Differential selectivity of cardiac neurons in separate intrathoracic autonomic ganglia

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Departments of 1Physiology and Biophysics and 2Physics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7; and 3Department of Physiology, University of South Alabama, Mobile, Alabama 36688

Armour, J. A., K. Collier, G. Kember, and J. L. Ardell. Differential selectivity of cardiac neurons in separate intrathoracic autonomic ganglia. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R939–R949, 1998.—Analyses of activity generated by neurons in middle cervical or stellate ganglia versus intrinsic cardiac ganglia were performed to determine how neurons in different intrathoracic ganglia, which are involved in cardiac regulation, interact. Discharges of 19% of intrathoracic extracardiac neurons and 32% of intrinsic cardiac neurons were related to cardiodynamics. Epicardial touch increased the activity generated by ~80% of intrinsic cardiac neurons and ~60% of extracardiac neurons. Both populations responded similarly to epicardial chemical stimuli. Activity generated by neurons in intrinsic cardiac ganglia demonstrated no consistent short-term relationships to neurons in extracardiac ganglia. Myocardial ischemia influenced extracardiac and intrinsic cardiac neurons similarly. Carotid artery baroreceptors influenced neurons in ipsilateral extracardiac ganglia. After decentralization from the central nervous system, intrinsic cardiac neurons received afferent inputs primarily from cardiac chemosensitive neurites, whereas middle cervical ganglion neurons received afferent inputs primarily from cardiac mechanosensory neurites. It is concluded that the populations of neurons in different intrathoracic ganglia can display differential reflex control of cardiac function. Their redundancy in function and noncoupled behavior minimizes cardiac dependency on a single population of intrathoracic neurons.

A population of intrinsic cardiac neurons, the parasympathetic postganglionic ones (3, 9, 12), receives direct inputs from medullary parasympathetic preganglionic neurons (10, 13). Another population of neurons within intrathoracic ganglia, including those on the heart, receives inputs from spinal cord sympathetic preganglionic neurons (5, 6, 10, 13). That cardiopulmonary sensory neurites can modify the activity generated by neurons in intrathoracic ganglia, including those on the heart, after chronic decentralization of such ganglia from the central nervous system has been interpreted as indicating that afferent neurons are present in intrathoracic ganglia (2, 7). Furthermore, it has been proposed that intrathoracic ganglia contain local circuit neurons (5, 6, 10, 13). Thus intrathoracic ganglia, including those within the heart, contain afferent neurons, local circuit neurons, and sympathetic efferent postganglionic neurons in addition to those parasympathetic efferent postganglionic neurons that are associated with the heart (1, 8).

The classical view of peripheral intrathoracic autonomic ganglia proposes that their synaptic junctions represent relay stations from the central nervous system to the end effectors on the heart, with little or no integrative capabilities. Recent data, including the identification of the multiple neuronal subtypes within the intrathoracic ganglia described above (1, 8), suggest instead that complex neuronal interactions can occur within the intrathoracic nervous system, which potentially may be critical for the maintenance of regional cardiac function (1, 8). Yet how the activity generated by various peripheral autonomic neurons is coordinated within and between separate intrathoracic ganglia remains a major unanswered question.

Coherence of activity has been shown to occur with respect to medullary and spinal cord neurons involved in cardiac regulation (14). Consistent coherence between neurons is indicative of principal and direct synaptic interconnections between those neurons or, conversely, the sharing by such neurons of common activation inputs. Whether intrathoracic neurons involved in cardiac regulation display coherence of their activity remains to be established. It also remains to be determined whether neurons in different intrathoracic ganglia involved in cardiovascular regulation receive similar types of cardiac afferent inputs. Furthermore, it is not known whether extrathoracic vascular mechanosensory axons, such as those in the carotid sinus, can selectively influence different populations of intrathoracic autonomic neurons involved in cardiac regulation. The present series of experiments were devised to evaluate how neurons in various intrathoracic ganglia responded to specific cardiovascular perturbations. This was done to determine how afferent information arising from cardiac and extracardiac sensory neurites is processed by various populations of intrathoracic neurons. The present study also investigated how various populations of intrathoracic neurons respond to transient coronary artery occlusion and the response characteristics of ischemia-sensitive neurons to discrete cardiac mechanical or chemical stimuli. The present experiments sought to determine how inputs from specific cardiovascular sensory neurites affect different populations of intrathoracic neurons when the intrathoracic nervous system is disconnected from the spinal cord and brain stem. Evaluation of the degree of short-term coordination between extracardiac and intrinsic cardiac neurons was performed by coherence analysis. In this manner we sought to characterize the putative inputs to and interactions among different populations of intrathoracic neurons involved in cardiac regulation.
MATERIALS AND METHODS

Adult mongrel dogs (n = 32) of either sex, weighing between 16 and 21 kg, were used in this study. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205] and were approved by the institutional animal care and use committees of Dalhousie University and the University of South Alabama.

General method. Canines were tranquilized with thiopental sodium (15–20 mg/kg iv) and then anesthetized with thiopental sodium (5 mg/kg iv to effect every 5–10 min for the duration of the surgical procedures). Noxious stimuli were applied to a paw throughout the experiments to ascertain the adequacy of the anesthesia. After anesthesia induction, the animal was intubated and positive-pressure ventilation was initiated and maintained with a Bird Mark 7A ventilator adequacy of the anesthesia. After anesthesia induction, the animal was intubated and positive-pressure ventilation was initiated and maintained with a Bird Mark 7A ventilator.

The activity generated by neurons in separate pairs of intrathoracic ganglia was studied in each animal. In all animals (n = 32) the activity generated by neurons in the ventral intraventricular ganglionated plexus was recorded concurrently with activity generated by neurons in either the left middle cervical (n = 24 dogs) or left stellate (n = 8 dogs) ganglion. For recording of neuronal activity from stellate or middle cervical ganglia, a tungsten microelectrode was inserted into either ganglion as described by us elsewhere (5, 6). The recording microelectrode had a 10-µm diameter, an exposed tip of 50 µm, and an impedance of 9–11 MW at 1,000 Hz. For recording of neuronal activity from the ventricular neurons described above (n = 32; i.e., all animals), the ventral pericardium was incised and retracted laterally to expose the fat on the ventral surface of the ventricles that contains the ventral septal component of the cranial medially ventricular ganglionated plexus (19). A circular ring of heavy-gauge wire was gently placed on the surface of this epicardial fat located on the ventral surface of the ventral intraventricular groove to minimize epicardial motion. This fat was explored with another tungsten microelectrode mounted on a micromanipulator at depths ranging from the surface of the fat to regions adjacent to cardiac musculature (10, 13). Proximity to cardiac musculature was indicated by increases in the amplitude of the ECG artifact. The indifferent electrodes were attached to structures adjacent to investigated ganglia. In this manner, activity generated simultaneously by neurons in intrathoracic intrinsic and extrinsic cardiac ganglia was recorded.

Signals from the extracardiac and intracardiac intrathoracic neurons were differentially amplified by separate Prince- ton Research model 115 amplifiers that had bandpass filters set at 300 Hz–10 kHz and amplification ranges of 100–500X. The output of these devices, further amplified (50–200X) and filtered (band width 100 Hz–2 kHz) by means of optically isolated amplifiers (Applied Microelectronics Instrument, Halifax, NS, Canada), were led to a Nicolet model 207 oscilloscope and to a Grass AM8 audio monitor. Activity generated by individual neurons was identified by the amplitude and shape of recorded action potentials. Separate loci in extracardiac and intracardiac intrathoracic ganglia were identified from which action potentials with signal-to-noise ratios >3:1 were recorded, individual units being identified by the amplitude and configuration of their action potentials. With the use of these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage, but rather records action potentials generated by cell bodies and/or dendrites (10, 13). We did not attempt to activate intrathoracic neurons by means of electrical stimuli applied to the nerves associated with the ganglia investigated, because such stimuli induce long-term changes in the activity patterns generated by such neurons (5, 6, 13).

Interventions. To determine if altered respiratory mechanics affected neuronal activity, positive-pressure respiration was discontinued for 30 s. Five minutes after resumption of positive-pressure respiration, pulmonary inflation pressure was elevated (e.g., from 10 to 25 mmHg) for brief periods of time and then respiratory rate was altered, being reduced and then increased for 60 s. To determine if altered cardiovascular states affected intrathoracic neuronal activity, the inferior vena cava and then the descending thoracic aorta were partially occluded individually for 5–10 s. Isoproterenol (0.2 µg/kg iv) was then administered as a bolus, and, after the preparation had returned to a basal state, dobutamine (0.25 µg/kg iv) was administered as a bolus. Each of the above interventions was repeated at least once.

Mechanosensitive neurites in various ventricular epicardial loci were activated by touching the ventricular epicardium with a saline-soaked cotton swab. Once the extent of the ventricular epicardial region associated with an identified sensory field was determined, chemicals were applied to it for 60–100 s using 1 × 1-cm gauze squares soaked with 0.5 ml of each respective chemical. Sensory fields were washed for 30 s with normal saline (~2 ml/s) after each chemical was removed, with at least 5 min elapsing before the
next intervention. The following chemicals were applied to identified epicardial sensory fields: veratridine (5 × 10− 6 g), substance P (1 µM), adenosine (1 µM), and ATP (1 µM). The order of their application varied among experiments. Chemicals that initially induced neuronal responses were reapplied to the same epicardial locus at least twice to verify reproducibility of induced responses. Gauze squares soaked with room-temperature normal saline were also applied to identified epicardial sensory fields to determine whether neuronal responses elicited by epicardial chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

After the interventions described above were completed, the left ventral descending coronary artery was occluded for 1–2 min by means of a silk ligature snare placed around the artery ~1 cm from its origin. Ten minutes after this intervention was discontinued, the circumflex coronary artery was occluded for 1–2 min. When these interventions had been completed, the cervical vagosympathetic complexes in the neck were severed. All of the various interventions described above that had elicited responses were then repeated. Subsequently, all connections between stellate ganglia and the spinal cord were cut, thereby decentralizing the intrathoracic autonomic ganglia from the central nervous system. All of the interventions described immediately above were repeated at a final time. The order of neuronal decentralization (severing of the vagi or stellate ganglion decentralization) was randomized among the animals.

Data acquisition and analysis. The activity generated by intrinsic cardiac and extracardiac intrathoracic neurons was recorded simultaneously along with a lead II ECG, left atrial and left ventricular chamber pressures, right and left ventricular intramyocardial pressures, and aortic pressure using an Astro-Med model MT 9500 eight-channel rectilinear recorder (Astro-Med, West Warwick, RI). Data were stored on VHS tape (T120 Scotch, 3M Canada, London, Ontario) using a videocassette recorder (A. R. Vetter, model 820, Rebersburg, PA) for later analysis.

Cardiac indexes derived from 10 consecutive cardiac cycles were analyzed before and during peak responses elicited by each intervention. The means (± SE) of these cardiac indexes were calculated from these data. Spontaneous fluctuations of cardiodynamics were minimal during control periods, heart rate varying <5 beats/min and systolic pressure fluctuating <5 mmHg. Thresholds for classifying induced cardiovascular changes were chosen to be greater than these ranges. Action potentials generated at a given locus were counted for 30-s periods to establish average activity immediately before and during maximal responses elicited by each intervention. If required, analysis of activity generated by two or more individual units was performed by means of a window discriminator (Hartley Instrumentation Development Laboratories, Baylor College of Medicine, Houston, TX).

Fluctuations in the amplitude of action potentials generated by a unit varied by <10 µV over several minutes; action potentials retained the same configurations over time. Thus action potentials recorded in a given locus with the same configuration and amplitude (<± 10 µV) were considered to be generated by a single unit. Action potentials with signal-to-noise ratios > 3:1 were analyzed. The threshold for neuronal activity changes was taken as a change of >20% from baseline values. Neuronal activity responses elicited by each intervention were evaluated by comparing activity generated immediately before each intervention with data obtained at the point of maximum change during the intervention. Data were expressed as means ± SE. One-way analysis of variance and paired t-test with Bonferroni correction for multiple tests were used for statistical analysis. A significance value of P < 0.05 was used for these determinations. Coherence of activity generated by neurons in different ganglia was determined as a cross-correlation of the mean corrected wave forms recorded from the two different populations of intrathoracic neurons (Acknowledgment III for the MP100WS by Biopac Systems, Goleta, CA). The coherence of neuronal activity recorded from two ganglia simultaneously was evaluated over 1– to 5-min periods. A continuous analysis of heart rate and peak left ventricular systolic pressure was likewise performed over the same time periods to compare the relative alterations in activity generated by two populations of intrathoracic neurons with concomitant changes induced in cardiac indexes.

RESULTS

Spontaneous activity generated by neurons in different ganglia. Nineteen percent of spontaneously active neurons in extracardiac intrathoracic ganglia and 32% of those in intrinsic cardiac ganglia displayed activity that occurred preferentially during specific phases of the cardiac cycle. Other intrathoracic neurons generated respiratory-related activity (14% of identified extracardiac and 8% of identified intrinsic cardiac neurons), with the remaining neurons generating sporadic activity.

Coherence (cross correlation) was evaluated for the activity generated by neurons in different (extracardiac versus middle cervical or stellate) ganglia to determine possible interactions that might occur between neurons therein. When coherence was employed to compare the timing of activity generated by neurons in two ganglia, no consistent short-term (ms) relationship of extracardiac to intracardiac neuronal activity was identified (Figs. 1B and 2). This included those occasions when bursting of activity occurred after epicardial application of a chemical (Fig. 1) or after systemic administration of a positive inotropic agent. Sometimes bursts of activity with periodicities of 10–30 s were generated by neurons in both studied ganglia. The bursts of activity generated by the separate populations of neurons were usually out of phase with one another, not phase shifted (Figs. 1 and 2). Although coherence analysis did identify isolated instances of correlated activity (correlation coefficient more than ±0.5), overall activity patterns exhibited minimal correlation, as demonstrated by correlation coefficients of less than ±0.3, even when applied to data recorded continuously for up to 5 min (Fig. 1). Thus there were no consistent short-term interactions between extrinsic and intrinsic cardiac neurons overall as assessed by coherence analysis, even when evaluating neuronal activity that displayed cardiovascular or respiratory-related activity.

Spontaneous activity generated by intrathoracic neurons changed after acute decentralization of the intrathoracic nervous system. In some instances, spontaneous activity was suppressed (Fig. 3A), whereas in other instances it increased (Fig. 3B) after acute decentralization. Thus overall there was no significant change in basal activity generated by intrathoracic neurons after decentralization of the intrathoracic nervous system in...
nonstimulated states. Moreover, cardiac and respiratory-related activity continued to be generated by a subpopulation of intrathoracic neurons after acute decentralization, as were slow cyclic bursts of neuronal activity (Fig. 3).

Carotid sinus stimulation. When mechanical stimuli were applied to the ipsilateral carotid bulb, neurons in the middle cervical ganglia were activated (16 ± 3 to 28 ± 6 impulses/min; P < 0.001) in 16 of 24 dogs, whereas those in stellate ganglia were activated (33 ± 9 to 45 ± 15 impulses/min; P < 0.05) in 6 of 8 dogs. In contrast, touching a carotid bulb activated intrinsic cardiac neurons (12 ± 2 to 35 ± 8 impulses/min) in only 5 of 32 dogs studied; thus the effects of this intervention on identified intrinsic cardiac neurons were insignificant overall. In a few instances, touching a carotid bulb activated previously inactive intrinsic cardiac neurons while concurrently suppressing the ongoing activity generated by neurons in an extracardiac ganglion (Fig. 4B). Investigated intrathoracic neurons were not affected when the contralateral carotid bulb or the right and left carotid artery below the carotid bulb were lightly touched. Acute decentralization of the ipsilateral thoracic sympathetic, not parasympathetic, nervous system eliminated intrathoracic neuronal responses elicited by touching the ipsilateral carotid bulb.

Bilateral carotid artery occlusion, proximal to the carotid sinus, reflexly increased left (106 ± 6 to 126 ± 8 mmHg; P < 0.001), but not right (28 ± 3 to 30 ± 4 mmHg), ventricular intramycardial systolic pressures. Left ventricular chamber systolic pressure (126 ± 5 to 135 ± 7 mmHg; P < 0.001) also increased during bilateral carotid occlusion, whereas heart rate and left atrial systolic pressure did not. This intervention simultaneously increased activity generated by middle cervical (13 ± 3 to 30 ± 6 impulses/min; P < 0.002) and intrinsic cardiac (19 ± 4 to 55 ± 19 impulses/min; P < 0.04) neurons in 11 animals, suppressing neuronal activity in 8 other animals (middle cervical ganglion neurons: 54 ± 18 to 41 ± 12 impulses/min, P < 0.02; intrinsic cardiac neurons: 44 ± 11 to 14 ± 13 impulses/min, P < 0.01). In the remaining five animals, intrinsic cardiac ganglion and middle cervical ganglion neuronal activity was not affected by this intervention.

Epicardial mechanical stimuli. Gentle mechanical distortion of epicardial sensory fields modified the activity generated by neurons in middle cervical and intrinsic cardiac ganglia, but not stellate ganglia overall (Tables 1 and 2). Repeated mechanical stimuli induced rapid neuronal responses, which waned quickly after the stimulus was removed (Fig. 4A). Most identified epicardial afferent neurites were located on the ventral and lateral surfaces of the left ventricle (cranial 2/3) and the right ventricular conus, with lesser numbers being located on the right ventricular sinus. A subpopulation of investigated neurons was also activated when sensory neurites on the thoracic aorta (4

Fig. 1. Cardiovascular and neuronal variables recorded before and after application of veratridine to a locus on the right ventricular sinus epicardium (applied between arrows). A: bradycardia and a fall in left ventricular systolic pressure (LVP; top) were induced initially by this intervention. Activity generated by intrinsic cardiac neurons (middle) increased, whereas that generated by left middle cervical ganglion neurons (LMCG; bottom) decreased immediately after veratridine application. About 75 s after veratridine application, the activity generated by both populations of neurons started to increase above baseline levels. At that time bursting of activity occurred about every 25 s in each population of neurons, activity being generated in a reciprocal fashion by these two neuronal populations. B: cross-correlation of the activity generated by these 2 populations of neurons derived from neuronal data obtained over the entire recording period. Most of the time, no tight correlation of activity generated by these 2 populations of neurons occurred (correlation coefficients of less than ±0.3), with occasional and fortuitous relationships occurring randomly when correlation coefficients transiently exceeded ±0.5.
dogs) or inferior vena cava (8 dogs) were touched. Interrupting the connections between the central nervous system and intrathoracic ganglia modified the responsiveness of intrathoracic neurons to local mechanical stimuli. For instance, after acute decentralization of the intrathoracic nervous system epicardial touch still increased the activity generated by neurons in middle cervical ganglia but not the activity generated by intrinsic cardiac neurons (Fig. 5).

Altered cardiovascular dynamics. Modification of cardiac preload and afterload alters systemic and cardiac hemodynamics. When the inferior vena cava was tem-

Fig. 2. A: continuous record (250 s) of ongoing heart rate and LVP, as well as the activity generated by intrinsic cardiac and middle cervical (MCG) neurons (data derived from Fig. 1). These indexes changed when veratridine was placed on a right ventricular epicardial locus (between arrows above MCG neuronal activity tracing). B: activity generated by each population of neurons during two 60-s periods was subjected to cross-correlation analysis (as indicated by horizontal lines below the MCG neuronal trace): i) during the control state and ii) once the veratridine-activated state had fully developed. Minimal coherence between the activity generated by these 2 populations of neurons was detected either during control or chemically stimulated states. Note that, whereas epicardial application of veratridine resulted in enhanced activity generated by both populations of neurons, such activity was out of phase with one another and, as such, failed to show consistent short-term correlation as evaluated by coherence analysis. bpm, Beats/min.

Fig. 3. Effects of severing the cervical vagosympathetic complexes on activity generated by neurons in ventral intraventricular ganglionated plexus (intrinsic) and LMCG for 2 representative animals. For one animal (A), severing the right (first arrowhead) and left (second arrowhead) cervical vagosympathetic complexes resulted in cessation of activity generated by neurons in intrinsic and LMCG. In another animal (B), when the cervical vagosympathetic complexes were severed, activity generated by neurons in intrinsic cardiac and MCG increased. For the animal depicted in B, note the cyclic nature of the activity patterns (~0.1 Hz) generated by neurons in both intrathoracic ganglia after severing the cervical vagosympathetic complexes. For both animals, LVP increased in a transient fashion after cervical vagosympathetic transection. Calibration bars: vertical bar beside LVP trace = 100 mmHg; vertical bar beside neuronal recordings = 0.5 mV. Horizontal bar in B = 10 s.
porarily occluded, thereby reducing venous return, right and left ventricular systolic pressures decreased. The activity generated by ventral intraventricular neurons increased in 13 of the 24 animals (24 ± 4 to 37 ± 11 impulses/min) and decreased in 2 of these animals as a consequence of inferior vena cava occlusion. Intrinsic cardiac neuronal activity changes were frequently most pronounced immediately after this occlusion terminated (Fig. 6). The activity generated by middle cervical ganglion neurons changed in 14 of 24 dogs when the inferior vena cava was occluded. When the descending aorta was partially occluded, left ventricular intramyocardial and chamber systolic pressures increased (Table 2). This intervention activated most intrinsic cardiac neurons (Table 2). The activity generated by middle cervical ganglion neurons was either enhanced (n = 13 dogs; 29 ± 7 to 83 ± 23 impulses/min, P < 0.1) or depressed (n = 11 dogs; 26 ± 2 to 7 ± 2 impulses/min; P < 0.1), depending on the population studied. In intact preparations, stellate ganglion neuronal activity was reduced during partial occlusion of the aorta (Table 2). Modification of cardiac preload and afterload a second time induced neuronal and cardiovascular responses, that were similar to those induced the first time. After acute decentralization of the intrathoracic nervous system, intrinsic cardiac but not extracardiac neurons were still responsive to the partial occlusion of the inferior vena cava or descending aorta (Table 2).

The augmentation in cardiac function induced by isoproterenol was accompanied by increases in the activity generated by intrinsic cardiac and middle cervical ganglion neurons, but not by stellate ganglion neurons (Tables 1 and 2). In contrast, dobutamine increased cardiac indexes as well as the activity generated by intrinsic cardiac neurons but not extracardiac neurons (Tables 1 and 2). Redadministration of these positive inotropic agents in the intact state consistently induced similar neuronal and cardiovascular responses. The enhancement in regional cardiac function induced by isoproterenol and dobutamine was minimally affected by decentralization of the intrathoracic nervous system; likewise, augmentation in intrinsic cardiac neuron activity was maintained (Fig. 5, Table 2). In contrast, middle cervical ganglion neurons were virtually unresponsive to isoproterenol after acute decentralization of the intrathoracic nervous system (Fig. 5).

Epicardial chemical stimuli. Neuronal responses initiated by epicardial chemical stimuli took time to develop and lasted, on average, 2.8 min after removal of the chemical. Although cardiovascular variables were

Table 1. Responses elicited by various interventions on cardiovascular variables and spontaneously activity generated by intrinsic cardiac and MCG neurons of 24 dogs

<table>
<thead>
<tr>
<th>Intervention</th>
<th>HR, beats/min</th>
<th>LAP, mmHg</th>
<th>RV IMP, mmHg</th>
<th>LV IMP, mmHg</th>
<th>LVP, mmHg</th>
<th>MCG Activity, impulses/min</th>
<th>Intrinsic Activity, impulses/min</th>
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<tbody>
<tr>
<td>Control</td>
<td>123 ± 3</td>
<td>7 ± 1</td>
<td>27 ± 1</td>
<td>110 ± 6</td>
<td>127 ± 3</td>
<td>20 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Epicardial touch</td>
<td>120 ± 3</td>
<td>7 ± 1</td>
<td>27 ± 2</td>
<td>109 ± 7</td>
<td>124 ± 4</td>
<td>22 ± 6</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>120 ± 3</td>
<td>7 ± 1</td>
<td>27 ± 2</td>
<td>109 ± 7</td>
<td>124 ± 4</td>
<td>41 ± 9*</td>
<td>35 ± 7*</td>
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<tr>
<td>Veratridine</td>
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<td>28 ± 2</td>
<td>110 ± 5</td>
<td>126 ± 3</td>
<td>26 ± 6</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>122 ± 3</td>
<td>7 ± 1</td>
<td>26 ± 2</td>
<td>110 ± 7</td>
<td>121 ± 4</td>
<td>43 ± 12</td>
<td>32 ± 6*</td>
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<tr>
<td>Substance P</td>
<td>122 ± 3</td>
<td>7 ± 1</td>
<td>26 ± 2</td>
<td>112 ± 6</td>
<td>132 ± 4</td>
<td>20 ± 3</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>122 ± 3</td>
<td>8 ± 1</td>
<td>30 ± 4</td>
<td>116 ± 7</td>
<td>133 ± 5</td>
<td>47 ± 10*</td>
<td>29 ± 5*</td>
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<tr>
<td>Adenosine</td>
<td>119 ± 3</td>
<td>8 ± 1</td>
<td>26 ± 1</td>
<td>111 ± 4</td>
<td>133 ± 4</td>
<td>31 ± 5</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>119 ± 3</td>
<td>8 ± 1</td>
<td>26 ± 1</td>
<td>107 ± 5</td>
<td>130 ± 3</td>
<td>28 ± 5</td>
<td>17 ± 5</td>
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<tr>
<td>Respiration off</td>
<td>119 ± 4</td>
<td>8 ± 1</td>
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<td>113 ± 6</td>
<td>128 ± 4</td>
<td>21 ± 7</td>
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<tr>
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<td>224 ± 14*</td>
<td>166 ± 5*</td>
<td>31 ± 11</td>
<td>53 ± 13*</td>
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</table>

Values are means ± SE (n = 24 dogs). Changes in heart rate (HR), left (LAP) atrial systolic pressures, right and left ventricular intramyocardial systolic pressures (RV IMP and LV IMP, respectively), left ventricular chamber systolic pressure (LVP), and neuronal activity are tabulated. Responses elicited by touching epicardial sensory fields (epicardial touch) or sensory field application of veratridine, substance P, or adenosine are presented, as well as the results obtained during cessation of respiratory (Respiration off) and after systemic administration of isoproterenol or dobutamine. MCG, middle cervical ganglion. *P < 0.05 control vs. intervention.
not changed overall during epicardial application of chemicals, owing to the small quantities of chemical applied to the localized region of the epicardium (Tables 1 and 2), in a few instances cardiac augmentation did occur (Fig. 1). Neurons in all ganglia studied were activated when substance P or purinergic agents were applied to epicardial loci (Tables 1 and 2). Most intrinsic cardiac and middle cervical ganglion neurons studied were activated by epicardial application of more than one chemical (Table 1). Of the chemicals studied, stellate ganglion neurons responded in a consistent fashion only to epicardial application of purinergic agents (Table 2). Reapplication of chemicals to previously responsive epicardial loci induced similar neuronal responses. Epicardial application of gauze squares soaked with room-temperature normal saline elicited no neuronal responses.

After acute decentralization, epicardial application of chemicals induced neuronal responses less frequently, the activity generated by extracardiac neurons being more profoundly suppressed than that of intrinsic cardiac neurons. For example, with the nervous system intact, epicardial application of substance P increased the activity generated by intrinsic cardiac ganglion neurons and middle cervical ganglion neurons by 146 and 59%, respectively (Table 1). After acute decentralization, middle cervical ganglion neurons were minimally affected by epicardial application of substance P, whereas induced changes in intrinsic cardiac neuronal activity were halved (Fig. 5). Similarly, the responses induced by epicardial application of veratridine (Fig. 5) or purinergic agents were attenuated after acute decentralization of the intrathoracic nervous system.

Altered respiratory dynamics. Neurons that generated respiratory-related activity increased their discharge frequency when respiratory rate or respiratory pressure pressure increased. The activity generated by these neurons decreased when respiratory rate or respiratory pressure was reduced or respiration ceased (Table 2). The activity of neurons generating respiratory-related activity increased in 5 of 32 dogs when loci in right- or left-sided pulmonary tissue were distorted gently. The respiratory-related activity generated by neurons in the remaining 27 dogs was modified by gentle epicardial mechanical stimuli.

Coronary artery occlusion. Transient coronary artery occlusion increased the activity generated by neurons in intrinsic cardiac ganglia (Table 2), whereas the activity generated by middle cervical ganglion neurons increased in 12 dogs and decreased in 7 dogs (no change overall). Stellate ganglion neurons were not affected by transient myocardial ischemia (Table 2). The majority of ischemia-sensitive neurons was sensitive to both mechanical and chemical stimuli. In general, activity generated by ischemia-sensitive neurons was not en-
hanced on reperfusion. Cardiovascular variables were unaffected overall by the transient myocardial ischemia employed (Table 2). After acute decentralization, neurons in some animals were modified by transient coronary occlusion but not with enough frequency to affect total activity overall (Table 2).

**DISCUSSION**

The results of the present experiments indicate that cardiodynamics are regulated by a hierarchy of peripheral autonomic neurons functioning as nested feedback loops. In addition to the well-known reflex control loops for cardiac regulation that use cardiopulmonary and arterial baroreceptor afferent axon inputs to central neurons, Fig. 7 illustrates the emerging concept of major intrathoracic reflex feedback loops that regulate autonomic neuronal outflows to the heart. Within this hierarchy, certain afferent axons on the heart and major intrathoracic vessels exert predominant effects on the outflows from the intrinsic cardiac nervous system, whereas others exert their feedback effects via ascending projections to neurons in intrathoracic extracardiac ganglia or the central nervous system. The efficacy of the afferent inputs to neurons in these various locations depends on where the neurons are located, as well as the origins and characteristics of their sensory inputs. Many of the afferent inputs to intrathoracic neurons arise from intrathoracic cardiovascular mechanical and/or chemical sensory axons. A separate and smaller population of intrathoracic neurons receives afferent inputs primarily from pulmonary mechanosensory endings, these neurons generating respiratory-related activity. Other neurons generate sporadic activity, which is not related to cardiac or respiratory dynamics in the time or frequency domains. A small population of intrathoracic neurons, which are primarily located in extracardiac ganglia, receives indirect inputs from ipsilateral carotid artery mechanosensory neurites mediated via central neuronal interconnections.

Neurons identified in most intact intrinsic cardiac and middle cervical ganglia, but not stellate ganglia, were affected by mechanical stimuli applied to ventricular epicardial loci (Tables 1 and 2). In accord with the fact that the majority of afferent neurons associated with cardiac and major vascular mechanosensory neurites is slow adapting when exposed to relatively constant mechanical stimuli (4, 11, 18), intrathoracic neurons responded rapidly and in a relatively consistent manner to sustained epicardial mechanical stimuli (Fig. 4A). Intrinsin cardiac neuronal responses elicited by epicardial mechanical stimuli were due in part to the connectivity of intrathoracic neurons with central neurones, as few of these neurons responded to such stimuli after acute decentralization (Fig. 5). In contrast, extracardiac intrathoracic neurons were still responsive to epicardial mechanical stimuli after acute decentralization of the intrathoracic nervous system. That a population of intrathoracic neurons still responded to epicardial stimuli after acute decentralization of their ganglia suggests that some identified intrathoracic neurons...
were either afferent ones or ones closely associated with them. These data also indicate that some intrathoracic neurons receive preferential inputs from cardiac mechanosensory neurites when functioning independently of central neurons.

Intrathoracic neuronal responses elicited by epicardial chemical stimuli likewise demonstrated differential effects after acute decentralization of the intrathoracic nervous system. Intrinsic cardiac neuronal responses elicited by epicardial chemical stimuli were attenuated and those generated by extracardiac neurons were virtually eliminated after decentralization (Fig. 5). The activity generated by most identified intrathoracic neurons increased when substance P, purinergic agents, or veratridine was applied to circumscribed epicardial loci in the intact state. Because of the relatively low concentrations of each chemical employed, neuronal activity changes usually occurred without alteration in monitored cardiac indexes (Tables 1 and 2). Thus, in confirmation with previous reports (11, 18), epicardial application of a chemical did not directly affect underlying cardiomyocyte contractile behavior in a detectable fashion. That is to say, the neuronal responses elicited by epicardial chemical application were primarily due to activation of chemosensory axons rather than being secondary to altered regional mechanics.

The majority (74%) of the intrathoracic neurons studied were affected by alterations in respiratory dynamics (Tables 1 and 2). Respiratory-induced changes in atrial or ventricular systolic pressures can alter the activity generated by cardiac mechanosensory afferent neurons (18). In agreement with that, most intrathoracic neurons that generated respiratory-related activity were influenced by touching the epicardium rather than pulmonary tissues. Thus the respiratory-related activity generated by many studied intrathoracic neu-
rons appeared to be due to respiratory-dependent alterations in cardiac mechanics. Only 16% of the neurons that generated respiratory-related activity received inputs from pulmonary mechanosensory neurites, as determined by touching pulmonary tissues.

When left ventricular and ascending aortic pressures were elevated by partially occluding the descending aorta, the activity generated by neurons in each ganglion studied changed (Table 2). Isoproterenol induced changes in the activity generated by intrinsic cardiac and middle cervical ganglion neurons, but not stellate ganglion neurons. These data are in accord with the fact that more neurons in the former two ganglia as opposed to the latter ganglion received inputs primarily from cardiac as opposed to aortic mechanosensory axons (2, 6, 7) because isoproterenol augments left ventricular systolic pressure, but not aortic pressure. Whereas intrinsic cardiac neuronal responses induced by positive inotropic agents were similar before and after acute decentralization, few middle cervical and stellate ganglion neurons responded to cardiovascular changes induced by positive inotropic agents after removal of central neuronal input. These data indicate that the effects exerted on extracardiac neurons during positive inotropic states were due in large part to enhanced input from central nervous system neurones, which presumably were influenced reflexly by arterial mechanosensory axons responding to elevations in arterial pressure.

In accord with previous reports (5, 6, 17), brief periods of coronary artery occlusion modified the activity generated by many of the intrathoracic ganglia studied. Activity generated by such ischemia-sensitive neurons varied, depending on where the neurons were located and the location of the sensory neurites that affected them. As occurs with respect to epicardial afferent neurons in dorsal root ganglia (18), but not nodose ganglia (11), the majority of ischemia-sensitive intrathoracic neurons was sensitive to mechanical and chemical stimuli. The activity generated by ischemia-sensitive intrathoracic neurons apparently depended to a greater extent on chemosensory inputs than on mechanosensory inputs, because left ventricular end-systolic (Tables 1 and 2) and end-diastolic pressures were relatively unaffected by the circumscribed and transient coronary artery occlusions employed. That ischemia-induced neuronal responses depended in part on the connectivity of intrathoracic neurons with central neurons (Table 2) is in accord with data obtained when specific mechanical or chemical stimuli were applied to epicardial sensory fields.

The results of the present experiments indicate that populations of neurons located in separate intrathoracic ganglia demonstrate minimal short-term (ms) interactions. Although sympathetic efferent preganglionic neurons directly input to a subpopulation of neurons in extrinsic (5, 6) and intrinsic (10, 13) cardiac ganglia, these neurons do not appear to represent the major determinant of coordinated neuronal activity among populations of neurons in various intrathoracic ganglia. Similarly, a small population of intrinsic cardiac neurons receives direct inputs from parasympathetic efferent preganglionic neurons (8, 9, 13). The likelihood that most intrathoracic neurons involved in cardiac regulation function relatively independent of one another suggests that they act as part of a distributive overlapping set of nested neural feedback networks. The functional selectivity of the behavior expressed by neurons in different intrathoracic ganglia modulating cardiodynamics indicates that there is a redundancy of function among intrathoracic neurons that are involved in regulating the heart.

Coordination of autonomic outflows from intrathoracic neurons to cardiomyocytes depends to a large extent on sharing of inputs from higher centers along with interactions among neurons in and between various peripheral ganglia. The sharing of cardiopulmonary afferent information acting through both intrathoracic and brain stem/spinal cord feedback loops permits an overall coordination of effector control that does not depend on successive synapses functioning solely as deterministic relay stations. The bursting of neuronal activity with 10- to 20-s time periodicities, even among neurons in intrathoracic ganglia disconnected from central neurons (Fig. 3B), suggests that complex network interactions can occur among the various populations of peripheral autonomic neurons, even when functioning independent of central neuronal input. Irrespective of the underlying pattern generators that regulate basal autonomic neural outflow, the data presented in this report support the concept of the diversity of afferent feedback in cardiac control.

Data obtained in the present experiment also indicate the underlying neuronal substrate contained within the intrathoracic nervous system, its various populations of neurons displaying selective responses to activation of specific sensory inputs. Activation of either chemo- or mechanosensory neurites induced brisk neuronal responses in all thoracic neuronal populations investigated, even when such populations were disconnected from central neurons. It is proposed that such sensory inputs are used in the autonomic neuronal reflex control networks that involve short (intrathoracic cardiac nervous system), medium (middle cervical and stellate ganglia), and long (spinal cord and brain) nested feedback loops (Fig. 7). Such an arrangement would maximize the potential for the maintenance of coordinated efferent autonomic neural outflow while providing the flexibility necessary for beat-to-beat regulation of efferent outflow to the heart. Therefore, the loss of the long feedback loop mediated by central neurons would not jeopardize cardiac regulation, as is evident in acutely (10, 13) or chronically (2) decentralized intrathoracic cardiac nervous system preparations.

Owing to the limited sample size of the populations of neurons in the various ganglia studied, our inability to find coherence of activity generated by neurons in distinct and separate intrathoracic ganglia does not exclude the possibility that direct neuronal interactions exist. Nevertheless, the relative lack of coherence between activity generated by the separate and distinct...
populations of intrathoracic neurons studied does indicate the functional and divergent selectivity of most intrathoracic neuron populations that are involved in cardiac regulation. Our data also indicate the importance of the varied afferent information that acts at multiple levels of the proposed network of nested feedback loops involved in the integrative control of regional cardiac function.

Perspectives

Neurons in different intrathoracic ganglia that are involved in cardiac regulation receive inputs primarily from cardiac mechanosensory and chemosensory neurites, with fewer inputs arising from neurites on major intrathoracic vessels. A small population of intrathoracic extracardiac neurones is influenced by sensory neurites associated with the ipsilateral carotid bulb mediated via central neurones. Fewer intrathoracic neurones are influenced by cardiac afferent stimuli when decentralized than in the intact state, indicating that many intrathoracic neurones involved in cardiac regulation depend in part on central neuronal connectivity. On the other hand, that some intrathoracic neurones were still influenced by cardiac sensory neurites in the acutely decentralized state implies that intrathoracic afferent neurones, which sense alterations in the mechanical and chemical milieu of the heart, can influence intrathoracic efferent neurones independent of central neurones. That neurones in different intrathoracic ganglia primarily exhibit noncoupled behavior, even when they are mutually entrained to cardiac events by cardiovascular afferent feedback, implies a redundancy of cardioregulatory control exerted by different populations of intrathoracic neurones. Furthermore, that different populations of intrathoracic neurones respond differently to similar cardiac interventions indicates that selective feedback mechanisms exist at successive levels of the intrathoracic nervous system. That neurones in different ganglia display functional dissimilarities also implies a minimal reliance of the heart at any time on any one population of peripheral autonomic neurones. The selective influence of each population of intrathoracic neurones on the heart likely depends on the nature and content of their sensory inputs. It is concluded that the intrathoracic nervous system acts as a distributive processor, using multiple nested feedback control loops to modulate cardiac function.

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