Angiotensin II potentiates adrenergic and muscarinic modulation of guinea pig intracardiac neurons

Allison E. Girasole¹, Christopher P. Palmer¹, Samantha L. Corrado¹, E. Marie Southerland²,
Jeffrey L. Ardell², and Jean C. Hardwick¹

¹Department of Biology, Ithaca College, Ithaca NY 14850, ²Department of Pharmacology,
College of Medicine, East Tennessee State University, Johnson City, TN 37614

To Whom Correspondence should be addressed:

Jean C. Hardwick, Ph.D.
Biology Department
Ithaca College
953 Danby Road
Ithaca, NY 14850
jhardwick@ithaca.edu

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angiotensin receptors, sympathetic
Abstract

The intrinsic cardiac plexus represents a major peripheral integration site for neuronal, hormonal, and locally-produced neuromodulators controlling efferent neuronal output to the heart. This study examined the interdependence of norepinephrine, muscarinic agonists, and angiotensin II (Ang II), to modulate intrinsic cardiac neuronal activity. Intracellular voltage recordings from whole mount preparations of the guinea pig cardiac plexus were used to determine changes in active and passive electrical properties of individual intrinsic cardiac neurons. Application of either adrenergic or muscarinic agonists induced changes in neuronal resting membrane potentials, decreased afterhyperpolarization duration of single action potentials and increased neuronal excitability. Adrenergic responses were inhibited by removal of extracellular calcium ions, while muscarinic responses were inhibited by application of TEA. The adrenergic responses were heterogeneous, responding to a variety of receptor-specific agonists (phenylephrine, clonidine, dobutamine, and terbutaline), although α-receptor agonists produced the most frequent responses. Application of Ang II alone produced a significant increase in excitability, while application of Ang II in combination with either adrenergic or muscarinic agonists produced a much larger potentiation of excitability. The Ang II-induced modulation of firing was blocked by the AT2 receptor inhibitor PD 123319 and was mimicked by the AT2 receptor agonist CGP-42112A. AT1 receptor blockade with telmasartin did not alter neuronal responses to Ang II. These data demonstrate that Ang II potentiates both muscarinic and adrenergic-mediated activation of intrinsic cardiac neurons, doing so primarily via AT2 receptor dependent mechanisms. These neurohumoral interactions may be fundamental to regulation of neuronal excitability within the intrinsic cardiac nervous system.
Introduction

Control of cardiac function is determined, in part, by the precise coordination of outputs from the sympathetic and parasympathetic branches of the autonomic nervous system. The specific efferent output from each branch is in turn controlled by integration of multiple inputs. In addition to inputs from preganglionic neurons, evidence indicates that sensory fibers and local circuit neurons (interneurons) modulate peripheral reflex processing (4; 6; 33). These peripheral autonomic ganglia can function interdependently with central components of the cardiac nervous system for precise beat-to-beat control of cardiac electrical and mechanical functions (4; 6). The intrinsic cardiac plexus, located within the cardiac tissue itself, exemplifies fundamental aspects of this coordinated communication.

The peripheral components of the cardiac nervous system, including the intrinsic cardiac ganglia, express a complex neurochemical profile (19; 33). Previous studies have shown that intracardiac neurons possess receptors for a variety of putative neuromodulators, including muscarinic receptors (7; 24; 38), adrenergic receptors (22; 33), neuropeptides (11; 17), and locally produced chemicals (34). Studies on the direct interactions between the sympathetic and parasympathetic neurons in this plexus are few (26), although the importance of this balance is integral to the normal function of the organism. For example, surgical disruption of the right atrial ganglionated plexus in dogs prevents parasympathetic-mediated bradycardia while maintaining parasympathetic-induced suppression of sympathetic inputs modulating chronotropic function (30; 35). This residual response is attributed to sympathetic/parasympathetic neural interactions mediated within the cardiac plexus (30).

Consequently, in the present study, our first objective was to examine the potential for intraganglionic interactions between sympathetic and parasympathetic efferent neurons by
determining the effects of both muscarinic and adrenergic neuromodulation on the output of intracardiac neurons. This included characterization of the potential contributions of $\alpha$ and $\beta$ adrenoreceptor subtypes to passive and active membrane properties of intrinsic cardiac neurons.

Previous studies have also demonstrated that neurohumoral interactions can impact functional cardiac control and that these responses are altered with chronic cardiac stress (6). For example, angiotensin II (Ang II) modulates peripheral autonomic neuronal function (15; 20). Ang II/autonomic interactions remodel with the progression of cardiac disease (2; 13) and, thus, represent novel targets to mitigate adverse disease progression in both cardiac tissues and within the associated cardiac nervous system (37). Stress-induced changes in cardiac tissues and associated neurohumoral regulators can be either detrimental or beneficial. For example, with renin-angiotensin system activation, increased AT$_1$ receptor activity is associated with increased risk of sudden cardiac death (14; 21), while increases in AT$_2$ receptor activation is considered cardioprotective (9; 25; 28). While Ang II is recognized as a putative neuromodulator of peripheral autonomic function, the potential effects of AT$_1$ and AT$_2$ receptors on active and passive membrane properties of efferent neurons of the intrinsic cardiac neurons have not been well characterized. In addition, the potential interactions between Ang II and adrenergic receptor systems on intrinsic cardiac neurons have not been considered either, even though both are co-activated during the progression of cardiac disease (6; 13; 30; 36). The potential for Ang II/adrenergic interactions to modulate intracardiac neurons underlie the second objective for the current studies. The data presented here demonstrate that Ang II potentiates both adrenergic and muscarinic-mediated activation of intrinsic cardiac neurons, primarily via AT$_2$ receptor dependent mechanisms.
Material and Methods

Animals: Hartley guinea pigs (male, 800-1000g, Charles River), were euthanized by CO₂ inhalation and exsanguination. All procedures were approved by the Institutional Animal Care and Use Committees of Ithaca College and were in accordance with the American Physiological Society Guiding principles for research involving animals and human beings (1).

The heart was removed and placed into ice-cold Krebs Ringer solution (mM: NaCl 121, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, glucose 8, aerated with 95% O₂/5% CO₂ for a pH of 7.4). The cardiac plexus, located in the epicardium of the atria, was dissected as previously described (17; 29). The region studied is located primarily in the wall of the left atrium underlying the area of the coronary sinus and was exposed by opening the atria and removing the overlying muscle and connective tissue. The isolated plexus was pinned to a Sylgard-lined 60 mm Petri dish and continuously superfused (6-8 mL/min) with 35-37°C Krebs Ringer. Norepinephrine (10⁻³ M), bethanechol (10⁻³ M), and histamine (10⁻³ M) were applied by local pressure ejection (6-9 psi, Picospritzer, General Valve Corp) through small tip diameter (5-10 μm) glass micropipettes positioned 50-100 μm from individual neurons. Up to two different drugs could be tested simultaneously by local pressure ejection. For multiple tests of responses in the same cell, the cells were washed with the circulating Krebs solution for several minutes between drug applications. Cells were tested to insure that all responses had returned to control levels prior to additional testing. Angiotensin II (100 nM) and the AT₂ agonist CGP-42112A (Sigma, 100 nM) were applied via the circulating bath solution. Adrenergic agonists phenylephrine (α₁ selective), clonidine (α₂ selective), dobutamine (β₁ selective), and terbutaline (β₂ selective) were applied by either local pressure ejection (10⁻³M) or by bath perfusion (10⁻³M).
µM). Angiotensin receptor inhibitors telmasartan (AT1 selective, Sigma, 1 µM) and PD 123319 (AT2 selective, Tocris Bioscience, 1 µM,) were applied by bath perfusion.

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

Single action potentials were evoked by depolarizing current injection (0.5-0.8 nA, 5 msec), averaged (5-6 individual recordings) and analyzed to determine the amplitude and duration (as determined by a return to the resting membrane potential) of the afterhyperpolarizing potential (AHP). Neuronal excitability was monitored by observing the response to a series of long depolarizing current pulses (0.1-0.6 nA, 500 msec). The number of evoked action potentials (AP) versus stimulus intensity was determined to assess changes in excitability.

Following characterization of the basic electrophysiological properties, induced changes in evoked AP frequency for each cell were assessed immediately following 1-2 sec application of either norepinephrine (NE) or the muscarinic agonist bethanechol. Each was applied by local pressure ejection immediately adjacent (~50-100 µm) to the recorded neuron, and doses were chosen that produce maximal or near maximal responses. Changes in action potential frequency versus stimulus intensity were determined to assess relative drug-induced changes in excitability of intrinsic cardiac neurons. Angiotensin II or specific angiotensin receptor agonists were added to the circulating bath solution and neuronal responses to NE, bethanechol, or histamine (applied
Inhibition of different ion channels was achieved through addition of known inhibitors to the bath solution, or omission of specific ions. Tetraethylammonium (TEA, 5 mM) and BaCl₂ (1 mM) were added to inhibit potassium channels. CsCl (2 mM) was added to inhibit the hyperpolarization-activated current, Iᵢₕ. 4-aminopyridine (1 mM) was added to inhibit the A-current (Iₐ). Calcium was removed from the Krebs solution, with MgCl₂ increased to 4 mM, to prevent influx of extracellular calcium. Solutions were superfused over the tissue and the neuronal responses to NE and bethanechol were measured before, during, and after application.

Statistical Analysis: Values are expressed as the mean ± standard error. Statistical significance was determined by Students t-test or ANOVA, with a p value less than 0.05 considered significant. Post hoc analysis was performed using Dunn’s method. Best fit lines for the frequency curves were generated using either a linear or single exponential relationship, which provided estimates of the R² values of 0.98 or greater.

Results

Adrenergic and muscarinic responses

Whole mount preparations of the guinea pig cardiac plexus were used to monitor individual neuronal responses to locally-applied norepinephrine (NE, 10⁻³M) or the muscarinic agonist, bethanechol (Beth, 10⁻³M). Intracellular voltage recordings from single neurons were
used to monitor both passive and active membrane responses. A total of 25 animals were used in this study and 140 individual cells were sampled.

Application of either NE or bethanechol induced a change in the resting membrane potential in the majority of cells. Approximately 100 cells were tested with NE and of those ~70% showed a change in membrane potential or evoked responses, whereas all 70 cells tested responded to bethanechol. The bethanechol response typically consisted of a rapid hyperpolarization (Figure 1A3, mean 6.7 ± 4 mV, 7.6 ± 4 sec, n=14), followed by a slow depolarization (mean 4 ± 2 mV, 39.7 ±10 sec). NE responses showed greater variability in the membrane responses, with approximately 60% showing a hyperpolarization (Figure 1A2, mean 4.8 ± 2 mV, 16 ± 8 sec, n=12) and 40% showing a depolarization (Figure 1A1, mean 2.8 ± 1mV, 19 ± 10 sec, n=9). Some neurons showed no measurable change in membrane voltage with NE application, while 6 cells showed a biphasic response.

Single action potentials were produced by injection of a depolarizing current pulse (0.5 - 0.8 nA, 5 msec) and averaged. Analysis of the afterhyperpolarization (AHP) phase of the action potential showed a decrease in AHP duration following application of either NE or bethanechol (Figure 1B). Prior to application, the average AHP amplitude was 15.8 ± 0.4 mV (n=37) with a duration of 260 ± 11 msec. Following NE application (n=14), the AHP amplitude was 15.6 ± 0.9 mV (not significantly different from control) with a duration of 196 ± 9 msec (p < 0.001 by t-test). Similarly, following bethanechol application (n=9), the AHP amplitude was 15.5 ± 1.0 mV (not significantly different) with a duration of 180 ± 12 msec (p < 0.001 by t-test).

Neuronal excitability was assessed by monitoring the number of action potentials produced by a series of depolarizing current steps (0.1-0.6 nA, 500 msec). As shown in Figure 1C (top panel), the majority of neurons fired only one or two action potentials at the onset of the
depolarizing stimulus. However, following either NE or bethanechol application, the same current pulse produced a significant increase in the number of evoked APs. In addition, bethanechol application consistently resulted in a greater increase in neuronal firing than NE (Figure 2).

**Ionic mechanisms underlying adrenergic and muscarinic responses**

Several potential ionic mechanisms could underlie the observed responses to NE and bethanechol. Previous studies of NE effects on intracardiac neurons of rats have demonstrated that NE can alter calcium currents (41), while other studies showed that NE can activate a non-specific cation channel (22). Several muscarinic receptor-mediated changes in ion currents have been described for intracardiac neurons, including inhibition of the m-current (32), regulation of the delayed rectifier potassium current (3), inhibition of calcium currents (12), and stimulation of intracellular calcium release (7). To examine whether any of these might contribute to the effects observed in these cells, a variety of inhibitors of different ion channels were added to the circulating Krebs solution. The ability of NE and bethanechol to increase neuronal firing in the presence or absence of these different inhibitors was then assessed. Barium (1 mM) was added to inhibit inwardly rectifying potassium currents, including the m-current (16; 18). Barium alone produced an increase in basal excitability levels. However, both NE and bethanechol were still able to further increase neuronal firing in the presence of 1 mM Ba^{2+} (n = 4 cells, Figure 3). Additionally, inclusion of either 1 mM 4-aminopyridine (to inhibit the A-current, 4 cells) or 2 mM CsCl (to inhibit Ih, 4 cells) did not significantly alter the effects of either NE or bethanechol, with all cells still showing an increase in evoked APs following NE or bethanechol application in the presence of the inhibitor (Figure 3). However, the addition of 5 mM TEA (to inhibit
potassium currents such as the delayed rectifier and some calcium-activated potassium channels (16; 18)) inhibited the ability of bethanechol to increase neuronal excitability (Figure 3, n=5 cells) while also inhibiting the bethanechol-induced hyperpolarization (data not shown). The NE-induced increase in excitability was still observed in all 4 cells tested in the presence of TEA (p<0.05 versus TEA alone by paired t-test). However, removal of extracellular calcium ions prevented the NE-induced increase in excitability, while the bethanechol-induced increase in evoked action potentials was still observed in all 4 cells tested (Figure 3, p<0.05 versus 0 Ca++ alone by paired t-test).

Characterization of adrenergic receptors

Specific adrenergic agonists were used to evaluate the receptor subtypes responsible for the observed NE responses. Agonists were applied either by local pressure ejection (10^{-3}M) or by inclusion in the circulating bath solution (10 µM). Action potentials were evoked in the absence and presence of the different adrenergic agonists. A cell was identified as responsive if the number of action potentials induced by step depolarization increased and if, following removal of the agonist challenge, that response returned to control levels. Cumulative data for the responding cells showed an evoked response of 2.8 ± 0.4 APs (n=20) at the maximal stimulus level in Krebs alone that increased to 4.5 ± 0.4 APs (n=20) in the presence the different adrenergic agonists (cumulative data for all agonists). Table 1 indicates the observed resting membrane potential response with agonist application for each of the responders and shows a heterogeneous expression of adrenergic receptors within the cardiac plexus. There was no apparent correlation between the detection of a membrane response, or the direction of the change, and the ability to increase evoked action potentials. Application of α-adrenergic agonists
produced the greatest percentage of responsive neurons (41% for α₁ and 38% for α₂) with β-adrenergic agonist application producing fewer responsive neurons (20% for β₁ and 17% for β₂). A total of 31 cells were challenged with at least two different agonists. Of those, 7 showed responses to 2 different agonists. In each case, the cells responded to the α₁ agonist and either β₂ (3 cells), β₁ (2 cells) or α₂ (2 cells).

**Angiotensin II modulation of responses**

Cardiac ganglion preparations were superfused with Ang II to determine its potential to directly modulate intracardiac neuronal activity. Ang II was applied via addition to the circulating bath solution (100 nM) and was allowed to circulate for several minutes. Single action potentials, neuronal excitability, and responses to NE and bethanechol were assessed in the presence and absence of Ang II. In addition, another known endogenous modulator of intracardiac neuron function, histamine (34), was also examined to determine if any effects of Ang II on neuronal activity were specific or relatively nonspecific to different potential neuromodulators. Ang II did not alter resting membrane potential or single action potential characteristics (data not shown). The number of evoked action potentials with increased depolarizing current pulses showed a small, but significant increase with Ang II at the highest stimulus intensity only (Figures 4B and 5). In the presence of Ang II, application of either NE or bethanechol produced a significantly greater number of action potentials in response to the same stimulus compared to control conditions (Figures 4 and 5). This response was readily reversible upon removal of Ang II, with excitability responses returning to levels observed prior to Ang II application. In contrast, Ang II did not alter the neuronal response to histamine. Comparison of the effects of Ang II at maximal stimulations for control, NE, bethanechol, and histamine shows
a significant increase in evoked action potentials in control, NE and bethanechol, but not with histamine (Figure 5).

Ang II works primarily via AT\textsubscript{1} and/or AT\textsubscript{2} receptors in the regulation of the cardiovascular system (28). While cardiomyocytes have both receptor sub-types, many of the induced functional cardiac responses to Ang II challenge are mediated indirectly via modulation of autonomic efferent function (15; 20). To determine which receptors were responsible for the effects observed in this preparation, we used both agonists and antagonists of the specific AT receptor subtypes. Telmasartin, an AT\textsubscript{1} receptor antagonist (1 µM), was added to the circulating Krebs solution alone and then in the presence of Ang II. Bethanechol-induced changes in neuronal firing were used to monitor Ang II-mediated effects. Telmasartin did not alter the Ang II-mediated increase in bethanechol-induced neuronal excitability in 4 different preparations (Figure 6), while inclusion of PD 123319, the AT\textsubscript{2} specific inhibitor (1 µM) inhibited the increase in evoked action potentials produced by bethanechol in the presence of Ang II (Figure 6, n=6). To further establish that AT\textsubscript{2} receptors could be mediating this augmenting response, CGP-42112A, an AT\textsubscript{2} specific agonist, was added to the bath solution (100 nM). Similar to the Ang II responses, addition of CGP-42112A also increased the number of depolarization-evoked action potentials following bethanechol application (n=4), as compared to control (Figure 6).

**Discussion**

Integration of information between the sympathetic and parasympathetic nervous system is crucial in the maintenance of cardiac function. The experiments presented here demonstrate that adrenergic neurotransmitters can increase output from intrinsic cardiac neurons via multiple adrenergic receptor subtypes, \(\alpha\)-adrenergic being the predominant type. In addition, local
stimulation with angiotensin II can also modulate intracardiac neuronal function, as well as enhance the increase in excitability induced by both adrenergic and muscarinic agonists. Thus, an increase in sympathetic activity and Ang II production can lead to a concomitant increase in intracardiac neuronal output to the heart. This may represent a feedback loop for parasympathetic blunting of sympathetic responses, or an integration of autonomic outputs within the intrinsic ganglion to modify overall cardiac function.

The guinea pig intracardiac neurons possess both nicotinic and muscarinic receptors (3; 38). The nicotinic receptors are responsible for fast synaptic transmission and lead to suprathreshold excitatory signals. The muscarinic receptors mediate slower responses and several different subtypes of muscarinic receptors are postulated to be expressed by these neurons (3; 7; 12). Thus, muscarinic receptor activation is a likely mechanism for modulation of neuronal output. Application of the muscarinic agonist bethanechol elicited a biphasic change in membrane potential, a shortening of action potential afterhyperpolarization, and an increase in the number of action potentials evoked by long depolarizing stimuli. This indicates that muscarinic receptor activation can produce an overall increase in neuronal output. The m2 receptor has been shown to increase potassium channel function in rat intracardiac neurons (3; 40) and to decrease calcium channel function (23). In addition Cuevas, et al (12) showed evidence for m4 receptor inhibition of calcium channel function in neonatal rat neurons. In the present study, bethanechol was still able to evoke an increase in neuronal firing in cells where the m-current was suppressed by the addition of Ba^{2+}. This suggests that mechanisms other than inhibition of Im contributed to the muscarinic regulation of neuronal excitability. We did find that the muscarinic response was inhibited by extracellular TEA, which would support either modulation of delayed rectifier channels or inhibition of calcium-activated potassium channels,
both of which are TEA-sensitive (18). In addition, a muscarinic-induced decrease in calcium currents could also lead to a decrease in calcium-activated potassium channels, which could result in the decrease in AHP duration seen with bethanechol application.

Previous studies of the intrinsic cardiac plexus have shown evidence for adrenergic regulation of intracardiac neurons (5; 26; 38; 41). Norepinephrine directly affected approximately 70% of guinea pig intrinsic neurons. NE application results primarily in membrane hyperpolarization, although some neurons show a depolarization. NE challenge also resulted in an inhibition of AHP duration, and an increase in evoked action potentials. There was no correlation, however, between the change in membrane potential and a change in evoked action potentials. The results from selective adrenergic agonist challenges indicate a heterogeneous expression of adrenergic receptors within the cardiac plexus, with approximately 41% of cells showing evidence for $\alpha_1$ receptors, 38% for $\alpha_2$ receptors, 20% for $\beta_2$ receptors, and 17% for $\beta_1$ receptors. In addition, a small percentage of neurons showed evidence for expression of multiple adrenergic receptors. The heterogeneity in adrenergic responses is further seen in the diverse membrane responses, even to a specific agonist challenge. However, by using specific ion channel blockers or removing ions, we were able to demonstrate that the NE-induced increase in evoked action potentials is consistently inhibited by the removal of extracellular calcium ions. Inhibition of potassium channels with $\text{Ba}^{2+}$, $\text{Cs}^+$, 4-AP, or TEA had no effect on the NE-induced increase in evoked action potentials. Thus, although specific receptor expression and membrane responses are heterogeneous, there appears to be a common ionic mechanism underlying the increase in neuronal excitability, with a requirement for extracellular calcium ions to initiate the response.
Increased activation of the sympathetic efferent nervous system is associated with increased production of renin and angiotensin II. Conversion of Ang I to Ang II occurs not only through plasma angiotensin converting enzyme activity (ACE), but also via cardiac tissue ACE and chymase produced by mast cells (31). The importance of elevated Ang II levels in cardiac disease is becoming increasingly clear and studies have shown evidence for negative outcomes associated with increased AT\(_1\) receptor activation (14; 21). Conversely, increased activation of AT\(_2\) receptors is associated with cardioprotection (9; 10; 25). While cardiomyoctes express AT\(_1\) and AT\(_2\) receptors, most of the functional changes (electrical and mechanical) induced by Ang II exposure to the heart \textit{in vivo} are mediated indirectly via induced alterations in autonomic efferent function (15; 20). Thus, we were interested in the potential regulatory effects of Ang II on intracardiac neuron function.

Application of Ang II to guinea pig intrinsic cardiac neurons results in a small, but significant increase in evoked action potentials at the highest stimulus levels. More pronounced was the synergistic effect of Ang II on the neuronal responses with either adrenergic or muscarinic receptor co-activation. NE application, in the presence of Ang II, resulted in a potentiation of the NE-induced increase in evoked action potentials. Similarly, bethanechol application in the presence of Ang II also produced a significantly greater increase in neuronal excitability. These results suggest that increases in cardiac interstitial levels of Ang II can result in enhanced output of intracardiac neurons to both adrenergic and muscarinic signals. However, Ang II did not result in a generalized increase in neuronal excitability since the response to histamine, a neuromodulator previously shown to increase evoked action potentials in these neurons (34), was not altered in the presence of Ang II.
The results from the characterization of the Ang II receptor sub-types on these cells suggest the expression of AT2 receptors. Application of the AT1 inhibitor telmarsartin did not alter the Ang II responses. The AT2-specific inhibitor PD 123319 did inhibit the Ang II-mediated potentiation of the bethanechol responses; while application of the AT2 agonist CGP 42112A mimicked the Ang II response. These results suggest that modulation of intrinsic cardiac neuronal output by Ang II is mediated via AT2 receptors.

**Perspectives and Significance**

Integration of sympathetic and parasympathetic efferent neuronal outputs to the heart can occur at the level of the postganglionic neurons (30; 35). The studies presented here show that intracardiac neurons of the guinea pig cardiac plexus can be modulated by both adrenergic signals and hormonal signals, such as angiotensin II. Both of these neuromodulators can produce an increase in neuronal output, and the combination of NE and Ang II actually produces an even higher evoked output from these neurons.

Given that chronic heart disease can result in increased sympathetic activity as well as increases in circulating and locally produced Ang II levels (6; 8; 13), these results suggest that the parasympathetic nervous system may compensate for these pathologies, in part, by increasing parasympathetic output. In fact, increased parasympathetic efferent output is correlated with protection against cardiac arrhythmias and sudden cardiac death in the setting of ischemic heart disease (27; 39). Future studies will focus on the impact of these different modulators on the output of intrinsic cardiac neurons with stimulation of fiber inputs. If these modulators are indeed producing functional changes in neuronal excitability, then this should be evident in changes in neuronal firing with synaptic stimulation. Future studies are needed to expand upon
the neurochemical/neurohumoral alterations within the cardiac nervous system and the cardiac
tissues they innervate during disease progression with the ultimate goal of developing strategies
to mitigate such adverse remodeling and thereby help preserve overall cardiac function.

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Reference List


therapeutic interference with the renin-angiotensin system in myocardial infarction? 


Table 1: Intracardiac neuron responses to adrenergic agonists

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<th>% of cells tested showing an increase in evoked APs</th>
<th>Observed Membrane Responses for Responding Neurons</th>
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<tr>
<td></td>
<td></td>
<td>Depolarization</td>
</tr>
<tr>
<td>Phenylephrine ($\alpha_1$)</td>
<td>41% (12 of 29 cells)</td>
<td>4 cells</td>
</tr>
<tr>
<td>Clonidine ($\alpha_2$)</td>
<td>38% (3 of 8 cells)</td>
<td>2 cells</td>
</tr>
<tr>
<td>Dobutamine ($\beta_1$)</td>
<td>17% (2 of 12 cells)</td>
<td>2 cells</td>
</tr>
<tr>
<td>Terbutaline ($\beta_2$)</td>
<td>20% (4 of 20 cells)</td>
<td>2 cells</td>
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The percentage of cells that showed an increase in evoked action potentials (APs) in the presence of the agonist is indicated and, for those cells that did induce an increase in excitability, the membrane response produced with agonist application is indicated.
Figure Legends

Figure 1: Neuronal responses to NE and bethanechol.

NE and bethanechol were applied by local pressure ejection (10^{-3} M, 1 sec, black circle indicates application) to individual intracardiac neurons. Panel A: NE application produced either a small depolarization (A₁), or a hyperpolarization (A₂) in the majority of cells tested. Bethanechol consistently produced a biphasic membrane response with a large hyperpolarization followed by a long, slow depolarizing phase (A₃). Panel B: Both bethanechol and NE application produced a shortening of the afterhyperpolarization duration phase of the action potential, with bethanechol producing a more significant inhibition. Panel C: The number of action potentials produced during a long depolarizing current pulse (0.4 nA, 500 msec) increased following application of either NE or bethanechol, with bethanechol consistently producing a greater increase in excitability. Resting membrane potentials; B -49 mV, C -40 mV.

Figure 2: Both NE and bethanechol increase neuronal excitability.

Neurons were stimulated with depolarizing current pulses (0.1-0.6 nA, 500 msec) and the number of action potentials produced at each intensity was determined prior to and following a 1 sec application of either NE or bethanechol. The points represent the mean ± SEM for each condition and the lines are the best fit curves. Control (n=140 cells) and bethanechol (n=65 cells) data were best fit with a single exponential curve and NE (n=98 cells) data was best fit with a linear curve (R²>0.98 for all fits). Statistically significant increases over control values for each stimulus intensity (*, p < 0.05 by ANOVA) are indicated.
Figure 3: Ionic mechanisms underlying the NE and bethanechol-induced increase in excitability.

Neuronal excitability was monitored prior to, and following, a brief (1 sec) application of either NE or bethanechol in control Krebs solution (A, left-sided panels), the same cell in a Krebs solution with 0 Ca\(^{2+}\) and 4 mM Mg\(^{2+}\) (A, middle panels), and a different cell in Krebs with 5 mM TEA (A, right-sided panels). Removal of extracellular calcium ions resulted in an inhibition of the NE-induced increase in excitability without altering the bethanechol-induced excitability. Conversely, addition of TEA had no effect on the NE-induced response but did suppress the bethanechol-induced increase in excitability. A comparison of evoked action potentials at the maximal stimulus intensity (panel B) for NE and bethanechol (Beth) in Krebs, 0 Ca\(^{2+}\), 5 mM TEA, 1 mM 4-aminopyridine (4-AP), 2 mM Cs\(^{+}\), and 1 mM Ba\(^{2+}\) (n=4 cells for each condition) shows significant inhibition of the bethanechol responses only in TEA and of the NE response only in 0 Ca\(^{2+}\) (*, p < 0.05 versus TEA or 0 Ca\(^{2+}\) alone by paired t-test). Cells tested with either Ba\(^{2+}\), Cs\(^{+}\), or 4-AP showed similar increases in evoked action potentials with both NE and bethanechol application compared to Krebs control responses. Resting membrane potentials panel A; Control Krebs -43 mV, 0 Calcium -48 mV, TEA -47 mV.

Figure 4: Angiotensin II increases neuronal responses to NE and bethanechol.

Panel A: Addition of 100 nM angiotensin II (AngII) to the circulating Krebs solution enhanced the increase in the number of action potentials produced with long depolarizing current pulses (0.6 nA, 500 msec) following application (1 sec) of either NE or bethanechol. Resting membrane potentials; Control -44 mV, NE -44 mV, bethanechol -54 mV. Panel B: For each condition (control, NE and bethanechol) the number of action potentials versus stimulus intensity was plotted with and without Ang II in the bath. Points represent the mean ± SEM at
each stimulus intensity. The lines represent the best fit curve to the data. For statistical analysis, a t-test was performed at each individual stimulus intensity, to compare the number of action potentials produced with and without Ang II and p values of 0.05 or less are indicated by the asterisk.

Figure 5: Angiotensin II increases neuronal excitability to NE and bethanechol, but not histamine.

The number of action potentials induced by a depolarizing current pulse (0.6 nA, 500 msec) was determined in the presence and absence of 100 nM Ang II in control conditions and following a brief (1 sec) application of either NE, bethanechol, or histamine. The bars represent the mean ± SEM of 10 or more cells for each condition. A statistically significant difference between the number of action potentials observed with and without Ang II was determined by t-test. Ang II alone increase neuronal excitability over control (*, p < 0.003), and also increased the response to NE and bethanechol (**, p<0.004), but had no significant effect on histamine responses.

Figure 6: Characterization of AT receptors.

The ability of Ang II to increase the evoked action potentials was tested with and without bethanechol application in control Krebs and Krebs containing either the AT₁ inhibitor telmasartin (1 µM), or the AT₂ inhibitor PD 123319 (1 µM). In addition, the effect of addition of the AT₂ agonist CGP-42112A (100 nM) on the bethanechol response was also tested. Neuronal excitability, tested at the maximum stimulus intensity (0.6 nA, 500 msec), was monitored prior to, and following a brief application (1 sec) of bethanechol. The bars represent the mean ± SEM
for each condition. The addition of Ang II potentiated the bethanechol-induced increase in
evoked action potentials in Krebs and telmasartin, but not in the presence of PD 123319 (* p <
0.05 by paired t-test). The AT2 agonist CGP-42112A alone was able to significantly increase the
bethanechol response to a similar level as Ang II. An example recording following bethanechol
application with and without CGP-42112A (0.3 nA, 500 msec, RMP -60 mV) is shown in Panel
B.
Number of Action Potentials

- Control
- Ang
- NE
- NE Ang
- Hist
- Hist Ang
- Beth
- Beth Ang

Statistical significance:
- *: p < 0.05
- **: p < 0.01
- n.s.: not significant