Remodeling of intrinsic cardiac neurons: effects of β-adrenergic receptor blockade in guinea pig models of chronic heart disease

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The intrinsic cardiac nervous system is a primary integration site for sympathetic and parasympathetic efferent activity, responding to descending central inputs, sensory afferents, and local circuit neuronal inputs (1). This system represents the final common pathway for the cardiac nervous system and has a primary role in beat-to-beat coordination of regional cardiac function (1). Previous studies in our laboratory demonstrated that neurons located in this plexus undergo remodeling with chronic heart disease, leading to increases in sensitivity to neuromodulators such as histamine and increased expression of neuronal nitric oxide synthase (11, 13). More recently, we showed that these intrinsic cardiac neurons are also synergistically modulated by norepinephrine (NE) and angiotensin II (ANG II) (8).

Since chronic heart disease leads to increases in the interstitial levels of both NE and ANG II within the heart (6, 10, 24), and because both of these substances are capable of modifying the output from intrinsic cardiac neurons, we wanted to determine whether the responses of intrinsic cardiac neurons to these modulators would be altered in models of chronic heart disease. In addition, since clinical treatment of chronic heart disease typically includes β-adrenergic receptor blockade (14), and β-receptor blockade mitigates adverse cardiac disease-induced remodeling of sympathetic neurons (10), we also wanted to determine the effects of chronic β-adrenergic blockade on disease-induced changes in intrinsic neuronal responses. Our hypothesis was that intrinsic cardiac neurons would demonstrate altered sensitivity to both NE and ANG II with both models of chronic heart disease due to the increased sympathetic outflow and elevated ANG II production. If remodeling of the intrinsic cardiac nervous system during cardiac disease progression requires stimulation of adrenergic receptors, chronic inhibition of β-receptors should alter these changes. To critically evaluate this hypothesis, we examined the effects of either chronic myocardial infarction (MI) or chronic pressure overload (PO) on neuronal responses to adrenergic agonists, muscarinic agonists, and ANG II, as well as the ability of intracardiac neurons to follow stimulation of presynaptic inputs. These experiments were done with and without chronic treatment with the nonspecific β-adrenergic antagonist timolol.

MATERIALS AND METHODS

Animals. Nine-week-old, male Hartley guinea pigs (Charles River), weighing between 500 and 650 g, were used in these chronic studies. Animals of the same age and weight were used as time control (sham) surgeries, in which the heart was visualized but not disturbed. Additional age-matched male Hartley guinea pigs were used for controls. All procedures were approved by the Institutional Animal Care and
Use Committees of East Tennessee State University and Ithaca College and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington DC, 1996).

Surgical induction of heart disease. As in previous studies, two models of heart disease were produced: chronic MI and chronic PO. The preparation and general surgical procedures were identical to those previously described (11, 13). A total of 17 MI animals were generated by ligation of the ventral descending coronary artery, and a total of 13 PO animals were produced by constricting the descending dorsal aorta by ~15–20%. Animals were allowed to recover for 8–10 wk before termination.

Drug treatments. For chronic treatment with the nonselective β-adrenergic agonist timolol, animals were anesthetized as previously described (11, 13) and surgically implanted with an Alzet osmotic pump under the skin 2 wk after the initial thoracic surgery. Timolol was delivered at a dosage of 2 mg·kg\(^{-1}\)·day\(^{-1}\). Vehicle controls were implanted with a pump containing only saline for 3–4 wk. The timolol pumps were replaced at 3- to 4-wk intervals, for a total drug treatment period of 42–50 days.

In vivo testing of timolol blockade. A subset of control animals were surgically implanted with either saline (n = 5) or timolol (n = 5) pumps as described under Drug treatments and maintained for 3 wk. After the 3-wk timolol treatment, guinea pigs were anesthetized and intubated. Core body temperature was maintained at 38.5°C with a circulating water heating pad. The right carotid artery was cannulated for blood pressure measurement, and heart rate was assessed from a lead II ECG. The right jugular vein was cannulated for agonist injections. Each animal was challenged with 1 μg/kg isoproterenol delivered via the jugular vein, and heart rates and blood pressures were determined.

Terminal experiments. Guinea pigs (800–1,000 g at termination) were euthanized by CO2 inhalation and exsanguination. The heart was removed, weighed, and placed into ice-cold Krebs Ringer solution (in mM: 121 NaCl, 5.9 KCl, 2.5 CaCl\(_2\), 1.2 MgCl\(_2\), 1.2 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), and 8 glucose, aerated with 95% O\(_2\)-5% CO\(_2\)) superfused (6–8 ml/min) with 35–37°C Krebs-Ringer. NE (Sigma, pH 7.4). The cardiac plexus, located in the epicardium of the atria, was stimulated with an extracellular electrode placed between the atria and the left ventricle. A bipolar electrode was placed on the epicardium of the right ventricle and used as a reference. NE and bethanechol (Sigma, 10\(^{-3}\) M) were applied by local pressure ejection (6–9 psi, Picospritzer, General Valve) through small-tip-diameter (5–10 μm) glass micropipettes positioned 50–100 μm from the individual neuron. For multiple tests of responses in the same cell, the cells were allowed to wash (via the circulating Krebs solution) for several minutes between applications, until the responses returned to control levels. ANG II was applied by inclusion in the circulating bath solution (100 nM, Sigma) and was applied for a total of 3–5 min.

Electrophysiological methods. Intracellular voltage recordings from intracardiac neurons were obtained with an AxoClamp 2B amplifier (Axon Instruments) from cells impaled with 2 M KCl-filled microelectrodes (40–80 MΩ). Data were collected, digitized, and analyzed using pClamp 8.2 (Axon Instruments). Individual neurons were used for an experiment if the membrane potential was ~40 mV or less and produced action potentials (APs) with an overshoot of at least 20 mV.

Neuronal excitability was monitored by observing the response to a series of long depolarizing current pulses (0.1–0.5 nA, 500 ms). For each cell, following characterization of the basic electrophysiological properties, changes in evoked AP frequency were assessed immediately following a 1- to 2-s application of either NE or bethanechol, and doses were chosen that produced maximal or near-maximal responses (8). Changes in AP frequency versus stimulus amplitude were determined to assess relative drug-induced changes in excitability.

To stimulate synaptic inputs, an extracellular electrode was placed on nerve fiber bundles leading to the ganglion containing the neuron of interest. Stimuli (2 ms, 0.1–10 V) were first given at a frequency of 0.5 Hz to elicit an orthodromic AP from the intracardiac neuron. Orthodromic responses were determined either by the ability to generate a subthreshold response or by the presence of a time delay between the stimulus artifact and the neuronal response, since it was not possible to obtain subthreshold excitatory postsynaptic potentials (EPSPs) in all cells. Suprathreshold stimuli were then given in 2-s trains at frequencies of 10, 20, and 30 Hz, and the number of APs produced by the neuron of interest was determined. Subthreshold EPSPs were analyzed by averaging individual events (5–20). The average trace was used to determine the amplitude and time constant of the decay determined from a single exponential fit.

### RESULTS

#### Evaluation of disease state. Two models of heart disease were utilized in this study: chronic MI and a chronic PO. As in previous studies (11, 13), the heart tissue was weighed at the time of euthanasia to assess cardiac hypertrophy, along with lung tissue weights to assess the relative disease state of the animals (Table 1). MI animals showed cardiac hypertrophy with no evidence of pulmonary edema, whereas PO animals showed even greater cardiac hypertrophy and increased fluid retention in the lungs. Several PO animals also had increased fluid in the pericardial sac at the time of termination.

<table>
<thead>
<tr>
<th>Recovery Time (days)</th>
<th>Body Weight, g</th>
<th>% Heart Weight</th>
<th>% Wet Lung Weight</th>
<th>% Dry Lung Weight</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>77 ± 6</td>
<td>948 ± 68</td>
<td>0.69 ± 0.03</td>
<td>0.50 ± 0.05</td>
<td>6</td>
</tr>
<tr>
<td>Sham + Timolol</td>
<td>81 ± 2</td>
<td>1,040 ± 42</td>
<td>0.66 ± 0.03</td>
<td>0.45 ± 0.04</td>
<td>6</td>
</tr>
<tr>
<td>MI</td>
<td>70 ± 4</td>
<td>934 ± 76</td>
<td>0.76 ± 0.05*</td>
<td>0.44 ± 0.03</td>
<td>6</td>
</tr>
<tr>
<td>MI + Timolol</td>
<td>75 ± 3</td>
<td>912 ± 53</td>
<td>0.73 ± 0.05*</td>
<td>0.43 ± 0.04</td>
<td>8</td>
</tr>
<tr>
<td>PO</td>
<td>68 ± 2</td>
<td>873 ± 60</td>
<td>0.92 ± 0.17†</td>
<td>0.67 ± 0.12†</td>
<td>7</td>
</tr>
<tr>
<td>PO + Timolol</td>
<td>58 ± 1</td>
<td>863 ± 44</td>
<td>1.01 ± 0.27*</td>
<td>0.87 ± 0.25*</td>
<td>6</td>
</tr>
</tbody>
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Values are means ± SE; n = number of animals. MI, myocardial infarction; PO, pressure overload. *P < 0.05 by ANOVA; †P < 0.05 versus MI by t-test.
that both NE and bethanechol produce an increase in evoked APs in guinea pig intracardiac neurons (8).

Application of NE produced a significantly greater frequency of evoked APs with increasing stimulus intensity in neurons from the chronic disease model animals compared with controls (Fig. 1). Data from sham surgical animals were not different from control animals, so that data have been pooled and is indicated on the graphs as controls (n = 98 cells). In both PO (n = 65 cells) and MI animals (n = 49 cells), the slope of the frequency of evoked APs versus stimulus intensity curve was significantly greater following NE application compared with baseline (Fig. 2). Although the slope of the frequency/intensity curves in disease models were not statistically different from controls with NE application, at the maximal stimulus intensity (0.5 nA), there was a significantly higher NE-evoked frequency in neurons derived from MI and PO animals compared with controls (ANOVA, P < 0.01, Fig. 3).

Addition of 100 nM ANG II to the circulating bath solution by itself did not alter the evoked frequency in either control preparations (n = 19) or MI tissues (n = 20). There was a small increase in frequency in neurons derived from PO animals with ANG II application (n = 26) (Fig. 3). The combination of NE application in the presence of ANG II produced an increase in evoked APs and an increase in the slope of the frequency-intensity curve in neurons from control animals (Figs. 1 and 2), confirming our previous results (8). In contrast, the addition of NE in combination with bath-applied ANG II did not produce a statistically significant increase in evoked AP

Fig. 1. Evoked action potential frequency versus stimulus intensity with adrenergic and muscarinic agonists. The frequency of evoked action potentials was determined with increasing stimulus intensity in neurons from control animals (A1, B1), chronic myocardiak infarction (MI) animals (A2, B2), and chronic pressure overload (PO) animals (A3, B3). Excitability was monitored in control Krebs solution (baseline), following local application of norepinephrine (NE), bethanechol (Beth), 100 nM ANG II with NE application (NE+ANG), and in ANG II with Beth application (Beth+ANG). Points represent the means ± SE of 212 or more cells (98 control, 65 PO, and 49 MI). The lines are a linear regression fit of the averaged data for each curve.
The effect of chronic treatment with the nonselective α-receptor blocker timolol was tested on both chronic MI and PO models at baseline and in response to NE, NE+ANG, Beth, or Beth+ANG II. The bars are means ± SE for the slopes of the regression for individual cells. Both NE and Beth consistently increased the slopes over baseline in all models tested (\( *P < 0.05 \), within-group comparison vs. baseline). The combination of ANG II with NE or Beth showed a significant increase in the slope only in control tissues (\( #P < 0.05 \), within-group comparison, significant difference NE vs. NE+ANG II).

Bethanechol application increases the frequency of evoked APs in intrinsically cardiac neurons (Fig. 1). The muscarinic-induced increase in the maximum evoked frequency and slope of the frequency/intensity curves was equivalent in neurons from control (\( n = 31 \) cells) and MI animals (\( n = 37 \) cells) (Figs. 2 and 3). Bethanechol produced a similar increase in the slope of the frequency/intensity curves in PO animals (Fig. 2). However, there was a significant increase in the maximum evoked AP frequency following bethanechol application in neurons from the PO preparations (\( n = 30 \) cells, \( P < 0.001 \) by ANOVA, Fig. 3). Addition of ANG II potentiated the maximum evoked bethanechol responses in control and MI neurons, but ANG II had no additional effect on bethanechol responses in cells from PO animals (Fig. 3). Addition of ANG II did not alter the slope of the frequency/intensity curves for any of the models tested (Fig. 2).

\( \beta \)-Adrenergic treatment and adrenergic responses. Chronic heart disease is often treated with \( \beta \)-adrenergic receptor blockers to mitigate the effects of excess sympathetic activation (7). The effect of chronic treatment with the nonselective \( \beta \)-adrenergic receptor blocker timolol was tested on both chronic MI (\( n = 8 \) animals) and PO models (\( n = 6 \) animals). Timolol (or vehicle) was delivered via an Alzet osmotic pump implanted under the skin 2 wk following the initial surgery, at a dosage of 2 mg·kg\(^{-1}\)·day\(^{-1}\). The total time period for timolol treatment in the surgical models was 42–50 days.

A subset of healthy animals was treated with timolol or saline vehicle implants for 3 wk to evaluate the efficacy of the \( \beta \)-receptor blockade. After 3 wk, heart rate and blood pressure were determined (under anesthesia) before and in response to the bolus injection of 1 µg/kg of isoproterenol. Timolol treatment resulted in a significant reduction in basal heart rate and blood pressure versus the saline implants (Fig. 4) and also reduced the increase in both HR and BP induced by the isoproterenol challenge.

In animals treated with timolol for 6–8 wk, there was no change in the relative weights of the heart or lungs, such that any hypertrophy or edema induced by the heart disease was still present in animals given timolol therapy (Table 1). Neuronal responses to NE and ANG II application were determined as before. It should be noted that timolol was not present in the solutions at the time of testing, and at least an hour had elapsed since hearts and associated intrinsic cardiac neurons were harvested from the animals. Control and sham surgical animals treated with timolol showed no differences in responses and that data were pooled (\( n = 57 \) cells). Chronic timolol treatment had no effect on NE-evoked responses in control animals (\( n = 44 \) cells, Fig. 5). In MI animals (\( n = 33 \) cells), the increased response to NE (versus untreated controls) was still observed in the timolol-treated preparations, and the ANG II responses (\( n = 12 \)) were also unchanged compared with MI alone (Fig. 6).

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**Fig. 2.** Changes in the slopes of the frequency-intensity curves with neuromodulators. The mean slope of regression fits to individual cells for control, MI, and PO models at baseline and in response to NE, NE+ANG, Beth, or Beth+ANG II. The bars are means ± SE for the slopes of the regression for individual cells. Both NE and Beth consistently increased the slopes over baseline in all models tested (\( *P < 0.05 \), within-group comparison vs. baseline). The combination of ANG II with NE or Beth showed a significant increase in the slope only in control tissues (\( #P < 0.05 \), within-group comparison, significant difference NE vs. NE+ANG II).

**Fig. 3.** Maximum frequency of evoked action potentials with neuromodulator application. The maximum evoked frequency of action potentials was determined for each cell (at 0.5 nA) with no modulators added (baseline) and following application of NE or Beth alone, or with ANG II included in the circulating bath. Bars represent means ± SE. NE and Beth consistently increased the maximum evoked frequency over baseline in control, MI, and PO tissues (\( *P < 0.05 \), within-group comparison vs. baseline, +\( P < 0.05 \), between-group comparison with the same agonist). By itself, ANG II increased the frequency over baseline only in the PO models (\( *P < 0.05 \)). The combination of ANG II and NE significantly increased the response to NE only in neurons from control animals (\( #P < 0.05 \), within-group comparison vs. NE alone). The combination of ANG II and Beth significantly increased the Beth response in neurons from control and MI preparations (\( *P < 0.05 \), within-group comparison vs. Beth alone).
In the PO animals, timolol treatment resulted in a significant inhibition in the evoked frequency in the presence of ANG II alone (n = 12 cells) compared with untreated PO neurons with ANG II (P < 0.05). NE responses in the neurons from PO animals (n = 18 cells) were unchanged with timolol treatment, and the combination of ANG II and NE (n = 12) were similar to that seen in MI; no significant differences but a trend toward reduced responses (Fig. 5).

Synaptic efficiency with chronic heart disease. The whole mount preparation of the cardiac plexus allows for the examination of synaptic efficacy within the intrinsic cardiac nervous system as well as the ability of specific neuromodulators to modify such responses. An extracellular focal electrode was used to stimulate the fiber bundles leading to the neurons used for voltage recording. Only orthodromic responses, as evidenced by a delay between the stimulus artifact and the neuronal voltage response, were used for analysis. Initially, low-frequency (0.5 Hz) stimulation was used to elicit subthreshold EPSPs. These could be observed in ~30% of cells tested. The remaining 70% of cells displayed a high safety factor, such that it was not possible to produce subthreshold responses. EPSPs were examined in control, MI, and PO preparations. No significant differences in EPSP amplitude, duration, or decay time constants were observed between control animals and preparations from disease models (Table 2).

The fiber tract stimulator was then set to suprathreshold stimulus intensity, and the neuronal responses to a 2-s train of stimuli at 10, 20, and 30 Hz was determined. The number of APs produced by the postsynaptic neuron during the 2-s stimulation was determined at each frequency. This output frequency was used as an index of synaptic efficacy. For neurons from control (n = 23) and MI preparations (n = 30), the cells responded with an output frequency of ~5–10 Hz, even as the stimulus frequency increased. In contrast, the output frequency of neurons from PO animals (n = 27) continued to increase with increasing stimulus frequency (Fig. 6).

Prior studies have shown that stimulation of muscarinic receptors can increase synaptic efficacy in autonomic neurons (15). Therefore, to determine whether blockade of muscarinic receptors could alter the synaptic efficiency in the intrinsic cardiac ganglion, atropine (1 µM) was added to the circulating bath solution, and the same series of train frequencies were tested in the control, PO, and MI preparations (n = 6 for each model). Atropine had no effect on control, MI, or PO responses (data not shown).

Fiber tract stimulation in preparations treated with timolol showed no differences in neurons from control (n = 12) or MI animals (n = 14) but did show a significant decrease in the output frequency in cells from PO animals treated with timolol (n = 12, Fig. 7). Examples of the responses of a cell from a control preparation, a PO animal, and PO with timolol treatment are shown in Fig. 7 (top). Addition of atropine (1 µM) also had no effect on the output frequency in the timolol-treated preparations (n = 6 or more for each model, data not shown).

DISCUSSION

Chronic heart disease produces significant remodeling of both cardiac tissues and neuronal systems within the heart (1, 7, 24). Neuronal changes include increases in sympathetic outflow, diminished central drive for parasympathetic preganglionics, and alterations in intrinsic cardiac neuronal responses (1, 2, 7, 24). The present study demonstrates that two different forms of chronic heart disease, myocardial infarction and pressure overload can produce alterations in intracardiac neurons that vary, depending on the form of disease. In addition, treatment with timolol, a nonselective β-adrenergic antagonist, can alter aspects of these induced neuronal changes, indicating that increased sympathetic inputs to the intrinsic cardiac neurons are responsible, in part, for a subset of the autonomic neuronal remodeling observed.

Two different models of chronic heart disease were induced in guinea pigs: MI and PO. The MI model is characterized by an initial ischemic event that leads to an infarct area of ~5–7% of the left ventricle (13). Animals show evidence of left ventricle hypertrophy but no pulmonary edema. PO animals show even greater hypertrophy, along with increased lung weights. Previous studies demonstrated that both of these models produce similar alterations in neuronal responses to histamine, an increase in neuronal nitric oxide synthase expression, and a proliferation in cardiac mast cell density (11, 13). The present study demonstrates that both models are also
associated with similar increases in neuronal sensitivity to NE and ANG II. However, there are also changes in intrinsic cardiac neuronal function that are specific to the disease model, particularly the dynamic interactions of adrenergic, cholinergic, and ANG II-dependent receptor mechanisms.

MI and PO animals at ~2 mo of cardiac disease progression show increased sensitivity to NE as evidenced by an increase in the evoked AP generation. Increases in sympathetic outflow have been documented with chronic heart disease (7, 18, 24). Changes in sympathetic innervation of the heart following MI show regional variation (16, 22), but in the atrial regions, where the neurons for this study are located, studies showed either no change in NE innervation or an upregulation of sympathetic innervations (16, 21). Even though the tissue damage produced by the MI is small and distant from the cardiac ganglion, and PO preparations. Neurons from MI and PO preparations showed no change in either the NE or ANG II responses in the MI preparations.

Both substance P and calcitonin gene-related peptide, which are found in cardiac sensory afferent fibers, can stimulate mast cell degranulation (20). These sensory fibers would be strongly stimulated during the initial response to both MI and PO induction and could serve as a trigger stimulus in the neuronal remodeling process. In the PO models, the chronic elevations in blood pressure could also result in prolonged afferent stimulation and a concomitant increase in RAS activation and local ANG II production. The combination of increased sympathetic activity and elevated ANG II levels could then lead to increased NE release and increased NE sensitivity. However, it is also possible that the elevation in ANG II levels within the cardiac ganglion produces a chronic potentiation of the NE response and a partial accommodation of ANG II-mediated effects. This possibility is supported by the blunting of the responses to the addition of ANG II to the bath solution in MI and PO preparations. Neurons from MI and PO preparations did not show a potentiation in the NE response with ANG II, which could be a result of chronically elevated ANG II levels, such that further stimulation by exogenously applied ANG II is not possible.

Neurons from PO animals also showed an increase in sensitivity to the muscarinic agonist bethanechol, which was not seen in neurons from MI animals. Again, ANG II failed to further potentiate this response in these neurons, as it normally does in control animals, providing further support for the hypothesis that there is chronic stimulation of the ANG II-potentiating mechanism in these animals. The increase in sensitivity to muscarinic agonists was only observed in the PO models, suggesting that this remodeling is specific to this form of cardiac disease.

β-Adrenergic receptor blockade is a standard therapy for patients with chronic heart disease (7, 18, 24). In the guinea pig, treatment with timolol for 3 wk via an osmotic pump produced a significant reduction in basal heart rate and blood pressure and blunted the response to isoproterenol infusion. The combination of induced disease, either MI or PO, coupled with timolol treatment for 6 wk that began 2 wk after the initial surgery, showed no change in either the NE or ANG II responses in the MI preparations.

In timolol-treated PO animals, we observed a reduction in the maximum evoked AP response to ANG II. A major

Table 2. Excitatory postsynaptic potentials in neurons from animals with chronic heart disease are unchanged

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI</th>
<th>PO</th>
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<tbody>
<tr>
<td>RMP, mV</td>
<td>-49.5 ± 7.9</td>
<td>-41.8 ± 5.8</td>
<td>-46.7 ± 9.1</td>
</tr>
<tr>
<td>EPSP amplitude, mV</td>
<td>6.8 ± 0.6</td>
<td>6.6 ± 0.6</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>6.0 ± 0.9</td>
<td>4.4 ± 0.6</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. RMP, resting membrane potential; EPSP, excitatory postsynaptic potential.
difference between the MI and the PO models is that the MI animals undergo a single ischemic event, followed by remodeling and stabilization. Moreover, because of extensive collateralization in the guinea pig myocardium, the percentage of left ventricular mass directly affected by the infarct is relatively small (~8% infarct (13)]. The PO animals, on the other hand, remain in an active and progressive disease state leading to congestive heart failure, and the stressor itself impacts the entire heart. The PO hearts also show greater levels of hypertrophy and pulmonary edema, which again suggests an ongoing and accelerating remodeling state compared with the MI animals.

Timolol treatment commenced 2 wk post-MI or -PO induction. This meant that the MI animals were able to undergo an initial remodeling period without β-receptor blockade. The PO animals had a similar time period without blockade. In both models, timolol was then present during the remaining 6 wk of disease progression. The differences we observed in the basal responses to ANG II with timolol treatment between the MI and PO models may represent the difference between an active disease state (where there is continuous stimulation of RAS, sensory afferents, and cardiac mast cells) and a compensated disease state (where sympathetic outflow may remain high, but the afferent pathways are not recruited). Thus, in the PO animals, timolol may reduce the activation of the RAS such that a decrease in ANG II response is observed. In a study in rabbits with MI, the elevation in ANG II following ischemia increases initially and then returns to near-control levels by 35 days (9). Alternatively, the characteristics of the imposed stressors may impact the observed neurohumoral adaptations. MI induces a heterogeneous stress, primarily localized to the left ventricle. In contrast, PO impacts large areas of both atrial and ventricular tissues in addition to primary arterial baroreceptors associated with the great vessels. Thus the ANG II response in the MI model is likely to be different from that seen with PO. This may account for the differences seen with timolol treatment on the ANG II modulation of neuronal excitability.

Increased sympathetic tone with chronic heart disease can produce an upregulation in the expression of β-receptors in the heart (18). However, chronic β-adrenergic blockade has been shown to increase cardiac sympathetic innervation (5), potentially through disruption of autoreceptor inhibition of axonal outgrowth. At the same time, there is evidence for reduced NE content within nerve fibers (22). In PO models, timolol treatment prevented the increase in synaptic efficiency seen with fiber tract stimulation. MI animals do not show this alteration in synaptic function, which could again be due to the static nature of the disease process in these animals compared with the PO models.

The mechanism underlying the change in synaptic efficiency in the PO models is still undetermined. Prior studies have shown that activation of muscarinic receptors at autonomic ganglia can increase synaptic gain to nicotinic signals (15). Indeed, intracardiac neurons showed increased sensitivity to muscarinic agonists in PO animals. However, addition of atropine to block muscarinic receptors did not alter the synaptic responses in this ganglion. Intrinsically changes in neuronal voltage sensitivity are also unlikely to be responsible. A change in voltage sensitivity should also been seen as an increase in the number of tonically active neurons with depolarizing current pulses, but there is no change in the relative percentage of tonic to phasic neurons with either model of chronic heart disease (11, 13). Since the amplitude of EPSPs is not changed in PO animals, this change is also not due to an increase in quantal content in the presynaptic terminals. Stimulation of the fiber bundles in this preparation leads to the activation of multiple inputs to the neurons. These would include preganglionic nicotinic synapses, sympathetic efferent neurons, afferent fibers, and interneurons. Any given cell may receive any combination of these inputs with fiber tract stimulation. Therefore, the change in synaptic efficiency could still be due to neuromodulator-induced changes in either presynaptic or postsynaptic function, but further studies will need to be done to elucidate this mechanism.

The fact that synaptic efficiency is increased in PO animals suggests that there may be an upregulation of local neuronal processing within the intrinsic cardiac nervous system as cardiac disease progresses. Alterations in neuronal excitability can create variable gates for signal processing in autonomic ganglia (3). Such an effect may represent an ongoing compensation to the increased sympathetic output, reduced central drive from

![Graph showing changes in synaptic efficacy with chronic pressure overload.](http://ajpregu.physiology.org/)
parasympathetic preganglionic inputs, and may underlie in part the development of pathology that could lead to increased risk for arrhythmia and pump failure. Alterations in parasympathetic output disrupts conduction of the pacemaker signal (21).

Increased sympathoexcitation is associated with increased incidence of sudden cardiac death and pump failure (7, 18, 23). The fact that timolol reversed this upregulation of synaptic efficacy demonstrates the functional impact of neuronal targets in effective management of chronic heart disease.

Perspectives and Significance

The current study examined the changes in signal processing within the intrinsic cardiac plexus in guinea pigs utilizing two different models of chronic heart disease as well as the ability of β-receptor blockade to mitigate these changes. The results suggest that there are significant changes in neuronal sensitivity to muscarinic agonists, NE, and ANG II with chronic heart disease, but the ability of β blockers to alter this sensitivity may be dependent on the active disease processes. Disease-induced increases in sympathetic activity, combined with increased ANG II levels, produces an increased sensitivity of intracardiac neurons to adrenergic agonists, with a loss of sensitivity to exogenously applied ANG II. This increased sensitivity to adrenergic agonists may reflect a compensatory response by parasympathetic efferent elements in the intrinsic cardiac ganglia to try to counteract sympathetic influence by enhancing output from parasympathetic neurons. One mechanism that might produce this increased sensitivity would be the chronic elevation in interstitial ANG II levels, leading to chronic potentiation of both adrenergic and muscarinic signaling.

β-Adrenergic blockade did not alter the increased adrenergic sensitivity. However, timolol treatment did reverse the increase in synaptic efficacy seen in the PO animals, suggesting that β-receptors are involved in regulating synaptic efficacy in a disease model with an active and progressive remodeling state. The remodeling of neuronal responses was dependent of the form of cardiac disease. Although some changes were similar in both disease models (increased adrenergic sensitivity and decreased ANG II effects), there were also changes that appeared to be specific to the particular form of cardiac disease. Ongoing, progressive disease leading to congestive heart failure, such as occurs in the PO models, induced increased muscarinic sensitivity and increased synaptic efficacy to levels not seen with the chronic MI models. The ability of pharmacological intervention to alter functional remodeling may be dependent on the dynamic nature of the disease process, since treatments of MI models showed little alteration in remodeling, while PO models were more responsive to adrenergic receptor blockade. Future studies should consider the dynamic interplay between cardiac stressors and neurohumoral responses, especially with respect to the timing and efficacy of targeted therapies to modify the cardiac disease process.

GRANTS

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REFERENCES


