Cardiac sympathectomy and spinal cord stimulation attenuate reflex-mediated norepinephrine release during ischemia preventing ventricular fibrillation

Jeffrey L. Ardell, … , J. Andrew Armour, Kalyanam Shivkumar


Rationale: Reflex-mediated sympathoexcitation is central to the pathogenesis of arrhythmias and heart disease; neuraxial modulation can favorably attenuate these cardiac reflexes leading to cardioprotection. Objective: The purpose of this study was to define the mechanism by which cardiac neural decentralization and spinal cord stimulation (SCS) reduces ischemia-induced ventricular fibrillation (VF) and sudden cardiac death (SCD) by utilizing direct neurotransmitter measurements in the heart. Methods and Results: Direct measurement of norepinephrine (NE) levels in the left ventricular (LV) interstitial fluid (ISF) by microdialysis in response to transient left anterior descending coronary artery occlusion (CAO: 15 min) in anesthetized canines. Responses were studied with: (i) intact neuraxis and were compared to those in which the (ii) intrathoracic component of the cardiac neuraxis (stellate ganglia), (iii) the intrinsic cardiac neuronal (ICN) system were surgically delinked from the central nervous system versus (iv) subjects with intact neuraxis subjected to pre-emptive SCS (T1-T3 spinal level). With an intact neuraxis, animals with exaggerated NE-ISF levels in response to CAO were at increased risk for VF and SCD. During CAO there was a 152% increase in NE level when the entire neuraxis was intact compared to 114% following intrathoracic neuraxial decentralization (removal of the stellates) and 16% increase following ICN decentralization, when the entire heart and ICN was delinked from the other levels of the neuraxis. During SCS, […]

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CARDIAC SYMPATHECTOMY AND SPINAL CORD STIMULATION ATTENUATE REFLEX-MEDIATED NOREPINEPHRINE RELEASE DURING ISCHEMIA PREVENTING VENTRICULAR FIBRILLATION

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Running Title: Neuraxial modulation and attenuation of norepinephrine release

Key Words: cardiac neuronal hierarchy; myocardial ischemia; norepinephrine; stellate ganglionectomy; spinal cord stimulation; ventricular arrhythmias

Word Count: 7139

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ABSTRACT

Rationale: Reflex-mediated sympathoexcitation is central to the pathogenesis of arrhythmias and heart disease; neuraxial modulation can favorably attenuate these cardiac reflexes leading to cardioprotection. Objective: The purpose of this study was to define the mechanism by which cardiac neuraxial decentralization and spinal cord stimulation (SCS) reduces ischemia-induced ventricular fibrillation (VF) and sudden cardiac death (SCD) by utilizing direct neurotransmitter measurements in the heart. Methods and Results: Direct measurements of norepinephrine (NE) levels in the left ventricular (LV) interstitial fluid (ISF) by microdialysis in response to transient left anterior descending coronary artery occlusion (CAO: 15 min) were performed in anesthetized canines. Responses were studied with: (i) intact neuraxis and were compared to those in which the (ii) intrathoracic component of the cardiac neuraxis (stellate ganglia), (iii) the intrinsic cardiac neuronal (ICN) system were surgically delinked from the central nervous system and (iv) subjects with intact neuraxis subjected to pre-emptive SCS (T1-T3 spinal level). With an intact neuraxis, animals with exaggerated NE-ISF levels in response to CAO were at increased risk for VF and SCD. During CAO there was a 152% increase in NE level when the entire neuraxis was intact compared to 114% following intrathoracic neuraxial decentralization (removal of the stellates) and 16% increase following ICN decentralization, when the entire heart and ICN was delinked from the other levels of the neuraxis. During SCS, CAO increased NE levels by 59%. Risk for CAO-induced VF was 38% in controls, 8% following total decentralization and 11% following SCS. Conclusions: These data indicate that ischemia related afferent neuronal transmission differentially engages central and intrathoracic sympathetic reflexes and amplifies sympathoexcitation. The resultant imbalances in regional ventricular NE release is associated with increased risk for VF and SCD. Surgical decentralization or SCS reduces this amplification of sympathoexcitation and resultant NE release and is associated with reduced VF and SCD.
INTRODUCTION

Acute myocardial ischemia (MI) can initiate lethal cardiac arrhythmias that are responsible for sudden cardiac death (SCD).(1) Local metabolic factors, including ionic shifts(1, 2), contribute to creating an arrhythmogenic substrate for arrhythmias. Vulnerability to MI arrhythmia induction is also powerfully modulated by the autonomic neural networks.(3-5) Cardiac afferent sensory transduction initiates reflexes within the cardiac nervous system(6) that ultimately regulate efferent sympathetic output to the heart. These reflexes result in the release of norepinephrine (NE) at the nerve terminals into the cardiac interstitium.(7-10) This reflex-mediated NE release can be highly arrhythmogenic at a time when the myocardium itself is highly susceptible to abnormal impulse formation and propagation.(3, 5, 11)

Pharmacological approaches to mitigate these consequences have focused on adrenergic receptor blockade which is an established therapy primarily targeted at the cardiac end-effectors.(12) The anti-arrhythmic efficacy of such pharmacological therapies to reduce SCD has limitations and address only one aspect of the pathophysiological mechanism leading to sympathoexcitation.(3, 5, 13) The contribution of cardiac innervation to the risk of VF, especially via the transduction of ischemia by neural afferents that cause reflex mediated sympatho-excitation, is not well understood.

The cardiac nervous system is comprised of a series of feedback control circuits, which are fundamental to dynamic cardiac control in health and disease.(14-16). New avenues for neuraxial therapeutic interventions can be understood with this framework.(17-19) Neuromodulation therapies are targeted at specific ‘nexus’ points of the cardiac nervous system such as the intrinsic cardiac nervous system,(17) extracardiac sympathetic ganglia,(19-21) spinal cord,(22-24) or vagosympathetic trunk.(25-28) These therapies have demonstrated myocardial salvage (24, 27, 29) and efficacy in arrhythmia control.(20, 21, 23) Neuraxial stabilization is further exemplified in the transplanted heart which contains a functional intrinsic cardiac nervous system(30, 31) and it ‘decentralized’ from the higher levels of the nervous system. In this setting ventricular fibrillation is rarely seen in transplanted hearts - even in the setting of acute ischemia.(32) The mechanistic basis of these clinical observations, especially why there is a reduction in sympathoexcitation with cardiac neural decentralization is not well understood. We sought to examine the effects of stepwise decentralization of the heart and spinal cord stimulation in separate sets of experiments with
the aid of direct neurotransmitter measurements of NE levels in the left ventricle interstitial fluid (ISF) space with the microdialysis technique.

We hypothesized that neural reflexes initiated by cardiac sensory transduction of ventricular ischemia are responsible for amplification of sympatho-excitation and resultant enhancement of regional cardiac NE levels leading to VF. We also hypothesized that stabilization of intra-thoracic reflex processing [by spinal cord stimulation (SCS) or surgical decentralization of the intrathoracic aspects of the cardiac nervous system from higher centers] can mitigate this amplification of sympatho-excitation during myocardial/ischemia reperfusion, thereby reducing regional ventricular NE release and thus decreasing the potential for VF.

RESULTS

Hemodynamic indices: Table 1 summarizes the hemodynamic data recorded before, during and after transient occlusion of the LAD coronary artery in each of the four animal groups studied. With the exception of the SCS neuromodulation group, LAD occlusion initiated minimal changes in heart rate. It decreased left ventricular (LV) systolic pressure (SP) and LV +dP/dt. It also increased LV end-diastolic pressure (DP). While SCS by itself did not alter baseline hemodynamic indices, it did mitigate depressions in LV +dP/dt associated with transient LAD occlusion.

Ventricular Fibrillation (Figure 1 & 2): In untreated control animals, transient (15 min) occlusion of the LAD coronary artery was associated with ventricular fibrillation in 38% of the animals (Group 1). This generally occurred within 3 minutes of reperfusion onset (Fig. 2, untreated) and was associated with surge in LV ISF catecholamine levels (109.8±25.0% change over baseline for survivors vrs. 458.3±63.6% change over baseline for animals that went into VF; p≤0.001). In a subset of animals with intact innervation (n=11), the circumflex artery was occluded and with VF onset primarily occurring 4-6 min into occlusion (red trace in Fig 3A). For the animals that experienced VF during circumflex CAO the ischemic zone had catecholamines levels double that of resistant animals (2037±161 pg/ml vs 1255±210 pg/ml, p=0.028) during that ischemic event. Acute decentralization of the intrathoracic nervous system from the influences of central neurons reduced the potential for ischemia induced
sudden cardiac death to 8% of control (occurring in 1 of 12 animals so tested, Fig 2 B) (Groups 2 and 3). Pre-emptive SCS also reduced the incidence of CAO induced VF in neurally intact preparations (1 of 9 animal during SCS+CAO so studied; Fig. 2 C) (Group 4). From group 4, 2 of 5 animals randomized to CAO first experienced SCD/VF at reperfusion. In Group 4 animals, randomized first to SCS+CAO and then one hour later to CAO alone, 5 of 6 animals survived the first ischemic challenge (c.f., in the presence of SCS). In response to the second (sham SCS) CAO, animals exhibited a 40% potential for SCD/VF. As detailed in the next section, the reduction in VF potential by sequential decentralization or active SCS was associated with mitigation of NE release during ischemia and reperfusion phases.

**Local LV interstitial NE release with an intact neuraxis:** Regional ventricular NE liberation was greatest during transition states (i.e., baseline to occlusion or occlusion to reperfusion) (Figure 3, panels A and B). When these data were sub-grouped retrospectively according to whether animals survived the ischemic insult (VF resistant; VF-R) versus those that did not survive (VF susceptible, VF-S), animals that died suddenly demonstrated enhanced NE levels within the ischemic zone during CAO and enhanced NE levels during reperfusion in all areas of the LV sampled (Fig. 3, panels C and D; Fig. 4 panel A). In intact preparations which survived the transient ischemic challenge, regional LV ISF NE increased in reflex response to LAD coronary artery occlusion/reperfusion - with progressively increasing contents when comparing normal to ischemic zones (703.3±74.0 to 1426.0±156.7 pg/ml, normal zone; 799.1±57.8 to 1933.1±183.8 pg/ml, ischemic zone; p=0.318 at baseline and p=0.047 during LAD CAO between zones).

**CAO-induced LV ISF NE following decentralization of the intrathoracic nervous system (Figure 4):** When the intrathoracic elements of the cardiac nervous system was surgically disconnected from the central nervous system (stellate ganglia decentralized and cervical vagosympathetic trunks cut bilaterally), transient CAO still reflexly increased NE contents in all 3 LV regions (ischemic, border and normal zones) (Fig. 4, panel B). However, such increase was reduced by ~25% across all zones when compared to data obtained in the intact neural state preparations. Following decentralization of the ICN, leaving it as the sole reflex controller of neuronal release, CAO reflex-induced release of NE into the non-ischemic and border zones was virtually eliminated. Residual ischemia induced NE release was identified within the ischemic zone (Fig. 4, panel C). Following ICN decentralization, no
Evidence of enhanced NE release was evident upon reperfusion in any of the zones evaluated.

**CAO-induced LV ISF NE following thoracic spinal cord stimulation (Figure 5):** Electrical stimulation of the dorsal columns of the high thoracic spinal cord (T1-T3) blunted myocardial ischemia-induced reflex induced release of NE into the ISF in all LV regions tested (149.3±33.9% vs 38.4±14.7% normal zone, p≤0.015; 206.8±34.1% vs 80.1±10.8, p≤0.001 ischemic zone; % change from basal levels). Pre-emptive SCS had no effect on basal hemodynamics but did mitigate the depression in LV contractility that was identified in control states in response to LAD CAO (Table 1). In contrast, in time controls similar regional ISF NE release and evoked hemodynamic responses were identified during repeat CAO (data not shown).

**DISCUSSION**

The major findings of this study are:

(i) Afferent sensory transduction of ventricular ischemia results in a reflex mediated amplification of sympathoexcitation.

(ii) The augmented NE release due to afferents engaging higher levels of the neuraxis and concomitant dispersion in regional NE levels in the heart that was associated increased incidence of VF.

(iii) Progressive ‘delinking’ of the heart from more rostral aspects of the neuraxis attenuates the amplification of sympathoexcitation by the higher regions.

(iv) Neuraxial modulation utilizing SCS mitigated MI-reflex induced augmentation of NE release; VF was reduced with preservation of contractile function.

**Cardiac Neurotransmission:**

The heart has a rich sensory innervation which transduces multiple physiological events that are transmitted to various levels of the neuraxis (Figure 1).(17, 33, 34) Afferent neural traffic is transmitted to the intrinsic neurons ganglionated plexus in the heart, stellate and middle cervical ganglia, the spinal cord (via dorsal root ganglia) with relays to higher centers via the spinoreticular track and by way of the vagosympathetic trunk to the nodose ganglia with neuronal projections to the nucleus tractus solitaries.(10, 30, 34, 35) This afferent information is processed by the ICNS, stellate/middle cervical ganglia, spinal cord and higher
centers to initiate the reflexes that ultimately result in activation of sympathetic efferent postganglionic neurons within intrathoracic extracardiac and intrinsic cardiac ganglia.(17, 33, 36) This reflex induced sympatho-excitation modulates cardiac chronotropy, dromotropy, inotropy and lusitropy.(37, 38)

Integrated control of cardiac indices by adrenergic efferent postganglionic neurons can be investigated locally by assessment of regional catecholamine release.(39-41) In this study interstitial fluid (ISF) levels of NE were determined in normal (remote), ischemic and border zone regions of the left ventricle in reflex response to transient, regional LV ischemia; levels of NE measured within the ISF are reflective of neuronal release, reuptake and diffusion.(39, 40, 42) This ISF-NE index of regional sympathetic activity was assessed in animals with i) intact neuraxis as compared to those in which ii) the intrathoracic component of the cardiac neuronal hierarchy was surgically disconnected from the central nervous system versus iii) those with intact neuraxis that had been pre-emptively treated with spinal cord stimulation (SCS: T1-T3 spinal levels). The results show that processing of cardiac afferents at each level of the neuraxis results in amplification of sympatho-excitation as evidenced by higher NE release and that targeted neuromodulation has the capacity to blunt such reflexes while preserving basal function.

**Hierarchy of Cardiac Neural Control:** Hierarchy for cardiac control functions as a series of nested feedback loops. Control is regulated by three major neuronal levels: i) the intrinsic cardiac nervous system, ii) extracardiac intrathoracic (stellate, middle cervical and mediastinal) ganglia and iii) the central nervous system (spinal cord, brainstem and higher centers).(17, 43-45) These three levels are capable of independent and interdependent reflex actions for control of regional cardiac function.(30, 35, 46, 47) The two lower level reflex circuits that are contained with the thoracic cavity (ICNS and stellate/middle cervical/mediastinal ganglia) are ‘cardio-centric’ and are primarily concerned with dynamic control of regional cardiac indices.(22, 30, 48, 49) Our study shows that their efferent output is delineated by level of the hierarchy involved.(10, 17, 36) ICNS evoked responses to transient myocardial ischemia reflect cardio-centric control restricted primarily to the ischemic zone and results in the least degree of amplification of sympathoexcitation.(49, 50) In contrast, intrathoracic-extracardiac (stellate/middle cervical ganglia dependent) reflex
responses to the ischemic stress involved coordination of adrenergic neurons that released NE throughout the left ventricle in reflex response to a focal ischemic stress. (22, 41, 42)

**Centrally mediated effects:** Superimposed upon these intrathoracic (peripheral) reflexes are those mediated by the central nervous system. Centrally mediated reflexes are essential to controlling not only cardiac function, but also blood flow demands throughout the entire body (37, 51, 52). In response to normal physiological stressors (e.g. orthostatic stress and dynamic exercise) changes in cardiovascular-related afferent inputs, along with somato-autonomic interactions, adjust cardiac output to meet whole body blood flow demand (37, 51, 52). Conversely, pathophysiological stressors such as acute MI have the potential to disrupt such control, sometimes with lethal consequences. (3, 4, 11, 51) Our data shows the highest degree of amplification of sympathoexcitation is seen when the higher centers are engaged. This also highlights the importance of measures such as sedation and general anesthesia for the management of ventricular arrhythmia storm. (53)

**Effect of Myocardial Ischemia:** Myocardial ischemia presents a novel, major stressor to the cardiac neuraxis. (51) It is known that visceral sensory information is integrated at multiple levels within the central nervous system, including the spinal cord (10, 44, 54) and brainstem. (36, 37, 43) From an integrated control perspective, afferent input (ischemia) elicits a differential response from the peripheral cardio-centric reflex loop (32, 50, 51) as compared to the response from central neural networks. (9, 10, 34, 43) Specifically, our data shows that the reflex response of the higher centers to sensory inputs from the ischemic myocardium is inherently pro-arrhythmic (amplification of sympatho-excitation) whereas when the central sites are delinked peripheral reflexes are less arrhythmogenic and are accompanied by a lower release of NE in the myocardium. It should be noted that there is still release within the ischemic zone even after ICN decentralization. This could be ‘cardio-cardiac reflex’ mediated or could reflect the local effects of extracellular K+ following myocyte death.

**Neuraxial Modulation/Clinical Relevance:** The results shown here are directly relevant to dorsal (high thoracic) SCS (55, 56) and decentralization of the intrathoracic aspects of the cardiac nervous system from the central nervous system (as seen with bilateral stellate ganglionectomy). (19-21) Previous data have demonstrated that SCS is effective in stabilizing reflex processing within extracardiac sympathetic ganglia (22) and the intrinsic cardiac ganglionated plexus, (57) while reducing the apoptotic potential of ischemic stressed
cardiomyocytes. Our results demonstrate that SCS blunted neuronal NE release into the LV interstitium with an associated reduced risk for SCD.

Classically, neuronal control of sympathetic outflows to the heart has been considered to be primarily under central neuronal command, with peripheral ganglia functioning solely as efferent neuronal relay stations. Extrapolating from that conceptual framework, decentralization of the intrathoracic elements of the cardiac nervous system would be expected to abolish all MI-induced catecholamine release, except for local effects mediated directly on adrenergic efferent postganglionic nerve terminals located within the ischemic zone. In contrast, our data shows that following decentralization of intrathoracic reflex control from the influences of central neurons, effective coordination of local catecholamine release across the ventricle is maintained (Fig. 4). In fact, the disparity in such release between the risk zone and normally perfused tissues actually decreased. At the same time, such a reduction in NE dispersion conferred a mortality benefit (Fig. 2). These results directly relate to the clinical setting of intractable ventricular tachycardia (VT) where stellate ganglia decentralization is effective in the management of VT storm.

**Limitations:** This study, being conducted in anesthetized animals, does not take into account the depressor effects of anesthetic agents on reflex regulation of cardiac NE release. However, general anesthesia does not fully inhibit centrally driven pro-arrhythmic events as evidenced by the efficacy of thoracic epicardial anesthesia and stellate ganglionectomy in the clinical setting. We did not directly record from intrathoracic extracardiac and intrinsic cardiac neurons concomitant with cardiac indices in this study. However, previous studies done by our group are consistent with the reduced adrenergic outflow noted in this study with SCS or decentralization. Finally, transection of the vagus also removes afferent neural signals from the heart which could have also regulated NE release and interrupted parasympathetic efferent projections to the heart which mitigates the potential for peripheral sympathetic/parasympathetic interactions. Preconditioning effects could also play a role in these situations. To minimize this, we performed repeat occlusions in animals that did not have VF and responses were similar. In the randomized order of CAO and SCS-CAO, in the 40% of animals that survived the SCS-CAO when done first experienced VF on CAO alone. This argues against preconditioning being a major factor for Group 4 (SCS).
Conclusion: This study indicates that regional ventricular ischemia activates central and intrathoracic (including those confined to the ICNS) reflexes that control cardiac sympathetic efferent postganglionic neurons which release NE into the ventricular interstitium. As summarized in the graphical abstract, the magnitude of regional release in response to the afferent transduction of ventricular ischemia is augmented and amplified by afferents engaging the higher levels of the neuraxis (stellates, spinal cord and the brain). Delinking the heart from each higher level of the neuraxis (by surgery or via bio electronically) was protective against the transient stress of CAO. This is analogous to the electrical stability of the transplanted human heart. (32, 65) These data provide a mechanistic basis to understand why procedures like cardiac sympathectomy (19-21) and SCS (23) are effective in arrhythmia control.

METHODS

Model: Thirty four mongrel dogs of either sex, weighing between 20 and 30 kg, were used in these studies. All experiments were performed in accordance with the guidelines for animal experimentation described in the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Academy Press, Washington DC, 2010. The Institutional Animal Care and Use Committee of East Tennessee State University approved these experiments.

Surgical preparation: Anesthesia was induced with sodium thiopental (6 mg/kg) and maintained with isofluorane (2%, inhalation therapy). A 5-Fr catheter transducer (Mikro-tip, Millar Inst.) was inserted into the left ventricular (LV) chamber via the right femoral artery to measure LV pressure and LV +dp/dt. Aortic pressure was monitored via a 5-Fr catheter transducer placed via the left femoral artery. Heart rate was monitored via a lead II electrocardiogram. A bilateral thoracotomy (fourth interspace) was performed. For the subsequent induction of transient regional ventricular ischemia, after exposing the heart, a pericardial cradle was formed and silk ligatures placed around the left anterior descending (LAD) coronary artery 1.5 cm from its origin (or the proximal circumflex coronary artery for the experiments on that territory). All hemodynamic data were digitized (Cambridge Electronic Design power 1401 acquisition system with Spike 2 software) for subsequent off-line analysis.

Neuromodulation treatment groups: Surgical decentralization of the intrathoracic cardiac nervous system: (Figure 1). Four different experimental groups evaluated the
central-peripheral interactions for modulation of NE levels in the LV ISF (interstitial fluid) in reflex response to transient coronary artery occlusion (CAO). Figure 1 summarizes the experimental design for selective neuraxial modulation/isolation. Group 1 (n = 11 dogs) had an intact cardiac neuraxis and served as untreated control for the other groups. For group 2 (n = 6 dogs), all neural connections between the stellate ganglia and spinal cord were severed bilaterally and both cervical vagosympathetic trunks transected. These surgical procedures decentralized neurons in stellate, middle cervical, mediastinal and intrinsic cardiac ganglia from the central nervous system.(66) For group 3 (n = 6 dogs), the stellate, middle cervical and mediastinal ganglia were excised surgically bilaterally, along with their entire intrathoracic extracardiac vagosympathetic complex.(30) This procedure removed all extracardiac neuronal connections (afferent and efferent) to the intrinsic cardiac nervous system, leaving it as the sole neuronal controller of cardiac indices.(17, 30)

**Neuromodulation treatment group: spinal cord stimulation: (Figure 1).** In group 4 (n=11), following induction of anesthesia, using techniques described previously,(22, 55) the epidural space was accessed via a Touhy needle and a four-pole electrode (Octrode, Advanced Neuromodulation Systems, Plano, TX) was advanced rostrally in the epidural space to the T1-T3 spinal cord level. The tip of the lead was positioned under fluoroscopy with the multi-pole electrode placed midway between mid-line and epidural region overlying left dorsal funiculus. T1-T3 SCS was delivered at a frequency of 50 Hz, duration of 200 µsec and current intensity of 90% motor threshold (MT), with the rostral pole as cathode. This level of stimulation corresponds to that utilized clinically to treat angina(55) and in both small and large animals to modulate intrathoracic autonomic reflex processing.(22, 57) Following SCS implant, the animal was rotated to the decubitus position, the chest opened, and the animals instrumented as detailed above. SCS MT levels were verified prior to and following completion of experimental protocol.

**Ventricular microdialysis.** Regional ventricular ISF NE levels were quantified using the microdialysis technique.(40-42) Six microdialysis probes were placed into the LV wall. As illustrated in Figure 2, two probes were inserted into the anterior wall region that was perfused by the LAD coronary artery (ischemic zone); two were inserted into the posterior-lateral LV wall. The remaining two probes were placed mid-way between the two other sets, a region that represented the border zone.
In each group, regional ventricular ischemia was induced by a 15 min period of left anterior descending coronary artery occlusion (CAO). Repeat occlusions were separated by at least 1 hour. In groups 2 & 3, surgical decentralization of intrathoracic vs intrinsic cardiac neurons was completed at least 1 hour prior to placement of the microdialysis probes. Dialysate was collected separately at baseline, during 3 min intervals throughout coronary artery occlusion and for 15 minutes following CAO. Concurrent changes in cardiovascular hemodynamic indices were recorded. In group 4 at least 1 hour separated arterial occlusions. All NE levels were assessed while the animals were still in sinus rhythm, we stopped collections that would have gone into the period of VF to avoid confounding effects of increasing NE levels due to VF induced sympathetic surge.

The treatment order (pre-emptive SCS + CAO vs CAO) was randomized. In the SCS group, T1-T3 SCS was delivered at 50 Hz, 200-µsec duration and at a current intensity of 90% MT (0.79±0.16 mA). SCS was initiated 15 min before coronary artery occlusion began and lasted for 36 minutes. SCS motor threshold was checked periodically and was found to not vary significantly from initial levels.

**Biochemical analyses:** Ventricular dialysate fluid collected from the three LV zones (normal, border and ischemic tissues) was analyzed for NE content during baseline and during each successive challenge. LV ISF NE concentrations were determined using radio-enzymatic-assay as described previously by our laboratory.(40, 67) Derived ISF levels for NE are reflective of regional sympathetic efferent postganglionic neuronal release, local catecholamine reuptake and diffusion from measurement sites.

**Statistical analyses:** Data are presented as mean±SE, ANOVA with post-hoc comparison (Holm-Sidak method) was used to compare hemodynamic and NE concentration data derived at baseline, LAD CAO and reperfusion. If data failed the normality test (Shapiro-Wilk), Kruskal-Wallis ANOVA on ranks was used with post-hoc comparison (Dunn’s Method). A p value of ≤ 0.05 was considered significant.

**Acknowledgment:** This study was supported by the NIH: Grants HL71830 (JLA), HL084261 (KS) & OT20D023848 (KS & JLA).
Author contributions: All authors contributed to production and presentation of the data and approved the final version of the manuscript.

Conflict of interest: University of California (UCLA) has patents developed by our group in the areas of cardiac catheter technology, embolism prevention technology, minimally invasive methods for cardiac interventions, cardiac neural diagnostics and therapeutics. None of these patents are directly related to this work.
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Figure 1: Experimental Design:
Summary of experimental protocol of coronary occlusion and sites of micodialysis for NE (norepinephrine collection). The left anterior descending coronary artery (LAD) is shown in this schematic. The levels of decentralization as related to primary elements of the neural hierarchy for cardiac control is shown. Responses in animals with i) intact neuraxis (Group 1) were compared to those in which the ii) intrathoracic component of the cardiac neuraxis was surgically disconnected from the central nervous system vs iii) those with intact neuraxis subjected to SCS (Group 4; T1-T3 spinal level). For group 2 decentralization the cervical vagosympathetic nerve trunks were transected and all connections from CNS to stellate ganglia transected. For group 3 decentralization, all connections to (efferent) and from (afferent) the heart were surgically interrupted. LCN-local circuit neurons; Sympath-Sympathetic; Parasym-Parasympathetic; Aff-afferent; T-thoracic spinal segment; C-cervical spinal segment; DRG-dorsal root ganglia; Gs and Gi – stimulatory and inhibitory guanine nucleotide binding protein.
Table 1: Hemodynamic response to transient coronary artery occlusion

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<th>Heart Rate</th>
<th>LVDP</th>
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<tr>
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<td>109.8 ± 8.5</td>
<td>2502.4 ± 160.1*</td>
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<tr>
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<tr>
<td>Reperfusion</td>
<td>99.0 ± 5.7</td>
<td>3.3 ± 0.6</td>
<td>98.8 ± 5.4*</td>
<td>2144.1 ± 175.7</td>
</tr>
<tr>
<td><strong>Intact: T1-T3 SCS</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>132.3 ± 5.6</td>
<td>1.3 ± 0.6</td>
<td>122.6 ± 8.6</td>
<td>2598.1 ± 215.3</td>
</tr>
<tr>
<td>SCS</td>
<td>135.0 ± 6.1</td>
<td>1.0 ± 0.6</td>
<td>125.2 ± 9.2</td>
<td>2760.0 ± 220.0</td>
</tr>
<tr>
<td>SCS+LAD CAO</td>
<td>140.3 ± 6.2*</td>
<td>3.4 ± 1.0*</td>
<td>122.2 ± 10.5</td>
<td>2552.6 ± 267.7</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>141.7 ± 6.2*</td>
<td>2.0 ± 0.8</td>
<td>123.0 ± 9.2</td>
<td>2589.3 ± 213.5</td>
</tr>
</tbody>
</table>

* p<0.04 versus baseline

Table 1: Hemodynamic response to transient coronary artery occlusion: Hemodynamic data derived in response to transient coronary artery occlusions (CAO) and reperfusion in each of the four experimental groups. * p≤0.05 versus baseline. [Repeated measure ANOVA with Holm-Sidak method for pairwise multiple comparison.]
Figure 2: Survival Following Coronary Artery Occlusion and Reperfusion: Panel A shows survival curves in animals with intact innervation in response to transient LAD (black lines) or circumflex CAO. Surgical decentralization of intra-thoracic ganglia of cardiac nervous system (panel B) and pre-emptive T1-T3 spinal cord stimulation (SCS, panel C) reduced mortality (% mortality) to 15’ occlusion (grey bar) of left anterior descending coronary artery (CAO) as compared to untreated controls. Intrathoracic decentralization curve includes both group 2 and 3. [Log Rank Sum test LAD CAO intact vs LAD CAO decentralized, p<0.05. Proportion of animal alive at 45 min of each intervention for SCS or Decentralized vs intact, p<0.05.]
Figure 3: LV Interstitial Fluid NE - VF resistant versus VF susceptible subjects: Changes induced in the interstitial levels of Norepinephrine (NE) (mean± SE) in response to 15 min LAD CAO (coronary artery occlusion) in LV tissues of the normal zone (panel A) compared to changes in the ischemic zone (panel B). Data are subdivided into those animals which tolerated the transient ischemic insult (VF resistant, VF-R; n=12) versus those that did not (VF susceptible, VF-S; n=8). Panels A and B summarize temporal changes in LV ISF NE from baseline to reperfusion phases (VF-S blue lines, VF-S red lines); panel C summarizes average LV ISF NE levels during baseline (BL), CAO and reperfusion. Panel D summarizes percent change from baseline during CAO and reperfusion phases for VF-R vs VF-S animals. * p≤0.01 vs baseline within sub-group (VF-R or VS-S); # p≤0.04 CAO vs reperfusion within sub-group; between group comparisons: + p≤0.05 VF-R vs VF-S; & p≤0.01 VF-R vs VF-S. [Panel C statistics: Repeated measure ANOVA with Holm-Sidak method for multiple pairwise comparison. Panel D statistics: Kruskal-Wallis Analysis of Variance on Ranks with Dunn’s methods for pairwise multiple comparisons.]
Figure 4: Left Ventricular Norepinephrine (NE) Release Following Neuraxial Decentralization: LV ISF NE levels in the ventral region perfused by the circumflex coronary artery (normal zone), LV mid-wall region (border zone) and the region perfused by the LAD coronary artery (ischemic zone) at baseline (closed circles), during LAD CAO (closed triangles) and reperfusion (closed squares). These data were derived from animals with intact neuraxis (panel A, Control, Group 1, n=7) vs those in which the intrathoracic nervous system was acutely decentralized (stellate decent: stellate ganglia decentralized and cervical vagosympathetic complexes cut) (panel B, Group 2, n=6) vs those in which the intrinsic cardiac nervous system (ICN) was surgically decentralized from all extracardiac neuronal inputs (panel C, ICN Decent, Group 3, n=6). *p≤0.01 from baseline; + p≤0.03 from baseline; # p≤0.05 from baseline. [Repeated measure ANOVA with Holm-Sidak method for pairwise multiple comparison.]
Figure 5: Coronary Artery Occlusion (CAO)-induced LV ISF NE changes following thoracic spinal cord stimulation: Changes induced in regional ventricular ISF NE levels in normal (left panel) vs ischemic (right panel) zones in reflex response to transient (15 min) LAD coronary artery occlusion (CAO) without (control, n=20) vs with pre-emptive neuromodulation (SCS, n=7). Note that SCS reduced ischemia induced reflex NE release in both the normal and ischemic zones. *p≤0.02 SCS-CAO vs untreated CAO. [Kruskal-Wallis Analysis of Variance on Ranks with Dunn’s methods for pairwise multiple comparisons.]