Interactions within the intrinsic cardiac nervous system contribute to chronotropic regulation

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Randall, David C., David R. Brown, A. Scott McGuirt, Gregory W. Thompson, J. Andrew Armour, and Jeffrey L. Ardell. Interactions within the intrinsic cardiac nervous system contribute to chronotropic regulation. Am J Physiol Regul Integr Comp Physiol 285: R1066–R1075, 2003. First published July 3, 2003; 10.1152/ajpregu.00167.2003.—The objective of this study was to determine how neurons within the right atrial ganglionated plexus (RAGP) and posterior atrial ganglionated plexus (PAGP) interact to modulate right atrial chronotropic, dromotropic, and inotropic function, particularly with respect to their extracardiac vagal and sympathetic efferent neuronal inputs. Surgical ablation of the PAGP (PAGPx) attenuated vagally mediated bradycardia by 26%; it reduced heart rate slowing evoked by vagal stimulation superimposed on sympathetically mediated tachycardia (25%). After combined RAGPx and PAGPx, vagal stimulation still reduced sympathetically mediated tachycardia by 36%. RAGP ablation (RAGPx) eliminated vagally mediated bradycardia, while retaining the vagally induced suppression of right atrial tachycardia (~83%). After combined RAGPx and PAGPx, vagal stimulation still reduced sympathetically-mediated tachycardia (~47%). After RAGPx alone and after PAGPx alone, stimulation of the vagi still produced negative dromotropic effects, although these changes were attenuated compared with the intact state. Negative dromotropic responses to vagal stimulation were further attenuated after combined ablation, but parasympathetic inhibition of atrioventricular nodal conduction was still demonstrable in most animals. Finally, neither RAGPx nor PAGPx altered autonomic regulation of right atrial inotropic function. These data indicate that multiple aggregates of neurons within the intrinsic cardiac nervous system are involved in sinoatrial nodal regulation. Whereas parasympathetic efferent neurons regulating the right atrium, including the sinoatrial node, are primarily located within the RAGP, prejunctional parasympathetic-sympathetic interactions regulating right atrial function also involve neurons within the PAGP.

intrinsic cardiac ganglia; sinoatrial node; inotropic; dromotropic

There is increasing evidence that the intrinsic cardiac nervous system (ICN) plays an active role in the regulation of cardiac function, with peripheral neuronal interactions contributing to the beat-to-beat regulation of regional cardiac function (2). The ICN contains a heterogeneous population of neurons that include parasympathetic, sympathetic, and afferent soma, with interconnecting local circuit neurons (LCN) (9, 12, 15, 35). A subset of these intrinsic cardiac neurons (~25%) receives direct inputs from extracardiac parasympathetic and sympathetic neurons (6, 9); the rest are dependent on sensory inputs and local circuit interconnections for their ongoing activity (2, 3). Previous studies have suggested that specific aggregates of intrinsic cardiac ganglia are preferentially associated with control of different cardiac regions (e.g., Refs. 3a, 22). For the canine heart, the cranial medial ventricular ganglionated plexus exerts predominant influence on ventricular contractility (17). Neural control of dromotropic function is primarily associated with the inferior vena cava-inferior atrial ganglionated plexus (3a). Regarding atrial rate, neurons contained within the ventral right atrial ganglionated plexus (RAGP) project along the sulcus terminalis to the sinoatrial (SA) node, where they play a key role in mediating direct parasympathetic inhibition of SA nodal rhythm (13). Neurons in other intrinsic cardiac ganglia besides the RAGP, specifically the posterior atrial ganglionated plexus (PAGP), may also contribute to the regulation of chronotropic function (27). Although the PAGP is anatomically close to the SA node, previous studies have indicated that it plays a minor role in directly mediating vagal bradycardia (27).

For neural control of the heart, peripheral interactions between sympathetic and parasympathetic efferent neurons occur at multiple sites (14, 19, 26). Moreover, with respect to cardiac effector neurons, the degree of functional interactions among them depends, in part, on preexisting sympathetic-parasympathetic efferent neuronal tone (25). For example, parasympa-
thetic efferent neuronal modulation of ventricular inotropic function becomes enhanced against a background of sympathetic efferent activity (5). Whether autonomic neuronal interactions mediated within the ICN demonstrate analogous tone-dependent interactions has not been determined.

We have previously analyzed the functional role of the RAGP and PAGP by quantifying the effect of their selective ablation (RAGPx and PAGPx) on patterned changes in heart rate (HR) evoked by a suddenly perceived behavioral stress test (30, 31). We found that surgical RAGPx blunted the initial (latency <3 s) HR acceleration to the stress; we attributed this initial HR increase to withdrawal of resting parasympathetic efferent neuronal tone (31). However, RAGPx did not significantly alter the more prolonged HR increase induced later on during the stress; we ascribed this slowly developed tachycardia phase to enhanced sympathetic efferent neuronal activity (31). Conversely, surgical removal of the PAGP differentially modulated the second phase of the cardioacceleration by primarily potentiating the effects of sympathetic efferent neurons innervating the SA node (30).

Ultimate control of atrial rate is dependent on the intrinsic properties of the SA nodal pacemaker cells, as modulated by autonomic inputs and humoral factors (2). Classically, the interactions between sympathetic and parasympathetic efferent postganglionic neurons controlling chronotropic function have been placed at the end-effector pacemaker cells; these have been ascribed to both prejunctional and postjunctional reciprocal interactions (25). However, recent studies have suggested that intrinsic cardiac neurons may also play an important role in mediating these interactions (34), i.e., intrinsic cardiac ganglia are more than passive efferent neuronal relay stations (8). Whereas RAGPx interrupts bradycardia associated with parasympathetic efferent preganglionic neuronal stimulation (3a), Furukawa et al. (19) reported that a small number of heretofore unidentified vagal efferent axons that are not contained within RAGP could mediate some of the interactive effects between cardiac parasympathetic and sympathetic efferent neurons. These investigators further speculated that RAGPx (which they referred to as the sinus rate-related parasympathetic nerves) could unmask presynaptic parasympathetic inhibition of any positive chronotropic effects produced by sympathetic efferent neuronal stimulation (19). In fact, McGuirt et al. (26) hypothesized that the most probable site for such residual prejunctional parasympathetic-sympathetic interactions in the canine is within the PAGP. The primary purpose of the present experiment was to test this hypothesis in the anesthetized dog. The findings of the present study support the hypothesis of McGuirt and colleagues.

METHODS

Subjects. Twelve adult mongrel dogs of either sex, weighing between 18 and 22 kg, used in these acute studies, were randomly assigned to one of two groups (n = 6 each). All experiments were performed in accordance with the guide-

lines for animal experimentation described in the "Guiding principles for research involving animals and human beings" (1). The Institutional Animal Care and Use Committee of the University of South Alabama approved the experiments.

Surgical preparation. The dogs were anesthetized with pentobarbital sodium (30 mg/kg iv, supplemented as needed; i.e., 8 mg/kg, if the animal responded to noxious stimuli or control arterial blood pressure increased). They were placed on positive-pressure respiration. The dog's body temperature was monitored continuously, and a heating pad was adjusted to maintain core temperature at 39°C. Arterial blood samples were taken periodically, blood gases and pH were measured, and bicarbonate was administered, as necessary, to maintain normal acid-base status. Arterial blood pressure was monitored via a catheter inserted into the femoral artery, advanced to the descending aorta, and attached to a Statham P23 ID pressure transducer. The heart was exposed via a transverse thoracotomy (T4–T5) and suspended in a pericardial cradle. Bipolar recording electrodes were inserted into the right atrium near the SA node and on the conus of the right ventricle to record atrial and ventricular electrograms, respectively. A second pair of atrial electrodes was placed on the right atrial appendage and used subsequently for pacing the heart. Right atrial function was assessed via pressure recordings from an isovolumic fluid-filled balloon placed into the chamber via an incision in the appendage; this balloon catheter was connected to a Statham P23 ID pressure transducer.

Extracardiac autonomic nerve inputs to the heart were isolated and prepared for electrical stimulation. The left and right cervical vagi were transected, and bipolar Teflon-coated wires with 1-mm bare tips were inserted into their distal ends. The vagi were then immersed in mineral oil. The left and right stellate ganglia were isolated, and all interconnections with the spinal cord were severed. Bipolar pin electrodes were inserted into each ganglion, and the electrodes and ganglia were coated with petroleum jelly to prevent desiccation. The bipolar stimulating electrodes were connected individually to S88 stimulators via SIU6 isolation units, and threshold voltages were established. For parasympathetic preganglionic efferent neuronal inputs to the ICN, each cervical vagus was stimulated at 20 Hz, 2 ms. Duration and voltage increased until a 10% bradycardia was evoked. For sympathetic efferent neuronal inputs, each stellate ganglion was stimulated at 4 Hz, 2 ms. Duration and voltage increased until a 10% tachycardia was evoked. Stimulus voltages were set to twice these threshold values, and this intensity of stimulation was utilized throughout the remainder of the study. The stability of the stimulations was assessed periodically to be certain that the nerve preparations remained viable. The animals were allowed to stabilize for 30 min after completion of instrumentation.

Cardiac responses to vagal stimulation and/or sympathetic stimulation were assessed before and after sequential and randomized ventral RAGPx and PAGPx. For RAGPx, fatty tissues were stripped from the ventral surface of the right pulmonary vein-right atrial complex (3a). For PAGPx, fatty tissue on the dorsal surface of the right atrium was removed, along with fat on the inferior surface of the right pulmonary artery as it crosses under the junction of the superior vena cava and right atrium (30).

Data acquisition. All data were recorded on chart paper by using a Grass model 7 polygraph. In four dogs, the signals were digitized at 200 Hz by using a DT2901 analog-to-digital converter and a personal computer using the ASYST data-acquisition system. In the other eight dogs, the data were digitized at 500 Hz by using a Pentium processor and a Data
Translation 2821F analog-to-digital converter. All data files were saved to disk for later analysis.

Experimental design and protocol. Table 1 summarizes the experimental design. For group 1 animals, the cardiac responses to autonomic stimuli were assessed before (stage 1) and after RAGPx (stage 2) and then after the subsequent removal of the PAGPx (stage 3). For group 2 dogs, the cardiac response to extracardiac efferent automatic neuronal stimulations were determined before (stage 1) and after PAGPx (stage 2) and then after combined (stage 3) PAGPx and RAGPx (i.e., the reverse of the other protocol). Figure 1 illustrates the two stimulation protocols used for both groups of dogs at all stages of the study (i.e., before and after sequential ablation of the respective intrinsic cardiac ganglionated plexuses). Each test consisted of a 90-s-long recording interval. For sequence A, the first 45 s served as a prestimulation control period. Bilateral stimulation of the cervical vagi (2 × threshold, 2-ms duration) was then performed from 45 to 75 s at frequencies of 0, 1, 3, 6, 9, 12, or 18 Hz, with the order of delivery being randomized among animals. To evaluate autonomic efferent neuronal modulation of dromotropic and right atrial inotropic function, independent of intrinsic rate changes, the atria were paced at 180 beats/min during the last 15 s for each vagal stimulation. In sequence B, the first 15 s represented a prestimulation control. Stimulation of stellate ganglia commenced at 15 s and was maintained for 60 s (until 75 s of the protocol). Concurrent vagal stimulation, at each frequency listed above, was then superimposed on the sympathetic stimulation starting at 45 s for 30 s (i.e., until 75 s). Atrial pacing at 180 beats/min was superimposed on the combined stellate and vagal stimulations between 60 and 75 s. In some cases, during combined sympathetic-parasympathetic stimulation, the dog’s HR exceeded that pacing rate, in which case pacing was not utilized. Sequences A and B were performed at stage 1 (with the ICN intact), stage 2 [after RAGPx (group 1) or after PAGPx (group 2)], as well as after ablation of both ganglia (stage 3; see Table 1).

Data analysis. Data were analyzed by using programs developed in house for the personal computer by using Microsoft Foundation Class. HR was determined by beat by beat with the use of the atrial electrogram recordings. Atrioventricular (AV) intervals (AVI) were determined by beat by beat as the time between the major deflections in the atrial and ventricular electrograms. An index of atrial inotropic function was determined by beat by beat as the difference between maximum and minimum intra-atrial balloon pressure. With the use of these beat-by-beat data, indexes of cardiac function were averaged across the 15-s vagal stimulation period (i.e., without pacing), as well as during the combination of vagal stimulation plus pacing. Sympathetic efferent neurally induced responses were averaged during the last 15 s of stellate ganglion stimulation (i.e., before onset of vagal stimulation), during the 15 s of combined stimulation of stellate ganglia and cervical vagal nerves, and finally during the last 15 s of combined autonomic stimulation plus pacing. Data are reported as means ± SE. Comparison of means within a group was performed with ANOVA and the least significant difference procedure. Comparison of means between groups at each specific combination of stimulation frequencies was performed with t-test utilizing heterogeneous and homogeneous variance versions, as appropriate. P levels of ≤0.05 were considered statistically significant.

RESULTS

Chronotropic responses before and after RAGPx and/or PAGPx. HRs during the initial 15 s of control are provided for both groups of dogs in Table 2 for each stage of the experiment. There were no statistically significant differences in atrial rates across groups or across stages of the study. With respect to animals in stage 1 (intrinsic cardiac ganglia intact), induced chronotropic responses to any combination of parasympa-

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<tr>
<td></td>
<td>Intrinsic cardiac ganglia intact</td>
<td>Intrinsic cardiac ganglia intact</td>
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<tr>
<td>Stage 1</td>
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<td>PAGPx</td>
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<td>Stage 3</td>
<td>Combined (RAGPx + PAGPx)</td>
<td>Combined (PAGPx + RAGPx)</td>
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RAGPx, right atrial ganglionated plexus ablation; PAGPx, posterior atrial ganglionated plexus ablation.

Table 2. Heart rate and atrioventricular interval during prestimulus baseline for each stage of intrinsic cardiac nervous system ablation

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<td>HRcontrol, beats/min</td>
<td>HRcontrol, beats/min</td>
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<tr>
<td>Stage 1</td>
<td>113 ± 8</td>
<td>124 ± 9</td>
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<td>Stage 2</td>
<td>116 ± 8</td>
<td>126 ± 11</td>
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<tr>
<td>Stage 3</td>
<td>123 ± 8</td>
<td>128 ± 12</td>
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AVls, ms

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<td>126 ± 12</td>
<td>123 ± 7</td>
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<tr>
<td>Stage 1</td>
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<td>119 ± 5</td>
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<tr>
<td>Stage 3</td>
<td>185 ± 19</td>
<td>195 ± 16</td>
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AVl, ms

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<th>Stage</th>
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<td>196 ± 15</td>
<td>198 ± 20</td>
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Values are averages ± SE for 15 s control before 0-Hz vagal stimulation trials. HRcontrol, heart rate during spontaneous rhythm; AVls, atrioventricular interval for spontaneous rhythm; AVl, atrioventricular interval when atria were paced at ~180 beats/min.

Fig. 1. Summary of experimental protocol. Bilateral stimulation of the cervical vagi (Parasymp. Stim.) with and without atrial pacing was conducted alone (A) or in conjunction with bilateral stimulation (4 Hz) of the stellate ganglia (Sympathetic Stimulation) (B). Both stimulation protocols were completed for all vagal stimulation frequencies (0, 1, 3, 6, 9, 12, and 18 Hz) in the decentralized, but intrinsic cardiac nervous system (ICN)-intact state (stage 1), following surgical ablation of the right atrial ganglionated plexus (RAGPx; group 1) or of the posterior atrial ganglionated plexus (PAGPx; group 2) in stage 2, and following combined removal of both sets of intrinsic cardiac ganglia (RAGPx + PAGPx; stage 3).
thetic and/or sympathetic nerve stimulation were similar among groups (group 1 vs. 2); the magnitudes of responses elicited by autonomic neuronal stimulation were stable with repeated trials.

Selective ablation of elements within the ICN disrupted functional neural modulation of regional cardiac function, effects differing depending on which neuronal populations were removed first. Selected recordings from a group 1 dog are shown in Fig. 2 for stimulations conducted first with the ICN intact (panels 1 and 2) and then after RAGPx (panels 3 and 4). Baseline HR was 105 beats/min. In stage 1 (ICN intact), stimulation of the cervical vagi reduced atrial HR (HRa) by 68 beats/min [compare with that evidenced in the right atrial electrogram (RAE) recording]. Note the pronounced negative dromotropic and inotropic responses that accompanied parasympathetic stimulation (panel 1). Bilateral stimulation of stellate ganglia increased HR to 190 beats/min in conjunction with increased regional contractile function and enhanced AV conduction (panel 2). Superimposing stimulation of the cervical vagi on the on-going stellate ganglion stimulation decreased the tachycardia response by 138 beats/min. Adding atrial pacing to these combined nerve stimulations revealed the occurrence of AV nodal blockade. Atrial contractile function was similarly suppressed by these activated parasympathetic efferent neuronal inputs.

Figure 2, panels 3 and 4, show the same subject’s responses to identical tests after RAGPx. Note, in particular, that stimulation of the vagus nerve alone minimally affected atrial rate (i.e., HRa slowed only 7 beats/min; see RAE in panel 3). The AV nodal block persisted, but to a lesser degree (i.e., third degree vs. second degree, pre- vs. postablation). Stimulation of the vagus nerves during on-going stellate ganglion stimulation (panel 4) still slowed atrial rate (from 192 to 137 beats/min), despite the elimination of the direct

Fig. 2. Induced changes in regional cardiac function in response to parasympathetic and/or sympathetic stimulation before (Intact, panels 1 and 2) and after RAGPx (panels 3 and 4). For this example, cervical vagi were stimulated at 6 Hz, and stellate ganglia at 4 Hz. Tests included stimulation of the vagi with and without atrial pacing (Vagus + Pace, panel 1) alone and when superimposed on a background of stellate ganglia stimulation (Stellate + Vagus + Pace, panel 2). RAGPx eliminated the bradycardia to sole vagal stimulation (Vagus + Pace, panel 3), but did not eliminate the heart rate slowing when the vagi were stimulated during concomitant stellate ganglia stimulation (panel 4). Autonomic modulation of dromotropic and inotropic (atrial and ventricular) functions was similarly maintained after RAGPx. RAE, right atrial electrogram; RVE, right ventricular electrogram; BP, arterial blood pressure; RA, pressure recorded from isovolumic balloon in right atrium; RV, right ventricular intramyocardial pressure; V_on, beginning of stimulation of the vagus nerves; P_on, beginning of atrial pacing at ~180 beats/min; S_on, beginning of stellate ganglia stimulation; off, termination of pacing and of stimulation of both the vagi and stellate ganglia.
vagal bradycardia by the RAGPx. However, the parasympathetic-induced 55 beats/min HR slowing during stellate stimulation was notably less than that which occurred [change (Δ) of 138 beats/min] during the comparable situation before RAGPx. For this animal in stage 3 trials (not shown, Fig. 2), vagal stimulation alone slowed atrial rate by only 4 beats/min; HR_a slowed by 19 beats/min (i.e., from 190 to 171 beats/min) when vagal stimulation was superimposed on stellate ganglion stimulation.

Figure 3 is identical to Fig. 2, except that this dog was a member of group 2. With the ICN intact (panels 1 and 2), stimulation of the cervical vagi (6 Hz) slowed HR by 61 beats/min (panel 1); superimposition of atrial pacing revealed a 2:1 AV nodal block. In panel 2, HR was increased to 189 beats/min by stellate ganglion stimulation; HR_a was reduced to 94 beats/min during superimposition of vagal stimulation (ΔHR_a = −95 beats/min). AV nodal blockade was evident during atrial pacing. After PAGPx (panels 3 and 4), in marked contrast to the data shown in Fig. 2, stimulation of the vagi still markedly slowed HR (HR reduced by 40 beats/min), although somewhat less than what occurred in the ICN-intact state. Thereafter, superimposing vagal stimulation during background stellate ganglion stimulation (panel 4) slowed HR by only 25 beats/min (i.e., from 189 to 164 beats/min). After subsequent RAGPx (stage 3; not shown, Fig. 3), sole vagal stimulation no longer slowed atrial rate, yet vagal stimulation superimposed on stellate stimulation continued to reduce that index (ΔHR_a = −25 beats/min).

Figure 4, top, summarizes the negative chronotropic responses elicited by parasympathetic efferent neuronal stimulation alone for groups 1 (left) and 2 (right) in dogs before (ICN intact, stage 1) and after RAPGx or PAGPx (stage 2) and subsequent to combined RAPGx + PAGPx (stage 3). RAGPx prevented the bradycardia induced by bilateral cervical vagal stimulation (Fig. 4, top left). In contrast, PAGPx produced only a modest effect on vagally induced slowing (Fig. 4, top right; compare solid circle and solid square data sets); these differences were significant in (only) three of six levels of stimulation. Between-group comparisons at stage 2 (RAPGx vs. PAGPx) indicated significant differences in the degree of bradycardia induced at all levels of parasympathetic efferent neuronal stimulation. Whereas the addition of PAGPx to the prior RAPGx (nearly superimposed lines, Fig. 4, top left)...

\[\text{Fig. 3. Induced changes in regional cardiac function in response to parasympathetic stimulation (6 Hz) and/or sympathetic stimulation before (Intact, panels 1 and 2) and after surgical PAGPx (panels 3 and 4). Presentation and notations are as in Fig. 2. PAGPx slightly reduced the bradycardia induced by vagal stimulation alone (panel 3) and attenuated the heart rate slowing elicited when vagal stimulation was superimposed on on-going sympathetic stimulation (panel 4).}\]
elicited no effect on the vagally mediated bradycardia, the addition of RAGPx to a preexisting PAGPx ablated any residual bradycardia induced by parasympathetic stimulation (compare solid square and solid triangle data sets; top right).

Figure 4, bottom, summarizes the chronotropic response to combined sympathetic and parasympathetic efferent neuronal stimulation for group 1 (left) and group 2 (right) for the three stages of the study. Even though RAPGx prevented bradycardia elicited by parasympathetic efferent neuronal stimulation (Fig. 4, top left), vagal stimulation in these animals continued to suppress the sympathetic-mediated tachycardia (bottom left), although to a lesser extent than what occurred in the ICN-intact state. PAGPx similarly blunted the negative chronotropic effects of vagal stimulation when superimposed against a background of sympathetic efferent neuronal activation (Fig. 4, bottom right). At stage 2, between-group comparisons of atrial rate responses induced during various combinations of sympathetic-parasympathetic neuronal stimulations showed significance at only one point (4 Hz, sympathetic + 12 Hz, parasympathetic). Whereas in group 1 animals the addition of PAGPx after RAGPx induced no further change in autonomic-induced changes in atrial rate (Fig. 4, bottom left, compare open circle and solid triangle data sets), in group 2 animals the addition of RAGPx to PAGPx further decreased HR slowing when vagal stimulation was superimposed on bilateral stellate ganglion stimulation (bottom right, compare solid square and solid triangle data sets). After combined RAGPx and PAGPx, a significant residual slowing of the sympathetic-induced tachycardia persisted in both groups at frequencies of parasympathetic stimulation >9 Hz.

AVI responses. Tables 2 and 3 summarize changes in parasympathetic efferent neuronal control of AV nodal function before and subsequent to RAGPx and/or PAGPx. During the prestimulation control period, neither AV-nodal intervals during spontaneous beats nor AVI during atrial pacing at ~180 beats/min were altered by either ganglion ablation (stages 1–3); between-group comparisons were similarly unaffected (Table 2). As evidenced in Figs. 2 and 3, parasympathetic efferent neurons prolonged AV conduction, an effect that is most appropriately evaluated at a constant atrial rate.

Table 3. Atrioventricular nodal function during bilateral stimulation of the cervical vagus nerves

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Entries indicate no. of animals experiencing indicated degree of atrioventricular nodal block.

Fig. 4. Frequency response curves for chronotropic responses to parasympathetic stimulation alone (top) or in combination with sympathetic stimulation (bottom) before (ICN intact, stage 1) and after surgical RAGPx (left; stage 2) or PAGPx (right; stage 2) and then after combined ablation of both intrinsic cardiac ganglia (stage 3). Chronotropic responses are indicated as percent change from prestimulation control levels (means ± SE). *P < 0.05 vs. ICN intact; †P < 0.05, stage 2 vs. stage 3 ablation; ‡P < 0.05, RAGPx vs. PAGPx (stage 2 between-group comparison).

Table 2. Atrioventricular nodal function during bilateral stimulation of the cervical vagus nerves

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<th>3 Hz</th>
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Table 3 demonstrates the number of animals that experienced various degrees of AV nodal block elicited with 3-, 9-, and 18-Hz vagal stimulation frequencies at each stage of the protocol for both groups; responses were categorized from no effect (none) to complete AV dissociation (3’s heart block). For animals in group 1, after RAGPx alone, vagal stimulation still produced negative dromotropic effects; these observed changes were attenuated compared with the ICN-intact state. This attenuated response trend was accentuated after stage 3, as 50% of the dogs showed no negative dromotropism after the combined ablation, even at supra-maximal stimulation levels (18-Hz stimuli). Group 2 dogs similarly exhibited a shift to lesser parasympathetic efferent neurally induced heart block with successive ablations of the PAGP and RAGP.

Effects of neural ablations on atrial contractile force. Panels 1 and 2 of Figs. 2 and 3 indicate that atrial pressure generation was depressed by vagal nerve stimulation in the ICN-intact state (compare right atrial tracing) without (panel 1) and with (panel 2) concurrent sympathetic efferent neuronal stimulation. Moreover, marked depression in atrial contractile function persisted after RAGPx (Fig. 2) or PAGPx (Fig. 3). Figure 5 summarizes the data demonstrating sympathetic-parasympathetic modulation of right atrial contractile function before (ICN intact) and after RAGPx (A) or PAGPx (B). These data indicate that selective extirpation of the RAGP or PAGP did not demonstrably alter the interactions that occur between the two efferent divisions of the autonomic nervous system with respect to control of right atrial contractile function. After combined RAGPx and PAGPx (stage 3), sympathetically induced increases in atrial contractile force were significantly reduced compared with stage 1 (+187 ± 33 vs. 90 ± 18%). The combined ablations also obtunded atrial inotropic responses elicited by 1- or 12-Hz vagal nerve stimulations that were imposed during background 4-Hz sympathetic efferent neural stimulation.

DISCUSSION

Neural control of SA nodal function involves the dynamic interplay between central and peripheral reflex control mechanisms (10). Whereas many of these interactions are afferent neuronal dependent (6, 8), the results of the present series of experiments indicate that the potential for efferent neuronal interactions exists at multiple points within the ICN that project to pacemaker cells vs. other atrial cardiomyocytes (19, 26). Moreover, the SA nodal pacemaker cells themselves contribute directly to sympathetic-parasympathetic interactions via postjunctional reciprocal G protein-dependent signal transduction cascades (14). Taken together, these data indicate that control of cardiac function resides in neural interactions within and between the peripheral and central nervous systems and the postjunctional sites of the heart.

Classically, intrathoracic autonomic ganglia have been regarded as a homogeneous aggregate of postganglionic efferent neurons that functioned solely as relay stations for parasympathetic preganglionic efferent inputs to cardiac myocytes (33). Thus proposed neural interactions occurring between sympathetic and parasympathetic efferent projections have been primarily restricted to presynaptic interactions occurring at end-effector junctions (25). It is now recognized that intrathoracic ganglia contain heterogeneous populations of efferent neurons, afferent neurons, and LCN (9, 12, 35). Indeed, a subset of intrinsic cardiac neurons can be modulated by parasympathetic and sympathetic efferent preganglionic inputs to the heart (9). Recently, it has been proposed that the ICN may subserve, in part, sympathetic-parasympathetic interactions (19, 26, 30).

The major novel finding of this study was that PAGPx significantly attenuates bradycardia induced by activated parasympathetic efferent preganglionic neurons superimposed on a background of increased sympathetic efferent neural inputs to the heart. Because
PAGPx exerted only modest effects on bradycardia induced by stimulating the cervical vagi alone, we hypothesize that a population of neurons within the PAGP, when activated, is capable of suppressing the ability of intrinsic cardiac sympathetic efferent neurons to modulate the SA node. Presumably this occurs via a presynaptic mechanism that may involve intrinsic cardiac LCN (2).

It is now well established that the neurons within the RAGP are the principal source for direct vagal inhibition of the SA node (e.g., Refs. 3a, 13, 20, 22). Activating the RAGP chemically (35) or electrically (15, 20) slows atrial rate. Chemical or surgical RAGPx effectively abolishes the bradycardia associated with sole vagal stimulation (3a, 18). Yet, even after RAGPx, cervical vagal stimulation still suppressed the sympathetically induced tachycardia; this residual attenuation was abolished by atropine (26). From these studies, McGuinty et al. (26) concluded that disrupting the RAGP eliminated direct vagal efferent neuronal control of the SA node, but left residual parasympathetic projections to sympathetic efferent neuronal elements innervating the SA node. These authors speculated that such residual parasympathetic-sympathetic interactions involved prejunctional mechanisms and that the most probable site for the parasympathetic neurons involved was “within the ganglionated plexus of the posterior right atrium.” These data also indicate that multiple intrinsic cardiac ganglia contribute to parasympathetic modulation of sympathetic inflows to the SA node.

In addition to the RAGP, the canine atria have at least three other major ganglionated plexuses that are associated with control of electrical and mechanical tissues of the heart (36). One of these, the PAGP, is anatomically close to the SA node (27); major sympathetic efferent projections to the SA node traverse this region (4). However, retrograde tracer techniques have shown minimal direct projections to the SA node, and bipolar electrical stimuli delivered to the PAGP fail to alter atrial rate (27). However, in the present study, the induced bradycardia to sole parasympathetic stimulation was affected by PAGPx. The attenuation of this response could reflect partial interruption of vagal preganglionic axons to the RAGP that traverse en passant the PAGP region (3a), or it may reflect functional interactions among neurons in the PAGP and RAGP with consequent modulation of the parasympathetic efferent neuronal projections to the SA node. With the use of discrete chemical stimuli, evidence exists for functional projections from the PAGP to the SA node, with resultant negative and positive evoked changes in right atrial function (35). Furthermore, surgical PAGPx has been shown to potentiate the sympathetically induced tachycardia evoked by an acute behavioral stress in dogs (30). Taken together, these data suggest that peripheral autonomic interactions mediated by neurons within the PAGP may depend, in part, on a substrate of background sympathetic efferent preganglionic neuronal discharge.

There is increasing evidence that the intrinsic cardiac ganglia contain the requisite elements to constitute a nervous network that plays an important role in the moment-to-moment matching of demands placed on the circulation by physiological, environmental, and psychological challenges (7). Very recent data, in fact, affirm an anatomic communication between the RAGP and the PAGP (23). Injecting a tracer into the cat RAGP resulted in 71.4 ± 6.8% of the retrogradely labeled neurons being loaded in PAGP. With respect to central nervous projections to the cardiac ganglia, retrogradely labeled neurons were found exclusively in the ventrolateral nucleus ambiguus after injection of the tracer into PAGP. This same investigator (23) also performed a dual-fluorescent retrograde neuronal tracer study to determine whether separate populations of vagal preganglionic neurons project to these two ganglionated plexi: one population projected to RAGP (51%), a second to PAGP (25%), and a third projected to both cardiac ganglia (24%). These findings establish a key criterion for coordination of cardiac function between intrinsic cardiac ganglia in cooperation with the central nervous system.

Although Priola et al. (29) concluded that these neural elements had only limited effects on cardiac perfor-
mance in the resting dog, they speculated that the intrinsic cardiac ganglia might be capable of significantly depressing cardiac function under conditions of elevated sympathetic tone, as would be encountered during exercise. Recent data have indicated that, even after cardiac transplantation, activity within the ICN is maintained and is essential for sustaining and regulating cardiac electrical and mechanical tissues (28).

Our progress in understanding the potential of the intrinsic cardiac ganglia is perhaps best illustrated by an intriguing model (2, 8) that proposes the existence of nested “circuits” involving elements both intrinsic and extrinsic to the heart, all of which regulate and coordinate regional cardiac function. A fundamental component of that model is that afferent and efferent neurons within peripheral ganglia interact to contribute to the dynamic regulation of cardiac function (8, 34). The data presented herein support that concept and enlarge upon it to indicate that neurons in the PAGP contribute to the regulation of SA nodal function not only via its parasympathetic efferent postganglionic neurons, but also via a presynaptic mechanism exerted on its sympathetic postganglionic neurons involved in regulating atrial pacemaker tissues. However, even after combined RAGPx and PAGPx, residual parasympathetic restraint on sympathetic inputs to the SA node still exists. Although sites for this residual interaction were not examined herein, future studies should consider the influences of the more dispersed aggregates of intrinsic cardiac ganglia interspersed within atrial muscle (36) and those previously identified in other atrial and ventricular ganglionated plexuses (35).

Perspectives

A model summarizing the organization and interactions that occur within the ICN to mediate efferent neuronal projections to the SA node is portrayed in Fig. 6. A major point of this concept is that interactions can occur among sympathetic and parasympathetic efferent neurons in various intrinsic cardiac ganglionated plexuses, as well as at the level of the medulla and postjunctionally at end-effector sites. For postjunctional autonomic interactions, Fig. 6 depicts the interdependence between G-coupled receptor systems onto adenyl cyclase, with the caveat that other SA nodal intracellular processes (e.g., involving cGMP) contribute to the integrated chronotropic response (16). With respect to neural interactions discussed in this paper, some sympathetic efferent postganglionic projections bypass the ICN to project directly to specific cardiac target tissues, whereas others project into the ICN to influence parasympathetic and sympathetic efferent postganglionic neurons in these ganglia that regulate specific target sites. Twenty-five percent of intrinsic cardiac neurons are directly influenced by such projections from these extracardiac neurons (9). The remaining neurons are afferent (~5% of population (3)) or are LCN (6, 9, 21) that provide a neural substrate for integration and interactions among intrinsic cardiac ganglia (34). Moreover, regulation of regional cardiac function likely involves different populations of intrinsic cardiac neurons (3a, 30, 35). Data from our group and others indicate that “direct” parasympathetic efferent neuronal inhibition of the SA nodal function depends primarily on neurons contained within the RAGP (3a, 31, 32). Data obtained in this study indicate that an “interactive” component of the interactions that occur between the two divisions of the autonomic nervous system is mediated by the PAGP and involves parasympathetic-mediated presynaptic inhibition of neurotransmitter release from cardiac sympathetic neurons. By this model, we do not intend to dismiss a participation of neurons within RAGP in mediating such an interaction; moreover, our data indicate that neurons in intrinsic cardiac ganglia distinct from the RAGP and PAGP also mediate vagal suppression of sympathetic efferent neuronal control of the SA node.

The primary function of the ICN is to maintain and coordinate regional cardiac function (2). In that role, it appears to act as a “low-pass filter” to smooth out spurious inputs from extracardiac sources and via short-loop afferent feedback to help maintain a balance between cardiac electrical and mechanical functions (7, 10). With separate and distinct aggregates of intrinsic cardiac neurons (e.g., those comprising the RAGP and PAGP) acting in concert with higher center neurons to regulate specific cardiac functions (e.g., SA node pacemaker activity), the cardiac nervous system exhibits the flexibility required to mediate rapid reflex control of specific cardiac functions. Disruptions of intrinsic cardiac ganglia have been associated with the progression of cardiac disease (11, 24). Destruction of specific elements within these ganglia may compromise overall neurohumoral control of the heart (3a, 31). Future studies must consider the role of the interdependent interactions that occur among neurons within these target organ ganglia in mediating neural control of regional cardiac function.

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DISCLOSURES

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