the reflex control of central sympathetic outflow in response to changes in blood volume. However, several subsequent findings indirectly suggested that vagal afferent input from the left ventricle might be more important than afferent input from the atria or lungs. In patients with aortic stenosis (particularly those with a history of syncope), forearm vaso-dilation was observed in response to leg exercise. This
paradoxical response was distinct from the exercise-induced forearm vasoconstriction observed in normal control subjects. These differences were felt to be related to activation of left ventricular vagal afferents, because greater exercise-induced increases in ventricular pressures were noted in aortic stenosis patients than in control subjects. Of interest, patients with mitral stenosis had increases in left atrial and pulmonary artery pressures during exercise, but they did not exhibit forearm vasodilation.

Marked abnormalities of vagal cardiopulmonary reflexes also have been observed in patients after cardiac transplantation and in dogs with transmural inferoposterior myocardial infarction. Because both cardiac transplantation and transmural myocardial infarction interrupt ventricular vagal afferent fibers, these reflex abnormalities could indicate that the left ventricle is the primary source of vagal afferent input. However, the relationship between this deafferentation and the abnormal reflexes is uncertain. We performed experiments designed specifically to assess the impact of left ventricular deafferentation on vagal cardiopulmonary reflexes. These experiments were also performed in dogs with sinoaortic denervation. Control of sympathetic nerve activity by the vagal cardiopulmonary reflex was measured before and after selective left ventricular deafferentation using epicardial application of phenol. We found that interruption of afferents from the entire left ventricle significantly attenuated cardiopulmonary reflex sensitivity. However, the magnitude of this effect was small, indicating that receptors in other parts of the cardiopulmonary region compensated for the loss of left ventricular afferent input.

In the present experiments, interruption of afferent input from the entire heart resulted in marked abnormalities of vagal cardiopulmonary reflexes. Taken in context with the results of our previous study, there are 2 possible interpretations for this finding. The results of the present study are consistent with the concept that the combined influence of atrial and ventricular receptors represents the primary source of afferent input for the vagal cardiopulmonary reflex. An alternative explanation that cannot be excluded is that receptors in the atria may be of primary importance. At least one previous study supports this latter possibility. In humans, Oren et al. measured cardiac dimensions by cine CT, right heart pressures, and either forearm vascular or muscle sympathetic nerve responses to low levels of lower-body negative pressure (LBNP, −10 mm Hg). As expected, LBNP elicited decreases in right heart pressures and reflex increases in forearm vascular resistance and muscle sympathetic nerve activity. On the basis of the cine CT observation that LBNP reduced left atrial size without a discernible change in ventricular volumes, the authors interpreted their results to suggest that left atrial receptors were primarily responsible for the reflex responses. However, ventricular pressures were not measured in this study. From a physiological and hemodynamic standpoint, it is almost certain that a fall in LAP induced by venous pooling would be associated with a decrease in ventricular diastolic pressures. Thus, a contribution of ventricular receptors to the reflex response elicited by LBNP cannot be excluded with certainty. It should also be noted that our study is not designed to assess the relative roles of atrial versus ventricular receptors in the reflex inhibition of sympathetic outflow in response to changes in blood volume.

Impaired vagal cardiopulmonary reflexes have been observed in other pathophysiological states, such as congestive heart failure. There are similarities between the abnormalities we have documented in this study and those that have been observed in congestive heart failure. However, the relationship between impaired vagal cardiopulmonary reflexes in congestive heart failure and alterations in vagal afferent input from the heart is uncertain. Previous studies have shown that the function of atrial myelinated receptors with vagal afferent fibers is impaired in experimental heart failure, but the effects of heart failure on other atrial or ventricular receptors with vagal afferent fibers have not been studied.

In summary, these experiments provide further insight into the relative importance of afferent input from the heart and lungs. The results demonstrate that pericardial lidocaine selectively abolishes reflexes mediated by cardiac receptors without affecting responses to activation of pulmonary receptors. The observation that pericardial lidocaine markedly attenuates renal sympathetic nerve responses to changes in blood volume shows that the loss of afferent input from the heart has major effects on the vagal cardiopulmonary reflex. This finding indicates that cardiac receptors are the primary source of afferent input for the control of sympathetic outflow by the vagal cardiopulmonary reflex. Pulmonary receptors alone cannot compensate for the loss of cardiac receptors and cannot preserve cardiopulmonary reflex responses to changes in blood volume.

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