Intrinsic cardiac nervous system in tachycardia induced heart failure

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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). 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 downregulation of cardiac myocyte β-adrenoceptor function (14, 21, 23) leading to alterations in the cardiomyocyte second messenger system (15), and 2) 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 pacing-induced heart failure; intrinsic cardiac neurons; atrial neuron; nicotinic receptors; parasympathetic efferent neurons; and sympathetic efferent neurons

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evolution of CHF (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 brain stem 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 rational basis for targeted and time-appropriate neurocardiac therapeutic strategies.

The primary objective of this study was to determine whether early-stage, pacing-induced heart failure modifies the function of the intrinsic cardiac nervous system and, if so, which elements within it become affected. 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. 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 then 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, whereas 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.

**MATERIALS AND METHODS**

Adult preconditioned mongrel dogs (n = 18) of either sex, weighing between 15 and 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 thiopental sodium (25 mg/kg iv); thereafter, anesthesia was maintained with isoflurane. In 10 of these animals comprising the experimental group, under sterile conditions, the right jugular vein was isolated via a midline neck incision, and a Swan-Ganz catheter was introduced through it into the main pulmonary artery. Cardiac output was determined via the thermodilution technique, and the values so obtained represented the prepping state during rest. The catheter was withdrawn, and a bipolar pacing electrode (model IS-1-B1-ATR; Medtronic, Minneapolis, MN) was inserted by using fluoroscopy such that the tip of the pacing electrode was positioned in the right ventricular apex. An implantable pacemaker (multiprogrammable pulse generator model SX5984, Medtronic, connected to these electrodes, was placed under the skin of the neck. The neck incision was then closed, and the animal was allowed to recover. Analgesic therapy (morphine, 0.5 mg/kg im) was given postoperatively at 8-h intervals for 24 h and as needed thereafter. Antibiotic (Cephalexin, 500 mg 2 times daily) therapy was administered for 5 days after surgery. Ventricular pacing was begun 2 wk after the pacemaker implant. The pacemaker was set to pace the ventricles at a rate of 240 beats/min (16); rapid ventricular pacing was maintained continuously thereafter for 2 wk. Continuous ventricular capture was ensured on a daily basis. The clinical status of each animal was monitored on a daily basis to detect signs of cardiac failure (ascites, dyspnea, fatigue, and 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 2 days after discontinuing 2 wk 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.** To compare cardiodynamic effects of locally applied nicotine, a group of eight sham-operated dogs was prepared. A midline neck incision was performed under aseptic conditions after induction of anesthesia as described in Induction of pacing-induced CHF. After 30 min, the neck incision was closed, and anesthesia discontinued. Pain and antibiotic therapy were then instigated as described in Induction of pacing-induced CHF for paced animals. Functional studies were performed in the sham-operated group 2 wk after surgery.

**Terminal Studies**

The dogs (pacing-induced CHF and sham-operated control groups) were anesthetized with thiopental sodium (15 mg/kg iv), supplemental doses (5 mg/kg iv) being provided every 5–10 min 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 fre-
ently 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.

After induction of anesthesia, dogs were intubated and placed on positive pressure ventilation by 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. After 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 saline (0.1 ml) to the anesthetized dog’s right atrial blood supply to determine local circuit neurons (5, 22).

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-Fr 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 drugs to the right atrial neurons of the sham-operated animals, because 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). To test the effects of systemically administered nicotine on neuronal and cardiovascular indexes, 0.1 ml of nicotine (100 μM) was injected into the aortic blood.

Because 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: α₁-adrenoceptor agonist phenylephrine (100 μM), α₂-adrenoceptor agonist clonidine (100 μM), β₁-adrenoceptor agonist dobutamine (100 μM), β₂-adrenoceptor agonist terbutaline (100 μM), and peptide angiotensin II (100 μM). Doses of these agonists (0.1 ml) used in this study have previously been shown to be capable of directly modifying intrinsic cardiac neuronal and resultant cardiodynamic responses by directly activating intrinsic cardiac neurons with minimal contributions from extracardiac neural elements (27, 31). To test the effects of systemically administered nicotine on neuronal and cardiovascular indexes, 0.1 ml of nicotine (100 μM) was injected into the aortic blood.

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 to minimize local motion of the atrial wall. This fat was explored with a tungsten microelectrode mounted on a micro-manipulator 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 1,000 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 an amplifier (model 113; Princeton Applied Research, Princeton, NJ) with band-pass filters set at 300 Hz to 10 kHz and with an amplification range of 100–500 times. The output of this device, further amplified (50–200 times) and filtered (band width 100 Hz–2 kHz) by means of an optically isolated amplifier (Applied Microelectronics Institute, Halifax, NS, Canada), was led to an oscilloscope (model 207; Nicolet, Madison, WI), an AM8 Audio Monitor (Grass Instrument, Quincy, MA), and a digital data converter and videotape recorder (model 3000; Vetter, Rebersburg, 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) and direct afferent inputs from cardiac mechano- and multimodal neurites (2) and extracardiac sensory neuron terminals (6, 43) that contact local circuit neurons.

Sensory inputs to intrinsic cardiac neurons. Ventricular mechanosensory inputs to identified right atrial neurites 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 (5 × 10⁻⁶ g; Sigma-Aldrich, St. Louis, MO) was applied to that site for 60 s by using 1 × 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 ~30 s with normal saline (~2 ml/s) after the veratridine-soaked pledget was removed with at least 5 min 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 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, because 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). To test the effects of systemically administered nicotine on neuronal and cardiovascular indexes, 0.1 ml of nicotine (100 μM) was injected into the aortic blood.

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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 mechanoreceptor activation (27). The order of administering these latter agents varied among experiments. To test their effects if they entered the systemic circulation in sufficient doses to modify monitored indexes, each agent was also administered individually into the aortic blood in the same doses 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-s periods via bipolar electrodes attached to a Grass Instrument 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 seven heart failure preparations, the ventral right atrial ganglionated plexus was removed and placed in a dish containing modified Krebs’ solution (in mM: 120 NaCl, 25 NaHCO3, 1 NaH2PO4, 5 KCl, 2 MgCl2, 2.5 CaCl2, and 11 d-glucose, 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 by 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 by using a mechanical three-axis micromanipulator; a sudden drop in potential at the electrode tip signaled cellular impalements. Transmembrane potentials were recorded in current-clamp mode by 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 0 V 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 0 V level confirmed.

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

To estimate whole cell resistance, hyperpolarizing current was injected through the recording electrode, and voltage responses to at least six 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 1–1/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 hyperpolarize to a baseline AHP amplitude below the neuronal membrane potential responses elicited by nicotine (100 μM dissolved in perfusate) were evaluated by local pressure application from the tip of a pipette placed within 100 μm of investigated ganglia.

Data Analysis

In vivo experimental data analysis. Cardiovascular indexes 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 cardio-dynamics were minimal during data collection periods before interventions: heart rate varying < 5 beats/min, and systolic pressure fluctuating < 5 mmHg. 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-s periods to establish average neuronal activity generated immediately before and during maximal responses elicited by each intervention. Fluctuations in the amplitude of APs generated by a unit varied by <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. Because 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 [ipm]). Data are expressed as means ± SE. One-way ANOVA and paired Student’s 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 in vitro experiments were played back from the tape into a personal computer through an analog-to-digital converter (Digidata 1200; Axon Instrument).
ments, Foster City, CA). These data were analyzed with Axon Instruments pCLAMP6 software. Data are presented as means ± SE. Pairwise comparisons between means were done by using two-tailed Student's t-test, adjusted for unequal number, with \( P < 0.05 \) for these comparisons.

**RESULTS**

**Overview of Failure Preparations**

Cardiac indexes (closed chest) were determined in the anesthetized state in the paced group before pacemaker implant and subsequently repeated in the terminal study. The average resting cardiac index before initiating the pacing protocol was 3.63 ± 0.49 l/min. Animals were paced for 2 wk; pacing was stopped 2 days before 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 l/min, a 54% decrease from control values. Ten animals entered the pacing protocol group; one of these animals died after instrumentation immediately after 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 nine paced animals.

As has been found in normal preparations (5), spontaneous activity was generated by, on average, three 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 APs identified at each site. During basal states, neurons at a locus in this ganglionated plexus generated an average aggregate activity of 41 ± 5 ipm. Overall, the level of intrinsic cardiac neuronal activity was relatively similar among animals before the initiation of the interventions.

**Transduction of Ventricular Mecha

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. Epidural mechanical stimuli immediately initiated an increase in neuronal activity in six animals [28 ± 6 to 73 ± 17 ipm, not significant (ns)]; it decreased neuronal activity in the other three animals (50 ± 8 to 15 ± 7 ipm, ns). After removal of the mechanical stimulus neuronal activity rapidly returned to control values. Epidural 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 indexes were unaffected by these mechanical interventions.

**Transduction of Ventricular Chemo

When veratridine was applied to these identified ventricular epicardial loci, neuronal activity increased

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**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 dogs with early-stage pacing-induced congestive heart failure (CHF)**

<table>
<thead>
<tr>
<th>Control Interventions</th>
<th>Responders</th>
<th>HR, beats/min</th>
<th>RAP, mmHg</th>
<th>RVSP, mmHg</th>
<th>PASP, mmHg</th>
<th>LVSP, mmHg</th>
<th>AoSP, mmHg</th>
<th>AoDP, mmHg</th>
<th>Change in Neuronal Activity, Δ ipm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardial touch</td>
<td>9</td>
<td>110 ± 4</td>
<td>10 ± 2</td>
<td>47 ± 5</td>
<td>42 ± 2</td>
<td>120 ± 7</td>
<td>125 ± 5</td>
<td>92 ± 6</td>
<td>43 ± 7*</td>
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<tr>
<td>Epicardial veratridine</td>
<td>9</td>
<td>110 ± 4</td>
<td>9 ± 1</td>
<td>47 ± 5</td>
<td>42 ± 2</td>
<td>124 ± 6</td>
<td>130 ± 5</td>
<td>92 ± 6</td>
<td>59 ± 8*</td>
</tr>
<tr>
<td>Nicotine, local artery (100 μM, 2 ml)</td>
<td>9</td>
<td>110 ± 4</td>
<td>10 ± 1</td>
<td>44 ± 6</td>
<td>40 ± 3</td>
<td>120 ± 9</td>
<td>125 ± 7</td>
<td>95 ± 7</td>
<td>2 ± 6</td>
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<tr>
<td>Nicotine, local artery (100 μM, 5 ml)</td>
<td>9</td>
<td>111 ± 4</td>
<td>11 ± 2</td>
<td>47 ± 6</td>
<td>44 ± 4</td>
<td>129 ± 10</td>
<td>130 ± 8</td>
<td>98 ± 7</td>
<td>45 ± 8*</td>
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<td>Angiotensin II, local artery</td>
<td>9</td>
<td>100 ± 5</td>
<td>10 ± 1</td>
<td>40 ± 5</td>
<td>40 ± 3</td>
<td>130 ± 8</td>
<td>130 ± 7</td>
<td>96 ± 6</td>
<td>48 ± 12*</td>
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<td>Phenylephrine, local artery</td>
<td>6</td>
<td>103 ± 4</td>
<td>8 ± 2</td>
<td>36 ± 6</td>
<td>40 ± 4</td>
<td>123 ± 13</td>
<td>134 ± 6</td>
<td>87 ± 11</td>
<td>42 ± 9</td>
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<td>3</td>
<td>103 ± 4</td>
<td>5 ± 1</td>
<td>42 ± 7</td>
<td>38 ± 8</td>
<td>118 ± 9</td>
<td>119 ± 10</td>
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<td>118 ± 9</td>
<td>131 ± 5</td>
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<td>112 ± 3</td>
<td>9 ± 2</td>
<td>39 ± 5</td>
<td>39 ± 5</td>
<td>119 ± 10</td>
<td>132 ± 5</td>
<td>103 ± 3</td>
<td>32 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 9 dogs. 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 impulses per minute (ipm). *Significant difference (P < 0.01) between baseline and intervention values. Monitored cardiovascular variables were unaffected by these interventions. HR, heart rate; RAP, right atrial systolic pressure; RVSP, right ventricular systolic pressure; PASP, pulmonary artery systolic pressure; LVSP, left ventricular systolic pressure; AoSP, aortic systolic pressure; AoDP, aortic diastolic pressure.
in seven dogs (e.g., Fig. 1; 34 ± 5 to 89 ± 12 ipm, \( P < 0.01 \)) and decreased in the other two dogs (85 ± 19 to 21 ± 2 ipm, ns). Veratridine changed absolute neuronal activity from baseline values by 59 ± 8 ipm 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 \( \mu \)M, 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 indexes 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 to induce neuronal activity change (\( \Delta 45 \pm 8 \) ipm; \( P < 0.01 \)) (Fig. 3). In those instances, neuronal activity increased (38 ± 3 to 95 ± 7 ipm; \( P < 0.01 \)) in six animals, whereas it decreased (48 ± 15 to 28 ± 12 ipm; ns) in the other three 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 (\( \Delta 48 \pm 12 \) ipm from baseline values; \( P < 0.01 \); Table 1) generated by neurons identified in

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

**Fig. 2.** Average changes in heart rate (A), right atrial contractile force (RACF; B), left ventricular intramyocardial systolic pressure (LV IMP; C) and right ventricle intramyocardial systolic pressure (RV IMP; D) 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 wk of cardiac pacing (2 wk Pace). Cardiodepressor responses are indicated by the 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-s duration). When administered at the same dosage in failure preparations, nicotine failed to elicit cardiac responses. \(* P < 0.01 \) comparing results in sham vs. paced preparations.

**Fig. 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 (arrow), activated identified neurons (bottom trace) without affecting heart rate (see ECG trace) or aortic blood pressure (AP).
was altered by local arterial administration of the α1-adrenoceptor agonist phenylephrine (Δ42 ± 9 ipm; P < 0.01), the β1-adrenergic agonist dobutamine (Δ59 ± 0 ipm; P < 0.01), and the β2-adrenergic agonist terbutaline (Δ82 ± 8 ipm; P < 0.01) (Table 1). The activity generated by identified neurons changed in only three dogs (Δ49 ± 7 ipm) when they were exposed to local arterial administration of the α2-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 indexes.

Intracardiac Autonomic Efferent Neuronal Inputs to Heart. In six 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 was activated after a fixed latency after 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 to 44 ± 10 beats/min; left side: 112 ± 6 - 63 ± 14 beats/min).

Intracellular Properties of Intrinsic Cardiac Neurons Derived from Paced Hearts.

Data obtained from 29 intrinsic cardiac neurons derived from seven paced hearts displayed two types of AP responses to intracellular depolarizing current pulses (1-s duration, intensity set to produce maximal number of APs) (Table 2). Fifty-nine percent (17 of 29) discharged one or two APs during depolarization, these neurons being classed as “phasic.” The remaining neurons (41%, 12 of 29) discharged APs 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 with control. Within groups, AP amplitude was greater in accommodating neurons, and between groups, mean AP and AHP amplitudes were increased in CHF animals compared with 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-induced CHF group. Nine of these neurons were phasic and seven 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). When the perfusate lacked Ca\(^2^+\) and contained 10 mM Mg\(^2^+\), these neurons were no longer affected by intraganglionic 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\(^2^+\)/high-Mg\(^2^+\) 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**

Effects of nicotine were evaluated in four phasic and four accommodating neurons derived from paced hearts. All of these neurons responded to nicotine similarly. Before nicotine application, APs were generated orthodromically by single-pulse nerve stimuli as well as by intracellular currents (Fig. 5Aa). Nicotine (100 μM; 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. 5Ab). 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. 5Ac).

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 after nerve stimulation (NS; Fig. 5Ba, arrow), but the responses elicited by intracellular current application were unaffected (Fig. 5Ba; ICS). Hexamethonium also eliminated the effects of nicotine on all neurons tested (Fig. 5Bb), including spike-blocking effects that nicotine had previously exerted during intracellular stimuli (Fig. 5Bc). 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 wk resulted in a reduction in resting cardiac output (~50%) 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 in 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, supports 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 in situ 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 on the nature of their inputs (6).

After 2 wk 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). Because 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 μM nicotine is 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 indexes 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 cardio-dynamics is curtailed such that it may become virtually nonexistent.

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 from normal preparations (24). Differences in whole cell resistances and AP amplitudes between 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 electrophysiologically properties of neurons derived from failing hearts (Table 2) compared with those derived from normal preparations (33). Atrial neurons from paced hearts exhibited decreased input resistances, as well as changes in their AHP 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, because 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 > 50% differentially remodeling 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 ner-
vous system to transduce cardiac sensory information as well as inputs from extracardiac efferent neurons. This remodeling does not appear to be a global effect inasmuch 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 presented herein indicate that neural remodeling accompanies and may, in some cases, 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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