Contrasting Reflex Effects of Chemosensitive and Mechanosensitive Vagal Afferents

SEIJI HIGUCHI, DONALD A. MORGAN, AND ALLYN L. MARK

SUMMARY Previous studies have identified two distinct types of cardiac vagal afferents, mechano-sensitive and chemosensitive. We tested the hypothesis that these two types of vagal afferents mediate different reflex sympathetic nerve responses. We compared effects of stimulation of chemosensitive and mechanosensitive vagal afferents on renal and adrenal sympathetic nerve activity in rats. In anesthetized, sinoaortic baroreceptor-denervated rats, we measured arterial pressure, heart rate, and renal and adrenal sympathetic nerve activity during intrapericardial administration of phenyl diguanide as a stimulus for chemosensitive afferents and during volume expansion and acute occlusion of the ascending aorta as stimuli for mechanosensitive afferents. Stimulation of chemosensitive afferents with phenyl diguanide (3, 10, and 30 μg/kg intrapericardially) caused decreases in renal sympathetic nerve activity (-36 ± 5, -52 ± 11, and -71 ± 5%; p<0.01) and in arterial pressure and heart rate but increased adrenal sympathetic nerve activity ( + 14 ± 27, +63 ± 21, and +83 ± 28%; p<0.05). These responses were abolished by vagotomy. Intrapericardial injection of saline vehicle did not change renal and adrenal sympathetic nerve activity. In contrast to the effects of phenyl diguanide, activation of mechanosensitive afferents by volume expansion with intravenous infusion of 6% dextran in 0.9% saline (Dextran 75) decreased both renal and adrenal sympathetic nerve activity. Stimulation of mechanosensitive afferents by acute occlusion of the ascending aorta also decreased both renal and adrenal sympathetic nerve activity. These results indicate that chemosensitive and mechanosensitive cardiac vagal afferents produce different reflex responses: Chemosensitive afferents increase and mechanosensitive afferents decrease adrenal sympathetic outflow.

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KEY WORDS  • phenyl diguanide  • vagotomy  • sinoaortic baroreceptor denervation

SINCE the concept of vagal depressor reflexes originating in the heart was introduced by von Bezold and Hirt1 in 1867, many studies have revealed that vagal afferent reflexes originating in cardiac sensory receptors participate in control of circulation in physiological2-6 and pathological states.7-11 These reflexes have been classified according to various characteristics including location of the receptors (e.g., atrial, ventricular, or pulmonary) and type of afferent fibers (e.g., myelinated or unmyelinated). There is substantial evidence that stimulation of unmyelinated cardiac vagal afferents produces inhibitory effects on sympathetic outflows, especially to the kidney.12

The Coleridges and their colleagues13-15 demonstrated that cardiac sensory receptors with unmyelinated vagal afferents are heterogeneous in terms of responsiveness to chemical and mechanical stimuli. According to their concept, there are two distinct types of unmyelinated vagal afferents, chemosensitive and mechanosensitive. These observations would seem to raise the possibility that chemosensitive and mechanosensitive afferents might subserve different reflex responses. However, most studies comparing chemosensitive and mechanosensitive cardiac vagal afferents have dealt with afferent recordings, and there has been no systematic comparison of the sympathetic nerve responses to activation of chemosensitive and mechanosensitive afferents.

In this study, we compared effects of stimulation of chemosensitive and mechanosensitive vagal afferents on renal and adrenal sympathetic nerve activity (SNA). We examined responses to intrapericardial administration of phenyl diguanide (PDG) as a stimulus...
for chemosensitive afferents and to graded volume expansion and aortic occlusion as stimuli for mechano-sensitive afferents.

Materials and Methods

General Procedures

Experiments were performed on 54 female Sprague-Dawley rats (Biolab, St. Paul, MN, USA) with body weights from 250 to 280 g. The care of the rats complied with the guiding principles of the American Physiological Society for animal experimentation. In all experiments, anesthesia was induced with methohexitol sodium (75 mg/kg intraperitoneally) and α-chloralose (50 mg/kg intravenously) and was maintained by α-chloralose (25 mg/kg/hr intra-arterially).

Polyethylene catheters were inserted into a femoral artery for recording of arterial pressure, into a ventral tail artery for withdrawal of blood, and into a femoral vein for administrations of fluids and drugs. In some experiments, a polyethylene catheter was inserted into the left ventricle through the right common carotid artery to measure left ventricular end-diastolic pressure (LVEDP). Heart rate was determined with a cardiofotometer triggered by the arterial pressure pulse. The trachea was cannulated, and animals breathed oxygen-enriched air. During open-chest experiments, animals were paralyzed with gallamine (4 mg/kg i.v. and supplements as needed) and ventilated artificially with a ventilator (Model 683, Harvard Apparatus, South Natick, MA, USA). Tidal volume was set at 10 ml/kg, and respiratory rate was adjusted to maintain arterial blood pH between 7.30 and 7.35. To prevent acidosis, small doses of NaHCO₃ (0.1–0.2 mM) were continuously infused. Body temperature was maintained between 36 and 38°C with a heating pad.

Sinoaortic baroreceptors were denervated according to the technique of Krieger. The effectiveness of denervation was confirmed by demonstrating the failure of phenylephrine-induced increases in mean arterial pressure of 30 to 50 mm Hg to elicit heart rate or sympathetic nerve responses.

Recordings of Sympathetic Nerve Activity

The left kidney and adrenal gland were exposed retroperitoneally. The sympathetic nerve branch to the kidney or the adrenal nerve branch was dissected free and placed on a thin, bipolar platinum electrode. When an optimal signal was obtained, the nerve on the electrode was isolated with a small amount of silicone rubber (Sil-Gel 604, Wacker-Chemie, München, West Germany). The neural signals were amplified by a Grass amplifier (Model P511, Quincy, MA, USA) with high and low frequency cutoffs of 1000 Hz and 100 Hz, respectively. The filtered neurogram was displayed on an oscilloscope (Model 5115, Tektronix, Portland, OR, USA) and monitored with an audio amplifier. The signals were also routed to a nerve traffic analyzer (Model 706C, University of Iowa Bioengineering, Iowa City, IA, USA), which counted nerve spikes exceeding the threshold voltage set just above the noise level. The time of the counter was set at 1 second, so that the impulse frequency was displayed as the number of spikes collected each second on a time-frequency histogram. The zero noise was expressed as the activity after injection of chlorisondamine (5 mg/kg i.v.) for renal SNA and as the postmortem activity for adrenal SNA.

Experimental Protocols

Protocol 1

Protocol 1 examined the effects of intrapericardial administration of PDG and volume expansion on renal SNA (n = 9) and adrenal SNA (n = 9). The thorax was opened through transisternal bilateral intercostal incision. A small hole was made in the pericardium through which a thin catheter (PE-10) was inserted into the pericardial space. After the thorax was closed and the rat was weaned from the ventilator, more than 30 minutes was allowed for stabilization before the protocols were begun.

PDG (3, 10, and 30 μg/kg) or vehicle was injected into the pericardial space in volumes of 25 μl. After responses, the pericardial space was flushed with 25 μl of saline.

Each injection was at least 10 minutes apart. To determine that the responses to intrapericardial injection of PDG were not mediated by spread of PDG into the pleural space, the distribution of pericardial injection of PDG was confirmed at the end of each experiment by an injection of dye (10 μl of Evans blue). In these experiments, we also examined responses to injection of PDG (30 μg/kg) into the pleural space.

Graded volume expansion was performed by infusion of 6% dextran in 0.9% saline (Dextran 75) into the femoral vein in 0.5-ml increments to a total of 3 ml. Increments in volume were administered in 15 to 30 seconds. After volume expansion, blood was withdrawn to restore LVEDP to preinfusion levels. After the vagal nerves were cut in the neck, injections of PDG and volume expansion were repeated.

Protocol 2

Protocol 2 examined the reproducibility of effects of intrapericardial administration of PDG and volume expansion on renal SNA (n = 5). In five rats, responses to intrapericardial injections of PDG and volume expansion were studied before and after sham vagotomy to examine the reproducibility of responses and to evaluate the effects of time and repeated administration of PDG and dextran.

Protocol 3

Protocol 3 studied the effects of intravenous administration of nitroprusside on renal SNA (n = 7). To determine whether increases in renal SNA during intrapericardial injection of PDG were due to a direct reflex effect of stimulation of chemosensitive cardiac vagal afferents or were secondary to decreases in arterial pressure, we examined the responses to decreases in arterial pressure produced by intravenous injection of nitroprusside (0.4–0.8 μg/kg).
Protocol 4

Protocol 4 studied the effects of aortic occlusion on renal SNA (n = 7) and adrenal SNA (n = 7). In these experiments, we examined responses to stimulation of mechanosensitive vagal afferents using occlusion of the ascending aorta, which distends the left ventricle and atrium. These experiments were performed in artificially ventilated open-chest rats. The pericardium was split, and a snare was placed around the ascending aorta. Occluding time was 5 seconds.

Protocol 5

Protocol 5 examined the effects of pericardial distention and stretching on renal SNA (n = 5) and adrenal SNA (n = 5). To determine if responses to intrapericardial injection of PDG were derived from the activation of pericardial as opposed to cardiac vagal afferents, we examined the responses to mechanical distention of the pericardium with inflation of a balloon in the pericardial space and with scratching the pericardium with forceps. These experiments were performed under artificial ventilation. The pericardial balloon was inflated for 30 seconds.

Data Analysis

The responses to PDG, saline, and aortic occlusion before versus after vagotomy were analyzed by paired t test. In comparing the responses to volume expansion before and after vagotomy, we employed the slope of linear regression. Data are presented as means ± SE. Statistical significance was considered as a p level below 0.05.

Results

Intrapericardial administration of PDG (3, 10, and 30 μg/kg) caused decreases in mean arterial pressure, heart rate, and renal SNA but increases in adrenal SNA (Figure 1; Tables 1 and 2). These responses were abolished by vagotomy (see Tables 1 and 2). Intrapericardial administration of PDG (30 μg/kg) tended to decrease renal SNA (-16 ± 8%) and increase adrenal SNA (+36 ± 20%), but these changes were not significant. Intrapericardial injection of vehicle (saline) did not change adrenal and renal SNA (see Tables 1 and 2). Graded volume expansion with dextran decreased both renal and adrenal SNA (Figure 2). These responses were eliminated by vagotomy (see Figure 2).

Acute occlusion of the ascending aorta increased left ventricular systolic (+82 ± 3 mm Hg) and diastolic pressure (+20 ± 1 mm Hg) and decreased both adrenal and renal SNA (Figure 3). These responses were abolished by vagotomy (see Figure 3).

Responses of arterial pressure, heart rate, and renal SNA to PDG (3, 10, and 30 μg/kg) did not differ before and after sham vagotomy (Table 3). Volume expansion also elicited similar responses in LVEDP and renal SNA before and after sham vagotomy. The slope of relating decreases in renal SNA to increases in LVEDP during volume expansion (change in renal SNA [%]/change in LVEDP [mm Hg]) was -4.5 ± 0.8 before sham vagotomy vs -3.8 ± 0.7 after sham vagotomy.

Intravenous injection of nitroprusside (0.4–0.8 μg) decreased mean arterial pressure to the same extent as that during intrapericardial administration of PDG (-38 ± 4 mm Hg for nitroprusside vs -38 ± 6 mm Hg for PDG, 30 μg/kg), but it did not significantly change adrenal SNA (+5 ± 4%).

Pericardial distention and scratching decreased mean arterial pressure but did not change heart rate, renal SNA (-1 ± 1% for distention and -2 ± 4% for

Table 1. Renal Sympathetic Nerve Responses to Intrapericardial Injections of Phenyl Diguanide and Saline Before and After Vagotomy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before vagotomy</th>
<th>After vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDG</td>
<td>PDG</td>
</tr>
<tr>
<td></td>
<td>3 μg/kg (n = 9)</td>
<td>10 μg/kg (n = 9)</td>
</tr>
<tr>
<td>Maximal change in renal SNA (%)</td>
<td>-36 ± 5*</td>
<td>-52 ± 11*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>129 ± 9</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>Before injection</td>
<td>-8 ± 2*</td>
<td>-22 ± 3*</td>
</tr>
<tr>
<td>Maximal change</td>
<td>480 ± 9</td>
<td>477 ± 9</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>-4 ± 1*</td>
<td>-10 ± 3*</td>
</tr>
<tr>
<td>Before injection</td>
<td>497 ± 9</td>
<td>477 ± 9</td>
</tr>
<tr>
<td>Maximal change</td>
<td>-4 ± 1*</td>
<td>-10 ± 3*</td>
</tr>
</tbody>
</table>
TABLE 2. Adrenal Sympathetic Nerve Responses to Intrapericardial Injections of Phenyl Diguanide and Saline Before and After Vagotomy

<table>
<thead>
<tr>
<th>Variable</th>
<th>PDG</th>
<th>PDG</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal change in adrenal SNA (%)</td>
<td>14 ± 27</td>
<td>63 ± 21*</td>
<td>83 ± 28*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before injection</td>
<td>133 ± 8†</td>
<td>131 ± 7†</td>
<td>134 ± 7†</td>
</tr>
<tr>
<td>Maximal change</td>
<td>-25 ± 6*</td>
<td>-26 ± 3†</td>
<td>-38 ± 6†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>509 ± 14</td>
<td>517 ± 15</td>
<td>501 ± 12</td>
</tr>
<tr>
<td>Maximal change</td>
<td>-6 ± 3</td>
<td>-5 ± 1*</td>
<td>-10 ± 4*</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. PDG = phenyl diguanide; SNA = sympathetic nerve activity.
*p<0.05, †p<0.01, compared with values after vagotomy.

scratching), or adrenal SNA (-4 ± 5% for distention and +1 ± 3% for scratching).

Discussion

The results of this study indicate that stimulation of chemosensitive and mechanosensitive vagal afferents produces contrasting reflex effects on efferent SNA, with increases in adrenal SNA during stimulation of chemosensitive afferents and decreases in adrenal SNA during stimulation of mechanosensitive afferents.

Sensory receptors with unmyelinated vagal afferents are present throughout the lungs in addition to the heart and great vessels. In this experiment, we administered PDG into the pericardial space to restrict its effects to the heart. Dye injected into the pericardial space at the end of experiment was localized to the pericardial space. Thus, we conclude that PDG administered into the pericardial space stimulated mainly chemosensitive cardiac vagal afferents.

We employed two methods to stimulate mechanosensitive vagal afferents. Volume expansion may activate vagal afferents in other locations (e.g., lungs) besides the heart. Aortic occlusion produces acute distention of the left ventricle and atrium and activates left-sided mechanosensitive cardiac vagal afferents. Taken together, the responses to these two stimuli constitute a reliable test of effects of stimulation of mechanosensitive cardiac vagal afferents, though we cannot completely exclude an effect of stimulation of mechanosensitive pulmonary vagal afferents.

Based on these considerations we suggest that the contrasting effects of intrapericardial PDG versus volume expansion or aortic occlusion on adrenal SNA reflect contrasting effects of chemosensitive and mechanosensitive cardiac vagal afferents.

We considered several alternative explanations for our results. These involve 1) arterial baroreceptors, 2) cardiac sympathetic afferents, 3) pericardial sensory receptors, and 4) pulmonary vagal afferents.

First, could the increase in adrenal SNA during PDG have been secondary to the decrease in arterial pressure and inhibition of arterial baroreceptors, and not to a direct reflex effect of activating chemosensitive vagal afferents? This possibility is excluded since the animals had undergone sinoaortic baroreceptor denervation, and decreases in arterial pressure during intravenous administration of nitroprusside failed to significantly increase adrenal SNA.

Second, Coleridge et al. have demonstrated the PDG and capsaicin administered intravenously stimu-
late chemosensitive vagal afferents, but it is also known that some chemicals, such as bradykinin and prostaglandins, activate cardiac sympathetic afferents and produce excitatory responses in arterial pressure, heart rate, and SNA. In seems unlikely, however, that the increases in adrenal SNA during PDG in our study derived from activation of cardiac sympathetic afferents, since the increases in adrenal SNA were abolished by vagotomy.

Third, we considered the possibility that the contrasting effects of volume expansion and intrapericardial PDG on adrenal SNA might be explained by stimulation of myocardial sensory receptors with volume expansion (decreasing adrenal SNA) and of pericardial receptors with PDG (increasing adrenal SNA). However, distending or scratching the pericardium failed to elicit reflex changes in renal or adrenal SNA. Thus, our results cannot be explained by activation with PDG of a distinct population of pericardial sensory receptors that increases adrenal SNA.

Fourth, could our results relate to contrasting effects of cardiac and pulmonary vagal afferents and not to a difference in chemosensitive and mechanosensitive vagal afferents? Specifically, one might suggest that intrapericardial PDG activates cardiac afferents, which increase adrenal SNA, and that volume expansion activates predominantly pulmonary afferents, which decrease adrenal SNA. We cannot exclude this possibility since we were unable to selectively activate chemosensitive pulmonary afferents subserved by the pulmonary circulation. However, intrapleural administration of PDG failed to decrease and, indeed, tended to increase adrenal SNA. In other words, stimulation of either cardiac or pleural chemosensitive vagal afferents increased or tended to increase adrenal SNA, whereas stimulation of mechanosensitive cardiopulmonary vagal afferents decreased adrenal SNA.

The physiological importance of our observations is uncertain. Specifically, we cannot state whether stimulation of chemosensitive cardiac vagal afferents is involved in responses to physiological and pathophysiological stimuli. However, we have recently found reflex sympathetic nerve responses to hemorrhage that bear an intriguing resemblance to the sympathetic nerve responses to stimulation of chemosensitive vagal afferents in this study. Specifically, in our previous study, we found that hemorrhage to an arterial pressure of 50 mm Hg in rats triggered vagal afferent reflexes that decreased renal SNA and increased adrenal SNA. It is conceivable that these reflex responses to hemorrhage result from the stimulation of chemosensitive vagal afferents activated by local circulatory factors released during hemorrhage. However, this suggestion and the physiological importance of chemosensitive cardiac vagal afferents remain speculative.

In summary, the results of this study indicate that stimulation of chemosensitive cardiac vagal afferents by PDG decreased renal SNA but increased adrenal SNA, while activation of mechanosensitive vagal afferents by two methods (volume expansion and aortic occlusion) decreased both renal and adrenal SNA. These results indicate that chemosensitive and mechanosensitive cardiac vagal afferents produce different reflex sympathetic nerve responses.

Acknowledgments
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References
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13. Coleridge HM, Coleridge JCG, Kidd C. Cardiac receptors in the dog, with particular reference to two types of afferent ending in the ventricular wall. J Physiol (Lond) 1964;174:323–339
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