Central vs peripheral neuraxial sympathetic control of porcine ventricular electrophysiology

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Abstract

Objective: Sympathoexcitation is associated with ventricular arrhythmogenesis. The aim of this study was to determine the role of thoracic dorsal root afferent neural inputs to the spinal cord in modulating ventricular sympathetic control of normal heart electrophysiology. We hypothesize that dorsal root afferent input tonically modulates basal and evoked efferent sympathetic control of the heart.

Methods: A 56-electrode sock placed on the epicardial ventricle in anesthetized Yorkshire pigs (n=17) recorded electrophysiological function; Activation Recovery Interval (ARI) and Dispersion in ARI, at baseline conditions and during stellate ganglion electrical stimulation. Measures were compared between intact states and sequential unilateral T1-T4 dorsal root transection (DRTx), ipsilateral ventral root transection (VRTx), and contralateral dorsal and ventral root transections (DVRTx).

Results: Left or right DRTx decreased global basal ARI; Lt.DRTx 369 ± 12 to 319 ± 13 ms (p < 0.01) and Rt.DRTx 388 ± 19 to 356 ± 15 ms (p < 0.01). Subsequent unilateral VRTx followed by contralateral DRx+VRTx induced no further change. In intact states, left (LSS) and right (RSS) stellate ganglion stimulation shortened ARIs (6±2% vs 17±3%), while increasing dispersion (+139% vs +88%,). There was no difference in magnitude of ARI or dispersion change with stellate stimulation following spinal root transections.

Conclusions: Interruption of thoracic spinal afferent signaling results in enhanced basal cardiac sympathoexcitability without diminishing the sympathetic response to stellate ganglion stimulation. This suggests spinal dorsal root transection releases spinal cord mediated tonic inhibitory control of efferent sympathetic tone while maintaining intrathoracic cardio-centric neural networks.
INTRODUCTION

Dynamic interactions between peripheral and central aspects of the cardiac nervous system are essential for the maintenance of adequate cardiac function (4, 6). Imbalances in autonomic neural processing are fundamental in the progression of cardiac pathology including ventricular arrhythmias (34, 35). Increased sympathoexcitation in ischemic and non-ischemic heart disease is associated with arrhythmia development (18). Correspondingly, modulation of sympathetic output via surgical sympathectomy, thoracic epidural anesthesia, or spinal cord stimulation has been demonstrated to be important therapeutic avenues for control of cardiac arrhythmias (13, 16, 17).

It is known that cardiac afferent neurons, projecting to high thoracic dorsal root ganglia, transmit tonic afferent neural input into the thoracic spinal cord to reflexly modulate efferent sympathetic outflow to the heart (11, 25, 29, 31, 38). Thoracic cardiac afferents likewise project to intrathoracic autonomic ganglia subserving intrathoracic short-loop feedback loops for cardiac control (7, 8). Intrathoracic reflexes works in conjunction with higher central control from the brainstem and spinal cord to provide dynamic autonomic neural regulation of cardiac electrophysiology (4, 6). However, what is not known is the effect that this tonic cardiac afferent signaling has on efferent sympathetic outflow and cardiac electrophysiological control in normal hearts. This is essential as a benchmark for subsequent studies in evaluating states of cardiac pathology. Further, with the emerging applications of neuromodulation and bioelectric medicine to treat cardiovascular diseases, the potential for central-peripheral neural interactions associated with electrical stimuli applied to specific nodes of the hierarchy for cardiac control needs be defined (14).

The aim of this study was to determine the role of cardiac afferent input to the spinal cord in modulating sympathetic control of ventricular electrophysiology in normal porcine hearts by examining the effects of sequential left and right dorsal root (afferent) and ventral (efferent) root
transection on ventricular electrophysiological response at rest and in response to bioelectric stellate ganglion stimulation. We hypothesize that dorsal root afferent input tonically modulates basal and evoked efferent sympathetic control of the heart.

METHODS

All animal experimental protocols were devised in accordance with guidelines set by the University of California Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Animal preparation:** Male and female Yorkshire pigs (n = 17) weighing 42 ± 3 kg were sedated with telazol (8-10 mg/kg, i.m.). Following intubation and initiation of positive-pressure ventilation, general anesthesia was induced and maintained with isoflurane (1-1.5%, inhalation) concomitant with intermittent boluses of fentanyl (1-3 µg/kg, i.v.). Following completion of surgery, anesthesia was changed to α-chloralose (50 mg/kg intravenous bolus administration followed continuous infusion at 10 mg/kg/hr, i.v.). Heart rate (HR) was monitored throughout the experiments via a standard limb lead electrocardiogram (ECG). The right femoral artery was catheterized for monitoring aortic blood pressure. To measure left ventricular (LV) pressure, a 5-Fr pig-tail 12-pole conductance pressure catheter was inserted into the LV chamber via the left carotid artery. This catheter was connected to a MPVS Ultra Pressure Volume Loop System (Millar Instruments, Houston, TX). The right internal jugular vein was cannulated to allow fluid replacement and drug administration. Arterial blood gases were evaluated hourly and adjustments of tidal volume and/or infusions of sodium bicarbonate were applied, as required, to maintain arterial blood gas homeostasis.

**Surgical Procedure:** Dorsal spinal laminectomy was performed in the prone position to expose the T1-T4 ventral and dorsal roots bilaterally. Animals were then placed in the supine
position and a midline sternotomy was performed to expose the heart and both stellate ganglia. After completion of surgery, animals were stabilized for 1 hour.

**Stellate ganglion sympathetic stimulation:** Each left (LSS) and right (RSS) stellate ganglion was stimulated individually via bipolar needle electrodes implanted in the ganglia that were connected to a Grass S88 Stimulator (Grass Co., Warwick, RI) via PSIU6 constant current isolation units. Square wave stimulation pulses (4 ms duration; 4 Hz frequency) were delivered individually to each ganglion. Stimulus threshold was defined as the stimulation current strength that was sufficient to elicit a 10% increase of left ventricular end-systolic pressure (LVESP) or heart rate. Stimulus intensity was increased to 1.5 times threshold for all subsequent stellate ganglion stimulations, while maintaining the 4 Hz frequency and 4 ms pulse width.

**Cardiac Electrophysiology: Activation recovery interval (ARI) and dispersion of ARI analyses:** A custom 56-electrode sock, placed over both ventricles, was attached to a Prucka CardioLab (GE Healthcare, Fairfield, CT) to identify regional activation recovery intervals. Global ventricular activation recovery intervals were calculated via customized software ScalDyn M (University of Utah, Salt Lake City, UT), as described previously (37). Briefly, localized ventricular epicardial activation times (AT) were measured from the beginning of the QRS complex to the first minimal dV/dt in the QRS complex. Localized epicardial recovery times (RT) were computed from the beginning of the QRS complex to the first maximal dV/dt of the T wave. Activation recovery intervals were derived from subtracting activation times (ATs) from these RTs. This parameter has been shown to correlate with local ventricular action potential durations (APDs) (36, 37). Global dispersion in ARI was calculated using the variance of all 56-electrode ARIs to identify spatial dispersion of regional ventricular epicardial repolarization.

**Experimental protocol:** The effects of sequential spinal transection, at rest and with left (LSS) or right (RSS) stellate ganglion stimulation, on hemodynamic parameters and ventricular electrical indices were recorded. Stellate ganglia were stimulated for 30-second periods, with
10-minutes separating each stimulation sequence in order to allow the return of hemodynamic and regional ventricular electrical indices to baseline values. Animals were randomized with either left or right unilateral root transection first. For the left-sided protocol group (n=9), the transection sequence was: 1) all spinal roots intact; 2) left T1-T4 dorsal roots cut (Lt. DRTx); 3) left T1-T4 ventral roots cut (Lt. VRTx); and 4) right T1-T4 dorsal and ventral roots cut (Rt. DVRx). For the right-side protocol group (n=8), the transection sequence was: 1) all spinal roots intact; 2) right T1-T4 dorsal roots cut (Rt. DRTx); 3) right T1-T4 ventral roots cut (Rt. VRTx); and 4) left T1-T4 dorsal and ventral roots cut (Lt. DVRx). To allow for stabilization for neural networks, a 60-minute observation period elapsed between each successive dorsal or ventral spinal cord root transection before stellate ganglion stimulation sequences were initiated.

**Statistical analysis:** Data are reported as mean ± standard error (SE). Repeated measures one-way analysis of variance with post-hoc correction for multiple hypothesis testing was performed for comparing electrophysiological and hemodynamic data obtained during baseline and stellate ganglion stimulations in each of the experimental conditions pre and post spinal root transection. Change in ARI and dispersion, by RSS and LSS, between four experimental conditions were also analyzed by repeated measures one-way analysis of variance. Stats were analyzed by using SigmaStat (version 3.1) and JMP (version 11). A p value less than 0.05 was considered to be statistically significant.

**RESULTS**

**Effects of thoracic spinal root transections on regional ventricular electrical indices.**

**Effects of sequential transection in the baseline (unstimulated) state:** Baseline global ventricular ARIs decreased following unilateral dorsal root transection; from 369 ± 12 to 319 ± 13 ms (p < 0.01) by Lt. DRTx and from 388 ± 19 to 356 ± 15 ms (p < 0.01) by Rt. DRTx. Figure 1 shows representative examples in baseline ARIs for both transection sequences. Subsequent
ipsilateral ventral root transections (Lt. VRTx or Rt. VRTx) or contralateral transections (Rt. DVRTx and LT. DVRTx) evoked minimal additional effects on ventricular ARI (Figure 1-3). In contrast, ventricular dispersion in ARI at baseline conditions remained relatively unaffected by any of these sequential spinal root transections (Figures 2 and 3).

*Activation recovery intervals and spatial dispersion in ARI elicited by stellate ganglion stimulation:* Left stellate ganglion (LSS) stimulation preferentially shortened ARIs particularly in the left lateral and posterior ventricular walls (Figure 4, middle panels). In contrast, right stellate ganglia (RSS) stimulation preferentially shortened ARIs in the right and anterior left ventricular walls (Figure 4, right panels). This regional alteration in ARI, induced by unilateral stellate ganglion stimulation, is consistent with our previous study (40). Unilateral T1-T4 dorsal root transections (Lt. DRTx or Rt. DRTx) shortened baseline ARI, however there was no change in the magnitude of ARI shortening with stellate stimulation and dorsal root transection (Figures 2, 3, 4). These ARI responses were not modified by subsequent unilateral ventral root transection, or by contralateral dorsal and ventral transection. Moreover, while enhancement of whole ventricular dispersion in ARI occurred during right or left stellate ganglion stimulation, alterations in this index elicited by ganglionic stimulation were not changed by any of the staged spinal transections (Figures 2 and 3).

*Effects of successive thoracic spinal root transections on hemodynamic measurements.* Basal heart rate was increased by T1-T4 dorsal root transection, with right or left unilateral transection (Figure 5). Subsequent root transection (ipsilateral VRTx and contralateral DVRx) evoked no further significant changes in basal heart rate. Positive chronotropic responses were evoked in response to right, but not left stellate stimulation (Figure 6). Right stellate evoked effects on heart rate were maintained following each of the successive root transections. Left stellate stimulation did not change heart rate during any stage of the successive root transections.
Indices of basal left ventricular (LV) inotropic function, as assessed by the first derivative of LV pressure (+dP/dt), were maintained following dorsal root transection, decreasing from baseline only with subsequent bilateral dorsal and ventral root transection. LVSP had no change after unilateral dorsal root transection but decrease from baseline with subsequent ventral root transections (Figure 5). LV pressure and inotropy increased with stellate stimulation and were unchanged with spinal root transections (Figure 6). Time control showed stable conditions throughout the duration of the experimental protocol with only minimal changes in HR, LVSP, and dP/dt max from baseline to end of protocol (HR 75–83 bpm, LVSP 90–81 mmHg, and dP/dt max 1247-1159 mmHg/s).

**DISCUSSION**

The primary findings of this study are; 1) Interruption of unilateral cardiac afferent input to the spinal cord increases basal sympathetic tone to the heart as demonstrated by a reduction in ARI duration and tachycardia. 2) Intrathoracic (cardio-centric) sympathetic control was functionally maintained following bilateral transection of dorsal and ventral spinal roots, as there was no difference in the magnitude of ARI shortening or increase in dispersion associated with subsequent stellate ganglion stimulation.

The results of this study suggest that dorsal afferent neural input into the spinal cord exerts tonic inhibitory control of basal efferent sympathetic output. Dorsal root transection interrupts the nerve fibers transmitting tonic afferent neural impulses from the heart (11). The cessation of this afferent input to the thoracic spinal cord resulted in a reduction in ARI duration and increased HR, indicating that afferent cardiac neural input tonically modulates efferent sympathetic signaling and release of this negative feedback signal results in increased cardiac sympathetic tone. ARI shortening occurred only after the initial dorsal root transection, with no additional changes seen after subsequent spinal root transections. Initial dorsal root transection
was also associated with an increase in heat rate with no additional changes seen with subsequent transections.

In this study, even partial loss of the cardiac afferent inputs, as with unilateral T1-T4 dorsal root transection, resulted in release of thoracic afferent-modulated basal sympathetic tone inhibition. An increase in sympathetic tone was identified following unilateral dorsal transection, regardless of whether it was left or right dorsal roots that were transected first. Interruption of dorsal afferent signaling from either side resulted in ARI shortening likely due to increased efferent signaling, similar to how patients with unilateral stroke or traumatic brain injury may experience paroxysmal sympathetic hyper-reactivity (32). It is also consistent with recent findings where unilateral cardiac infarctions evoked bilateral changes in stellate ganglion structure/function (2), an effect that is sympathetic afferent neuron mediated. While unilateral afferent transection increased basal sympathetic tone, there was no significant change in LV mechanical function likely due to compensatory neural inputs from the contralateral spinal nerve roots and intact baroreflexes (3, 4, 31).

The results of this study show that sympathetic cardio-centric control was functionally maintained following surgical spinal root transection, as there was no difference in the magnitude of ARI shortening or increase in dispersion associated with subsequent stellate ganglion stimulation, despite the difference observed in basal tone with spinal root transection. Stellate ganglion stimulation leads to an increase in cardiac sympathetic outflow leading to ARI shortening, increased dispersion in ARI, and increased LV inotropy independent of spinal cord modulation (29, 37). As previously reported, RSS, as compared to LSS, is associated with a greater chronotropic response (40). In this study, RSS was associated with significant increases in heart rate, whereas LSS was not. The components of cardiac neural reflexes exist at multiple levels, with cardio-cardiac reflexes mediated by neurons the intrinsic cardiac nervous system (5, 12), extra-cardiac intrathoracic sympathetic ganglia (6-8), spinal cord (17), brainstem (19) and higher centers (20). The results of this study support the concept that intrathoracic neural
networks are capable on their own of integrated cardiac control, even when disconnected from the rest of the nervous system (7, 28). This finding has important implications with respect to residual autonomic control of cardiac function in patients following therapeutic bilateral stellate decentralization (14, 34).

While ventricular electrophysiological changes were identified after unilateral dorsal root transection, mechanical changes were not observed until both dorsal and ventral roots were transected. With removal of bilateral afferent and efferent neural inputs to and from the spinal cord there was a significant reduction in LV dP/dt max and systolic blood pressure. This reduction in function may have been due to several reasons. 1) High thoracic dorsal root transection uncouples central-peripheral control elements in the cardiac neuroaxis. Following bilateral transection of the dorsal and ventral roots, central aspects of the cardiovascular control system have lost important afferent signals from the heart. Thus, the normal spinal/higher center modulation of sympathetic outflows transitions towards intrathoracic cardio-centric reflex control. Without central control, sympathetic efferent activity to the heart and peripheral vasculature is lost, including that which is necessary to support inotropic function and blood pressure. 2) The degree of changes seen in cardiac mechanical function might not follow a linear relationship with changes in ventricular electrical indices following spinal root transections, similarly to the differential relationship in sympathetic control of chronotropism and inotropism in subjects with spinal cord injury (24). 3) Vagal projections, both afferent and efferent, remained intact in our study. These residual neural circuits could have sub-served important reflex adjustments to the stress imposed by spinal cord root transections. Future studies should consider the potential ramifications of differential effects of targeted neuromodulation interventions on ventricular electrical versus mechanical function.

Study limitations
This study was designed to determine the effect of cardiac afferent signaling on ventricular electrical indices. As such, dorsal root transections were performed as the primary procedure, with sequential ventral root transections being performed later. The effects of ventral root transection alone remain to be evaluated. Furthermore, root transections were done in healthy preparations. It is known that chronic cardiac disease induces remodeling of peripheral and central aspects of the cardiac nervous system (2, 22, 23) and, as such, chronic disease models will need to be evaluated. Cardiac control via the cardiac nervous system involves both sympathetic and parasympathetic outflows. In this study the parasympathetic networks remained intact, although they too can be impacted by alterations in afferent inputs secondary to dorsal root transection (11, 15, 27). We have recently shown that the vagus can have profound influence on cardiac electrophysiology reflective of interactions between afferent and efferent components of cervical vagus (39). Future studies should consider the dynamic interplay between different levels of the hierarchy for cardiac control in response to the disease process - particularly as influenced by targeted neuromodulation based therapeutic approaches. Finally, general anesthesia can suppress sympathetic nerve activity and could have impacted our results, though we kept the concentrations of anesthetic agents constant and similar across all animals. This study was powered for the primary outcome of changes in ARI with spinal transection and therefore may not be adequately powered to detect all hemodynamic responses associated with nerve root transection or, for that matter, stellate ganglion stimulation.

Conclusion

We demonstrate that the dorsal root afferent inputs from the heart to the spinal cord exert tonic inhibitory control of efferent sympathetic tone to the heart. Interruption of thoracic spinal afferent signaling results in enhanced basal cardiac sympathoexcitability without mitigating the functional sympathetic response to direct stellate ganglion stimulation. This indicates that spinal dorsal root transection releases centrally mediated reflex modulation of
efferent sympathetic tone to the heart while maintaining intrathoracic cardio-centric neural networks. The mechanistic insight into spinal cord control of cardiac sympathoexcitation may aid in developing future therapeutic modalities aimed at spinal modulation of ventricular arrhythmias.

Significance and Relevance

The finding of functional intrathoracic cardio-centric sympathetic control is of important clinical relevance, especially as related to residual neural control of cardiac function after stellate ganglion decentralization for intractable ventricular tachycardia (13, 30, 33). Stellate ganglion decentralization has been shown to protect against ventricular arrhythmias in humans (1, 30, 34). This surgical procedure removes most, if not all, cardiac afferent inputs to spinal cord neurons and preserves most intrathoracic cardio-cardiac reflexes (10, 21), including those confined to the intrinsic cardiac nervous system (7, 9, 21). The data from our study demonstrate the functional capacity of intrathoracic neural networks to coordinate cardiac electrical and mechanical function - even when disconnected from the central nervous system. It is through understanding the inherent network interactions that exist throughout the cardiac neuroaxis that one can mechanistically understand its acquired adaptations to cardiac disease and thus devise novel autonomic regulatory approaches to optimize outcomes. Stellate decentralization for intractable VT (13, 30) and spinal cord stimulation for angina (26) are just some of the ongoing areas of clinically impactful manifestations of this principle.


Figure legends

Figure 1. Effects of the sequential T1-T4 root transection on the baseline Activation Recovery Intervals (ARI’s) in a representative animal. Top panels show left-right transection protocol while bottom panels show right-left protocol. Dorsal root transection shortened baseline ARI homogenously in both left and right sided protocols. Reduction in global ventricular ARI is seen after initial ipsilateral dorsal root transection.

Figure 2. Summary effects of sequential spinal root transections in left-sided experiment protocol on stellate ganglion stimulation induced changes in global ventricular activation recovery intervals along with dispersion in ARI. After left (T1-T4) dorsal root transections (Lt. DRTx), global ventricular ARI shortened. Subsequent transection of ipsilateral ventral roots (Lt. VRTx) and transection of contralateral dorsal and ventral roots (Rt. DVRTx) evoked no further effects on global ventricular ARI. Spinal cord dorsal and ventral root transections exerted no significant effects on overall ventricular dispersion. #: p<0.05 = difference in baseline from preceding condition. * p<0.05 = difference from baseline with stellate ganglion stimulation (left = LSS, right = RSS).

Figure 3. Summary effects of sequential spinal root transections in right-sided experiment protocol on stellate ganglion stimulation induced changes in global ventricular activation recovery intervals along with dispersion in ARI. After right (T1-T4) dorsal root transections (Rt. DRTx), global ventricular ARI shortened. Subsequent transection of ipsilateral ventral roots (Rt. VRTx) and transection of contralateral dorsal and ventral roots (Lt. DVRTx) evoked no further effects on whole heart ARI. Spinal cord dorsal and ventral root transections exerted no significant effects on overall ventricular dispersion. #: p<0.05 = difference in baseline from preceding condition, * p<0.05 = difference from baseline with stellate ganglion stimulation (left = LSS, right = RSS).
Figure 4. Representative ARI effects evoked by stellate ganglion stimulation prior to (intact) and following successive T1-T4 root transections. Unilateral T1-T4 dorsal root transections shortened baseline ARI; however there was no change in the magnitude of ARI shortening with stellate stimulation and unilateral dorsal root (Left=LDRTx), ventral root (LVRTx) or contralateral dorsal/ventral root transections (RDVRTx). Left stellate ganglion (LSS) stimulation preferentially shortened ARIs, particularly in the left lateral and posterior ventricular walls. In contrast, right stellate ganglia (RSS) stimulation preferentially shortened ARIs in the right and anterior left ventricular walls.

Figure 5. Hemodynamic data presented as mean ± standard error. A. Hemodynamic results from left dorsal root transection protocol, B. represents hemodynamic results from right dorsal root transection protocol. L/R DRTx = left or right dorsal root transection, VRTx = ventral root transection, DVRTx = dorsal and ventral root transection. * = p < 0.05 difference from baseline. Significance in differences between conditions are displayed above bar. ns = non-significant changes likewise indicated.

Figure 6. Hemodynamic data presented as mean ± standard error. A. Hemodynamic changes observed with left dorsal root transection - baseline as compared to LSS, B. Left dorsal root transection - baseline as compared to RSS, C. Right dorsal root transection - baseline as compared to LSS, D. Right dorsal root transection - baseline as compared to RSS. LSS = left stellate ganglion stimulation, RSS = right stellate ganglion stimulation, L/R DRTx = left or right dorsal root transection, VRTx = ventral root transection, DVRTx = dorsal and ventral root transection. * = p < 0.05 difference from baseline with LSS and RSS.