INTERACTIONS WITHIN THE INTRINSIC CARDIAC NERVOUS SYSTEM

CONTRIBUTE TO CHRONOTROPIC REGULATION

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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 attenuated vagally-mediated bradycardia by 26%; it reduced HR slowing evoked by vagal stimulation superimposed upon sympathetically-mediated tachycardia by 36%. RAGP ablation eliminated vagally-mediated bradycardia, while retaining the vagally induced suppression of sympathetic-mediated tachycardia (-83%). Following combined RAGP and PAGP ablation, vagal stimulation still reduced sympathetic-mediated tachycardia (-47%). After RAGPx alone, and after PAGPx alone, stimulation of the vagi still produced negative dromotropic effects, though these changes were attenuated compared to the intact state. Negative dromotropic responses to vagal stimulation were further attenuated after combined ablation, but parasympathetic inhibition of AV-nodal conduction was still demonstrable in most animals. Finally, neither RAGP nor PAGP ablation 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 SA node, are primarily located within the RAGP, prejunctional parasympathetic-sympathetic interactions regulating right atrial function also involve neurons within the PAGP.
INTRODUCTION

There is increasing evidence that the intrinsic cardiac nervous system 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 (1). The intrinsic cardiac nervous system contains a heterogeneous population of neurons that include parasympathetic, sympathetic, and afferent soma with interconnecting local circuit neurons (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 upon sensory inputs and local circuit interconnections for their ongoing activity (1,2). Previous studies have suggested that specific aggregates of intrinsic cardiac ganglia are preferentially associated with control of different cardiac regions (e.g., 3,22). 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 (3). Regarding atrial rate, neurons contained within the ventral right atrial ganglionated plexus (RAGP) project along the sulcus terminalis to the 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 pre-existing sympathetic-parasympathetic efferent neuronal tone (25). For example, parasympathetic efferent neuronal modulation of ventricular inotropic function becomes enhanced against a background of sympathetic efferent activity (5). Whether autonomic neuronal interactions mediated within the intrinsic cardiac nervous system 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 upon patterned changes in HR evoked by a suddenly perceived behavioral stress test (30,31). We found that surgical ablation of the RAGP blunted the initial (latency < 3 sec) HR acceleration to the stress; we attributed this initial HR increase to withdrawal of resting parasympathetic efferent neuronal tone (31). However, RAGP ablation 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 cardio-acceleration by primarily potentiating the effects of sympathetic efferent neurons innervating the SA node (30).

Ultimate control of atrial rate is dependent upon the intrinsic properties of the SA nodal pacemaker cells, as modulated by autonomic inputs and humoral factors (1). 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 pre-junctional and post-junctional 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). While ablation of the RAGP interrupts bradycardia associated with parasympathetic efferent preganglionic neuronal stimulation (3), 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 ablation of the RAGP (which they referred to as the sinus rate-related parasympathetic nerves SRRPN) 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 (26).

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 guidelines for animal experimentation described in the “Guiding Principals for Research Involving Animals and Human Beings” (Am. J. Physiol., Regul. Integr. Comp. Physiol. 283: R281-R283, 2002). 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; i.v.], 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 adjusted to maintain core temperature at 39°C. Arterial blood samples were taken periodically, blood gases and pH 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 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 established. For parasympathetic preganglionic efferent neuronal inputs to the intrinsic cardiac nervous system, each cervical vagus was stimulated at 20 Hz, 2 msec. duration and voltage increased until a 10% bradycardia was evoked. For sympathetic efferent neuronal inputs, each stellate ganglion was stimulated at 4 Hz, 2 msec. 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 minutes after completion of instrumentation.

Cardiac responses to vagal stimulation and/or sympathetic stimulation were assessed prior to and following sequential and randomized ablation of the ventral right atrial (RAGP) and the posterior atrial (PAGP) ganglionated plexuses. For RAGP ablation (RAGPx), fatty tissues were stripped from the ventral surface of the right pulmonary vein-right atrial complex (3). For PAGP ablation (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 SVC and right atrium (30).

**Data Acquisition:** All data were recorded on chart paper using a Grass Model 7 polygraph. In four dogs the signals were digitized at 200 Hz using a DT2901 A/D converter and a PC using the ASYST data acquisition system. In the other 8 dogs the data were digitized at 500 Hz using a Pentium processor and a Data Translation 2821F A/D converter. All data files were saved to disk for later analysis.
**Experimental Design and Protocol:** Table I summarizes the experimental design. For **Group 1** animals, the cardiac responses to autonomic stimuli were assessed before (Stage 1) and after RAGPnx (Stage 2) and then after the subsequent removal of the PAGPnx (Stage 3). For **Group 2** dogs, the cardiac responses to extracardiac efferent autonomic neuronal stimulations were determined before (Stage 1) and after PAGPnx (Stage 2) and then after combined (Stage 3) PAGPnx and RAGPnx (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 sec. long recording interval. For sequence A, the first 45s served as a pre-stimulation control period. Bilateral stimulation of the cervical vagi (2x threshold, 2 ms duration) was then performed from the 45 sec to 75 sec 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 bpm during the last 15 sec for each vagal stimulation. In Sequence B, the first 15 sec represented a pre-stimulation control. Stimulation of stellate ganglia commenced at 15s and was maintained for 60 seconds (until 75 seconds of the protocol). Concurrent vagal stimulation, at each frequency listed above, was then superimposed upon the sympathetic stimulation starting at 45 sec. for 30 seconds (viz., until 75 sec). Atrial pacing at 180 bpm was superimposed upon the combined stellate and vagal stimulations between 60 and 75 sec. In some cases during combined sympathetic-parasympathetic stimulation the dog’s heart rate exceeded that pacing rate, in which case pacing was not utilized. Sequences A and B were performed at Stage 1 (with the intrinsic cardiac nervous system intact), Stage 2 (after RAGPnx (Group 1) or after PAGPnx (Group 2)), as well as after ablation of both of ganglia (Stage 3; see Table I).

**Data Analysis:** Data were analyzed using programs developed in-house for the PC using Microsoft Foundation Class. HR was determined beat-by-beat using the atrial electrogram recordings. AV-intervals (AVI) were determined beat-by-beat as the time between the major
deflections in the atrial and ventricular electrograms. An index of atrial inotropic function was
determined beat-by-beat as the difference between maximum and minimum intra-atrial balloon
pressure. Using these beat-by-beat data, indices of cardiac function were averaged across the 15s
vagal stimulation period (i.e., without pacing) as well as during the combination of vagal
stimulation plus pacing. Sympathetic efferent neuronally induced responses were averaged
during the last 15 seconds of stellate ganglion stimulation (i.e., prior to onset of vagal
stimulation), during the 15s of combined stimulation of stellate ganglia and cervical vagal nerves
and finally during the last 15s of combined autonomic stimulation plus pacing. Data are reported
as mean ± 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 and smaller were considered
statistically significant.

RESULTS

Chronotropic responses before and after RAGPx and/or PAGPx. Heart rates during the
initial 15 sec. of control (HRcontrol) are provided for both groups of dogs in Table II 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 parasympathetic and/or sympathetic nerve
stimulation were similar among Groups (#1 vs. #2); the magnitudes of responses elicited by
autonomic neuronal stimulation were stable with repeated trials.

Selective ablation of elements within the intrinsic cardiac nervous system disrupted
functional neural modulation of regional cardiac function, effects differing dependent upon
which neuronal populations were removed first. Selected recordings from a Group 1 dog are
shown in Figure 2 for stimulations conducted first with the intrinsic cardiac nervous system (ICN) intact (left two panels) and then following RAGPx (right two panels). Baseline HR was 105 bpm. In Stage 1 (ICN intact), stimulation of the cervical vagi reduced atrial heart rate (HRa) by 68 bpm (c.f., evidenced in the right atrial electrogram (RAE) recording). Note the pronounced negative dromotropic and inotropic responses that accompanied parasympathetic stimulation (left panel). Bilateral stimulation of stellate ganglia increased HR to 190 bpm in conjunction with increased regional contractile function and enhanced AV conduction (second panel from left). Superimposing stimulation of the cervical vagi on the on-going stellate ganglion stimulation decreased the tachycardia response by 138 bpm. Adding atrial pacing to these combined nerve stimulations revealed the occurrence of AV-nodal blockade. Atrial contractile function was likewise suppressed by these activated parasympathetic efferent neuronal inputs.

The right two panels of Figure 2 show the same subject’s responses to identical tests after ablation of the RAGP. Note, in particular, that stimulation of the vagus nerve alone minimally affected atrial rate (i.e., HRa slowed only 7 bpm; c.f. RAE in panel 3). The AV-nodal block persisted, but to a lesser degree (i.e., third degree vs. second degree, pre- vs. post-ablation). Stimulation of the vagus nerves during on-going stellate ganglion stimulation (right panel) still slowed atrial rate (192 bpm to 137 bpm), despite the elimination of the direct vagal bradycardia by the RAGPx. However, the parasympathetic-induced 55 bpm HR slowing during stellate stimulation was notably less than that which occurred (?138 bpm) during comparable situation prior to RAGPx. For this animal in Stage 3 trials (not shown, Figure 2), vagal stimulation alone slowed atrial rate by only 4 bpm; HRa slowed by 19 bpm (i.e., from 190 bpm to 171 bpm) when vagal stimulation was superimposed upon stellate ganglion stimulation.
Figure 3 is identical to Figure 2, except that this dog was a member of Group 2. With the intrinsic cardiac nervous system intact (left 2 panels), stimulation of the cervical vagi (6 Hz) slowed HR by 61 bpm (left panel); superimposition of atrial pacing revealed a 2:1 AV-nodal block. In the second panel, HR was increased to 189 bpm by stellate ganglion stimulation; HR$_a$ was reduced to 94 bpm during superimposition of vagal stimulation (HR$_a$ = -95 bpm). AV-nodal blockade was evident during atrial pacing. After PAGPx (right two panels), in marked contrast to the data shown in Figure 2, stimulation of the vagi still markedly slowed HR (HR reduced by 40 bpm), though somewhat less than occurred in the ICN-intact state. Thereafter, superimposing vagal stimulation during background stellate ganglia stimulation (right panel) slowed HR by only 25 bpm (i.e., 189 to 164 bpm). Following subsequent RAGPx (Stage 3; not shown, Figure 3), sole vagal stimulation no longer slowed atrial rate, yet vagal stimulation superimposed upon stellate stimulation continued to reduce that index (HR$_a$ = -25 bpm).

The top panels of Figure 4 summarize the negative chronotropic responses elicited by parasympathetic efferent neuronal stimulation alone for Groups 1 (left) and 2 (right) in dogs prior to (ICN intact, Stage 1), following RAPGx or PAGPx (Stage 2) and subsequent to combined RAPGx + PAGPx (Stage 3). RAGPx prevented the bradycardia induced by bilateral cervical vagal stimulation (top left panel). In contrast, PAGPx produced only a modest effect on vagally induced slowing (top right panel; compare and data sets); these differences were significant in (only) 3 of 6 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, top left panel) elicited no effect on the vagally mediated bradycardia, the addition of RAGPx to a pre-existing PAGPx ablated any residual
bradycardia induced by parasympathetic stimulation (compare and data sets, top right panel).

The bottom panels of Figure 4 summarize the chronotropic response to combined sympathetic and parasympathetic efferent neuronal stimulation for Groups 1 (left panel) and 2 (right panel) for the three stages of the study. Even though RAPGx prevented bradycardia elicited by parasympathetic efferent neuronal stimulation (top left panel), vagal stimulation in these animals continued to suppress the sympathetic-mediated tachycardia (bottom left panel), although to a lesser extent than occurred in the ICN-intact state. PAGPx likewise blunted the negative chronotropic effects of vagal stimulation when superimposed against a background of sympathetic efferent neuronal activation (bottom right panel). 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 following RAPGx induced no further change in autonomic induced changes in atrial rate (bottom left panel, compare ? and data sets), in Group 2 animals the addition of RAGPx to PAGPx further decreased HR slowing when vagal stimulation was superimposed upon bilateral stellate ganglion stimulation (bottom right panel, compare and data sets). Following combined RAGP and PAGP ablation, a significant residual slowing of the sympathetic induced tachycardia persisted in both groups at frequencies of parasympathetic stimulation greater than 9 Hz.

**AV-Interval Responses:** Tables II and III summarize changes in parasympathetic efferent neuronal control of AV nodal function prior to and subsequent to ablation of the RAGP and/or PAGP. During the pre-stimulation control period, neither AV-nodal intervals during spontaneous beats (AVIₘ) nor AV-intervals during atrial pacing at ~180 bpm (AVIₚ) were altered.
by either ganglion ablation (Stages 1 to 3); between group comparisons were likewise unaffected (Table II). 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 III 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 (30 heart block). For animals in Group 1, after RAGPx alone vagal stimulation still produced negative dromotropic effects; these observed changes were attenuated compared to 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 supramaximal stimulation levels (18 Hz stimuli). Group 2 dogs likewise exhibited a shift to lesser parasympathetic efferent neuronally induced heart block with successive ablations of the PAGP and RAGP ganglionated plexuses.

Effects of Neural Ablations upon Atrial Contractile Force: The left two panels of Figures 2 and 3 indicate that atrial pressure generation was depressed by vagal nerve stimulation in the ICN-intact state (c.f., RA tracing) without (panel 1) and with (panel 2) concurrent sympathetic efferent neuronal stimulation. Moreover, marked depression in atrial contractile function persisted following RAGPx (Figure 2) or PAGPx (Figure 3). Figure 5 summarizes the data demonstrating sympathetic-parasympathetic modulation of right atrial contractile function prior to (intrinsic cardiac nervous system intact) and following RAGPx (top panel) or PAGPx (bottom panel). These data indicate that selective extirpation 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. Following combined RAGPx
and PAGPx (Stage 3), sympathetically induced changes in atrial contractile force were significantly reduced compared to Stage 1 (287±33% vs. 190±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 neuronal stimulation.

**DISCUSSION**

Neural control of SA nodal function involves the dynamic interplay between central and peripheral reflex control mechanisms (10). While 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 intrinsic cardiac nervous system that project to pacemaker cells versus other atrial cardiomyocytes (19,26). Moreover, the SA nodal pacemaker cells themselves contribute directly to sympathetic-parasympathetic interactions via post-junctional 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 post-junctional sites of the heart.

Classically, intrathoracic autonomic ganglia have been regarded as a homogeneous aggregate of post-ganglionic efferent neurons that functioned solely as relay stations for parasympathetic pre-ganglionic efferent inputs to cardiac myocytes (33). Thus, proposed neural interactions occurring between sympathetic and parasympathetic efferent projections have been primarily restricted to pre-synaptic interactions occurring at end-effector junctions (25). It is now recognized that intra-thoracic ganglia contain heterogeneous populations of efferent, afferent and local circuit neurons (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 intrinsic cardiac nervous system may subserve, in part, sympathetic-parasympathetic interactions (19,26,30). The major novel finding of this study was that ablation of the posterior atrial ganglionic plexus significantly attenuates bradycardia
induced by activated parasympathetic efferent preganglionic neurons superimposed upon a background of increased sympathetic efferent neuronal inputs to the heart. Since, ablation of the PAGP exerted only modest effects upon 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 sino-atrial node. Presumably this occurs via a pre-synaptic mechanism that may involve intrinsic cardiac local circuit neurons (1).

It is now well established that the neurons within the right atrial ganglionated plexus are the principal source for direct vagal inhibition of the SA-node (e.g., 3,13,20,22). Activating the RAGP chemically (35) or electrically (15,20) slows atrial rate. Chemical or surgical ablation of the RAGP effectively abolishes the bradycardia associated with sole vagal stimulation (3,18). Yet, even after RAGP ablation, cervical vagal stimulation still suppressed the sympathetically-induced tachycardia; this residual attenuation was abolished by atropine (26). From these studies, McGuirt, 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 3 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). Yet in the current study, the induced bradycardia to sole parasympathetic stimulation was affected by PAGP ablation. The attenuation of this response
could reflect partial interruption of vagal preganglionic axons to the RAGP that traverse *en passant* the PAGP region (3). 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. Using 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 ablation of the PAGP has been shown to potentiate the sympathetic 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 upon the circulation by physiological, environmental and psychological challenges (7). Very recent data, in fact, affirm an anatomical communication between the RAGP and the PAGP (23): Injecting a retrograde tracer into the cat RAGP resulted in 71.4 ± 6.8% of neurons in PAGP being labeled. With respect to central nervous projections to the cardiac ganglia, retrogradely labeled neurons were found exclusively in the ventrolateral nucleus ambiguous (Namb) after injection of the tracer into PAGP. This same investigator (23) also performed a dual fluorescent retrograde neuronal tracer study to determine if 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 co-ordination of cardiac function between intrinsic cardiac ganglia in co-operation with the central nervous system.

Although Priola, et al. (29) concluded that these neural elements had only limited effects on cardiac performance 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 intrinsic cardiac nervous system 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 (1,8) that proposes the existence of nested “circuits” involving elements both intrinsic and extrinsic to the heart, all of which regulate and co-ordinate 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 not only contribute to the regulation of SA nodal function via its parasympathetic efferent postganglionic neurons, but also via pre-synaptic mechanism exerted upon its sympathetic postganglionic neurons involved in regulating atrial pacemaker tissues. Yet, even after combined ablation of the RAGP and PAGP, residual parasympathetic restraint on sympathetic inputs to the SA node still exists. While 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 intrinsic cardiac nervous system to mediate efferent neuronal projections to the SA node is portrayed in Figure 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 post-junctionally at end-effector sites. For post-junctional autonomic interactions, Figure 6 depicts the interdependence between G-coupled receptor systems onto adenylyl 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 intrinsic cardiac nervous system to project directly to specific cardiac target tissues, others project into the intrinsic cardiac nervous system 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, (2)) or are local circuit neurons (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 (3,30,35). 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 (3,31,32). 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 pre-synaptic 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 intrinsic cardiac nervous system is to maintain and coordinate regional cardiac function (1). 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 (3,31). 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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FIGURE LEGENDS

Figure 1: Summary of experimental protocol. Bilateral stimulation of the cervical vagi (Parasymp. Stim.) with and without atrial pacing was conducted alone (panel A) or in conjunction with bilateral stimulation (4 Hz) of the stellate ganglia (Sympathetic Stimulation) (panel B). Both stimulation protocols were completed for all vagal stimulation frequencies (0, 1, 3, 6, 9, 12 and 18 Hz) in the decentralized, but ICN-intact state (Stage 1), following surgical ablation of the right atrial ganglionated plexus (Group 1, RAGPx) or of the posterior atrial ganglionated plexus (Group 2, PAGPx) in Stage 2, and following combined removal of both sets of intrinsic cardiac ganglia (Stage 3, RAGPx+PAGPx).

Figure 2. Induced changes in regional cardiac function in response to parasympathetic and/or sympathetic stimulation before (Intact, left two panels) and after surgical ablation of the right atrial ganglionated plexus (RAGPx; right two panels). 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,” leftmost panel) alone, and when superimposed on a background of stellate ganglia stimulation (“Stellate + Vagus + Pace,” second panel). 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 (rightmost panel). Autonomic modulation of dromotropic and inotropic (atrial and ventricular) functions were likewise maintained following RAGP ablation. 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 bpm. S_on = beginning of stellate ganglia stimulation. Off = termination of pacing and of stimulation of both the vagi and stellate ganglia.
Figure 3: Induced changes in regional cardiac function in response to parasympathetic stimulation (6 Hz) and/or sympathetic stimulation before (Intact, left two panels) and after surgical ablation of the posterior atrial ganglionated plexus (PAGPx; right two panels). Presentation and notations as in Figure 2. PAGPx slightly reduced the bradycardia induced by vagal stimulation alone (panel 3), and attenuated the HR slowing elicited when vagal stimulation was superimposed upon on-going sympathetic stimulation (panel 4).

Figure 4. Frequency response curves for chronotropic responses to parasympathetic stimulation alone (top panels) or in combination with sympathetic stimulation (bottom panels) before (ICN intact, Stage 1) and after atrial surgical ablation of the ventral right atrial ganglionated plexus (RAGPx, left panels, Stage 2) or posterior atrial ganglionated plexus (PAGPx, right panels, Stage 2) and then following combined ablation of both intrinsic cardiac ganglia (Stage 3). Chronotropic responses are indicated as percent change from prestimulation control levels (± SE). * p<0.05 versus ICN intact, + p<0.05 Stage 2 versus Stage 3 ablation, # p<0.05 RAGPx versus PAGPx (Stage 2 between group comparison).

Figure 5. Frequency response curves for atrial inotropic responses to parasympathetic stimulation in combination with sympathetic stimulation before (ICN intact, Stage 1) and after surgical ablation of the ventral right atrial (RAGPx, top panel, Stage 2) or posterior atrial (PAGPx, bottom panel, Stage 2) ganglionated plexus. Inotropic responses are indicated as percent of prestimulation control levels (± SE) as determined from the difference between minimum and maximum pressures derived from the intra-atrial balloon placed in the right atrium. While PAGPx slightly attenuated the positive inotropic response to sole sympathetic stimulation (panel B, left hand point), overall neither RAGPx nor PAGPx significantly altered the sympathetic-parasympathetic interactions for control of right atrial contractile function.
Figure 6. Schematic of proposed neural interactions occurring within the peripheral autonomic ganglia and at the nerve-effector junction for autonomic control of SA-nodal function. This model depicts the proposed sites for interactions in the efferent outflow to the heart. While neurons within RAGP directly project to and inhibit SA-node function, neurons within PAGP are responsible, in part, for the interaction between sympathetic and parasympathetic control of atrial rate via a presynaptic inhibition of neurotransmitter release from the sympathetic fibers. Additional intrinsic cardiac ganglia, not located within RAGP or PAGP, also contribute to the parasympathetic modulation of sympathetic input to the SA node.
Table I: *Experimental Design*

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<tr>
<th>Stage</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Stage 1</td>
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<td>Intrinsic cardiac ganglia intact</td>
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<tr>
<td>Stage 2</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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Table II: Heart Rate and AV-Interval during Pre-Stimulus Baseline for each stage of ICN ablation.

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<tr>
<td></td>
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<td>Stage 3</td>
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<td>Stage 2</td>
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Average ± SE for 15 sec. control prior to 0 Hz vagal stimulation trials. HR_{control} = heart rate during spontaneous rhythm. AVI_S = AV-interval for spontaneous rhythm. AVI_P = AV-interval when atria were paced at 180 bpm.
Table III: AV-nodal function during bilateral stimulation of the cervical vagus nerves

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Entries indicate number of animals experiencing indicated degree of AV-nodal block
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

Graph showing the relationship between atrial contractile force (% baseline) and parasympathetic stimulation frequency (Hz) for different groups:
- RAGPx
- ICN intact

Graph demonstrates a decrease in atrial contractile force as parasympathetic stimulation frequency increases.
Higher Centers

Sympathetic Efferent Soma

LCN

Sympathetic Efferent Soma

Parasympathetic Efferent Soma

Spinal Cord

Medulla

Brainstem

Spinal Cord

Extracardiac Intrathoracic Ganglia (Stellate, Middle Cervical)

Intrinsic Cardiac Ganglionated Plexus

Sinoatrial node

ATP cAMP

Figure 6