Visceral afferent activation-induced changes in sympathetic nerve activity and baroreflex sensitivity

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Visceral afferent activation-induced changes in sympathetic nerve activity and baroreflex sensitivity. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1780–R1791, 1999.—The following experiments were done to determine whether changes in baroreflex sensitivity evoked by cervical vagus nerve stimulation are due to sympathoexcitation mediated by the parabrachial nucleus. The relative contribution of cardiopulmonary and general gastric afferents within the cervical vagus nerve to the depression in baroreflex sensitivity are also investigated. Male Sprague-Dawley rats anesthetized with thiobutabarbital sodium (50 mg/kg) were instrumented to measure blood pressure and heart rate or for the continuous monitoring of renal sympathetic nerve activity. Baroreflex sensitivity was measured using bolus injections of phenylephrine. Electrical stimulation of the cervical vagus (with or without the aortic depressor nerve) or the abdominal vagus nerve produced a significant increase in renal nerve activity and a decrease in baroreflex sensitivity. Both of these effects were blocked after the microinjection of lidocaine into the parabrachial nucleus before nerve stimulation. Therefore, we conclude that an increase in the activity of cardiac, pulmonary, or general gastric afferents mediated the increased sympathetic output and decreased baroreflex sensitivity via a pathway involving the parabrachial nucleus.

cervical vagus; abdominal vagus; renal sympathetic activity; parabrachial nucleus; lidocaine

EXPERIMENTALLY INDUCED myocardial infarction has provided further insight into the relationship between baroreflex sensitivity (BRS) and cardiovascular disease (32). After ligation of the left descending coronary artery in dogs, BRS was significantly depressed (5) and plasma norepinephrine was significantly elevated. The mechanism for these effects has been suggested to be mediated by an increase in vagal afferent activity due to the presence of necrotic and noncontractile segments of the myocardium. Such abnormalities would result in the mechanical distortion of sensory receptor endings and, therefore, produce an abnormal increase in the activity of cardiac vagal afferent fibers (5).

Using a model of direct cervical vagal afferent activation in the rat, we have shown that electrical stimulation of the entire vagus nerve bundle for 2 h resulted in a significant depression in BRS (the ability of the reflex to defend against a pressor challenge) that occurred concurrently with a significant increase in plasma norepinephrine (27). It therefore appeared that this model of prolonged cervical vagal stimulation produced an altered autonomic output characteristic of several cardiovascular pathologies (1, 3, 8, 13, 14, 22, 29–32). Furthermore, we have shown that bilateral lesion of a central nucleus involved in autonomic regulation, namely, the parabrachial nucleus (PBN) of the pons, completely abolished the increase in plasma norepinephrine levels and decreased BRS observed after cervical vagus nerve stimulation (27).

The role of the PBN in the integration and relay of visceral afferent information to the forebrain has been well established. Evidence has also demonstrated that prolonged vagal afferent stimulation results in significant changes in the release of glutamate and the immunostaining intensity of various modulatory neuropeptides within the PBN (25). These peptides in the PBN were shown to significantly enhance or depress glutamatergic neurotransmission of visceral afferent information to other forebrain autonomic nuclei (24). Taken together, this evidence has led us to the conclusion that increased vagal afferent activity produces significant changes in the PBN resulting in the altered processing of visceral information, which, in turn, results in changes characteristic of an abnormal autonomic output.

The cervical vagus nerve contains afferents that relay information from mechanoreceptors located on the surface of the aorta and carotid arteries, myocardial cells, and also general visceral afferents to the central nervous system from the lung and abdomen (16, 21). Baroreceptors located in the wall of the aortic arch send afferents via the aortic depressor nerve (ADN), which joins the cervical vagus bundle (15). These vagal afferents enter the central nervous system and terminate within the nucleus of the solitary tract (7, 19). Vagal afferent information is then topographically relayed to the PBN and then to other forebrain nuclei for the integration and coordination of autonomic and behavioral responses (4).

Although evidence has been provided demonstrating that stimulation of the entire cervical vagus bundle results in an increase in plasma norepinephrine levels and a decreased BRS, the individual contribution of the major visceral afferents within this nerve bundle (baroreceptor, cardiopulmonary, and general gastric) was not investigated. Therefore, renal sympathetic nerve activity, which is a direct measure of sympathetic tone, along with BRS, will be examined before and after 2 h of electrical stimulation of afferent nerves contained within the vagus: the cervical vagus nerve (including and excluding the ADN), the ADN alone, and the...
EXPERIMENTAL PROCEDURES

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Prince Edward Island Animal Care Committee.

General surgical procedures. Experiments were performed on a total of 40 male Sprague-Dawley rats (Charles River, Montreal, PQ, Canada) weighing 250–290 g. For all animals, food and tap water were provided ad libitum, except food was removed 24 h before surgery. Rats were anesthetized with thiobutabarbital sodium (Inactin; Research Biochemicals, Natick, MA; 50 mg/kg ip), which provides a stable plane of anesthesia in male rats for up to 9 h. A polyethylene catheter (PE-50; Clay Adams, Parsippany, NJ) was inserted into the right femoral artery to monitor blood pressure and heart rate and into the right femoral vein (PE-10) for the intravenous administration of drugs. Arterial blood pressure was measured using a pressure transducer (model P23 ID, Gould, Cleveland, OH) connected to a polygraph (model 2200S, Gould). Heart rate was determined from the pulse pressure with use of a Gould tachograph (Biotach). An endotracheal tube was inserted, and when necessary animals were ventilated with room air (65 strokes/min, 2.5 ml tidal volume) to facilitate respiration.

Isolation and electrical stimulation of visceral afferent nerves. In groups 1–4 (n = 8/group), the right cervical vagus nerve (including the ADN) was isolated through a midline cervical incision. In two of these groups the right ADN was isolated from the vagus bundle and either stimulated (ADN group) or cut (barodenervated vagus group). In group 5 (n = 8) a midline incision was made below the diaphragm, and the right abdominal vagus was isolated free of the surrounding tissue, including the esophagus. After isolation, all nerves were placed on stainless steel electrodes and fixed in place with dental impression material (Basilex, Ash Temple, Bedford, NS, Canada). In all cases the cervical vagus and ADN (groups 1–4) or abdominal vagus (group 5) was crushed distal to the stimulating electrode, permitting activation of visceral afferents only. The stimulus intensity used to activate cervical and abdominal vagal and ADN afferents (0.5–1.5 mA, groups 1–3 and 5) was determined using a 5-s train of pulses (50 Hz, 2-ms pulse duration) to produce a maximal reflex decrease in heart rate (70–110 beats/min) and blood pressure (30–40 mmHg). After determination of a maximal stimulus intensity, the nerves were stimulated intermittently (1 s on-2 s off cycle) for 2 h. The 1 s on-2 s off cycle of 2-h duration was chosen to prevent visceral afferent activation-induced adaptation observed with sustained visceral afferent activation (17, 25). In group 4 (nonstimulated controls, n = 4) the cervical vagus nerve was isolated and crushed distally, and a stimulating electrode was fixed in place, but no current was passed through the electrodes.

Peribarachral microinjections. After nerve isolation in groups 1–5, the animals were placed in a Kopf (Tujunga, CA) stereotaxic frame, and small burr holes were drilled bilaterally through the temporal-occipital bone to allow for the stereotaxic insertion of a 30-gauge stainless steel, 1-µl Hamilton microsyringe into the PBN according to coordinates obtained from a stereotaxic atlas of the rat brain (23). In four animals from each group, bilateral microinjections of the anesthetic lidocaine (1%, 300 nl/side, Vetoquinol Canada, Joliette, PQ, Canada) were made into the PBN to reversibly interrupt neurotransmission. Previous work in our laboratory has provided evidence to show that lidocaine injected into the PBN at this volume and concentration has an ~1-mm-diameter spread zone. It is therefore likely that all injections that terminate within the PBN would spread throughout most of the nucleus, regardless of their exact location. Such injections into the PBN have been shown to result in the blockade of cardiovascular responses for ~2 h (26). The timing of the lidocaine microinjections was to prevent the effects of the sustained afferent stimulations but, at the same time, allow blood pressure and heart rate, as well as functionality of the PBN, to return to control before BRS testing. The remaining animals from each group (n = 4) received bilateral microinjections of saline (0.9%, 300 nl/side) into the PBN. All injections were made 5 min before the beginning of nerve stimulation.

Renal nerve recording. The right kidney was exposed using a retroperitoneal approach and with the aid of an operating stereomicroscope, a renal nerve branch was isolated from the surrounding tissue. A bipolar platinum electrode was used to record nerve activity. The electrode was secured to the nerve with use of dental impression material (Basilex). The multiunit nerve activity was first amplified and recorded with a 100-Hz to 3-kHz band pass and 60-Hz notch filter by a preamplifier (model P15, Grass, Warwick, RI). The signal was then further amplified (Gould Universal Amplifier) before being sent to an oscilloscope (BK Precision Instruments, Chicago, IL). In addition, the signal was sent to a microcomputer for display and analysis with use of the Integrated Program for Electrophysiological Experiments (IPEE) software program (ConradYim Software, Etobicoke, ON, Canada). Significant changes in nerve activity were quantified by the IPEE program as the number of spikes per bin (2 ms/bin width), which are 1 SD above baseline. Renal sympathetic activity was recorded for 200 s at 30 min before and immediately before the beginning of nerve stimulation and 30, 60, 90, and 120 min after the 2 h of nerve stimulation. Background noise levels were determined by the intravenous infusion of hexamethonium chloride (Sigma Chemical, St. Louis, MO; 2 mg/kg) at the conclusion of the experimental period. The residual signal was subtracted as noise in calculations of absolute values for sympathetic nerve activity.

Baroreflex activation and BRS plot. The cardiac baroreflex was evoked using bolus intravenous injections of increasing concentrations of the α1-adrenergic receptor agonist phenylephrine hydrochloride (PE; Sigma Chemical). The changes in blood pressure and heart rate in response to activation of the cardiac baroreflex with use of PE (0.025, 0.05, and 0.1 mg/kg) were monitored 30 min before and 30 and 120 min after termination of the nerve stimulation protocol.

The peak changes in mean arterial pressure and heart rate evoked by each concentration of PE were plotted against each other to provide an index of BRS. Regression analysis was carried out on 12 data points for each time period. Regression lines were obtained by the least-squares method, and the slopes of the lines before and after vagal stimulation were calculated. This plot of BRS was used to analyze changes in the slope (sensitivity) of the baroreflex function curves before and after nerve stimulation and microinjections of lidocaine or saline into the PBN. The determination of BRS after 2 h of complete cervical vagal stimulation, as well as the effect of
prior bilateral microinjection of lidocaine into the PBN, on the
bradycardia (21 ± 3 beats/min, P > 0.05) was observed (Fig. 1, A, B1, and B2). This enhanced PE-induced pressor response returned to prestimulated levels 2 h after the end of the stimulation (P > 0.05; Fig. 1, A, B1, and B2).

Renal nerve activity recovered to prestimulation values when measured 120 min after stimulation (16 ± 4 spikes/bin, P > 0.05; Fig. 1, C and D, Table 2).

When the changes in mean arterial pressure were plotted against the changes in heart rate in response to the concentrations of PE used to test the baroreflex, the slope of the regression line obtained 30 min after termination of the vagal stimulation was significantly decreased (0.30 ± 0.06 beats·min⁻¹·mmHg⁻¹, P < 0.05; Fig. 1E) compared with the slope of the regression line before stimulation (0.51 ± 0.01 beats·min⁻¹·mmHg⁻¹; Table 2). The slope of the BRS returned to prestimulated values after an additional 90 min (0.49 ± 0.01 beats·min⁻¹·mmHg⁻¹, P > 0.05; Table 2).

In animals receiving lidocaine microinjections (n = 4) into the PBN before cervical vagal stimulation, baroreflex testing 30 and 120 min after termination of the stimulation did not demonstrate a significant change in the pressor response (P > 0.05 for 30 and 120 min) or reflex bradycardia (P > 0.05 for both time points; Fig. 2, A, B1, and B2). Consequently, when the changes in mean arterial pressure were plotted against the changes in heart rate in response to the various concentrations of PE, the slope of the regression line obtained 30 min after termination of the cervical vagal stimulation was 0.49 ± 0.04 beats·min⁻¹·mmHg⁻¹, which was not significantly different from that obtained before the stimulation (0.47 ± 0.01 beats·min⁻¹·mmHg⁻¹; Fig. 2E). The slope of the BRS remained not significantly different from the prestimulated value for up to 120 min after stimulation (0.48 ± 0.01 beats·min⁻¹·mmHg⁻¹, P > 0.05). In addition, no significant changes in renal nerve activity were observed at any time after termination of the vagal stimulation (P > 0.05 for 30, 60, 90, and 120 min after stimulation; Fig. 2, C and D). The PE-induced changes in mean arterial pressure, the

RESULTS

In all animals receiving PE injections (0.025, 0.05, and 0.1 mg/kg) before nerve stimulation or sham stimulation (n = 40), the respective changes in mean arterial pressure, heart rate, and the slope of the BRS plot (average slope = 0.51 ± 0.03 beats·min⁻¹·mmHg⁻¹) were not significantly different from each other (P > 0.05 for each parameter). Bilateral microinjection of lidocaine (n = 20) or saline (n = 20) into the PBN before stimulation did not significantly alter baseline mean arterial pressure or heart rate (P > 0.05 for both). Furthermore, BRS and renal sympathetic nerve activity were unchanged for the duration of the experimen-
tal time course in all nonstimulated animals (n = 8, P > 0.05 for both measures at all time points; figure not shown). Mean arterial pressure and heart rate changes before, during, and after electrical stimulation of the complete cervical vagus (n = 8), ADN alone (n = 8), barodenervated cervical vagus nerve (n = 8), or abdom-
nal vagus nerve are shown in Table 1. Also, the 30-min poststimulation cardiovascular parameters were not significantly different from prestimulated values (Table

Table 1. MAP and HR before, during, and after 2 h of electrical stimulation of visceral afferent nerves

<table>
<thead>
<tr>
<th></th>
<th>Cervical Vagus Nerve</th>
<th>Barodenervated Cervical Vagus Nerve</th>
<th>Abdominal Vagus Nerve</th>
<th>Aortic Depressor Nerve</th>
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<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>30 min before</td>
<td>105 ± 16</td>
<td>104 ± 18</td>
<td>106 ± 18</td>
<td>105 ± 12</td>
</tr>
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<td>60 min during</td>
<td>68 ± 18*</td>
<td>86 ± 5*</td>
<td>110 ± 22</td>
<td>88 ± 4*</td>
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<tr>
<td>30 min after</td>
<td>99 ± 19</td>
<td>101 ± 21</td>
<td>104 ± 12</td>
<td>106 ± 10</td>
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<tr>
<td>HR, beats/min</td>
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<tr>
<td>30 min before</td>
<td>410 ± 22</td>
<td>408 ± 18</td>
<td>404 ± 19</td>
<td>399 ± 20</td>
</tr>
<tr>
<td>60 min during</td>
<td>288 ± 9*</td>
<td>388 ± 7*</td>
<td>410 ± 22</td>
<td>384 ± 8*</td>
</tr>
<tr>
<td>30 min after</td>
<td>408 ± 18</td>
<td>410 ± 11</td>
<td>406 ± 16</td>
<td>404 ± 12</td>
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</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate. *Significantly different from 30 min before.

The PE-induced changes in mean arterial pressure, the
reflex changes in heart rate, and the depressed slope of the BRS curves after electrical stimulation of the complete cervical vagus after lidocaine or saline microinjection into the PBN are similar to those previously observed (27).

ADN stimulation and parabrachial microinjections. In all animals receiving saline (n = 4) or lidocaine microinjections before ADN stimulation, baroreflex testing 30 and 120 min after termination of the stimulation did not result in any significant changes in the PE-induced pressor response (39 ± 7 and 40 ± 8 mmHg, respectively, P > 0.05 for both time points) or the reflex bradycardia (20 ± 4 and 19 ± 5 beats/min, respectively, P > 0.05 for both time points; figure not shown). The slopes of the regression lines (BRS) obtained 30 and 120 min after termination of the ADN stimulation were 0.50 ± 0.04 and 0.49 ± 0.05 beats·min⁻¹·mmHg⁻¹, respectively, which were not significantly different from that obtained before the stimulation (0.51 ± 0.01 beats·min⁻¹·mmHg⁻¹, P > 0.05 for both time points; Table 2). In addition, no significant changes in renal nerve activity were observed at any time after termination of the ADN stimulation (average activity 524 ± 5, 22 ± 7, 26 ± 7, and 24 ± 7 spikes/bin at 30, 60, 90, and 120 min after 2 h of cervical vagus nerve stimulation. E: change in slope of baroreflex function before and after nerve stimulation. Vertical and horizontal lines represent SE, and all regression lines had $r^2 > 0.9$ (n = 12). *Significantly different (P < 0.05) from prestimulated values. Each symbol represents an average value obtained from 4 animals.

Fig. 1. Effects of cervical vagus nerve stimulation and saline injection into parabrachial nucleus (PBN) on cardiovascular and sympathetic nerve responses. A: a representative arterial blood pressure (mmHg) and heart rate (beats/min) response from 1 animal after a bolus intravenous injection of phenylephrine (PE; 0.1 mg/kg) as indicated by vertical arrows. Cardiovascular responses to PE administration were taken from a continuous record from an experimental animal 30 min before and 30 and 120 min after bilateral microinjection of saline into PBN and cervical vagus nerve stimulation. B: average changes in mean arterial pressure (MAP) and heart rate (HR) in response to PE 30 min before and 30 and 120 min after termination of cervical vagus nerve stimulation. C: a representative sample of renal sympathetic nerve activity (RSNA, in spikes/bin, 2-s bin width) 30 min before and 30 and 120 min after cervical vagus nerve stimulation. D: average change in RSNA 30 min before and 30, 60, 90, and 120 min after 2 h of cervical vagus nerve stimulation. E: change in slope of baroreflex function before and after nerve stimulation.
Table 2. BRS and RSNA before and after 2 h of electrical stimulation of visceral afferent nerves

<table>
<thead>
<tr>
<th></th>
<th>Cervical Vagus Nerve</th>
<th>Barodenervated Cervical Vagus Nerve</th>
<th>Abdominal Vagus Nerve</th>
<th>Aortic Depressor Nerve</th>
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<tbody>
<tr>
<td>BRS, beats·min⁻¹·mmHg⁻¹</td>
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<tr>
<td>30 min before</td>
<td>0.51 ± 0.01</td>
<td>0.49 ± 0.01</td>
<td>0.49 ± 0.04</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>30 min after</td>
<td>0.30 ± 0.06*</td>
<td>0.31 ± 0.06*</td>
<td>0.29 ± 0.06*</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>120 min after</td>
<td>0.49 ± 0.01</td>
<td>0.41 ± 0.07</td>
<td>0.52 ± 0.05</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>RSNA, spikes/bin</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>30 min before</td>
<td>19 ± 5</td>
<td>17 ± 5</td>
<td>20 ± 5</td>
<td>24 ± 5</td>
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<td>30 min after</td>
<td>345 ± 23*</td>
<td>374 ± 24*</td>
<td>225 ± 19*</td>
<td>22 ± 7</td>
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<tr>
<td>120 min after</td>
<td>16 ± 4</td>
<td>22 ± 5</td>
<td>21 ± 5</td>
<td>24 ± 7</td>
</tr>
</tbody>
</table>

*Significantly different from 30 min before.

Values are means ± SE. BRS, baroreflex sensitivity; RSNA, renal sympathetic nerve activity (bin width = 2 s).

The 2 h of nerve stimulation in animals receiving a microinjection of saline (0.9%, 300 nl/side) into the PBN before nerve stimulation (n = 4), a significantly enhanced pressor response (80 ± 8 mmHg, P < 0.05) independent of a significant change in the reflex bradycardia (20 ± 4 beats/min, P > 0.05) was observed. The enhanced pressor response recovered when measured 120 min after nerve stimulation (P > 0.05), and the reflex bradycardia remained not significantly different from the prestimulated value (P > 0.05; Fig. 3, A, B1, and B2).

Recordings of renal nerve activity obtained 30 min (Fig. 3, C and D, Table 2) after termination of the stimulation were significantly enhanced (average activity = 374 ± 24 spikes/bin at 30 min after stimulation, P < 0.05) compared with prestimulated levels (17 ± 5 spikes/bin, Table 2). This increase in renal sympathetic nerve activity remained significantly elevated for the next 60 min after stimulation (369 ± 7 and 389 ± 7 spikes/bin at 60 and 90 min, P < 0.05 for both time points). Renal nerve activity recovered to prestimulation levels when measured 120 min after stimulation (22 ± 5 spikes/bin, P > 0.05; Fig. 3, C and D, Table 2).

When the changes in mean arterial pressure were plotted against the changes in heart rate in response to various concentrations of PE, the slope of the regression line obtained 30 min after termination of the vagal stimulation was significantly decreased (0.31 ± 0.06 beats·min⁻¹·mmHg⁻¹, P < 0.05) compared with the slope of the regression line before stimulation (0.49 ± 0.01 beats·min⁻¹·mmHg⁻¹; Fig. 3E, Table 2). The slope of the BRS returned to prestimulated values after an additional 90 min (0.41 ± 0.07 beats·min⁻¹·mmHg⁻¹, P > 0.05; Table 2).

In animals receiving lidocaine microinjections before stimulation of the barodenervated cervical vagus (n = 4), baroreflex testing 30 and 120 min after termination of the stimulation resulted in no significant change in the pressor response (35 ± 8 and 39 ± 7 mmHg, P > 0.05 for both time points) or reflex bradycardia (21 ± 4 and 23 ± 5 beats/min, P > 0.05 for both time points; Fig. 4, A, B1, and B2). The slope of the regression line (BRS) obtained 30 min after termination of the nerve stimulation was 0.54 ± 0.04 beats·min⁻¹·mmHg⁻¹, which was not significantly different from that obtained before the stimulation (0.50 ± 0.05 beats·min⁻¹·mmHg⁻¹, P > 0.05). Also, the slope of the BRS was not significantly different from the prestimulated value when measured at 120 min (0.49 ± 0.04 beats·min⁻¹·mmHg⁻¹, P > 0.05; Fig. 4E). In addition, no significant change in renal nerve activity was observed at any time after termination of the vagal stimulation (average activity = 23 ± 4, 27 ± 5, 22 ± 7, and 25 ± 6 spikes/bin at 30, 60, 90, and 120 min after stimulation, respectively, P > 0.05 for all time points; Fig. 4, C and D).

Abdominal vagus nerve stimulation and parabrachial microinjections. Intravenous injection of PE before saline microinjections into the PBN and abdominal vagal stimulation produced a dose-related increase in mean arterial pressure and a reflex decrease in heart rate (Fig. 5A). When the baroreflex was evoked 30 min after the 2 h of abdominal vagal stimulation, a significantly enhanced pressor response (81 ± 8 mmHg, P < 0.05) independent of a significant change in the reflex bradycardia (19 ± 4 beats/min, P > 0.05) was observed (Fig. 5, A, B1, and B2). This enhanced PE-induced pressor response returned to prestimulated levels 2 h after the end of the stimulation (38 ± 7 mmHg, P > 0.05). The reflex bradycardia remained not significantly different from the prestimulation value (22 ± 6, P > 0.05; Fig. 5, A, B1, and B2).

Renal nerve activity recorded 30 min (Fig. 5, C and D, Table 1) after termination of the abdominal vagal stimulation was significantly enhanced (average activity = 225 ± 19 spikes/bin at 30 min after stimulation, P < 0.05) compared with prestimulated levels (20 ± 5 spikes/bin). This increase in renal nerve activity remained significantly elevated for the next 60 min after stimulation (220 ± 18 and 228 ± 19 spikes/bin at 60 and 90 min, P < 0.05 for both time points). Renal nerve activity recovered to prestimulation values 120 min after stimulation (21 ± 5 spikes/bin, P > 0.05; Fig. 5, C and D, Table 2). The increase in renal nerve activity after abdominal vagus nerve stimulation was significantly less than the increase observed at 30 min after cervical vagus nerve and barodenervated cervical vagus nerve stimulation (P < 0.05 compared with both nerve stimulation paradigms).

When the changes in mean arterial pressure were plotted against the changes in heart rate in response to various concentrations of PE, the slope of the regression line obtained 30 min after termination of the abdominal vagal stimulation was significantly decreased (0.29 ± 0.06 beats·min⁻¹·mmHg⁻¹, P < 0.05; Fig. 5E) compared with the slope of the regression line before stimulation (0.49 ± 0.04 beats·min⁻¹·mmHg⁻¹; Fig. 3E, Table 2). The slope of the BRS returned to approximately prestimulated values after an additional 90 min (0.52 ± 0.05 beats·min⁻¹·mmHg⁻¹, P > 0.05; Table 2).

In animals receiving lidocaine microinjections before abdominal vagal stimulation (n = 4), baroreflex testing 30 and 120 min after termination of the stimulation was 0.54 ± 0.04 beats·min⁻¹·mmHg⁻¹, which was not significantly different from that obtained before the stimulation (0.50 ± 0.05 beats·min⁻¹·mmHg⁻¹, P > 0.05). Also, the slope of the BRS was not significantly different from the prestimulated value when measured at 120 min (0.49 ± 0.04 beats·min⁻¹·mmHg⁻¹, P > 0.05; Fig. 4E). In addition, no significant change in renal nerve activity was observed at any time after termination of the vagal stimulation (average activity = 23 ± 4, 27 ± 5, 22 ± 7, and 25 ± 6 spikes/bin at 30, 60, 90, and 120 min after stimulation, respectively, P > 0.05 for all time points; Fig. 4, C and D).
indicated no significant changes in the pressor response (36 ± 6 and 35 ± 7 mmHg, respectively, P > 0.05) or reflex bradycardia (20 ± 5 and 22 ± 6 beats/min, respectively, P > 0.05; Fig. 6, A, B1, and B2). The slope of the regression line (BRS) obtained 30 min after termination of the abdominal vagal stimulation was 0.49 ± 0.04 beats·min⁻¹·mmHg⁻¹, which was not significantly different from that obtained before the stimulation (0.51 ± 0.05 beats·min⁻¹·mmHg⁻¹, P > 0.05; Fig. 6E). The slope of the BRS remained unchanged compared with the prestimulated value for up to 120 min after stimulation (0.50 ± 0.01 beats·min⁻¹·mmHg⁻¹, P > 0.05). In addition, no significant changes in renal nerve activity were observed at any time after termination of the abdominal vagal stimulation (average activity = 13 ± 6, 19 ± 7, 15 ± 7, and 18 ± 6 spikes/bin at 30, 60, 90, and 120 min, respectively, P > 0.05 for all time points; Fig. 6, C and D).

Histological verification of cannula placement. Figure 7 is a composite diagram indicating the microinjection sites of lidocaine (n = 20) and saline (n = 20) in the PBN. For clarity, only unilateral sites are shown; however, only animals that received accurate bilateral injections were used in this investigation. The lidocaine injection sites of those animals where the tip of the microinjector was located outside the PBN (n = 10; Fig. 7) are also included; however, the data were not used in this study. The results from such animals showed that the lidocaine injections outside the PBN (~1–1.5 mm from the PBN border) did not alter the nerve stimulation-induced changes in BRS or renal nerve activity (data not shown).
DISCUSSION

This study demonstrated that prolonged activation of afferents contained within the cervical vagus, barodenervated cervical vagus, and abdominal vagus nerves produced a decrease in BRS and an increase in renal sympathetic nerve activity. The observed effects on these vagus nerves were only observed after increases in blood pressure. However, the selective activation of baroreceptor afferents contained within the ADN did not significantly affect sympathetic tone. Activation of the ADN in conjunction with the other afferents within the cervical vagus resulted in a time-dependent decline in the magnitude of the enhanced sympathetic response (Fig. 1D). Such a time-dependent decrease in the sympathetic system output was not observed when the ADN was excluded from the stimulation paradigm (barodenervated vagus; Fig. 2D). This enhanced level of sympathetic activity was, in all cases, correlated to an increase in the PE-induced pressor response and, consequently, a depressed BRS. Furthermore, this investigation has provided evidence demonstrating that the PBN mediated the enhanced sympathetic tone and depressed BRS, inasmuch as reversible PBN lesions completely abolished both of these visceral afferent stimulation-induced effects.

The fact that lidocaine microinjected into the PBN before stimulation of the entire cervical vagus (including ADN) was capable of blocking the altered BRS and sympathetic outflow is consistent with a previous report (27). However, because catecholamine turnover studies were not performed in the previous investigations where an increase in plasma norepinephrine was
measured after a cardiac pathology, a change in sympathetic tone could only be inferred (9, 27, 29, 31). The possibility does exist, however, that the pattern of enhanced renal nerve activity observed after visceral afferent stimulation might have represented a tonic rather than a cardiac cycle-related change. However, examination of poststimulation renal nerve recordings showed that the changes in sympathetic tone remained entrained to the cardiac cycle (6–8 Hz).

Another point of interest was the fact that a significant increase in renal nerve activity observed after termination of the vagal stimulation was unaccompanied by significant changes in blood pressure. It is quite possible that our vagal stimulation, in addition to causing sympathoexcitation, also caused the release of neurohumoral factors into the circulation. Hartikainen and colleagues (13) noted an increase in plasma norepinephrine, renin activity, atrial natriuretic peptide, and endothelin-1 after myocardial infarction in humans. Again, no significant change in blood pressure or heart rate of these subjects was observed after the infarction had occurred (13). These authors noted that only the increase in plasma norepinephrine was correlated with the depressed BRS. Because we did not measure any neurohumoral levels in this study, we cannot rule out their participation in blood pressure regulation after vagal stimulation or in attenuating BRS in our model. However, the fact that the attenuated BRS and sympathoexcitation were blocked after the microinjection of lidocaine into the PBN suggests that if these humoral factors are released in our model, they likely act through the PBN to mediate their effects.
The present investigation demonstrates that selective ADN stimulation does not significantly contribute to the enhanced sympathetic tone or the depressed BRS observed after complete cervical vagus nerve stimulation. The lack of significant changes in autonomic tone observed after selective stimulation of the ADN is supported by results from other laboratories. For example, it has been demonstrated that, after experimentally induced coronary artery occlusion in the dog model of myocardial infarction, plasma catecholamine levels were significantly elevated and BRS was significantly depressed (9, 31). Subsequently, it was demonstrated that vagotomy prevented these changes while selective barodenervation was without effect. It was therefore suggested that the contribution of afferents within the ADN to altered plasma norepinephrine levels and BRS in this cardiac pathology was, at best, minimal (9). Interestingly, changes in BRS particularly during or shortly after diagnosis of coronary atherosclerosis would implicate the aortic baroreceptors in mediating the central component of this effect. However, an investigation into the changes in the function of isolated aortic mechanoreceptors in vitro found that changes in BRS were not reversed after restoration of vascular distensibility (6). These authors concluded that other independent factors, such as a central defect in modulating the baroreflex or the release of several neurohumoral substances into the circulation, must have mediated the effect. Further evidence to rule out the contribution of baroreceptors in mediating changes in BRS comes from studies which showed that a reduction in arterial blood pressure with antihyperten-
sive therapy (which unloads baroreceptors and therefore decreases ADN activity) was not effective in blocking the depressed BRS observed in elderly hypertensive patients (10). All these lines of evidence suggest that an increase in baroreceptor afferent activity is not involved in mediating autonomic changes that result in a decreased BRS. The present results, where sympathetic tone and BRS were also unchanged after selective ADN activation, confirm the validity of our experimental model in causing autonomic and cardiovascular changes similar to those observed after several cardiac pathologies.

Of particular interest was the finding that, after stimulation of the abdominal vagus nerve only, a significant increase in sympathetic tone and decreased BRS were observed. The increase in renal sympathetic nerve activity was, however, significantly less than that observed after cervical vagus nerve or barodenervated cervical vagus nerve stimulation. Although the abdominal vagus-induced increase in sympathetic tone was significantly smaller, activation of general gastric afferents produced a decrease in BRS comparable to that observed after cervical vagus nerve or barodenervated cervical vagus nerve stimulation. Gieroba and Blessing (11), using parameters for abdominal vagal afferent stimulation similar to that used in the current study, demonstrated that neurons in cardiovascular and autonomic nuclei, such as the nucleus of the solitary tract, lateral PBN, and rostral ventrolateral medulla, were strongly excited. Interestingly, the abdominal vagus-induced excitation of the neurons in the rostral ventrolateral medulla was reduced by a preceding stimulation.
of ADN afferents (12). In the present study, cervical vagal stimulation, including the ADN, activated cardiopulmonary and general gastric afferents and resulted in a larger increase in sympathetic tone than stimulation of the abdominal vagus nerve alone. However, the magnitude of the increased sympathetic tone significantly decreased at each successive time point, whereas in the two stimulation paradigms where the ADN was not stimulated (barodenervated and abdominal vagus) the renal sympathetic nerve activity remained significantly elevated for an extended period of time. This might be explained via a mechanism similar to that described by Gieroba and colleagues (12) whereby tonic or stimulation-induced increases in ADN afferent activity would attenuate the central processing of general gastric afferents, thus modifying any changes in sympathetic tone. It is reasonable to speculate that tonic cardiovascular information originating from the myocardium has some degree of “priority” over afferent information from the gastrointestinal tract, and thus one function of ADN afferents would be to influence the abdominal vagus-induced excitability of the rostral ventrolateral medulla and subsequent sympathetic output. This postulation may underlie the mechanism responsible for gastric afferent activation-induced cardiac instability and even sudden cardiac death, which may occur after abdominal surgery or in cases of functional bowel disorders and visceral pain (20, 21, 33). Direct stimulation of the abdominal vagus nerve may mimic the output of such pathologies and thereby override the protective effects of ADN afferent activity, resulting in a depressed BRS.

Our results provide direct evidence that the PBN is involved in mediating the central connectivity between particular vagal afferents and the sympathetic preganglionic cell group responsible for the altered sympathetic output. Lidocaine, which reversibly interrupts neurotransmission, when microinjected into the PBN did not alter baseline cardiovascular parameters but did completely block the enhanced sympathetic tone observed after cervical and abdominal vagus nerve stimulation. This is consistent with our previous findings on the role of the PBN in mediating the increase in plasma norepinephrine levels and decrease in the sensitivity of the baroreflex after cervical vagal stimulation (27). Previous evidence also demonstrated that stimulation of the cervical and abdominal vagus nerves resulted in significant neurochemical changes in the PBN (25). These neurochemicals in the PBN were shown to significantly modulate glutamatergic transmission between the nucleus of the solitary tract and the forebrain (24). In addition, significant differences in monoamine levels were observed in the PBN of the cardiomyopathic hamster compared with two strains of control hamsters (2). These results suggest that the PBN, as part of the central autonomic network, has a major role in the integration of ascending visceral information before transmission to other forebrain autonomic nuclei. Finally, this study demonstrates that, in addition to cervical vagal stimulation-induced autonomic changes, the PBN mediates the enhanced sympathetic response after general gastric afferent activation.

Perspectives
Testing of the cardiac baroreflex has gained acceptance as a diagnostic tool in the clinical assessment of cardiovascular disease (22, 28). Alterations in BRS have proven to be reliable indicators of an individual’s susceptibility to cardiovascular pathologies such as hypertension, congestive heart failure, and myocardial
infarction (1, 8, 13, 14). Measurement of BRS in patients after their first myocardial infarction revealed a decreased BRS and an increased sympathetic tone (22, 29, 32). Consequently, these individuals were shown to have a significantly higher risk (~70%) for a second cardiovascular accident or the onset of cardiac arrhythmias, which might result in sudden cardiac death (18, 30, 31).

Alterations in autonomic tone play an important role in the onset of sudden cardiac death, after and independent of cardiovascular disease (18). In particular, ventricular arrhythmias, resulting from sympathetic hyperactivity in the absence of sufficient vagal efferent tone, have been implicated as the causal factor in many cases of sudden cardiac death (3, 8, 29). The results presented here suggest that an increase in cardiac, pulmonary, or general gastric afferent activity to the central nervous system can evoke such autonomic imbalance. Further identification of the selective afferents within these nerves (e.g., atrial or pulmonary stretch, gastric distention) may increase our understanding of the role of these afferents in cardiovascular pathology-induced sympathoexcitation and arrhythmogenesis.

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