Chronic decentralization of the heart differentially remodels canine intrinsic cardiac neuron muscarinic receptors

F. M. SMITH, 1 A. S. McGUIRT, 3 D. B. HOOVER, 4 J. A. ARMOUR, 2 AND J. L. ARDELL 4
1Department of Anatomy and Neurobiology and 2Department of Physiology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada; 3Department of Physiology, University of South Alabama, Mobile, Alabama 36688; and 4Department of Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee 37614-1708

Received 11 April 2001; accepted in final form 17 July 2001

Smith, F. M., A. S. McGuirt, D. B. Hoover, J. A. Armour, and J. L. Ardell. Chronic decentralization of the heart differentially remodels canine intrinsic cardiac neuron muscarinic receptors. Am J Physiol Heart Circ Physiol 281: H1919–H1930, 2001.—The objective of the study was to determine if chronic interruption of all extrinsic nerve inputs to the heart alters cholinergic-mediated responses within the intrinsic cardiac nervous system (ICN). Extracardiac nerve inputs to the ICN were surgically interrupted (ICN decentralized). Three weeks later, the intrinsic cardiac right atrial ganglionated plexus (RAGP) was removed and intrinsic cardiac neuronal responses were evaluated electrophysiologically. Cholinergic receptor abundance was evaluated using autoradiography. In sham controls and chronic decentralized ICN ganglia, neuronal postsynaptic responses were mediated by acetylcholine, acting at nicotinic and muscarinic receptors. Muscarine- but not nicotine-mediated synaptic responses that were enhanced after chronic ICN decentralization. After chronic decentralization, muscarine facilitation of orthodromic neuronal activation increased. Receptor autoradiography demonstrated that nicotinic and muscarinic receptor density associated with the RAGP was unaffected by decentralization and that muscarinic receptors were tenfold more abundant than nicotinic receptors in the right atrial ganglia in each group. After chronic decentralization of the ICN, intrinsic cardiac neurons remain viable and responsive to cholinergic synaptic inputs. Enhanced muscarinic responsiveness of intrinsic cardiac neurons occurs without changes in receptor abundance.

intracardiac ganglia; intracellular recording; muscarinic receptors; nicotinic receptors; autoradiography

NEUROHUMORAL CONTROL of the heart is dependent on the dynamic interactions of end-cardiac effectors with reflexes occurring within intrinsic and extracardiac ganglia and the central nervous system (4, 5, 34). Altered neuronal input to the intrinsic cardiac nervous (ICN) system is associated with compromised cardiac control that can lead to cardiac dysrhythmias (6, 7, 21). After complete interruption of all extracardiac neuronal inputs (i.e., decentralization), neurons in the ICN system continue to generate spontaneous activity that is dependent on intracardiac sensory inputs (3, 4, 7). Even in the transplanted heart, the ICN system continues to operate, albeit in a modified fashion, to regulate regional cardiac function reflexively (24).

After interruption of central neuronal inputs to autonomic ganglia, internal synaptic reorganization occurs (33). After decentralization, guinea pig inferior mesenteric ganglia neurons become more dependent on synaptic inputs from peripheral enteric receptors (22). However, the nature of decentralization-induced changes within the intracardiac nervous system is not known. Chronically interrupting preganglionic inputs to peripheral autonomic neurons affects their active and passive membrane properties (14, 15). Presumably, receptor densities and function, or patterns of synaptic connectivity, may also change in individual intrinsic cardiac neurons after removal of preganglionic neuronal inputs. In such a state, acetylcholine (ACh) “super-sensitivity” has been reported in mammalian postganglionic sympathetic neurons (11) and in principal neurons of the amphibian heart (23, 25, 27). However, not all autonomic neurons display increased sensitivity after chronic decentralization (10, 16). Whether the function of cholinergic receptors associated with intrinsic cardiac neurons is altered after chronic decentralization remains unknown.

This study focused on the following goals: 1) to determine the responses of intrinsic cardiac neurons to cholinergic agents after long-term removal of their extracardiac neuronal inputs; 2) to investigate whether synaptic neurotransmission within the ICN system is modified after decentralization; and 3) to determine whether chronic decentralization of intrinsic cardiac neurons alters their nicotinic or muscarinic receptor densities. For this purpose, transmembrane potential recordings were used to characterize the in vitro responses of sham-operated or chronically decentralized canine ventral right atrial neurons to specific cholinergic receptor agonists or antagonists and to evaluate changes in synaptic neurotransmission within these intrinsic cardiac ganglia. In addition, the density of
nicotinic and muscarinic receptors associated with such neurons was quantified by receptor autoradiography to correlate receptor abundance with local electrophysiological properties and synaptic function.

**MATERIALS AND METHODS**

Adult mongrel dogs (n = 51) of either sex, weighing 17–27 kg, were used in the experiments. All experimental procedures in this study conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Revised 1996) and were approved by the animal care and use committees of East Tennessee State University and the University of South Alabama.

**Chronic Decentralization of ICN**

Thirty mongrel dogs of either sex were pretreated with cephalolin sodium (1 g im) and anesthetized with pentobarbital sodium (30 mg/kg iv). Supplemental doses were provided as needed to maintain a surgical level of anesthesia. Under aseptic conditions and positive pressure ventilation, a left T4-T5 thoracotomy was performed. Decentralization of the ICN system was achieved by dissection around the major intrapericardial vessels, as described previously (5). Interruption of the extracardiac nerve inputs to the heart was confirmed by the loss of chronotropic and dromotropic responses to supramaximal stimulation of the right and left vagosympathetic complexes (10 V, 5 ms, 20 Hz) and ansae subclavia (10 V, 5 ms, 10 Hz). The thoracic cavity was then closed and residual air was withdrawn. Analgesic therapy (Buprenex; 0.2 mg im) was given postoperatively at 8-h intervals for 24 h and as needed thereafter. Antibiotic (Cephalexin; 500 mg 2× daily) therapy was administered orally for 7 days after surgery. The animals were allowed to recover for 3–4 wk. Twenty-one additional animals underwent the same aseptic thoracic surgical procedures except that no intrapericardial dissections were performed. Postoperative care for these sham-operated animals was identical to that of animals with chronically decentralized ICNs. Neurons from the hearts of these animals were treated as controls for comparison with neurons from decentralized hearts.

**Experimental Protocols**

Three to four weeks after surgery, the dogs were anesthetized with thiopental sodium (15 mg/kg iv). Supplemental doses (5 mg/kg iv) were provided every 5–10 min throughout the surgery. Noxious stimuli were applied periodically to achieveelectrical stimulation of the heart. The thoracic cavity was then closed and residual air was withdrawn. Analgesic therapy (Buprenex; 0.2 mg im) was given postoperatively at 8-h intervals for 24 h and as needed thereafter. Antibiotic (Cephalexin; 500 mg 2× daily) therapy was administered orally for 7 days after surgery. The animals were allowed to recover for 3–4 wk. Twenty-one additional animals underwent the same thoracic surgical procedures except that no intrapericardial dissections were performed. Postoperative care for these sham-operated animals was identical to that of animals with chronically decentralized ICNs. Neurons from the hearts of these animals were treated as controls for comparison with neurons from decentralized hearts.

**Drugs and Neurochemicals**

Pharmacological agents (Sigma; St. Louis, MO) were freshly dissolved in small volumes of perfusate on the day of the experiment. ACh chloride, nicotine, and muscarine were applied by local pressure ejection from a four-barrel pipette tip placed adjacent to investigated neurons. The pressure and duration of drug ejection from the pipette barrels were controlled via a Picospitzer (model II, General Valve; Fairfield, NJ). Hexamethonium chloride and atropine sulfate were administered in the perfusate.

**Electrophysiology Data Analysis**

Selected portions of the recorded data 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 the use of pCLAMP6 software (Axon Instruments). Numerical data are presented as means ± SE. Pairwise comparisons between
means were done using Student’s two-tailed t-test, with \( P \leq 0.05 \) for all comparisons.

**Cholinergic Receptor Autoradiography**

Tissues were cut into 20-μm-thick sections with the use of a microtome cryostat at −20°C and transferred to microscope slides coated with chrome alum-gelatin. Sections were collected in sets of three with adjacent sections placed on separate slides. The first section in each set was stained with hematoxylin and eosin and used to locate cardiac ganglia. Slides containing adjacent sections were stored at −80°C for subsequent autoradiographic analysis. Muscarinic and nicotinic receptors were evaluated by studying tissue sections containing intrinsic cardiac ganglia. Muscarinic receptors were identified by \( l^{-}[3H] \) quinuclidinyl benzilate (\( [3H] \) QNB; 49 Ci/mmol, NEN Life Science Products; Boston, MA), as described previously (18). \( 125I \)-labeled epibatidine (\( [125I] \) IPH; 1,200 Ci/mmol, NEN Life Science Products) was used to label nicotinic receptors (12). To determine total binding for muscarinic receptors, sections were incubated in phosphate-buffered saline (pH 7.4) containing 1 nM \( [3H] \) QNB for 2 h at room temperature. Nonspecific binding was established from adjacent sections incubated in the presence of 1 μM atropine. Nicotinic receptor labeling was accomplished by incubating sections in a buffered solution containing 0.5 nM \( [125I] \) IPH for 1 h at room temperature. Nonspecific binding of this radioligand was determined in the presence of 300 μM nicotine. After incubation in both protocols, the slides were washed in buffer at 4°C, dipped in cold distilled water, drained, and dried without heat with the use of an electric fan.

Radioligand binding sites were identified by film autoradiography with Hyperfilm-3H (Amersham; Arlington Heights, IL). Autoradiographic microscales for the appropriate radionuclide enabled quantitative evaluation of receptor binding. Film exposure times at 4°C were 5 wk for \( [3H] \) QNB and 1 day for \( [125I] \) IPH. On completion of the autoradiography, all labeled sections were stained with hematoxylin and eosin to identify ganglia and other tissues in the autoradiograms. A microcomputer-assisted imaging device (Imaging Research) was used for quantitative evaluation of film autoradiograms. Specific binding of each radioligand was determined as the difference between total and nonspecific binding, and mean binding data are

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**Fig. 1. Effects of acetylcholine (ACh) on intrinsic cardiac neurons from sham control (Control) and decentralized (DCX) hearts.** In A–D, the short horizontal bar under the traces indicates application of a 10-ms pulse of ACh (1 mM) from the tip of a pipette placed near the ganglion. A: for control hearts, ACh depolarized a control intrinsic cardiac neuron, evoking a short burst of action potentials (APs) at the start of depolarization. ACh depolarized the chronically DCX neuron more than the control one, evoking a longer-lasting burst of APs. During the repolarization phase, the membrane potential began to oscillate with APs being discharged on oscillatory peaks. B: for control hearts, hexamethonium (100 μM for 5 min in perfusate) reduced the amplitude of ACh-induced depolarization relative to the control state; no APs were generated. For DCX, hexamethonium reduced the amplitude of ACh-induced depolarization; AP discharge was facilitated during plateau phase of response. C: for control, atropine (10 μM, 5 min in perfusate) reduced the amplitude of ACh-induced depolarization minimally; it did not prevent a burst of APs during the initial phase of depolarization. For DCX, atropine reduced the amplitude of ACh-induced depolarization and prevented repetitive AP firing during the plateau phase of the depolarization; it eliminated oscillations in membrane potential during the repolarization phase. D: combined hexamethonium-atropine blockade eliminated responses to ACh. Resting membrane potentials are shown to the left of traces in A. Because of a sampling error, the APs elicited during the transition from one sampling bin to the next appear to be truncated in A–C. Vertical bar, 15 mV; horizontal bar, 5 s.
presented as femtomoles per milligram of tissue (means ± SE). Analysis of variance was used to determine if there were significant differences among the experimental and control groups; where significant F values occurred, differences between specific means were analyzed with the Newman-Keuls test.

RESULTS

Intracellular recordings were made from 98 intrinsic cardiac neurons. Thirty-seven neurons were sampled from nine hearts with intact cardiac innervation (sham-operated animals); data obtained from these neurons represent control data for the purposes of this study. Sixty-one neurons were sampled from twelve hearts after chronic decentralization of the ICN system. Decentralization was confirmed in vivo before the removal of cardiac tissue by the lack of cardiac responses to supramaximal electrical stimulation of vagus nerves and the ansae subclavia (data not shown).

Effects of Cholinergic Agonists and Antagonists

ACh. Local pressure application of ACh affected intrinsic cardiac neurons from both control and decentralized hearts (Fig. 1). ACh depolarized neurons from control hearts by a mean value of 10 ± 3 mV (n = 12), usually eliciting multiple action potentials (APs) on the rising phase of the depolarization (Fig. 1A). After the membrane potential reached a plateau, no further spontaneous APs were generated. Also, APs could not be evoked by direct intracellular current injection. Membrane potentials returned to resting level by 15 ± 2 s after ACh application.

After chronic decentralization, ACh evoked a more rapid and higher-amplitude depolarization of membrane potential than occurred in control neurons (Fig. 1A). Moreover, such depolarization was accompanied by the generation of more APs on the rising phase than occurred in control neurons. During the plateau phase, no spontaneous APs occurred; direct intracellular current application likewise failed to evoke APs. Mean peak amplitude of depolarization of the membrane potential during the plateau phase was 17 ± 3 mV (n = 12), a value significantly greater than that in control neurons. In decentralized neurons, as membrane potentials began to repolarize after the plateau phase, oscillations in the membrane potentials occurred with a periodicity in the range of 2–4 s (mean 2.5 ± 0.2 s; Fig. 1A). When the amplitude of the positive-going peaks of these oscillations reached threshold for regener-
erative responses, APs were elicited. Oscillations continued in some neurons for up to 2 min after ACh application; oscillatory amplitude declined and AP generation ceased as membrane potentials returned to resting levels. The mean duration of the ACh-induced depolarization in decentralized neurons was $1.2 \pm 0.4$ min, five times longer than that identified in control neurons.

In the two groups of intrinsic cardiac neurons, activation of both nicotinic and muscarinic receptors contributed to the effects of ACh. To differentiate these effects, neuronal responses to ACh were recorded before and after the addition of hexamethonium (100 μM) and atropine (10 μM) to the perfusate. In neurons from control hearts, hexamethonium reduced the amplitude (to $5 \pm 2$ mV) but did not affect the duration ($14 \pm 2$ s) of ACh-evoked depolarization significantly. Hexamethonium also slowed the initial phase of depolarization and eliminated AP generation (Fig. 1B). However, in decentralized neurons, overall hexamethonium had no effect on either depolarization amplitude ($14 \pm 2$ mV) or duration ($1.1 \pm 0.4$ min). Furthermore, these neurons generated APs not only during the initial phase of the depolarization but throughout the plateau phase (Fig. 1B).

Atropine exerted little effect on the amplitude ($8 \pm 2$ mV) or duration ($15 \pm 2$ s) of ACh-induced depolarization in control neurons (Fig. 1C). APs were discharged during the initial phase of the depolarization, but these were fewer in number compared with the preatropine response. After chronic decentralization, atropine reduced the depolarization evoked by ACh (to $10 \pm 2$ mV) without affecting duration ($1.0 \pm 0.4$ min). In these neurons, although APs occurred on the rising phase of the depolarization, they were prevented from occurring during the plateau phase (Fig. 1C). In both groups of neurons, ACh-induced effects were eliminated by concurrent application of hexamethonium and atropine (Fig. 1D).

Nicotine. Nicotine depolarized neurons from control (Fig. 2A) and decentralized (Fig. 3A) hearts. In both cases, APs could be generated during the initial phase of the depolarization. During the plateau phase of these responses, spontaneous APs were not evident, nor could intracellular current injection evoke APs. In both groups of neurons, ACh-induced effects were eliminated by concurrent application of hexamethonium and atropine (Fig. 1D).

Fig. 4. Effects of muscarine (Musc, arrows) on a sham control neuron elicited before (A) and during (B) atropine exposure. Neuron was stimulated with depolarizing and hyperpolarizing ICS (1-s duration) before and just after application of Musc (100 μM over 15 ms) by pressure ejection from a nearby pipette tip. Changes in whole cell membrane conductance were inferred from changes in membrane potential responses to hyperpolarizing pulses. A, left: before application of Musc, depolarizing stimulation evoked 1 AP; there was no anodal break firing after hyperpolarization. Musc depolarized the membrane potential and facilitated multiple AP generation during depolarizing currents (middle). Membrane potential was returned to the resting level by steady intracellular current injection (*). In this state, a hyperpolarizing current pulse evoked a greater change in membrane potential than occurred before Musc application (indicating a decrease in conductance); an AP occurred at the anodal break. Responses to intracellular stimulation after washout of Musc (right) were similar to those elicited before Musc application. B: atropine (10 μM, 5 min in perfusate) eliminated Musc-induced depolarization and prevented muscarinic-induced changes in neuronal responses to ICS application. Vertical bars, 10 mV or 0.5 nA; horizontal bar, 3 s.
Muscarine. Muscarine modified membrane potential, whole cell conductance, and neuronal excitability of both control and decentralized neurons. Muscarine depolarized control neurons by 11 ± 2 mV for a mean duration of 2.0 ± 0.1 min (Fig. 4A), while also reducing mean whole cell conductance by 30 ± 4%. Depolarizing current pulses with amplitude sufficient to produce one AP before muscarine application (Fig. 4A) evoked multiple (2–3) APs in the presence of muscarine (Fig. 4A). In addition, anodal break firing at the termination of hyperpolarizing pulses occurred during but not before muscarinic application (Fig. 4A). After membrane potentials had returned to the resting levels, depolarizing and hyperpolarizing stimuli evoked responses (Fig. 4A) similar to those elicited before muscarine administration. No spontaneous APs were evoked during either the rising or plateau phases of muscarine-induced depolarization. In decentralized neurons, muscarine induced a rapid depolarization of cardiac neurons with a mean peak depolarization amplitude (21 ± 3 mV) that was significantly greater than the corresponding value found in control neurons. Furthermore, APs were generated during both the rising and plateau phases of depolarization (Fig. 5A) in decentralized neurons. When membrane potential began to repolarize, oscillations occurred with a mean periodicity of 2.4 ± 0.3 s; bursts of APs occurred at the positive-going peaks of these oscillations (Fig. 5A). Mean duration of the muscarine-induced depolarizations (2.6 ± 0.2 min) was significantly longer in decentralized compared with sham control neurons. Muscarine also decreased whole cell conductance by 65 ± 5%, a significant increase over the corresponding value in control neurons. Atropine eliminated all muscarine-induced effects in control and chronic decentralized neurons (Figs. 4B and 5B).

In response to prolonged intracellular depolarizing currents, intrinsic cardiac neurons exhibited two types of discharge patterns. One class that was designated “accommodating” produced multiple APs with decrementing rate over time during prolonged intracellular depolarization (Fig. 6A, inset). The other class of neurons, designated “phasic,” generated a single AP at the onset of the prolonged depolarization (Fig. 6B, inset). The majority of chronically decentralized intrinsic car-
diac neurons (72%) displayed phasic behavior. These two classes of neurons displayed different firing patterns in response to muscarine. After decentralization, mean amplitudes of muscarine-induced depolarization were not significantly different between accommodating (22 ± 3 mV) and phasic neurons (20 ± 2 mV), yet the AP firing rate induced by muscarine was greater in accommodating cells (Fig. 6). Moreover, the peak amplitude of muscarine-induced membrane potential oscillations was greater in accommodating neurons (Fig. 6) than in phasic neurons. In fact, some phasic neurons tested with muscarine did not discharge any APs during membrane oscillations (Fig. 6B).

Activation of muscarinic receptors increased the excitability of intracardiac neurons. In control neurons with phasic firing behavior, anodal break firing was evoked before muscarine application (Fig. 4A). After muscarine, control phasic neurons exhibited multiple APs during depolarizing current injections and hyperpolarizing stimulation evoked anodal break firing after exposure to muscarine (Fig. 4A). In decentralized phasic neurons, muscarine induced an increase in excitability that was proportionally greater (Fig. 7) than the excitability increase displayed by control phasic neurons in response to this agent. Depolarizing stimuli applied before muscarine exposure generated single APs, whereas hyperpolarizing stimuli evoked no anodal break firing (Fig. 7). After muscarine application, depolarization evoked continuous AP firing (Fig. 7) and anodal break firing was evident after hyperpolarizing current injection (Fig. 7). Atropine abolished all muscarine-induced neuronal responses (Fig. 7).

Muscarinic Modulation of Synaptic Activity

Single-pulse electrical stimulation of nerves connected to ganglia under study showed that synaptic inputs were present on ~90% of neurons sampled from control hearts and ~80% of neurons from decentralized hearts. Muscarine had no detectable effect on neurotransmission to control neurons (data not shown). In
contrast, synaptic activation of decentralized neurons was facilitated by muscarine in the four neurons tested. Decentralized neurons responded to single-pulse stimulation of an interganglionic nerve with one orthodromically mediated AP (Fig. 8A). The same stimulus, repeated in the presence of muscarine, evoked multiple APs (Fig. 8B).

Cholinergic Receptor Autoradiography

Autoradiography done on RAGP tissue taken from 12 control and 18 decentralized hearts showed that binding of [3H]QNB to muscarinic receptors and [125I]IPH to nicotinic receptors was highly specific in both groups (Fig. 9). Over 90% of [3H]QNB binding was blocked by atropine (Fig. 9F), whereas nonspecific binding of [125I]IPH, determined in the presence of nicotine, was undetectable (Fig. 9C). There was a marked difference in the distribution and abundance of binding sites for the muscarinic and nicotinic radioligands in control and decentralized RAGP. Specific binding sites for [3H]QNB were associated primarily with ganglia (shown histologically by arrows in the micrographs of Fig. 9, A and D) and, to a lesser extent, the adjacent myocardium (Fig. 9E). However, [125I]IPH binding appeared to be restricted to ganglia with no binding observed in myocardial cells (Fig. 9B). Table 1 shows that muscarinic receptors were approximately tenfold more abundant than nicotinic receptors in ganglia from both groups and that the density for each type of receptor was unaffected by chronic decentralization.
DISCUSSION

Whereas decentralization of the ICN system intercepts all extracardiac inputs to these ganglia, neural connections are still present. The results of this study show that cholinergic receptor-mediated responses of neurons in the ICN system remain viable after chronic removal of inputs from neurons in extracardiac intrathoracic ganglia and the central nervous system. Chronic decentralization appears to differentially remodel cholinergic function within the ICN system, preferentially facilitating muscarinic mechanisms in this system. As such, synaptic communication among intrinsic cardiac neurons is maintained after chronic decentralization with neuronal responses to ACh being augmented.

The effects of decentralization on the response characteristics of peripheral autonomic neurons to ACh reported in previous studies are conflicting, ranging from supersensitivity to a marked reduction in sensitivity. Decentralization of both sympathetic and parasympathetic neurons is most frequently reported to result in changes in neuronal membrane potential and conductance in response to ACh application (13, 16, 23, 25, 26, 28, 30–32). In contrast, some neurons have been reported to display less sensitivity to ACh after decentralization (15) or no change in ACh sensitivity (17). The present findings support evidence that the majority of autonomic neurons display increased sensitivity to ACh after they are decentralized.

ACh activates both nicotinic and muscarinic receptors in intracardiac neurons before and after chronic decentralization (Figs. 1–5). Activation of nicotinic receptors alone, either by ACh in the presence of atropine, or by nicotine, evoked similar depolarization in control and decentralized neurons (Figs. 1C, 2, and 3). Brief bursts of APs were generated at the start of depolarizations induced by ACh. During the nicotinic-induced plateau phase of depolarization, evoked and spontaneous APs were eliminated (Figs. 1C, 2, and 3) in both control and ICN-decentralized neurons. Nicotine-induced spike blockade in autonomic ganglia has been previously described (35). These data indicate that intrinsic cardiac neuronal nicotine receptor function is maintained after chronic decentralization of these neurons.

The exaggerated responses to ACh displayed by intracardiac neurons after chronic decentralization were mediated primarily by enhanced muscarinic receptor function. The amplitude and duration of muscarine-induced responses were significantly greater in decentralized (Figs. 5A, 6, and 7) than control (Fig. 4) neurons. Furthermore, muscarine produced greater increases in whole cell resistance in chronically decentralized than intact neuronal preparations (Figs. 4 and 5). Moreover, activation of muscarinic receptors either by ACh (Fig. 1) or by muscarine (Figs. 5 and 6) evoked rhythmic oscillations in neuronal membrane potentials. This novel observation was dependent on the neuronal membrane potential because resetting this potential to resting levels by current clamping the cells caused the oscillations to cease. In such instances, oscillations resumed after the current clamp was terminated (asterisk in Fig. 5A).

The ICN system contains neurons exhibiting phasic and accommodating firing behaviors (4, 29). The baseline properties of these types of neurons influence their responses to neurochemical challenge. After chronic decentralization, the excitability of both phasic and accommodating neurons was increased in the presence of a muscarinic agonist. Muscarine, at a dose that produced high-frequency APs and high-amplitude membrane potential oscillations in accommodating neurons, produced lower-amplitude oscillations sometimes without AP discharge in phasic neurons (Fig. 6). Thus accommodating neurons were more excitable than phasic neurons in the presence of muscarine. However, at higher doses of muscarine, some phasic neurons also generated APs during these oscillations (data not shown). In phasic
neurons, muscarine enhanced the firing frequency during intracellular depolarization and facilitated anodal-break firing after hyperpolarizing current injection (Fig. 7).

The mechanisms underlying muscarine-induced oscillations in decentralized intrinsic cardiac neuron membrane potentials remain unknown. These oscillations could result from synaptic interactions among various local intrinsic cardiac neurons that are dependent on muscarinic receptors. In this regard, pyramidal neurons in the guinea pig hippocampus display muscarinically evoked depolarization accompanied by membrane oscillations with associated AP burst firing (8). When synaptic transmission is disrupted in the hippocampus, activation of muscarinic receptors still evokes neuronal depolarization such that membrane potential oscillations and rhythmic firing cease (8). Given that the intrinsic cardiac neural network con-
contains a heterogeneous population of neurons that include afferent, efferent, and local circuit neurons (3, 4, 7), each with complex neurochemistry and multiple interconnections, it is feasible that local network interactions are responsible for the muscarine-induced oscillations and rhythmic APs so generated.

Muscarine-induced oscillations in membrane potentials may also be due to factors intrinsic to individual neurons. In that regard, multiple muscarinic receptor types have been associated with intrinsic cardiac neurons (19, 20). Thus it is possible that muscarine activates more than one receptor subtype on these neurons. Activation of different receptor subtypes can produce different cellular responses: M1 receptors are excitatory, whereas M2 receptors are inhibitory, in intrinsic cardiac neurons (1, 2, 36). If these subtypes were coactivated by muscarine, alternating depolarization and hyperpolarization could result that would lead to membrane oscillations.

A major role for muscarinic receptors in the peripheral autonomic nervous system is modulation of synaptic efficacy (9). An important observation arising from our study was the fact that muscarine facilitated neurotransmission within chronically decentralized intrinsic cardiac ganglia (Fig. 8). We propose that this enhancement of synaptic activity represents a major determinant of the ongoing spontaneous activity generated by neurons within chronically decentralized intrinsic cardiac ganglia (3) or those in transplanted hearts (24). It is proposed that enhancement of muscarinic synaptic mechanisms therein may be critical for maintaining the function of intracardiac reflex loops after removal of extracardiac neuronal inputs. One obvious consequence of this remodeling would be to increase the efficacy of synaptic communication among neurons as the ICN system reorganizes after the chronic loss of its extracardiac neuronal inputs.

Decentralization-induced increases in efficacy of muscarinic receptor neurotransmission within the ICN system may reflect an upregulation of the number of postjunctional cholinergic receptors or an increase in the efficiency of intracellular receptor-effector coupling. Employing autoradiographic analysis of receptor binding properties, no change in the number of muscarinic or nicotinic receptors associated with intrinsic cardiac neurons was observed (Table 1, Fig. 9). Given these data, the observed increase in muscarinic receptor-mediated responses in chronically decentralized preparations likely reflects changes in receptor-effector coupling efficiency.

Perspectives. Regional control of cardiac function is dependent on the coordination of activity generated by neurons within intrathoracic autonomic ganglia and the central nervous system. The hierarchy of nested feedback loops therein provides precise beat-to-beat control of regional cardiac function (4). Within the hierarchy of intrathoracic ganglia and nerve interconnections, complex processing takes place that involves summation of sensory inputs, preganglionic inputs from central neurons, and intrathoracic ganglionic reflexes activated by local cardiopulmonary sensory inputs (4, 34). The activity of neurons within intrathoracic autonomic ganglia is likewise modulated by circulating hormones, chief among them being circulating catecholamines and angiotensin II (4, 6). The progressive development of cardiac disease is associated with remodeling of these neurohumoral control mechanisms (4, 6, 7). Whereas much has been learned about cardiomyocyte adaptations, sparse information exists regarding understanding the role of afferent and efferent neuronal interactions within the central and peripheral nervous system and between the cardiac nervous system and the cardiac myocytes during the evolution of cardiac disease.

Data presented in this study demonstrate that intrinsic cardiac neurons retain their capacity to interact after removal of extracardiac neuronal inputs and that their local cholinergic inputs are maintained. The ICN system, once disconnected from other intrathoracic and central neurons, adapts to this loss with changes in synaptic efficacy. Furthermore, cholinergically mediated excitation of chronically decentralized intrinsic cardiac neurons becomes enhanced primarily via potentiation of muscarinic receptor-mediated neurotransmodulation. The net effect of this remodeling is an upregulation of neurotransmission within the ICN system. This may effectively increase information processing within decentralized intracardiac reflex loops, compensating in part for the loss of inputs from extracardiac neurons. Data presented herein may help to explain how the ICN system remodels in the decentralized (3) or transplanted (24) state to sustain and modulate cardiac electrical and mechanical function.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-58140 (to J. L. Ardell), by a Medical Research Council of Canada grant (to F. M. Smith and J. A. Armour), and by the New Brunswick Heart and Stroke Foundation (to F. M. Smith). F. M. Smith was a Research Scholar of the Heart and Stroke Foundation of Canada for a portion of this study.

Table 1. Effect of chronic decentralization on the density of muscarinic and nicotinic receptors in cardiac ganglia of the ventral right atrial ganglionated plexus

<table>
<thead>
<tr>
<th>Receptor/Radioligand</th>
<th>Acute decentralization</th>
<th>Chronic decentralization</th>
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<tr>
<td>Muscarinic/[3H]QNB</td>
<td>80.8 ± 11.9(8)</td>
<td>74.2 ± 6.1(12)</td>
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<tr>
<td>Nicotinic/[125I]IPH</td>
<td>7.01 ± 2.03(4)</td>
<td>5.22 ± 0.51(6)</td>
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Values are means ± SE; n values are in parentheses. [3H]QNB, l-[3H]quinclidinyl benzilate; [125I]IPH, 125I-labeled epibatidine. A single value for specific binding was determined for each animal based on readings obtained from multiple sections through ganglia. Estimated tissue equivalent (dry weight) provided with autoradiographic microscales was used as the denominator for specific binding units.
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