THE INTRINSIC CARDIAC NERVOUS SYSTEM in TACHYCARDIA INDUCED
HEART FAILURE

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ABSTRACT

Objective: To test the hypothesis that early stage heart failure differentially affects the intrinsic cardiac nervous system’s capacity to regulate cardiac function. Methods: Following 2 wks of rapid ventricular pacing, in 9 anesthetized canines, cardiac and right atrial neuronal function were evaluated in situ in response to enhanced cardiac sensory inputs, stimulation of extracardiac autonomic efferent neuronal inputs and close coronary arterial administration of neurochemicals that included nicotine. Right atrial neuronal intracellular electrophysiological properties were then evaluated in vitro in response to synaptic activation and nicotine. Intrinsic cardiac nicotine sensitive neuronally induced cardiac responses were also evaluated in 8 sham-operated, unpaced animals. Results: Two weeks of rapid ventricular pacing reduced the cardiac index by 54%. Intrinsic cardiac neurons of paced hearts maintained their cardiac mechano- and chemo-sensory transduction properties in vivo. They also responded normally to sympathetic and parasympathetic preganglionic efferent neuronal inputs, as well as to locally administered α- or β-adrenergic agonists or angiotensin II. The dose of nicotine needed to modify intrinsic cardiac neurons was 50 times greater in failure compared to normal preparations. That dose failed to alter monitored cardiovascular indices in failing preparations. Phasic and accommodating neurons identified in vitro displayed altered intracellular membrane properties compared to control, including decreased membrane resistance, indicative of reduced excitability.

Conclusions: Early stage heart failure differentially affects the intrinsic cardiac nervous system’s capacity to regulate cardiodynamics. While maintaining its capacity to transduce cardiac mechano- and chemosensory inputs, as well as inputs from extracardiac autonomic efferent neurons, intrinsic cardiac nicotine sensitive local circuit neurons differentially remodel such that their capacity to influence cardiodynamics becomes obtunded.
Introduction

Alterations in sympathetic and parasympathetic efferent neuronal control of cardiac function have been implicated in animal models of congestive heart failure (CHF), as well as in human heart failure. Generally, it has been thought that there is a progressive blunting in the capacity of sympathetic efferent neurons to support the failing heart (14; 18). Three primary functional alterations have been reported to occur secondary to a generalized, tonic enhancement of sympathetic efferent neuronal activity in CHF: 1) increased release of norepinephrine from sympathetic efferent postganglionic nerve terminals that over time induces down-regulation of cardiac myocyte β-adrenoceptor function (14; 21; 23), 2) leading to alterations in the cardiomyocyte second messenger system (15) and 3) the eventual depletion of norepinephrine stores in intracardiac sympathetic efferent postganglionic nerve terminals secondary to their tonic excitation (17). Recently it has been reported that ventricular myocyte cell surface β-adrenoceptor density and affinity are similar in tachycardia-induced (30) and genetically derived (37) models of heart failure to those identified in normal hearts. Presumably that is why exogenously administered β-adrenergic agonists can augment heart rate and ventricular inotropy in a canine model of tachycardia-induced heart failure (16).

Altered parasympathetic efferent neuronal control of the failing heart has also been ascribed to changes occurring at the level of the cardiac myoneural junction (11; 19). However, parasympathetic efferent preganglionic axons, when stimulated electrically, induce bradycardia and reduce ventricular contractile force in the canine model of tachycardia-induced heart failure to degrees that approximate those identified in normal preparations (38). Moreover, ventricular myocyte cell surface cholinergic receptor affinity and numbers are similar in this model of heart failure to those identified in normal ventricular tissues (38).
Recent data have suggested that synaptic function within cardiac ganglia may be compromised during the evolution of congestive heart failure (12), with important consequences for cardiac control. We have proposed that the intrinsic cardiac nervous system comprises afferent, parasympathetic efferent, sympathetic efferent and interconnecting local circuit neurons that interact locally as well as with neurons in higher centers to reflexly coordinate regional cardiac behavior (1; 6). Local circuit neurons represent the vast majority of functionally identified neurons in the mammalian intrinsic cardiac nervous system. We have defined this population of neurons as those that do not project their axons outside their respective ganglia/ganglionated plexus to either the target organ or to central neurons (6). We have proposed that the functional connectivity that exists among the different populations of intrathoracic neurons, reflective of shared inputs from the spinal cord and brainstem neurons, resides primarily with its local circuit neurons (6; 9). We have also proposed that this population acts to exert an overall stabilizing influence on the intrinsic cardiac nervous system to mitigate imbalance among efferent neurons innervating disparate cardiac regions in the presence of pathology (9).

There is mounting evidence that early treatment of heart failure may minimize the long term detrimental effects of such pathology (20; 29). To date, the capacity of the intrinsic cardiac nervous system to transduce sensory inputs from the failing heart, along with inputs from central efferent cholinergic and adrenergic neurons in the control of residual heart function, is unknown. Understanding the synergism between neuronal and myocyte remodeling during early stage heart failure might provide a rationale basis for targeted and time appropriate neurocardiac therapeutic strategies.

The primary objective of this study was to determine if early stage, pacing-induced heart failure modifies the function of the intrinsic cardiac nervous system and, if so, which elements
within it become affected. In order to do this, we evaluated the capacity of the failing heart’s intrinsic nervous system to transduce the cardiac mechanical and chemical milieu. We also determined its capacity to transduce inputs from central autonomic efferent neurons involved in controlling cardiodynamics. Then the capacity of selected components of the intrinsic cardiac nervous system, in particular its nicotine sensitive local circuit neurons, to influence failing heart status was investigated *in vivo*. Finally, we assessed the response characteristics of intrinsic cardiac neurons derived from the failing heart to nicotinic synaptic inputs *in vitro*. The results of this study indicate that while the intrinsic cardiac nervous system is capable of transducing the mechanical and chemical milieu of the failing heart as well as inputs from extracardiac autonomic efferent neurons, the capacity of its nicotinic sensitive local circuit neurons to transduce these inputs remodels such that their ability to influence cardiodynamics becomes obtunded early on during the evolution of CHF.

**Methods**

Adult preconditioned mongrel dogs (*n* = 18) of either sex, weighing between 15-22 kg, were employed in this study. 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 committees of Dalhousie University, East Tennessee State University and the University of Montreal approved the experiments.

*Survival Surgical Procedures.*

*Induction of pacing-induced CHF.* Animals were anesthetized with sodium thiopental (25 mg/kg iv); thereafter, anesthesia was maintained with isoflurane. In 10 of these animals that constituted
the experimental group, under sterile conditions the right jugular vein was isolated via a midline neck incision and a Swan Ganz catheter introduced through it into the main pulmonary artery. Cardiac output was determined via the thermodilution technique, the values so obtained representing the pre-pacing state during rest. The catheter was withdrawn and a bipolar pacing electrode (Medtronic Inc, #IS-1-B1-ATR) was inserted using fluoroscopy such that the tip of the pacing electrode was positioned in the right ventricular apex. An implantable pacemaker (multiprogrammable pulse generator SX5984, Medtronic Inc., Minneapolis, Minn.), connected to these electrodes, was placed under the skin of the neck. The neck incision was then closed and the animal allowed to recover. Analgesic therapy (morphine: 0.5 mg/kg i.m.) was given post-operatively at 8-hr intervals for 24 hr and as needed thereafter. Antibiotic (Cephalexin, 500 mg 2x daily) therapy was administered for 5 days after surgery. Ventricular pacing was begun 2 weeks following the pacemaker implant. The pacemaker was set to pace the ventricles at a rate of 240 beats/minute (16); rapid ventricular pacing was maintained continuously thereafter for 2 weeks. Continuous ventricular capture was assured on a daily basis. The clinical status of each animal was monitored on a daily basis to detect signs of cardiac failure (ascites, dyspnoea, fatigue, lack of appetite); these signs were never apparent for this group of animals. Some weight gain occurred in each of these animals. In the paced group, functional experiments were performed two days after discontinuing 2 weeks of ventricular pacing. This was done to minimize any direct effects on the intrinsic cardiac nervous system of exogenous electrical stimuli delivered via the pacing electrodes.

Sham-operated control animals. In order to compare cardiodynamics effects of locally applied nicotine, a group of 8 sham-operated dogs were prepared. A midline neck incision was performed under aseptic conditions after induction of anesthesia as described above. After 30 min, the neck incision was closed and anesthesia discontinued. Pain and antibiotic therapy were
then instigated as described above for paced animals. Functional studies were performed in the sham-operated group 2 weeks following surgery.

**Terminal studies**

The dogs (pacing induced CHF and sham-operated control groups) were anesthetized with sodium thiopental (15 mg/kg iv), supplemental doses (5 mg/kg iv) being provided every 5-10 minutes throughout the surgery. After all surgery was completed, anesthesia was changed to α-chloralose (20-35 mg/kg iv) that was provided in hourly doses or more frequently as needed for the duration of the terminal experiments. Noxious stimuli were applied periodically to a paw throughout the experiments to ascertain the adequacy of the anesthesia. When neuronal activity was recorded, spontaneous activity was suppressed for 5-10 min after bolus injections of α-chloralose due to the neuronal depressor effects of this agent. Therefore, at least 10 min were allowed to elapse after such injections before recordings proceeded.

Following induction of anesthesia, dogs were intubated and placed on positive pressure ventilation using a Bird Mark 7A respirator driven with an air-oxygen mixture to maintain blood gases within the physiological range. For the paced group, a Swan-Ganz catheter was inserted into the pulmonary artery via the left jugular vein and cardiac output was determined by thermodilution (average of three recordings). These cardiac outputs served as closed chest paired measurements for those obtained at pacemaker lead implant. Following determination of cardiac output, a catheter was placed in the aortic root via one femoral artery to record aortic pressure and to administer pharmacological agents into the systemic circulation. Then a femoral vein was catheterized to deliver normal saline and the anesthetic agent to each animal throughout the experiments. A bilateral thoracotomy was made through the fourth intercostal space. A PE50 catheter was inserted via the right atrial appendage into the right ventricular chamber. Left
ventricular chamber pressure was monitored via a # 6 French Cordis catheter was inserted into that chamber via the other femoral artery. The latter 2 catheters and the catheter in the aortic root were attached to Bentley Trantec (Irvine, CA) model 800 pressure transducers.

In order to administer chemicals to the somata of neurons in the right atrial ganglionated plexus of sham operated and pacing-induced heart failure preparations, a 24-French catheter was inserted into the lumen of the right coronary artery. The cannula was threaded proximally (retrograde to blood flow) to position its tip cranial to the origin of the arterial branch supplying blood to the ventral right atrial ganglionated plexus. This placement was confirmed by gentle palpation of the cannula tip through the artery wall. The cannula was then fixed to the arterial wall with cyanoacrylate adhesive. PE-15 tubing was then attached to this cannula with a stopcock at its other end to permit the administration of chemicals into the local arterial blood supply of neurons in the right atrial ganglionated plexus. Hemodynamic indices remained unaffected by the placement of this cannula. Post-mortem examination of appropriate catheter placement was confirmed by injecting methylene blue dye through this catheter.

Intrinsic cardiac neuronal activity recorded in vivo

The right atrial ganglionated plexus on the ventral surface of the right atrium (40) was exposed in the pacing-induced CHF group. A circular ring of heavy-gauge wire was placed gently on epicardial fat containing this neuronal plexus in order to minimize local motion of the atrial wall. This fat was explored with a tungsten microelectrode mounted on a micromanipulator at depths ranging from the surface of the fat to regions adjacent to cardiac musculature (8). A tungsten microelectrode (250 µm shank diameter; impedance 9-11 MΩ at 1000 Hz) was employed. Proximity of its tip to cardiac musculature was indicated by increases
in the amplitude of the recorded ECG artifact. The indifferent electrode was attached to pericardium adjacent to the heart.

Electrical signals generated by right atrial neurons were amplified differentially by a Princeton Applied Research model 113 amplifier with bandpass filters set at 300 Hz to 10 kHz and with an amplification range of 100-500X. The output of this device, further amplified (50-200X) and filtered (band width 100 Hz-2 kHz) by means of an optically isolated amplifier (Applied Microelectronics Institute, Halifax, N.S., Canada), was led to a Nicolet model 207 oscilloscope, a Grass AM8 Audio Monitor and a digital data converter and videotape recorder (Model 3000, A. R. Vetter Co., Rebersberg, PA). The activity generated by the individual neurons was identified by the amplitude and shape of recorded action potentials (APs). Loci were identified in the right atrial ganglionated plexus from which APs with signal to noise ratios greater than 3:1 could be recorded. These techniques permit the recording of APs generated by local neuronal somata and/or neurites rather than axons of passage (8). Efferent neurons in this ganglionated plexus are primarily associated with control of sinoatrial nodal function (3; 35; 40), receiving inputs from extracardiac sympathetic and parasympathetic efferent neurons (22), direct afferent inputs from cardiac mechano- and multimodal neurites (2) and contains a substantial population of interneurons defined as local circuit neurons (5; 22).

Sensory inputs to intrinsic cardiac neurons. Ventricular mechanosensory inputs to identified right atrial neurons were activated by touching various right and left ventricular epicardial loci gently with a saline soaked cotton swab. Once the extent of a ventricular epicardial region associated with identified sensory neurites was determined, veratridine (5x10^{-6} gm; Sigma-Aldrich) was applied to that site for 60 seconds using 1 cm x 1 cm gauze squares soaked with 0.5 ml of the chemical. Veratridine is a Na^+ channel modifier that activates cardiac chemosensory neurites in a reproducible manner (35). Sensory fields were washed for about 30
seconds with normal saline (~2 ml/sec) after the veratridine soaked pledget was removed, at least 5 minutes being allowed to elapse before the next intervention. Veratridine was reapplied to an active 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 veratridine were due to vehicle effects or the mechanical effects elicited by gauze squares.

**Local chemical administration.** Chemicals, obtained from Sigma-Aldrich Inc (Oakville, Ont., Can.), were dissolved in 0.9% saline and administered (0.1 ml) into the local arterial blood supply of identified right atrial neurons. Nicotine (100 µM) was administered in bolus doses of 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ml into the local coronary arterial blood supply of right atrial neurons of failing hearts. Nicotine (100 µM) was applied in 0.1 ml doses to the right atrial neurons of the sham-operated animals since this dosage represents a suprathreshold one to elicit neuronal and resultant cardiodynamic responses by directly activating intrinsic cardiac neurons with minimal contributions from extracardiac neural elements (27; 31). In order to test the effects of systemically administered nicotine on neuronal and cardiovascular indices, 0.1 ml of nicotine (100 µM) was injected into the aortic blood.

Since catecholamines and angiotensin play major roles in the etiology of CHF (1; 18; 20), the other chemicals tested in the pacing induced heart failure model included: the alpha1-adrenoceptor agonist phenylephrine (100 µM); the alpha2-adrenoceptor agonist clonidine (100 µM); the beta1-adrenoceptor agonist dobutamine (100 µM); the beta2-adrenoceptor agonist terbutaline (100 µM); and the peptide angiotensin II (100 µM). The doses of these agonists (0.1 ml) used in this study have previously been shown to be capable of directly modifying intrinsic cardiac neuronal activity when applied to them via their local arterial blood supply without entering the systemic circulation in sufficient quantities to modify distant tissues such as cardiac
myocytes, thereby preventing intrinsic cardiac neuronal responses secondary to cardiac mechano-receptor activation (27). The order of administering these latter agents varied among experiments. In order to test their effects if they entered the systemic circulation in sufficient doses to modify monitored indices, each agent was also administered individually into the aortic blood in the same doses as used in close arterial injections to identified neurons.

*Electrical stimulation of extrinsic cardiac efferent inputs.* Right and left stellate ganglia and cervical vagosympathetic trunks were stimulated (5 V, 4 ms, 10 Hz) individually for 20-second periods via bipolar electrodes attached to a Grass SD-9 square wave stimulator. Enough time was allowed to elapse between these interventions for hemodynamic variables to return to baseline values.

*Intracellular recordings from failing heart intrinsic cardiac neurons in vitro*  
Immediately after completing the *in situ* experiments in 7 heart failure preparations, the ventral right atrial ganglionated plexus was removed and placed in a dish containing modified Krebs' solution (composition in mM: NaCl, 120; NaHCO₃, 25; NaH₂PO₄, 1; KCl, 5; MgCl₂, 2; CaCl₂, 2.5; D-glucose, 11; pH 7.4) equilibrated with 95 % O₂/5 % CO₂ gas at room temperature. Most of the residual atrial muscle was trimmed away and the tissue was transferred to a recording chamber (5 ml volume), pinned to the silicone rubber chamber floor and superfused with modified Krebs' solution at 36°C at a flow rate of 5-10 ml/min. With the aid of a dissecting microscope, ganglia in the underlying fat were exposed by blunt dissection. Ganglia were mechanically stabilized via a small metal platform placed under it.

Pipette electrodes made from standard borosilicate capillary tubing were drawn to fine tips using a micropipette puller (Model P87, Sutter Instruments, Novato, CA); these electrodes, when filled with 3 M KCl, had resistances of 50-80 MΩ. Electrodes were advanced through the
ganglion sheath using a mechanical 3-axis micromanipulator; a sudden drop in potential at the electrode tip signaled cellular impalements. Transmembrane potentials were recorded in current-clamp mode using a standard intracellular amplifier (Model 1600, A-M Systems, Everett, WA). Before penetrating a ganglion, microelectrode resistance was nulled with the amplifier's bridge-balancing circuitry and amplifier offset; electrode tip potentials were nulled to establish zero volt levels relative to the bath reference electrode. The reference electrode consisted of a pipette containing 1% agar dissolved in 3 M KCl with its tip immersed in the bath solution; this was connected to the amplifier by Ag wire coated with AgCl. Transmembrane electrical potentials were determined as the difference between the bath reference potential and the intracellular electrode potential. At the end of trials on each neuron, the electrode was withdrawn into the bath and the zero voltage level confirmed.

Neurons were activated intracellularly by directly injecting current through the recording electrode using voltage-to-current conversion circuitry in the amplifier, driven by rectangular pulses generated by a Model S-88 Grass Instrument Co. (Quincy, MA) stimulator. Nerves connecting to ganglia containing identified neurons were then stimulated using bipolar Ag wire electrodes connected to a second stimulator via a constant-current photo-isolation unit (Grass Model PSIU6). Current and voltage waveforms, monitored on an oscilloscope, were recorded for later analysis in digital format on videotape (Model 3000, A. R. Vetter Co.). Thereafter, the nicotine receptor antagonist hexamethonium chloride (100 \mu M) was applied to these preparations via the bathing medium.

In order to estimate whole-cell resistance, hyperpolarizing current was injected through the recording electrode and voltage responses to at least 6 different current intensities recorded for each neuron. Resistance was estimated from the slope of a plot relating hyperpolarizing current intensity against magnitude of voltage displacement from the resting level. Neuronal time
constant, estimated from membrane responses to small (0.1 nA) hyperpolarizing currents, was calculated as the time for the membrane potential to change by \(1 - \frac{1}{e}\) of the final steady-state potential value obtained during hyperpolarization. Whole-cell membrane capacitance was calculated as the quotient of the time constant and input resistance. AP duration was measured at half the amplitude of the AP; AP firing threshold was derived from the membrane potential value at the inflection point during the rapidly rising phase of the AP. Afterhyperpolarization (AHP) duration was estimated as the time for the membrane potential to repolarize to half maximum AHP amplitude. The membrane potential responses elicited by nicotine (100 \(\mu\)M dissolved in perfusate) were evaluated by local pressure application from the tip of a pipette placed within 100 \(\mu\)m of investigated ganglia.

Data analysis

In vivo experimental data analysis: Cardiovascular indices derived from 10 consecutive cardiac cycles in sham operated and pacing-induced heart failure preparations were analyzed before and during peak responses elicited by each intervention. Spontaneous fluctuations in cardiodynamics were minimal during data collection periods prior to interventions; heart rate varying less than 5 beats/min and systolic pressure fluctuating less than 5 mm Hg. Thresholds for determining whether cardiovascular changes had been induced were thus chosen to be greater than these ranges. For the paced group, APs generated at an active locus in the right atrial ganglionated plexus were counted for 30-second periods in order to establish average neuronal activity generated immediately prior to and during maximal responses elicited by each intervention. Fluctuations in the amplitude of APs generated by a unit varied by less than 0.1 mV over several minutes; the waveforms of these APs retained their configurations over time. Thus, APs recorded in a given locus with the same configuration and amplitude (± 0.1 mV) were
considered to be generated by a single unit. Only those APs recorded with signal-to-noise ratios greater than 3:1 were analyzed. The magnitudes and directionality of the neuronal 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. Since chemicals induced either excitatory or depressor neuronal activity responses, depending on the preparation studied, responses derived from all active neurons identified in each animal were also normalized to absolute alterations from baseline values (impulses per minute). Data are expressed as means ± SE. One-way ANOVA 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 comparisons.

*In vitro experimental data analysis*: Selected portions of the recorded data obtained during the *in vitro* experiments were played back from the tape into a personal computer through an analog-to-digital converter (Digidata 1200, Axon Instruments, Foster City, CA). These data were analyzed with Axon Instruments pCLAMP6 software. Data are presented as means ± SE. Pairwise comparisons between means were done using student’s two-tailed t-test, adjusted for unequal n, with p < 0.05 for these comparisons.

**Results**

*Overview of failure preparations*. Cardiac indices (closed chest) were determined in the anesthetized state in the paced group prior to pacemaker implant and subsequently repeated in the terminal study. The average resting cardiac index before initiating the pacing protocol was 3.63±0.49 liters per minute. Animals were paced for 2 weeks; pacing was stopped 2 days prior to initiation of the terminal studies. The average cardiac index obtained in the closed-chest, paced group after the pacing was discontinued and just before instrumenting the animals for
performing the functional studies was 1.65±0.18 liters per minute, a 54% decrease from control values. Ten animals entered the pacing protocol group; one of these animals died following instrumentation, immediately following administering 1 ml of room temperature saline into the arterial blood supply of the ventral right atrial ganglionated plexus. Therefore, the paced data presented herein include those obtained from the other 9 paced animals.

As has been found in normal preparations (5), spontaneous activity was generated by, on average, 3 neurons in a locus of the right atrial ganglionated plexus in each paced animal. The activity generated by individual neurons was readily discerned by the heights and configurations of the action potentials identified at each site. During basal states, neurons at a locus in this ganglionated plexus generated an average aggregate activity of 41±5 impulses per min (ipm). Overall, the level of intrinsic cardiac neuronal activity was relatively similar among animals prior to the initiation of the interventions.

Transduction of ventricular mechanosensory stimuli. When sensory fields on the epicardium of the right or left ventricles of previously paced hearts were touched, the activity generated by neurons identified in the right atrial ganglionated plexus changed by 43±7 ipm (Table 1). Overall, the basal and neural-evoked activity to cardiac mechanical stimuli did not show a direct phase relationship to the cardiac cycle. Epicardial mechanical stimuli immediately initiated an increase in neuronal activity in 6 animals (28±6 to 73±17 ipm, ns); it decreased neuronal activity in the other 3 animals (50±8 to 15±7 ipm, ns). Following removal of the mechanical stimulus neuronal activity rapidly returned to control values. Epicardial mechanosensory fields that affected identified neurons were located on the outflow tract of the right ventricle (n = 7 animals) and the cranial, ventral surface of the left ventricle (n = 3 animals). Monitored cardiovascular indices were unaffected by these mechanical interventions.
Transduction of ventricular chemosensory stimuli. When veratridine was applied to these identified ventricular epicardial loci, neuronal activity increased in 7 dogs (Fig. 1; 34±5 to 89±12 ipm, p < 0.01) and decreased in the other 2 dogs (85±19 to 21±2 ipm, ns). Veratridine changed absolute neuronal activity from baseline values by 59±8 impulses per minute overall (p < 0.01, Table 1). Veratridine did not affect monitored cardiovascular variables (Table 1).

Local arterial administration of chemicals: In sham-operated animals (n=8), close arterial administration of nicotine (100 µM, 0.1 ml) into the blood supply of the ventral right atrial ganglionated plexus induced a biphasic response that consisted of an initial rapid bradycardia and negative inotropic response (atrial and ventricular) followed by positive chronotropic and inotropic responses (Fig. 2). In the paced group (n=9), cardiovascular indices were unaffected when that dose of nicotine was administered in a similar fashion to right atrial neurons (Table 1, Fig. 2) or into the systemic circulation. The dose of nicotine that was locally administered via close coronary arterial injections to failure preparations had to be increased 50-fold in order to induce neuronal activity change (delta 45±8 ipm; p < 0.01) (Fig. 3). In those instances, neuronal activity increased (38±3 to 95±7 ipm; p < 0.01) in 6 animals while decreasing (48±15 to 28±12 ipm; ns) in the other 3 animals. Neuronal activity changes induced in failure preparations by this large dose of nicotine were not accompanied by alterations in recorded cardiovascular variables (Table 1).

Local arterial administration of angiotensin II altered the activity (delta 48±12 ipm from base line values; p < 0.01; Table 1) generated by neurons identified in each failure preparation, increasing neuronal activity in 7 dogs (31±9 to 87±15 ipm; p < 0.01) while decreasing it in the other 2 dogs (40±10 to 17±5 ipm; ns). The activity generated by neurons in 6 animals was altered by local arterial administration of the alpha1-adrenoceptor agonist phenylephrine (delta 42±9 ipm; p < 0.01), the beta1-adrenergic agonist dobutamine (delta 59±0 ipm; p < 0.01) and the
beta$_2$-adrenergic agonist terbutaline (delta 32±8 ipm; p < 0.01) (Table 1). The activity generated by identified neurons changed in only 3 dogs (delta 49±7 ipm) when they were exposed to local arterial administration of the alpha$_2$-adrenoceptor agonist clonidine (Table 1). Owing to the low dose of neurochemicals so administered, monitored cardiovascular variables were not affected by local arterial administration of these pharmacological agents. Corresponding systemic administration of these agonists in the doses utilized for close arterial administration failed to alter neuronal activity or monitored cardiovascular indices.

Extracardiac autonomic efferent neuronal inputs to the heart: In 6 chronically paced animals, right or left stellate ganglion stimulation increased heart rate by 56% and 43%, respectively. Right or left stellate ganglion stimulation increased left ventricle systolic pressure generated by failing hearts (+65% and 82%, respectively). When the right or left stellate ganglion was stimulated, the activity generated by neurons identified in paced animals increased over time (Fig. 4) such that activities reached 230% (23±7 to 76±18 ipm) and 107% (28±9 to 58±19 ipm), respectively, above control values before terminating these stimuli. The activity generated by identified neurons gradually returned to baseline values after terminating these stimuli. None of the recorded neurons were activated after a fixed latency following application of such electrical stimuli.

Stimulation of the right or left cervical vagosympathetic trunks enhanced the activity generated by right atrial neurons in the failure preparations by 105% and 87%, respectively. None was activated after a fixed latency by electrical stimuli delivered to vagal efferent axons. The neuronal activity responses so induced took time to evolve and lasted after the termination of the vagal stimuli. Neuronal activity changes induced by vagal stimulation were accompanied by bradycardia (right side: 119±6 - 44±10 beats/minute; left side: 112±68 - 63±14 beats/minute).
Intracellular properties of intrinsic cardiac neurons derived from paced hearts. Data obtained from 29 intrinsic cardiac neurons derived from 7 paced hearts displayed two types of action potential (AP) responses to intracellular depolarizing current pulses (1 s duration, intensity set to produce maximal number of action potentials) (Table 2). Fifty-nine % (17 of 29) discharged one or two APs during depolarization, these neurons being classed as "phasic". The remaining neurons (41 %, 12 of 29) discharged action potentials at high initial rates that decremented with time during the depolarization current pulses; these neurons were classed as "accommodating". The membrane properties and response characteristics of these neurons were sorted according to their firing behavior (Table 2) and compared with data from normal control animals in a previous study from our laboratories (33). The functional distribution of identified neurons, phasic to accommodating, was similar between control and CHF groups. The mean resting membrane potentials for all populations of neurons from both groups were similar. Membrane capacitance was similar for phasic and accommodating neurons (phasic = 186±28 pF, accommodating 175±25 pF). The neuronal time constant in accommodating neurons (12.6±1.6 ms) significantly exceeded that in phasic neurons (7.1±1.3 ms). The mean whole-cell resistance of accommodating neurons was significantly greater than that of phasic neurons in both groups, but for both populations of neurons it was reduced in the CHF group compared to control. Within groups, AP amplitude was greater in accommodating neurons and between groups mean AP and afterhyperpolarization (AHP) amplitudes were increased in CHF animals compared to control. While AHP duration for accommodating neurons was longer in control animals, it was differentially reduced in CHF animals to a level similar to that of phasic neurons.

Single-pulse stimuli delivered to nerves connected to intrinsic cardiac ganglia containing studied neurons elicited depolarizing responses in 16 of 19 neurons derived from the pacing-induce CHF group. Nine of these neurons were phasic and 7 were accommodating; responses
elicited by nerve stimulation were similar in both types of neurons. Fourteen neurons exhibited graded levels of depolarization in response to graded increases in nerve stimulus intensity. The magnitude of depolarizations elicited in 12 of these neurons exceeded threshold for AP generation (Fig. 5 Ai). When the perfusate lacked Ca\(^{++}\) and contained 10 mM Mg\(^{++}\), these neurons were no longer affected by intra-ganglionic nerve stimulation indicating that responses to nerve stimulation were orthodromically mediated. Two of the neurons that responded to nerve stimulation did not display graded depolarizations in response to graded stimulus intensities, and these neurons generated APs that arose abruptly from their resting membrane potentials once nerve stimulation intensity exceeded threshold. Responses elicited by these neurons were not eliminated in low-Ca\(^{++}\)/high Mg\(^{++}\) perfusate; furthermore, these neurons generated APs that followed high-frequency nerve stimulation (>50 Hz). Such responses were likely the result of antidromic activation of their axons in the nerves being stimulated. All responses to nerve stimulation were depolarizing in nature.

*Nicotine-induced neuronal responses in vitro.* The effects of nicotine were evaluated in 4 phasic and 4 accommodating neurons derived from paced hearts. All of these neurons responded to nicotine similarly. Prior to nicotine application, APs were generated orthodromically by single-pulse nerve stimuli as well as by intracellular currents (Fig. 5 Ai). Nicotine (100 \(\mu\)M, applied for 50 - 200 ms via pressure-ejection from the adjacent pipette tip) induced membrane depolarization (range 10-20 mV; average amplitude, 16±3 mV). When such depolarization exceeded firing thresholds, multiple APs were generated during the depolarization rising phase (Fig. 5Aii). The neuronal membrane became fully depolarized within a minute after exposure to nicotine. As depolarization peaked, blockade of AP generation occurred; this blockade lasted longer than the membrane repolarization duration (Fig. 5Aiii).
In the presence of hexamethonium (100 µM applied to the bath for 5 min) postsynaptic neuronal responses in all neurons tested were no longer elicited following nerve stimulation (Fig. 5 Bi, NS arrow), but the responses elicited by intracellular current application were unaffected (Fig. 5 Bi; ICS). Hexamethonium also eliminated the effects of nicotine on all neurons tested (Fig. 5Bii), including spike-blocking effects that nicotine had previously exerted during intracellular stimuli (Fig. 5 Biii). The doses of hexamethonium employed in vitro did not affect the resting membrane potentials of identified cells; nor did it affect neuronal responses to direct intracellular current application or antidromically-mediated neuronal activation.

Discussion

Rapidly pacing the canine heart for 2 weeks resulted in a reduction in resting cardiac output (about 50%) that was accompanied by a differential remodeling of the capacity of nicotine sensitive elements within its intrinsic cardiac nervous system to modulate regional cardiodynamics. The fact that these animals did not exhibit elevated resting heart rates, decreased mean aortic blood pressure or significant ascites at the time the functional studies were performed indicates that the paced animals utilized in this study were in early stage heart failure (10; 16).

The pacing protocol employed in this study did not substantially alter the capacity of the intrinsic cardiac nervous system to transduce the mechanical and chemical milieu of the heart (Table 1). That epicardial mechanical stimuli either increased or decreased the activity generated by identified neurons, depending on the neuron studied, is in agreement with how the cardiac mechanical milieu is transduced by intrinsic cardiac (35) or extracardiac (6) afferent neurons in normal dogs. That the intrinsic cardiac nervous system of failing hearts retains this
capacity is in contrast to what occurs with respect to the reduced capacity of cardiac afferent neurons in extracardiac ganglia to transduce the failing heart’s milieu (41).

Cardiac responses elicited as a consequence of stimulating right or left sided extrinsic autonomic efferent neuronal inputs (stellate ganglia or vagosympathetic trunks) in these early stage pacing-induced heart failure preparations were similar in nature to those identified the sham-operated preparations, as well as those reported in intact preparations (3; 4; 16). These data indicate that neurotransmission from extrinsic cardiac nerves to intrinsic cardiac efferent neurons and thence to the myocardium (the direct "throughput" pathways from CNS to myocyte) appears to be functionally intact in early stage heart failure (c.f., Fig. 4). Right atrial neurons identified in failing hearts were excited by extracardiac parasympathetic or sympathetic efferent neuronal inputs, but not after fixed latencies (Fig. 4). In other words, most intrinsic cardiac neurons identified in these paced preparations received indirect inputs from extracardiac sympathetic and parasympathetic efferent neurons. That identified neurons were activated in a multisynaptic fashion in those instances, along with the fact that enhancement of activity persisted well after terminating such stimuli, support the concept that most identified neurons were not efferent postganglionic neurons; this is in accord with what occurs in normal preparations (22).

Nicotinic neurotransmission also plays a significant role in the local processing of cardiac sensory information within the intrinsic cardiac nervous system. It has been hypothesized that this occurs in part via its local circuit neurons (8; 22), interposed as they are between cardiac sensory and motor neurons (1; 5; 6). Neurons identified on failing hearts that did respond reflexly to activation of ventricular sensory neurites did so without exhibiting the cardiac-related phasic activity associated with primary afferents (2; 36) and thus were indirectly modulated via stochastic neural interactions mediated within the cardiac nervous system (28). In other words,
neurons identified \textit{in situ} in this study did not receive direct afferent neuronal inputs. From these data it can be surmised that the majority of identified neurons were local circuit in nature (5).

It has been proposed that most of the activity identifiable in intrinsic cardiac ganglionated plexuses on the beating heart originates from local-circuit neurons (~70\% of the entire population) (6). That this neuronal population transduced ongoing sensory inputs from the failing heart in a normal manner (8; 22; 35) implies that the function of the latter is preserved early on in this model of heart failure. Excitatory and inhibitory synapses are present in intrinsic cardiac ganglia (5; 6). That excitatory or inhibitory responses were elicited from identified neurons transducing the various epicardial mechanical or chemical stimuli is in accord with previous data indicating that intrinsic cardiac local circuit neurons transduce similar cardiac stimuli in a differential manner depending upon the nature of their inputs (6).

After two weeks of rapid ventricular pacing, the response characteristics of intrinsic cardiac neurons to locally administered β-adrenergic agonists, phenylephrine or angiotensin II (Table 1) were also similar to those reported to occur in normal preparations (7; 25; 27). As the integration and processing of cardiac sensory information by the intrinsic cardiac nervous system may involve excitatory and inhibitory inputs (5), multiple and directionally opposite responses to varied stimuli might be expected given the varied nature of their responses to cardiac afferent or efferent neuronal inputs (1; 5).

Nicotinic receptor-dependent cholinergic synapses within the intrinsic cardiac nervous system play a key role in regional cardiac regulation (13; 26; 27; 32). When 0.1 ml of 100 \(\mu\text{M}\) nicotine is administered into the regional arterial blood supply of right atrial neurons in normal dogs their activity either increases or decreases, depending on the sub-population of neurons studied (27). Accompanying such nicotine induced neuronal responses in normal preparations are immediate parasympathetic efferent neuron mediated negative chronotropic and inotropic
responses that are followed by sympathetic efferent neuron mediated increases in heart rate and contractility (27; 31). The biphasic nicotine-induced cardiac responses also occurred in sham-operated animals reported herein (Fig. 2). When nicotine was administered in the same dose into the regional arterial blood supply of right atrial neurons in the heart failure preparations, monitored cardiodynamic indices were not affected. Failing heart intrinsic cardiac neurons also failed to respond to nicotine administered into their local arterial blood supply in doses that elicit responses from intrinsic cardiac neurons of normal preparations (27). Neuronal activity only changed when the dose of nicotine that was administered to right atrial neurons was increased to 50 times greater than that needed to activate neurons in normal canine preparations (31); in contradistinction to normal preparations, monitored cardiovascular indices were unaffected by this higher nicotine dose. These data indicate that not only does the sensitivity of populations of intrinsic cardiac neurons to exogenously administered nicotine become obtunded in heart failure, but also the capacity of such neurons to influence cardiodynamics is curtailed such that it may become virtually non-existent.

In order to assess whether these in situ nicotine induced responses reflect corresponding functional changes among individual cholinergic intrinsic cardiac neurons, we explored the intracellular properties of these neurons to nicotinic inputs in vitro. As occurs in normal preparations (33; 39), phasic and accommodating neurons were identified in ganglia derived from the CHF preparation and in the same proportion (33). Membrane capacitance, an approximate index of relative cell size being proportional to membrane surface area, was similar in phasic and accommodating neurons from failure preparations as those derived normal preparations (24). Differences in whole-cell resistances and AP amplitudes among phasic and accommodating neurons were also similar to those reported for normal preparations (33).
However, even at this early stage of CHF significant differences were evident in selective electrophysiological properties of neurons derived from failing hearts (Table 2) compared to those derived from normal preparations (33). Atrial neurons from paced hearts exhibited decreased input resistances, as well as changes in their afterhyperpolarization properties. Decreases in their input resistance would have the overall effect of decreasing their excitability and therefore likely suppress their information processing capabilities. Interestingly, phasic and accommodating neurons were differentially affected by early stage CHF since AHP duration was only altered in accommodating neurons. Given the limitation of studying acutely extirpated neurons, specifically their lack of spontaneous activity, it is difficult to relate in a direct manner these data to those derived from the in situ model. Having stated that fact, data derived from extirpated neurons do support the contention that neurons within the failing heart’s intrinsic cardiac nervous system functionally remodel early during the evolution of CHF.

Perspectives: Two-weeks of rapid cardiac pacing that depresses resting cardiac output by over 50% differentially remolds the capacity of nicotine sensitive local circuit neurons in the intrinsic cardiac nervous system to influence cardiodynamics. Such remodeling is attended by retention of the capacity of the intrinsic cardiac nervous system to transduce cardiac sensory information as well as inputs from extracardiac efferent neurons. This remodeling does not appear to be a global effect in as much as its angiotensin II sensitive neurons function normally in such a state. This neural remodeling does appear to target intrinsic cardiac cholinergic sensitive neurons early on in the progression of CHF, specifically nicotinic sensitive ones. Alterations in intrinsic cardiac neuronal muscarinic receptor function may also contribute to the altered responsiveness within the intrinsic cardiac nervous system (12; 32). Data present herein indicates that neural remodeling accompanies and may in some case precede myocyte and matrix
remodeling (34) in the evolution of CHF. Understanding the interplay and synergism between these two processes can potentially provide new therapeutic targets for effective clinical management of such pathologies.

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References


7. **Armour JA.** Canine intrinsic cardiac neurons involved in cardiac regulation possess $\alpha_1, \alpha_2, \beta_1$ and $\beta_2$ adrenoreceptors. *Can J Physiol Pharmacol* 13: 277-284, 1996.


Figure 1. The effects of epicardial application of veratridine on the ECG, left ventricular chamber pressure (LVP) and right atrial neuronal activity after 2 weeks of rapid cardiac pacing. A few seconds after applying a veratridine soaked pledget to the right ventricular conus epicardium (indicated by arrow blow), right atrial neuronal activity (bottom trace) increased. Recorded cardiovascular variables were unaffected by this intervention.

Figure 2. Average changes in heart rate, right atrial contractile force (RACF), left ventricular intramyocardial systolic pressure (LV IMP) and right ventricle intramyocardial systolic pressure (RV IMP) induced by administering nicotine (100\(\mu\)M; 0.1 ml) into the local arterial blood supply of the right atrial ganglionated plexus of 8 sham operated dogs (Sham) and the 9 dogs subjected to 2 weeks of cardiac pacing (2 week Pace). Cardiodepressor responses are indicated by the solid black bars and augmentor responses by the shaded bars. In the sham-operated animals, the same dose of nicotine induced a biphasic response with initial negative chronotropic and inotropic responses being followed by augmentor responses (~60 sec duration). When administered at the same dosage in failure preparations, nicotine failed to elicit cardiac responses. * = p < 0.01 comparing results in sham versus paced preparations.

Figure 3. Nicotine (100 \(\mu\)M, 5 ml), when administered into the arterial blood supply of the right atrial ganglionated plexus of a pacing-induced heart failure preparation (at arrow below), activated identified neurons (bottom trace) without affecting heart rate (see ECG trace) or aortic blood pressure (AP).
**Figure 4.** Effects of left stellate ganglion stimulation on cardiac neurons and heart function after 2 wks of rapid ventricular pacing. Aortic (AP) and left ventricular (LVP) systolic pressures increased soon after beginning stellate ganglion stimulation (between arrows below). Enhancement of neuronal activity became evident once stimulation ceased (removal of stimulation artifact). Neuronal activity remained elevated thereafter for about 30 seconds.

**Figure 5.** Effects of nicotine on membrane potential, postsynaptic responses to nerve stimulation and intracellular activation *in vitro* of a phasic neuron contained within the ventral right atrial ganglionated plexus obtained from a paced heart. **Ai:** Synaptic mediated neuronal response to single-pulse stimulation (left trace, 0.5 ms pulse duration, 600 μA; NS, arrow) of a nerve connected to ganglion under study, AND action potential induced by intracellular stimulation (right trace, 50 ms pulse duration, 0.34 nA delivered through recording electrode; ICS). **Aii:** Nicotine (200 ms pressure pulse, 100 μM, delivered to ganglion from nearby pipette; NIC at arrow) depolarized the neuron and evoked action potentials (AP) on the rising phase of the depolarization; further AP generation was blocked during the peak phase of depolarization (level of resting membrane potential indicated by dotted line). **Aiii:** Membrane potential 1 min post-nicotine application. Note that both synaptically- (NS) and intracellularly (ICS) -evoked APs remained blocked. **B panels:** in same neuron as in A, but with bath application of hexamethonium (HEX, 100 μM). **Bi:** HEX blocked response to synaptically mediated activation (NS) but not to intracellular stimulation (ICS). **Bii:** Nicotine-induced depolarization and AP generation were eliminated by hexamethonium. **Biii:** 1 min post-nicotine application, in continued presence of hexamethonium, synaptic activation was blocked while intracellularly evoked AP generation (ICS) remained effective. For all traces, vertical bar represents 15 mV. For A (i), (iii) and B (i), (iii): horizontal bar represents 10 ms; for A (ii) and B (ii): horizontal bar
represents 1 sec. The zero voltage level is shown to the left of traces in A (i) and B (i). Nerve stimulation artifacts in A (i, iii) and B (i, iii) have been truncated for the sake of clarity.

**Table 1.** Intrinsic cardiac neuronal effects elicited by mechanical or chemical (veratridine) stimuli applied to selected ventricular epicardial loci, as well as by close coronary arterial administration of nicotine (two doses), angiotensin II, phenylephrine, clonidine, dobutamine and terbutaline is summarized for all 9 dogs with early stage pacing-induced CHF. For these dogs basal activity averaged 41±5 ipm. Changes in neuronal activity from baseline values are expressed as absolute values of the differences between activity recorded before and after application of each intervention expressed as Δ ipm (impulses per minute). * indicates a significant difference (p < 0.01) between baseline and intervention values. Monitored cardiovascular variables were unaffected by these interventions.

**Table 2.** *In vitro* membrane properties and characteristics of intracellularly evoked responses of intrinsic cardiac neurons obtained from control animals (33) and from canines in early stage pacing-induced CHF. Neurons were categorized according to whether their AP firing behavior was phasic or accommodating in response to 1 sec duration intracellular depolarizing currents delivered through the recording electrode. AP, action potential; AHP, after-hyperpolarization following AP; RMP, resting membrane potential; \( R_{in} \), whole-cell input resistance. Data are expressed as means ± SE. * indicates significant difference (p<0.05) between accommodating and phasic neurons in control or CHF groups. # indicates significant difference (p<0.05) for specific cell types (phasic or accommodating) between control and CHF animals.
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