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Dynamic remodeling of the guinea pig intrinsic cardiac plexus induced by chronic myocardial infarction

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Abstract

Myocardial infarction (MI) is associated with remodeling of the heart and neurohumoral control systems. The objective of this study was to define time-dependent changes in intrinsic cardiac (IC) neuronal excitability, synaptic efficacy, and neurochemical modulation following MI. MI was produced in guinea pigs by ligation of the coronary artery and associated vein on the dorsal surface of the heart. Animals were recovered for 4, 7, 14, or 50 days. Intracellular voltage recordings were obtained in whole mounts of the cardiac neuronal plexus to determine passive and active neuronal properties of IC neurons. Immunohistochemical analysis demonstrated an immediate and persistent increase in the percentage of IC neurons immunoreactive for neuronal nitric oxide synthase. Examination of individual neuronal properties demonstrated that afterhyperpolarizing potentials were significantly decreased in both amplitude and time course of recovery at 7 days post-MI. These parameters returned to control values by 50 days post-MI. Synaptic efficacy, as determined by the stimulation of axonal inputs, was enhanced at 7 days post-MI only. Neuronal excitability in absence of agonist challenge was unchanged following MI. Norepinephrine increased IC excitability to intracellular current injections, a response that was augmented post-MI. Angiotensin II potentiation of norepinephrine and bethanechol-induced excitability, evident in controls, was abolished post-MI. This study demonstrates that MI induces both persistent and transient changes in IC neuronal functions immediately following injury. Alterations in the IC neuronal network, which persist for weeks after the initial insult, may lead to alterations in autonomic signaling and cardiac control.

Keywords

Intrinsic cardiac nervous system; Myocardial infarction; Neuromodulation; Angiotensin II; Norepinephrine; Bethanechol

1. Introduction

Cardiac pathology induces changes in both the heart and in the neurohumoral control systems that modulate it (Armour, 2008). For the cardiac nervous system, remodeling can occur at multiple levels, from the intrinsic cardiac nervous system (ICN) (Hardwick et al.,

Author contributions

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2008, 2009; Hopkins et al., 2000) to extracardiac intrathoracic ganglia (Nguyen et al., 2012) and extending up to central neural processing circuits associated with the baroreflex (Zucker et al., 2012). Neurohumoral responses are likewise altered, including the renin–angiotensin–aldosterone system (RAS) and circulating catechol-amines (Dell'Italia, 2011; Gonzalez et al., 2009; Mill et al., 2011). Previous work from our laboratory demonstrated long-term (2 months) changes in intrinsic cardiac (IC) neuron excitability, including changes in network synaptic efficacy, resulting from either chronic myocardial infarction (Hardwick et al., 2008) or chronic pressure overload (Hardwick et al., 2009). Although the immediate consequences of myocardial ischemia/infarction have been evaluated on intrathoracic aspects of neuronal processing within the cardiac nervous system (Armour et al., 1998; Waldmann et al., 2006), there is little information concerning how the intrinsic cardiac plexus adapts in the initial days to weeks following myocardial infarction. This is especially critical because clinical studies have found that this time frame is associated with a high risk for lethal arrhythmias (Billman, 2006; Chugh et al., 2008).

Previous studies in animal models have shown that the remodeling process subsequent to MI is most dynamic during the first 7 days following injury, being stabilized by approximately 14 days post-MI (Ahonen et al., 1975; Crow et al., 2004; Dobaczewski et al., 2010). We hypothesized that enhanced afferent feedback to the ICN (Wang et al., 2008), induced by myocardial infarction, leads to rapid remodeling of the intracardiac neuronal network. This increase in afferent input can arise due to multiple changes within the heart. These include an altered ventricular substrate produced by apoptosis and establishment of the infarct substrate (Crow et al., 2004), stress-induced changes in the collagen matrix (Dobaczewski et al., 2010), disruptions in regional cardiac contraction (Antoni et al., 2011; Mollema et al., 2007), and disruptions in central and peripheral nerve networks for cardiac control (Ahonen et al., 1975; Chen et al., 2001; Ewert et al., 2008; Mill et al., 2011). Changes in local feedback systems within the intrinsic cardiac nervous system are also subjected to changes in centrally derived preganglionic inputs, with enhanced activity from sympathetic projections and lesser inputs from the parasympathetic nervous system (Mill et al., 2011; Zucker et al., 2012). Finally, there are local neurohumoral changes that include an increase in local production of Ang II (Dell'Italia, 2011). All of these factors can result in imbalances in efferent outflows and contribute to the evolution of cardiac pathologies (Armour, 2008).

The current study examined MI-induced remodeling of guinea pig IC neurons during the early stage (4 to 14 days post-MI) versus later stage (7 weeks) adaptations to injury. Our hypothesis is that remodeling of the neuronal network begins immediately following injury and continues to adapt during the days to weeks following injury. Because no one has examined remodeling of the intrinsic cardiac plexus during these early time points, we wanted to define the time-specific changes in IC neuronal excitability, synaptic efficacy, and the dynamics of IC neuromodulation. Our data indicate that although some short-term changes in intrinsic properties and synaptic efficacy were observed at early time points, these parameters returned toward control by 2 weeks post-MI. Conversely, altered sensitivity to neuromodulators and phenotypic changes were observed immediately and maintained throughout the extended recovery time period.

2. Materials and Methods

2.1. Surgical induction of heart disease

Using techniques detailed previously (Hardwick et al., 2008, 2012), myocardial infarction (MI) was surgically induced by ligation of the ventral descending coronary artery and associated vein on the left ventricle just distal to the first diagonal branch, in 9-week-old male Hartley guinea pigs (Charles River), weighing between 500 and 650 g. Postoperative care was analogous to that detailed previously (Hardwick et al., 2012). All procedures were

approved by the Institutional Animal Care and Use Committees of Ithaca College and East Tennessee State University and were in accordance with the *Guide for the Care and Use of Laboratory Animals*, National Academy Press, Washington DC, 2002. Following MI, animals (4–6 for each condition) were recovered for 4, 7, 14, or 50 days. Sham surgeries (in which the heart was visualized but with no coronary occlusion) were allowed to recover for 7 days post-surgery. In addition, age-matched animals without surgery were used as controls.

2.2. Terminal experiments

Animals were euthanized by CO₂ inhalation and exsanguination. 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 was dissected as previously described (Hardwick et al., 1995). The region studied is located primarily in the region of the intra-atrial septum and the left atrium, an area not directly affected by the infarction. The atria is opened and the overlying muscle and connective tissue is removed, with the intrinsic cardiac network exposed. Following dissection, the tissue was continuously superfused (6–8 mL/min) with 35–37 °C Krebs Ringer. Adrenergic (norepinephrine (NE), Sigma, 10⁻³ M) and muscarinic (bethanechol (Beth), Sigma, 10⁻³ M) agonists were applied by local pressure ejection (6–9 psi, Picospritzer, General Valve Corp.) through small tip diameter (5–10 µm) glass micro-pipettes positioned 50–100 µm from the individual neuron. Angiotensin II (Ang II) was applied in the circulating bath solution (100 nM, Sigma) for 3–5 min. 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.

2.3. Electrophysiological methods

Intracellular voltage recordings from intracardiac neurons were obtained as previously described (Girasole et al., 2011). Single action potentials were produced by brief depolarizing current injections (0.5–0.9 nA, 3–5 ms). Individual traces were averaged and analyzed to determine afterhyperpolarization (AHP) amplitudes. AHP durations were analyzed to determine the time needed to reach 50% from the peak of the AHP to the resting membrane potential. Neuronal excitability was monitored by observing the response to a series of long depolarizing current pulses (0.1–0.6 nA, 500 ms) before and following a brief (1–2 s) application of either NE or bethanechol. Changes in the number of action potentials and regression analysis of frequency versus stimulus intensity curves were used as a measure of changes in relative neuronal excitability. Following the characterization of neuronal responses to the agonists (NE and bethanechol), angiotensin II (Ang II, 10⁻⁷ M) was added to the bath solution and neuronal responses in the presence and absence of agonist (NE or bethanechol) application were characterized in combination with Ang II.

To stimulate synaptic inputs, an extracellular electrode was placed on nerve fiber bundles leading to the ganglion containing the neuron of interest (Hardwick et al., 2012). Orthodromic responses to fiber tract stimulation (0.1–10 V, 2 ms duration) 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. Suprathreshold stimuli were then given in 2 s trains at frequencies of 10, 20, and 30 Hz, and the number of action potentials produced by the neuron of interest was determined.

2.4. Immunohistochemical analysis

Following physiological experiments, the cardiac ganglion preparations were fixed in Zamboni's (2% paraformaldehyde and 0.1% picric acid) overnight at 4 °C and stained with

antibodies to nNOS and MAPII as previously described (Hardwick et al., 2008). The antibodies used were mouse anti-MAPII (1:500, Sigma), rabbit anti-nNOS (1:500, Cayman), donkey anti-rabbit Rh (1:500), and donkey anti-mouse FITC (1:500, all from Jackson Immunoresearch). The percentage of nNOS cells was determined by counting the total number of MAPII-immunoreactive cells and then determining the number of nNOS-immunoreactive cells.

2.5. Statistical analysis

Values are expressed as the mean $\pm$ standard error. Statistical significance was determined by Student's *t*-test or ANOVA followed by post hoc testing using the Holm–Sidak or Dunn test, with a *p* value less than 0.05 considered significant.

3. Results

As was shown in previous studies, surgically induced MI in the guinea pig produced a left ventricular infarction that encompasses approximately 8% of the ventricular tissue (Hardwick et al., 2008). In these studies, chronic MI (~2 months recovery) increased expression of neuronal nitric oxide synthase (nNOS) in neurons (Hardwick et al., 2008) and altered responses to adrenergic agonists and angiotensin II (Hardwick et al., 2012). The current study examined these parameters during early phases of recovery and remodeling in response to the stress imposed by myocardial infarction.

Immunohistochemical analysis was done on whole mount preparations of the guinea pig cardiac plexus stained with antibodies for both microtubule associated protein II (MAPII) and nNOS (Fig. 1A). MAPII was used to identify the total number of neurons in a preparation. The mean value for MAPII staining was 597 ± 305 cells ($n = 33$), with no significant difference in MAPII staining between treatments. Because of the variability in the number of neurons per preparation, the number of neurons that also stained with nNOS was normalized to a percentage of the total number of neurons in a given tissue. The percentage of IC neurons with nNOS expression following MI showed a time-dependent increase (Fig. 1B). The increase in nNOS expression is apparent at 4 days after MI and reaches a plateau at 14 days recovery at levels 3 times that of control. This increase in nNOS is maintained out to 50 days post-MI (* $p = 0.006$ versus control by ANOVA).

Intrinsic neuronal properties were examined at each of the recovery periods following MI (4–50 days) as well as in sham surgical animals at 7 days recovery. There were no significant changes in resting membrane potential or input resistance versus control animals (data not shown). Neurons from sham surgical animals at 7 days recovery ($n = 31$) demonstrated a significant increase in the AHP amplitude versus control (non-surgical) tissues (Fig. 2B), but there was no significant difference in the total AHP duration (data not shown). In animals with MI, there was a small but significant decrease in the amplitude of the afterhyperpolarizing potential (AHP, Fig. 2B) at 7 days post-MI ($n = 36$) versus controls ($n = 48$), 14 days ($n = 45$), and 50 days post-MI ($n = 74$). The duration of the AHP was analyzed by measuring the time from the peak of the AHP to 50% recovery of that amplitude to resting membrane potential. Sham surgical tissues showed no significant change in 50% AHP duration (Fig. 2C versus non-surgical controls. However, cells from MI animals at 4, 7, and 14 days recovery showed significant decreases in the recovery time to 50% of AHP amplitude (Fig. 2C, * $p < 0.001$ by ANOVA). By 50 days post-MI, there was no longer a significant difference in the 50% recovery time.

Chronic MI produces an increase in IC neuron sensitivity to adrenergic agonists, as seen in the increase in the number of evoked action potentials in response to depolarizing currents (Hardwick et al., 2008). Neuronal excitability in the absence of agonist application

(baseline) was unchanged from control animals at all post-MI time points (data not shown), and these data were pooled. With the application of NE, an increase in evoked action potentials with increasing stimulus intensity (vs. baseline, in the absence of NE) was observed at 4, 7, and 14 days recovery (Fig. 3A). There were no significant differences between the different recovery times in the frequency–intensity (*F/I*) curves in the presence of NE. Regression analyses of the slopes of the *F/I* curves for each condition show an increase in the slope of the curves with NE application in all MI animals when compared with NE application in healthy animals (Fig. 3B). In addition, an analysis of the maximal responses for each condition shows that NE increases evoked action potentials over baseline in all cases (Fig. 3C; $^{\#}p < 0.01$ versus baseline by ANOVA), and that MI produces a further increase in the NE-induced increase in excitability as early as 4 days post-MI. This change remains elevated at all subsequent time points (Fig. 3C, $^*p < 0.01$ versus NE in control animals by ANOVA). Sham surgical animals were no different from control (non-surgical) tissues.

Muscarinic agonists exert major neuromodulating effects on IC neurons, and these effects can be altered in pathophysiological states (Bibevski and Dunlap, 2011; Dunlap et al., 2003; Smith et al., 2001). Bethanechol increased neuronal excitability over baseline levels following MI (Fig. 4A). Analysis of the *F/I* curves shows that the increase in excitability is essentially equivalent to that seen in untreated animals (Fig. 4B). An examination of the maximal responses confirms that bethanechol application increased the maximal evoked neuronal excitability under all conditions (Fig. 4C; $^{\#}p < 0.01$ versus baseline by ANOVA). However, at 7 days post-MI and in shams at 7 days post-surgery, the maximal bethanechol-induced change in evoked action potentials was significantly reduced ($^*p < 0.01$ versus other bethanechol conditions by ANOVA).

Angiotensin II potentiates the responses to both NE and bethanechol in guinea pig IC neurons in normal animals (Girasole et al., 2011). With chronic MI (8–10 weeks post-MI), this potentiation is no longer seen (Hardwick et al., 2012). When Ang II was combined with NE application in the early recovery times, no potentiation of the NE response was observed at any of the time points tested (Fig. 5A). When Ang II was combined with bethanechol application post-MI, a significant increase in the bethanechol response was observed only in 7 days post-MI preparations (Fig. 5B).

In previous studies, neuronal responses to the high-frequency stimulation of inputs are unchanged in chronic MI (8–10 weeks) preparations as compared with controls but did show increased synaptic efficacy in animals with chronic pressure overload (8–10 weeks) (Hardwick et al., 2012). Synaptic efficacy was evaluated at various time points post-MI by measuring the responses to the stimulation of fiber tract bundles leading to the neuron of interest with a 2-s suprathreshold train at 10, 20, and 30 Hz. Although the responses at 4 and 14 days post-MI were not significantly different from each other or from shams, the synaptic evoked responses observed at 7 days post-MI were significantly increased (Fig. 6).

4. Discussion

Cardiac remodeling following myocardial infarction has been shown to produce increased sympathetic drive to the heart and an increase in activation of the renin angiotensin system (RAS) (Dell'Italia, 2011; Li et al., 2004; Triposkiadis et al., 2009). In addition, studies in humans and dogs have shown a concomitant decrease in parasympathetic function following MI that is associated with negative outcomes (Porter et al., 1990; Vanoli et al., 2008). In the guinea pig, remodeling of the intrinsic cardiac neurons with chronic MI includes increased adrenergic sensitivity and increased expression of nNOS within intracardiac neurons (Hardwick et al., 2008). In order to better characterize the dynamics of this remodeling

process, the present study examined the time course of these changes in the earlier time periods following induction of MI.

Phenotypic and functional changes in the intrinsic cardiac plexus were observed within 4 days after MI. A significant increase in adrenergic sensitivity became apparent at 4 days post-MI and persisted for up to 7 weeks. Previous studies have shown a reduction in adrenergic innervation to the infarct site, with an upregulation in adrenergic fibers in other regions of the heart and in sympathetic ganglia (Li et al., 2004; Nguyen et al., 2012; Parrish et al., 2010). The rapid and sustained upregulation in intracardiac neuronal sensitivity to norepinephrine likely represents an adaptive response to the elevated sympathetic drive. The majority of the neurons in the guinea pig intrinsic cardiac ganglia studied here are cholinergic, postganglionic parasympathetic neurons (Parsons, 2004). A small number of tyrosine-hydroxylase containing small intensely fluorescent cells (SIF cells) can be observed in the guinea pig cardiac plexus (Mawe et al., 1996), but these cells are significantly smaller than the principal cells. Nevertheless, these adrenergic neurons may play an important role in the integrated efferent control of regional cardiac function, especially during times of stress (Armour, 2008). The increase in IC neuronal excitability with norepinephrine may be part of an adaptive IC network response to increase the functional parasympathetic postganglionic output in response to the MI-induced decrease in central vagal command, thereby counterbalancing the sympathetic activation. In that regard, it is known that significant parasympathetic-sympathetic interactions occur within the intrinsic cardiac nervous system to modulate efferent outflow to the heart (McGuirt et al., 1997; Randall et al., 1998, 2003).

Previous studies in our laboratory also showed that Ang II can potentiate the NE responses in untreated animals (Girasole et al., 2011). However, in animals with chronic heart disease, the ability of Ang II to potentiate the NE response was lost (Hardwick et al., 2012). A similar result was seen in this study even at the early stages of recovery from MI. Ang II no longer potentiated the increase in evoked action potentials with NE application at any of the recovery time points tested. This may be due to the chronic elevation in cardiac Ang II levels produced by increased stimulation of the RAS system and local production of Ang II (Dell'Italia, 2011), such that exogenous application of Ang II had minimal additional effect.

In healthy animals, muscarinic agonists such as bethanechol also increase neuronal excitability. A similar increase in excitability with bethanechol application was observed at 4 days post-MI, and also at the later time points (14 days and 50 days). However, at 7 days post-MI, the muscarinic response was significantly less than that seen in either healthy animals or the other post-MI time points tested. Sham surgical animals at 7 days of recovery also demonstrated a decrease in muscarinic sensitivity, suggesting that this could represent a generalized response to the surgery, rather than a specific response to the MI. In addition, Ang II potentiation of the bethanechol responses were only observed in control animals and at the 7-day post-MI recovery point. There was no potentiation of the bethanechol-induced increase in excitability by Ang II at 4 days, 14 days, or 50 days recovery. Because the Ang II-induced potentiation of NE was not seen at any point following MI, in contrast to the maintained muscarinic and Ang II interactions at 7 days post-MI, this suggests a differential remodeling of adrenergic and muscarinic neuromodulatory influences on the IC networks in response to the ischemic event.

Ischemia leads to activation of local afferent fibers and activation of cardiac mast cells (Mackins et al., 2006). This in turn leads to the release of multiple cytokines and the local production of Ang II. Several studies have shown that these molecules can induce changes in expression of angiotensin receptors, as well as expression of different NOS isoforms in

myocytes (Isbell et al., 2007). In the current study, there was an upregulation in the percentage of neurons expressing nNOS following MI. The expression of nNOS within intracardiac neurons shows a time-dependent increase following MI that stabilizes at an approximate 3-fold increase over control levels by 14 days, and this increase is maintained for weeks afterward. The ganglia examined in our experiments were located in the left atria, in the region of the coronary sinus and the interatrial septum (Mawe et al., 1996). Studies by Dawson, et al. (Dawson et al., 2008) found a decrease in nNOS levels in ganglia located in the guinea pig right atria 3 days post-MI. However, regional differences in IC neuronal remodeling are likely, particularly when the injury is confined to a specific locus. Ganglia located in the right atria project primarily to the SA node and most likely mediate chronotropic effects (Harrison et al., 2005). Conversely, the interatrial ganglia, which are the focus of this study, are thought to project primarily to the AV node and mediate regulation of conduction velocity (Harrison et al., 2005). In addition, afferent stimulation within the infarcted area may alter intracardiac ganglia receiving specific inputs from this region differently than ganglia from non-injured areas (Huang et al., 1993; Waldmann et al., 2006). There is little evidence for direct projection from intracardiac ganglia to the left ventricular myocardium, where the ischemic event occurs (Hoover et al., 2004). Thus, neuronal remodeling in the cardiac plexus must be mediated by a combination of afferent stimulation and chemokine release.

Some distinct changes were observed at 7 days of recovery that were not seen at other time points. The stimulation of the fiber bundles innervating the intrinsic cardiac neurons was utilized to study communication to the intrinsic cardiac neurons. Previous studies found that in both control animals and animals with chronic MI, the postsynaptic neurons were able to follow an input frequency up to approximately 10–15 Hz. Higher-frequency inputs did not result in higher-frequency postsynaptic outputs. However, neurons from animals with chronic pressure overload were able to follow the input frequency up to ~30 Hz with much higher efficacy (Hardwick et al., 2012). In the acute recovery from MI, there was significant alteration in synaptic efficacy at 7 days post-MI, where higher output frequencies were observed with fiber tract stimulation. This time point correlates with a significant decrease in the AHP amplitude and duration with single action potentials. Increases in AHP durations are associated with lower firing rates in motor neurons (Manuel et al., 2006; Wienecke et al., 2009) and parasympathetic neurons in the nucleus ambiguus (Lin et al., 2010). Similar to the brainstem neurons, the AHP duration in the IC neurons is due primarily to Ca^{2+} -activated potassium channels (Jelson et al., 2003). Sham animals demonstrated a significant increase in AHP amplitude with no change in AHP recovery times and showed normal responses to fiber tract stimulation. However, at 7 days post-MI, there was a significant decrease in both AHP amplitude and the time course of recovery to baseline, along with an increase in synaptic efficacy. The increase in firing rates evident at 7 days post-MI disappears by 14 days, at which point the AHP amplitude has returned to control values. Whether the change in AHP conductance alone can account for the change in synaptic responses requires further investigation. These data do indicate that there are specific alterations in the conductances associated with single action potentials following MI. The response to surgery alone produces an apparent increase in K_{Ca} channel function, as seen by the increase in AHP amplitude. Following MI, at the same recovery point, there is a significant decrease in AHP amplitude and time to 50% recovery of AHP amplitude, suggesting a decrease in K_{Ca} conductance. This alteration appears to diminish as recovery progresses, such that by 50 days post-MI, no significant differences are seen in the action potentials in either sham animals (Hardwick et al., 2008) or MI animals.

Neurons at 7 days post-MI also demonstrated a decrease in muscarinic sensitivity, but this decrease was also observed in the sham animals. Sham animals demonstrate normal synaptic responses and previous studies demonstrated that atropine, a muscarinic antagonist, did not

alter postsynaptic output in either control or chronic heart disease models (Hardwick et al., 2012). The unique subset of changes observed at the 7-day post-MI recovery point warrant further study of the causes, and consequences of these changes. We believe that the changes in neuronal function at 7 days represent an early phase of neuronal network remodeling that has the potential for increased instability of the autonomic control pathways. Although the guinea pig model does not demonstrate altered survival rates, studies in both humans and canines show increased risk for negative outcomes in the initial recovery period. In human and dog studies, patients stratify into three separate groups in response to ischemic stress: one group that does not survive the initial insult, a second group that survives the initial MI insult but is at high risk for sudden cardiac death in response to a second ischemic/infarct stress, and the final group that compensates in response to MI stress (Billman, 2006; Chugh et al., 2008; Schwartz et al., 1992).

The cardiac nervous system is adaptive in response to imposed stress. This study demonstrates the progression of neuronal remodeling in terms of overall IC network excitability and neuromodulatory influences that control its integrated function. The short-term augmentations in synaptic efficacy post-MI returns toward baseline levels as the ventricular substrate stabilizes, while the augmenting influences of Ang II on IC neurons diminishes. The net effect of such changes would exert restraining influences on nested feedback loops contained within the intrinsic cardiac nervous system and allow for more effective control of regional cardiac function. Future studies should evaluate targeted neuromodulation therapies in order to assess whether it is possible to mitigate adverse remodeling in order to preserve cardiac function.

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Fig. 1. nNOS expression with recovery from MI. Whole mounts of the cardiac ganglion were labeled with antibodies for nNOS (1:500) and MAPII (1:500). MAPII staining was used to determine the total number of neurons in the tissue. Panel A shows representative staining from a control preparation and a preparation at 50 days post-MI. The percentage of neurons that were immunoreactive for both nNOS and MAPII was determined in control preparations and in animals following MI at 4, 7, 14, and 50 days recovery (panel B). The dashed line indicates the MI surgery. The points represent the mean $\pm$ SEM of 4–6 animals for each condition. Following MI (black circles), there was significant increase in nNOS expression versus controls (no surgery, open circle) at 4, 14, and 50 days post-MI (* $p < 0.04$

by ANOVA), but there was no significant difference ($p > 0.05$) at 7 days post-MI versus control. Scale bar = 20 μm .

Fig. 2.

Alterations in afterhyperpolarizing potentials with recovery from MI. (A) Example recordings of single action potentials from an IC neuron from a control preparations versus a sham at 7 days post-surgery (A₁) and 7 days post-MI versus 50 days post-MI (A₂). RMPs were -48 mV for control and sham and -45 mV for 7 days and 50 days. (B) AHP amplitudes were differentially increased in sham surgery 7-day recovery animals and selectively decreased at 7 days post-MI induction. (C) AHP durations were differentially altered post-MI. The time needed for the voltage to return to 50% of the peak of the AHP was determined for each treatment. The dashed line indicates the MI surgery. Sham treatment (gray triangle) had no significant effect on half time of recovery versus controls (open circle). Conversely, MI animals (black squares) showed a significant decrease in the time to 50% of peak amplitude at 4, 7, and 14 days ($p < 0.001$ versus control by ANOVA). By 50 days post-MI, there was no significant difference versus control.

Fig. 3.

Adrenergic modulation of neuronal excitability following MI. Evoked action potential number with increasing stimulus intensity (0.1–0.6 nA, 500 ms) was determined by intracellular voltage recordings from intrinsic cardiac neurons in control preparations, shams, and in preparations at 4, 7, 14, and 50 days post-MI. (A) The frequency–intensity curve for evoked action potentials in the early recovery periods (4, 7, and 14 days) indicates that NE application (1 s) increased neuronal excitability following MI, with no differences between the recovery time points. Baseline excitability was unchanged at all recovery time points, so these data were pooled. Points represent the mean $\pm$ SEM from ~ 20 cells for each condition. (B) Regression analysis of individual *F/I* curves were used to determine the slope for each condition. MI induces an increase in the slope at all time points as compared with controls. (C) Comparison of the maximal responses under all conditions (at 0.6 nA) shows a significant increase in evoked action potentials with NE application in control (NE), shams, and post-MI preparations (4, 7, 14, and 50 days; # $p < 0.01$ versus baseline by ANOVA). Following MI, the responses to NE are significantly greater than those seen in control and sham animals at all recovery time points (* $p < 0.05$ versus control NE by ANOVA).

Fig. 4.

Muscarinic modulation of neuronal excitability following MI. Evoked action potentials were also increased following bethanechol application (1 s) with increasing stimulus intensities (A) at 4, 7, and 14 days following MI. Points represent the mean $\pm$ SEM from 17–20 cells. (B) Regression analyses of individual *F/I* curves were used to determine the slope for each condition. There was no significant change in the slopes following MI, although there is a trend toward reduced responses at 7 days post-MI. (C) The maximal responses (at 0.6 nA) show a significant increase in the number of action potentials with bethanechol application versus baseline under all conditions ($^{\#}p < 0.01$ versus baseline by ANOVA). The maximal responses with bethanechol application in sham preparations and at 7 days post-MI were significantly lower than all other bethanechol conditions ($^{*}p < 0.05$ by ANOVA).

Fig. 5.

Loss of angiotensin II neuromodulation responses on IC neurons following MI. Angiotensin II (100 nM) added to the circulating bath (gray bars) potentiates the number of evoked action potentials (maximal responses at 0.6 nA) following either NE application (A) or bethanechol application (B) in control animals ($^{\#}p < 0.05$ versus either NE alone (white bars), or bethanechol alone (white bars) by paired t -test). Following recovery from MI, the addition of Ang II had no effect on the norepinephrine responses (NS by paired t -test) at any of the time points tested. The potentiation of the bethanechol response was observed at 7 days post-MI ($^{\#}p < 0.05$ versus bethanechol alone at 7 days by paired t -test), but there was no significant difference at either 4 or 14 days of recovery.

Fig. 6.

Changes in synaptic efficacy following MI. Fiber bundles synapsing with the intrinsic cardiac neurons were stimulated with an extracellular electrode (0.1–10 V, 2 ms) for 2 s at a frequency of 20 Hz. Recordings from preparations at 4, 7, and 14 days recovery from MI are shown in panel A. The output frequency produced in the neuron from the 7-day recovery period was greater than that seen at 4 or 14 days of recovery. Panel B shows summary data from multiple cells, including shams. Points are the mean $\pm$ SEM ($n = 6$ or more cells). The output frequency with fiber tract stimulation was significantly greater in neurons from 7-day recovery preparations at 20 and 30 Hz compared with neurons from shams, 4 and 14 days recovery (** $p < 0.001$ by ANOVA). Resting membrane potentials: 4 days, -50 mV; 7 days, -61 mV; 14 days, -45 mV.