Increased susceptibility to ventricular arrhythmias is associated with changes in Ca^{2+} regulatory proteins in paraplegic rats

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Rodenbaugh, David W., Heidi L. Collins, Dustin G. Nowacek, and Stephen E. DiCarlo. Increased susceptibility to ventricular arrhythmias is associated with changes in Ca^{2+} regulatory proteins in paraplegic rats. Am J Physiol Heart Circ Physiol 285: H2605–H2613, 2003.—Paraplegia may increase susceptibility to ventricular arrhythmias by altering the autonomic control of the heart. Altered cardiac autonomic control has been documented to change the expression of genes that encode cardiac Ca^{2+} regulatory proteins. Therefore, we tested the hypothesis that paraplegia alters cardiac electrophysiology with concomitant changes in Ca^{2+} regulatory proteins in a manner that increases the susceptibility to ventricular arrhythmias. To test this hypothesis, intact (n = 10) and paraplegic (n = 6) male Wistar rats were chronically instrumented to measure atrioventricular (AV) interval, sinus cycle length, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle (cSNRT), Wenckebach cycle length (WCL), and the electrical stimulation threshold to induce ventricular arrhythmias. In addition, relative protein abundance and mRNA expression for sarco(endoplasmic reticulum Ca^{2+} ATPase (SERCA), phospholamban, and the Na/Ca exchanger were determined in intact (n = 8) and paraplegic (n = 8) rats. Paraplegia significantly (P < 0.05) reduced AV interval (−25%), sinus cycle length (−24%), SNRT (−28%), cSNRT (−53%), WCL (−19%), and the electrical stimulation threshold to induce ventricular arrhythmia (−48%). Paraplegia significantly increased the relative protein abundances of SERCA (45%) and the Na/Ca exchanger (40%) and decreased phospholamban levels (−28%). In contrast, only the relative mRNA expression of the Na/Ca exchanger was increased (25%) in paraplegic rats. These data demonstrate that paraplegia enhances cardiac electrophysiological properties and alters Ca^{2+} regulatory proteins in a manner that increases susceptibility to ventricular arrhythmias.

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heart, 2) electrical stimulation threshold to induce ventricular arrhythmias, and 3) relative protein and mRNA abundances of cardiac Ca\(^{2+}\) regulatory proteins in intact and paraplegic rats.

METHODS

Experimental Subjects

All surgical and experimental procedures that involved animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Studies to determine cardiac electrophysiological parameters were initially conducted on 10 male Wistar rats (intact group). Subsequently, six of the intact rats underwent complete spinal cord transection between the fourth and fifth thoracic vertebrae (T\(_4\),T\(_5\); paraplegic group). The remaining four rats underwent sham spinal cord transection and served as time controls (intactTC group). A separate group of 16 male Wistar rats was randomly divided into sham spinal cord transected (intact, n = 8) and T\(_4\)-T\(_5\) spinal cord transected (paraplegic, n = 8) groups. Whole heart homogenates from these groups were used to determine relative differences in mRNA expression and protein abundance for cardiac Ca\(^{2+}\) regulatory proteins.

Surgical Procedures

Instrumentation. All surgical procedures were performed using aseptic surgical techniques. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip), and supplemental doses (10–20 mg/kg ip) were administered if the rat regained the blink reflex or responded during the surgical procedures. The heart was approached via a left thoracotomy through the third intercostal space. Teflon-coated silver wire electrodes were sutured 2–3-mm apart on the surface of the left ventricle and atrium as previously described (42). At least 1 wk was allowed for recovery (25). During the recovery period, the rats were handled, weighed daily, and acclimatized to the laboratory and investigators. Subsequently, the animals were anesthetized as described, and three insulated stainless steel electrocardiogram (ECG) electrodes were sutured subcutaneously on the ventral side of the thorax. In addition, a telemetry device (PhysioTel PA-C40, Data Sciences International) was implanted as previously described (5, 6, 42). The sensor of the telemetry device, which was located within the tip of a catheter, was inserted into the abdominal aorta for continuous non-tethered recording of pulsatile arterial blood pressure via radiotelemetry.

Spinal cord transection. After anesthesia was induced, rats were positioned prone over a thoracic roll that slightly flexed the trunk. The T\(_4\) and T\(_5\) vertebrae were exposed via a midline dorsal incision. The underlying spinal cord between T\(_4\) and T\(_5\) was completely transected through the intervertebral space as previously described (5, 6, 42). In this way, the vertebral column remained stable and intact because the vertebrae were not disturbed. Sympathetic innervation to the heart is derived from preganglionic fibers that exit the central nervous system at the first through fourth thoracic levels of the spinal cord (3). Transection was between the fifth and sixth thoracic levels of the spinal cord; therefore, supraspinal control of cardiac sympathetic activity remained intact. The completeness of the transection was confirmed by visual inspection of the lesion site. During the acute recovery period (~1 wk), all rats were handled at least twice daily. During these periods, visual inspections and physical manipulations were performed to detect and prevent pressure sores. In addition, the urinary bladder was voided by manual compression and all animals were weighed. After this acute recovery period, rats required only daily inspection, and bladders did not require manual compression. In addition, body weight was recorded at least every other day to determine the overall health of the animals. Specifically, body weight before any intervention averaged 576 ± 19 g. At the completion of the studies, the body weights of paraplegic (406 ± 19 g) and intact time-control rats (435 ± 11 g) were not significantly different. At day 7 posttransection, the rats received a motor-activity score using criteria described previously (51). The motor-activity score was assessed by placing the animal on a paper-covered table and observing spontaneous motor activity for 1 min. Motor scores ranged from 0 to 5. A motor score of 5 indicates normal walking, whereas a score of 0 indicates no weight-bearing or spontaneous movement in the hind limbs. All rats had a motor score of 0, which indicates no weight bearing. Upon completion of the studies, the site of the spinal lesion was confirmed by autopsy (23). Time-control (intactTC) rats underwent identical surgical procedures; however, the spinal cord was not transected.

Experimental Procedures

Cardiac electrophysiology. Conscious, unrestrained rats were studied in their home cages (area, ~13,350 cm\(^2\)) for all experiments. Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. Subsequently, the atroventricular (AV) interval, sinus cycle length, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle length (cSNRT), Wenckebach cycle length (WCL), and electrical stimulation threshold to induce ventricular arrhythmias were determined. Upon completion of these studies, rats were randomly assigned to the paraplegic or time-control groups. All protocols were repeated 4 wk after spinal cord transection (paraplegic group) or sham spinal cord transection (intactTC group).

The AV interval, sinus cycle length, SNRT, and cSNRT were determined during atrial pacing at a frequency of 8.4 Hz for 5-s durations (48, 50). The AV interval was measured as the time from the last paced stimulus to the onset of the QRS complex (Fig. 1A). The SNRT was measured as the time from the last paced stimulus to the onset of the P wave (Fig. 1B). To control for differences in sinus rate, SNRT was normalized to resting heart rate by subtracting the sinus cycle length from the SNRT (cSNRT = SNRT − sinus cycle length). Sinus cycle length was determined from at least 60 consecutive cycles after the pacing period when normal sinus rhythm resumed. A 60-s period was allowed to elapse between each successive pacing.

The WCL was determined during incremental increases in atrial pacing frequency (48, 50). The WCL was defined as the minimum cycle length that failed to conduct through the AV node as indicated by missed ventricular contraction. Missed ventricular contractions were detected by both the ECG and the arterial pressure waveform (Fig. 2B). The WCL is an index of AV nodal conduction whereby increases in the WCL represent depressions in AV nodal conduction and decreases in WCL represent enhancements in AV nodal conduction.

The electrical stimulation threshold to induce ventricular arrhythmias was determined as previously described (42). A MacLab programmable stimulator delivered trains of pulses through the ventricular stimulating electrodes (frequency, 50 Hz; duration, 10 ms). The current delivered was recorded via an amp meter (model 22-805, Radio Shack) in series with the ventricular stimulating electrode. The amperage was re-
corded online with a personal computer (Gateway 2000). The intensity of the trains was increased in 10^{-10}/H_{9262} increments every 10 s. The electrical stimulation threshold to induce ventricular arrhythmias was determined as the minimum current that caused ventricular arrhythmias (42). Ventricular arrhythmias were identified on the ECG as rapid, wide QRS complexes with concomitant decreases in arterial pressure. Normal sinus rhythm appeared on termination of the stimulation without the use of defibrillation shocks.

Molecular Cardiology

Sample preparation for Western analysis. Seven weeks after spinal cord transection or sham spinal cord transection, intact and paraplegic rats were quickly decapitated without anesthetic to avoid the confounding influences of anesthetics on the cardiovascular system including direct chronotropic effects, activation of the renin-angiotensin system, and/or activation of cardiovascular reflex phenomena (24, 35, 40). The hearts were rapidly removed, rinsed clean of clots, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent Western blotting and real-time PCR analysis.

Each heart was subsequently pulverized to a fine powder using a liquid nitrogen-cooled stainless steel mortar and ceramic pestle (Fisher Scientific). Total protein was extracted from each heart sample by first homogenizing [for 4 × 10 s, using a handheld homogenizer (Fisher Scientific)] the heart powder in a buffer (pH 7.5) that contained (in mM) 20 MOPS, 100 NaCl, and 2 EDTA along with a protease inhibitor cocktail (Roche Diagnostics). SDS was added to the homogenate for a final concentration of 1% SDS. The samples were then sonicated and vortexed (3 × 1 min) to disrupt any remaining cells. The resulting homogenate was centrifuged (10,000 relative centrifugal force units for 5 min) to pellet any debris. The supernatant was removed and used for determination of total protein concentration using a DC protein assay (Bio-Rad) and subsequent Western blot analysis.

Quantitative Western blot analysis. SDS-PAGE immunoblot conditions, the protein dependency of immunoc hemical
detection, and linear correlations between optical density and protein content were first determined as previously described (Ref. 47; see Appendix Fig. 1 online at http://ajpheart.physiology.org/cgi/content/full/00319.2003/DC1). Using this method, we determined the optimal amount of protein for immunoblot analysis to be 10 μg of protein for all of the proteins of interest.

Protein samples were solubilized in Laemmli sample buffer that contained 350 mM dithiothreitol (Sigma) for a final protein concentration of 1 μg/ml. Lanes were loaded with equal amounts of protein, electrophoresed on 10% SDS-PAGE gels, and transferred onto Trans-Blot nitrocellulose membranes (Bio-Rad). After the transfers, each membrane was stained with amido black to confirm adequate and uniform transfer of protein. For the immunoreactions, commercially available antibodies were used according to manufacturers’ conditions with corresponding dilutions: α-actin (1:1,000 dilution; A2172, Sigma); sarco-(endo)plasmic reticulum (SERCA)2 (1:1,000 dilution; MA3-919, Affinity Bioreagents); phospholamban (1:2,000 dilution; 05-205, Upstate Biotechnology); and Na/Ca exchanger (1:1,000 dilution; p11-13, Swant). For detection, secondary mouse (NA931V) or rabbit (NA934V) peroxidase-conjugated antibodies (1:10,000 dilution; Amersham) were used accordingly. Detection was performed using the Western Lightning enhanced chemiluminescence system (Perkin Elmer). Exposures were digitally captured, and the amount of protein in each band was determined by densitometry using Kodak ID 3.5 software. An internal standard (α-actin), calculated using the internal standard (α-actin), was used to correct for potential differences in loading and transfer. Data were normalized by expressing all values relative to the average (intact) value determined for each blot.

mRNA preparation and cDNA synthesis. Poly A+ mRNA was extracted from pulverized whole heart sample using the Dynabeads mRNA Direct Kit (Dynal Biotech) for rapid isolation of mRNA. The mRNA was isolated and eluted from the beads in 10 mM Tris·HCl (20 μl) according to the manufacturer’s instructions. To quantify the concentration of extracted mRNA, the RiboGreen RNA Quantitation Kit (Molecular Probes) was used. The concentration of mRNA for each sample was determined according to the manufacturer’s instructions. First-strand cDNA synthesis was performed in a 20-μl volume using the SuperScript II First-Strand Synthesis System for RT-PCR (Invitrogen; Carlsbad, CA) according to the manufacturer’s RT protocol.

Oligonucleotide primers. Four different pairs of primers (sense and antisense) were designed using published mRNA sequences for α-actin, SERCA, phospholamban, and the Na/Ca exchanger using Primer3 software (45). Primer information is listed on Table 1. All custom primer sequences were purchased from Invitrogen.

Real-time PCR. The iCycler iQ Real-Time PCR Detection System (Bio-Rad) was used for amplification and data collection and analysis. First, the real-time PCR conditions and sensitivity were optimized as previously described (2). Briefly, cycle threshold (CT) data for 10-fold serially diluted samples were collected and plotted as a function of the log of the dilution factor for each sample. The efficiency of each PCR reaction was determined from the slope of the line generated (see Appendix Fig. 2 online). The average real-time PCR reaction efficiency for all primers was 95 ± 2%. To control for spurious results, melt-curve data collection and analysis were also performed at the completion of each experiment using the manufacturer’s described cycle conditions (see Appendix Fig. 3A online). In addition, real-time PCR products were electrophoresed on a 3% agarose gel and visualized by ethidium bromide to verify PCR product size as well as purity (see online Appendix Fig. 3B).

All reactions were performed using iQ SYBR Supermix (2×, Bio-Rad) optimized for real-time PCR according to manufacturer’s instructions. Specifically, reactions were performed using 25-μl volumes that contained master mix (1×), forward or reverse primers (500 nM), sample cDNA (5 μl), and DNase-free water (1.5 μl) in a 96-well, thin-wall PCR plate that was sealed with optical quality sealing film. PCR reaction conditions included a denaturation step of 3 min at 95°C, followed by 45 cycles of 15 s at 95°C and 60°C step. Relative differences in mRNA were standardized to the internal standard (α-actin), calculated using the comparative 2^−ΔΔCt method (30), and normalized by expressing values relative to the average (intact) value.

Data Analysis

All data were expressed as means ± SE. A one-way ANOVA with post hoc Bonferroni t-tests was used to compare AV interval, sinus cycle length, SNRT, cSNRT, WCL, mean arterial blood pressure, heart rate, and electrical stimulation threshold to induce ventricular arrhythmias for intact, paraplegic, and intactTC rats. Student’s unpaired t-tests were used to compare relative differences in protein abundance and mRNA expression for SERCA, phospholamban, and the Na/Ca exchanger in intact and

Table 1. Primers used for real-time PCR

<table>
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<tr>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
<th>Coding Sequence</th>
<th>Product Length, bp</th>
<th>NCBI Accession No. (rat)</th>
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<td>α-Actin</td>
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<tr>
<td>Forward</td>
<td>CGGCGGCGGCGGCGGCGG</td>
<td>325–344</td>
<td>130</td>
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<td>Reverse</td>
<td>CGGCGGCGGCGGCGGCGG</td>
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<td>Phospholamban</td>
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<td>Forward</td>
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<td>155–174</td>
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<tr>
<td>Reverse</td>
<td>CTCTCTCTCTCTCTCTCT</td>
<td>246–265</td>
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<td>Na/Ca exchanger</td>
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SERCA2, sarco(endo)plasmic reticulum 2.

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paraplegic rats. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Cardiovascular Physiology

Mean arterial pressure values were not different between the intact and paraplegic rats (95 ± 3 vs. 90 ± 3 mmHg, respectively). However, paraplegic rats had significantly higher heart rates (444 ± 23 vs. 326 ± 10 beats/min). Mean arterial pressure values and heart rates were not significantly different between intact (95 ± 3 mmHg and 326 ± 10 beats/min) and intactTC (97 ± 4 mmHg and 321 ± 3 beats/min) rats.

Figure 3 presents the cardiac electrophysiological parameters that were determined in intact, paraplegic, and intactTC rats. Paraplegia significantly reduced all cardiac electrophysiological parameters. Specifically, the paraplegic rats had lower values for AV interval (45 ± 2 vs. 60 ± 2 ms), sinus cycle length (137 ± 6 vs. 181 ± 4 ms), SNRT (151 ± 5 vs. 211 ± 4 ms), cSNRT (14 ± 3 vs. 30 ± 3 ms), and WCL (85 ± 2 vs. 104 ± 3 ms). The cardiac electrophysiological parameters were not significantly different for intact and intactTC rats (Fig. 3).

Figure 4 presents the electrical stimulation threshold to induce ventricular arrhythmias in intact, paraplegic, and intactTC rats. Paraplegia significantly reduced the electrical stimulation threshold to induce ventricular arrhythmias (235 ± 26 vs. 449 ± 26 μA). Importantly, the ventricular arrhythmia threshold values in the intactTC and intact rats were not significantly different (438 ± 83 vs. 449 ± 26 μA, respectively).

Molecular Cardiology

Figure 5 presents relative differences in protein abundance for SERCA, phospholamban, and the Na/Ca exchanger in intact and paraplegic rat hearts. Paraplegia significantly increased the relative abundance of SERCA (45%) and Na/Ca exchanger (40%) proteins, whereas relative phospholamban protein abundance was significantly decreased (~28%).

Figure 6 presents relative differences in mRNA expression for SERCA, phospholamban, and the Na/Ca exchanger in intact and paraplegic rat hearts. Relative Na/Ca exchanger mRNA expression was significantly increased (25%) in paraplegic rats. In contrast, relative mRNA expression was virtually identical in intact and paraplegic rats for SERCA (101 ± 6 vs. 110 ± 6%, respectively) and phospholamban (101 ± 5 vs. 99 ± 7%, respectively).

DISCUSSION

In this study, we examined the relationship between cardiac electrophysiological parameters (see Fig. 3), changes in cardiac Ca$^{2+}$ regulatory proteins (see Fig. 5), and susceptibility to ventricular arrhythmias in...
Intact and paraplegic rats (see Fig. 4). Paraplegia was associated with alterations in the abundance of cardiac Ca\textsuperscript{2+} regulatory proteins. Importantly, these molecular changes were associated with an increase in the intrinsic excitability and conductive properties of the heart as well as a reduced electrical stimulation threshold to induce ventricular arrhythmias. These results are consistent with clinical reports that suggest an increased susceptibility to cardiac arrhythmias (4, 11, 15) as well as alterations in the electrocardiograms of individuals with spinal cord injuries (27, 33). Furthermore, these results extend a recent report that documents a lower electrical stimulation threshold to induce ventricular arrhythmias in hypertensive paraplegic rats (42).

Sympathetic innervation to the heart is derived from preganglionic fibers that exit the central nervous system at the first through fourth thoracic levels of the spinal cord (3). In this study, the spinal cord was transected between the fifth and sixth thoracic levels of the spinal cord. Therefore, supraspinal control of cardiac sympathetic activity remained intact. The sympathetic nervous system affects cardiac electrophysiology over seconds to minutes by activating \(\alpha\)- and \(\beta\)-adrenergic receptors. \(\beta\)-Adrenergic receptor stimulation, which increases intracellular cAMP levels, also increases heart rate, AV nodal conduction, and contractile force and shortens atrial and ventricular refractoriness. \(\beta\)-Adrenergic receptor stimulation enhances the plateau phase of the action potential by increasing current through L-type Ca\textsuperscript{2+} channels, while repolarization is accelerated due to an increase in both the delayed cardiac rectifier K\textsuperscript{+} current and the Cl\textsuperscript{−} current. Thus \(\beta\)-adrenergic receptor stimulation may shorten or prolong action potential duration depending on whether effects on Ca\textsuperscript{2+} currents or K\textsuperscript{+}/Cl\textsuperscript{−} currents predominate. \(\beta\)-Adrenergic receptor stimulation also causes more rapid pacemaker activity in the sinus node by shifting activation of the pacemaker current to more positive potentials. \(\alpha\)-Adrenergic receptor stimulation enhances cardiac contractility due to Ca\textsuperscript{2+} influx. Furthermore, \(\alpha\)-adrenergic stimulation enhances the development of afterdepolarizations and triggered beats. In this situation, multiple ionic mechanisms are involved, and elevated intracellular Ca\textsuperscript{2+} concentration is a common feature. \(\alpha\)-Adrenergic stimulation results in reduction of the electrical stimulus threshold to induce ventricular arrhythmias (4, 11, 15) as well as alterations in the electrocardiograms of individuals with spinal cord injuries (27, 33). Furthermore, these results extend a recent report that documents a lower electrical stimulation threshold to induce ventricular arrhythmias in hypertensive paraplegic rats (42).

Fig. 5. Representative Western blots for sarco(endo)plasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA; A), phospholamban (PLM; B), and Na/Ca exchanger (NCX; C) using two intact and two paraplegic rats as well as means ± SE quantified relative abundance for each protein of interest in intact \((n = 8)\) and paraplegic \((n = 8)\) male Wistar rat hearts. Paraplegia increased the relative abundance of SERCA (by 45%) and Na/Ca exchanger (by 40%) with a concomitant decrease in phospholamban (−28%); \(*P < 0.05\), intact vs. paraplegic rats.

Fig. 6. Relative differences in mRNA abundance for SERCA (A), phospholamban (B), and Na/Ca exchanger (C) in intact \((n = 8)\) and paraplegic \((n = 8)\) male Wistar rat hearts. Paraplegia increased the relative abundance of Na/Ca exchanger mRNA (25%). There was no significant difference in mRNA expression for SERCA and phospholamban. \(* P < 0.05\), intact vs. paraplegic rats.
induce ventricular fibrillation as well as increased likelihood of spontaneous ventricular arrhythmias. β-Adrenergic receptor blockade and enhanced parasympathetic tone inhibit these effects and are known to be protective against ventricular arrhythmias and sudden death (9, 46, 52, 53). Importantly, there is substantial evidence to document increased sympathetic activity above the level of the spinal cord transaction (32, 42). For example, paraplegia increases cardiac sympathetic tonus in hypertensive rats (42). Furthermore, paraplegic rats (34, 42, 43) and humans (7, 17, 22, 36) have elevated heart rates. In addition, increased cardiac sympathetic activity directly increases the intrinsic excitability and conductive properties of the heart in a manner similar to that observed in paraplegic rats. For example, infusion of epinephrine decreased the effective refractory period and increased AV nodal conduction and heart rate (37). Importantly, administering a β-adrenergic receptor antagonist inhibited the responses to epinephrine. Conversely, perturbations that lower sympathetic activity and/or raise parasympathetic activity slow the conductive properties and intrinsic excitability of the heart (48, 50). Taken together, these results suggest that the altered cardiac electrophysiological parameters and increased susceptibility to ventricular arrhythmias in paraplegic rats may be due in part to increased cardiac sympathetic activity.

Adrenergic signals also alter cardiac electrophysiology over the time course of hours by affecting the expression and abundance of cardiac Ca²⁺ regulatory proteins. Previous reports have documented that adrenergic receptor stimulation enhances the expression of the Na/Ca exchanger in vivo and in vitro (12, 13). For example, Na/Ca exchanger message and protein abundance were increased in the presence of the β-adrenergic receptor agonist isoproterenol (12). The authors demonstrated that the increased Na/Ca exchanger expression was mediated via cAMP-dependent increases in the transcription initiation rate. The increased abundance of the Na/Ca exchanger may be secondary to increases in intracellular Ca²⁺ and not the direct action of β-adrenergic receptor stimulation, because the increased abundance occurred with increased intracellular Ca²⁺ (12). The authors also demonstrated a functional significance for the adrenergic receptor-mediated increase in the Na/Ca exchanger. That is, the increased expression of the Na/Ca exchanger was associated with increases in the amplitude and decay rate of the Ca²⁺ transient. Adrenergic receptor stimulation, via cAMP-dependent pathways, also regulates the function and expression of phospholamban and SERCA (38). Specifically, cAMP response-element modulators regulate the expression of SERCA, whereas cAMP-dependent phosphorylation of phospholamban directly affects SERCA function (38). Thus multiple adrenergic receptor-stimulated cAMP pathways may be affecting the abundance as well as the function of the Ca²⁺ regulatory proteins examined in this study.

The increased susceptibility to ventricular arrhythmias in paraplegic rats may occur via alteration of the expression of genes that encode proteins critical to myocyte Ca²⁺ homeostasis. The ability of cardiac myocytes to maintain cytosolic Ca²⁺ concentration within a tightly controlled range is crucial for cardiac electrical stability (12). For example, it is well documented that reductions in phospholamban and/or increases in SERCA protein abundance result in an increased SERCA Ca²⁺ load (18). The SERCA Ca²⁺ overload may produce spontaneous Ca²⁺ releases and thereby lead to ectopic activity. Similarly, triggered beats occur more frequently in the presence of increased heart rate (44). Elevated intracellular Ca²⁺ may also close gap junctions, decreasing cell-to-cell coupling, and thereby decreasing action potential conduction directly provoking arrhythmias. Thus we propose that increased cardiac sympathetic activity, higher heart rate, and changes in Ca²⁺ regulatory proteins in paraplegic rats favor conditions of Ca²⁺ overload, which increase the likelihood for ventricular arrhythmias.

The increase in Na/Ca exchanger protein abundance in paraplegic rats was paralleled by an increase in the expression of Na/Ca exchanger mRNA. In contrast, the changes in SERCA and phospholamban protein abundance in paraplegic rats were not associated with parallel changes in mRNA expression. Several reports have documented similar findings whereby changes in protein abundance were not associated with changes in mRNA expression. For example, Na/Ca exchanger protein abundance but not mRNA expression was significantly increased in ventricular myocytes from infarcted hearts (14). In heart failure, SERCA2A protein abundance was reported unchanged despite significantly reduced mRNA expression (10). Thus up- or downregulation of protein abundance does not necessarily parallel a change in mRNA expression. A lack of correlation between mRNA expression and protein levels may reflect in part differences in the rates of synthesis and/or degradation of mRNA and protein (31). Additionally, regulation of modulation factors may compensate for changes in the total amount of protein expressed (1).

Limitations

In this study, we document a relationship between indirect indices of increased cardiac sympathetic activity, changes in cardiac Ca²⁺ regulatory proteins, and increased susceptibility to ventricular arrhythmias in paraplegic rats. This study does not document a cause-and-effect relationship. In addition, other mechanisms certainly contribute to the changes reported in this study. Thus additional studies designed to examine molecular mechanisms that mediate the increased susceptibility to ventricular arrhythmias in paraplegic rats merit consideration.

In conclusion, this is the first study to demonstrate an increased susceptibility to ventricular arrhythmias with concomitant changes in cardiac electrophysiological parameters and the abundance of Ca²⁺ regulatory
proteins in a chronic cardiac model of paraplegia. The data suggest that the increased susceptibility to ventricular arrhythmias may be due to sarc(o)endomembrane proteins in a chronic model of paraplegia. The increased susceptibility to spontaneous Ca$^{2+}$ release. As a result, individuals with paraplegia may have resting membrane potentials closer to threshold and thus be more susceptible to ventricular arrhythmias (41).

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**DISCLOSURES**

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**REFERENCES**


