Cardiac spinal deafferentation reduces the susceptibility to sustained ventricular tachycardia in conscious rats

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Lujan HL, Krishnan S, DiCarlo SE. Cardiac spinal deafferentation reduces the susceptibility to sustained ventricular tachycardia in conscious rats. Am J Physiol Regul Integr Comp Physiol 301: R775–R782, 2011. First published June 15, 2011; doi:10.1152/ajpregu.00140.2011.—The response to myocardial ischemia is complex and involves the cardio-cardiac sympathetic reflex. Specifically, cardiac spinal (sympathetic) afferents are excited by ischemic metabolites and elicit an excitatory sympathetic reflex, which plays a major role in the genesis of ventricular arrhythmias. For example, brief myocardial ischemia leads to ATP release, which activates cardiac spinal afferents through stimulation of P2 receptors. Clinical work with patients and preclinical work with animals document that disruption of this reflex protects against ischemia-induced ventricular arrhythmias. However, the role of afferent signals in the initiation of sustained ventricular tachycardia has not been investigated. Therefore, we tested the hypothesis that cardiac spinal deafferentation reduces the susceptibility to sustained ventricular tachycardia in adult (12–15 wk of age), conscious, male Sprague-Dawley rats. To test this hypothesis, the susceptibility to ventricular tachycardias produced by occlusion of the left main coronary artery was determined in two groups of conscious rats: 1) deafferentation (bilateral excision of the T1-T5 dorsal root ganglia) and 2) control (sham deafferentation). The ventricular arrhythmia threshold (VAT) was defined as the time from coronary occlusion to sustained ventricular tachycardia resulting in a reduction in arterial pressure. Results document a significantly higher VAT in the deafferentation group (7.0 ± 0.7 min) relative to control (4.3 ± 0.3 min) rats. The decreased susceptibility to tachyarrhythmias with deafferentation was associated with a reduced cardiac metabolic demand (lower rate-pressure product and ST segment elevation) during ischemia.

Cardiovascular risks; ischemia; dorsal root ganglia

SUDDEN CARDIAC DEATH (SCD) causes more than 350,000 deaths annually in the United States (1, 76, 78) and is most commonly caused by ventricular tachyarrhythmias that culminate in ventricular fibrillation (VF) (6, 28). The most common cause of SCD is acute myocardial ischemia. Multiple ischemic metabolites, including ATP, thromboxane A2, serotonin (5-hydroxytryptamine), histamine, lactic acid (protons), reactive oxygen species, and bradykinin are released during myocardial ischemia and reperfusion and stimulate cardiac spinal afferents (5, 24–27, 45, 53, 70, 71) leading to angina pectoris and excitatory cardiac-cardiovascular reflex responses (35, 45, 49). The excitatory cardiac-cardiovascular reflex plays a major role in the genesis of ventricular arrhythmias (59). Disruption of this excitatory sympathetic reflex has been used successfully to reduce arrhythmias in high-risk patients with structural heart disease (8), following myocardial infarction (67), patients with long QT syndrome (19, 62), and patients with catecholaminergic polymorphic ventricular tachycardia (19, 75), as well as in animal models during coronary artery occlusion (58, 59, 63, 65). Importantly, all of these previous studies, with the exception of Schwartz et al. (59), have disrupted both the afferent and efferent limbs or only the efferent limb of the excitatory sympathetic reflex (43). In contrast, Schwartz et al. (59) documented that disruption of the afferent limb, in anesthetized and vagotomized dogs and cats, decreased the absolute number of ectopic beats elicited by brief occlusion and reperfusion of the circumflex and/or the anterior descending coronary artery. However, surrogate endpoints for VF, such as the absolute number of ectopic beats, may be misleading (2) and not representative of VF or SCD (69). Thus, to date, the effects of disruption of the afferent limb on the response to coronary occlusion-induced VF have not been studied.

Based on these clinical and experimental results, we tested the hypothesis that cardiac spinal deafferentation reduces the susceptibility to sustained ventricular tachycardia initiated by occlusion of the left main coronary artery. Conscious, chronically instrumented rats were studied to negate the confounding effects of anesthetic agents and surgical trauma.

MATERIALS AND METHODS

Surgical Procedures

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of Wayne State University, Detroit, MI. The studies conformed to the American Physiological Society guidelines and principles for research involving animals. Two groups of adult male Sprague-Dawley rats were studied: 1) deafferentation (n = 6, bilateral excision of the T1-T5 dorsal root ganglia) and 2) control (sham deafferentation; n = 6). All surgical procedures were performed using aspetic surgical techniques. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), atropinized (0.05 mg/kg ip), intubated, and prepared for aseptic surgery. Supplemental doses of pentobarbital sodium (10–20 mg/kg ip) were administered if the rat regained the blink reflex or responded during the surgical procedures.

Radiotelemetry implantation. After anesthesia was induced, a telemetry device [PhysioTel C50-PXT (Data Sciences International) pressure, temperature, and electrocardiogram] was implanted in all rats as previously described (17, 55), and a catheter was placed in the intraperitoneal space for the infusion of fluids and drugs. Specifically, the transmitter body, which contains the thermistor, and the intraperitoneal catheter were placed in the intraperitoneal space through a ventral abdominal approach. The intraperitoneal catheter was exteriorized on the dorsal aspect of the neck. The pressure sensor of the telemetry device, located within the tip of a catheter, was inserted into the descending aorta for continuous, nonthethered, recording of pulsatile arterial blood pressure. The electrical leads from the telemetry device were placed in a modified lead II configuration by placing the negative electrode slightly to the right of the manubrium and the positive electrode at the anterior axillary line along the 5th intercostal space. A minimum of 1 wk was allowed for recovery and for the

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animals to regain their presurgical weight. During the recovery period, the rats were handled, weighed, and acclimatized to the laboratory and investigators.

Thoracotomy procedures. After the recovery period, the animals were anesthetized as described above, and the hearts were approached via a left thoracotomy through the 4th intercostal space. A coronary artery occluder was made from an atraumatic needle holding 5.0-gauge prolene suture (Ethicon). The needle and suture were passed around the left main coronary artery 2–3 mm from the origin by inserting the needle into the left ventricular wall under the overhanging left atrial appendage and bringing it out high on the pulmonary conus. The needle was cut from the suture, and the two ends of the suture were then exteriorized and secured at the back of the neck. The tubing was filled with a mixture of Vaseline and mineral oil to prevent a pneumothorax. At least 1 wk was allowed for recovery. During the recovery period, the rats were handled, weighed, and acclimatized to the laboratory and investigators.

Spinal deafferentation. After anesthesia was induced, rats were intubated and positioned prone over a thoracic roll that slightly flexed the trunk. The T1-T4 thoracic vertebrae were exposed via a midline dorsal incision, and the spinous processes and laminae were removed. Subsequently, the T1-T5 dorsal root ganglia were carefully isolated and excised bilaterally. Identical procedures were used for the control rats; however, the dorsal root ganglia were not cut. Cell bodies of afferent fibers from the heart are located in the dorsal root ganglia of spinal segments T1-T5 (59). Therefore, excision of dorsal root ganglia from T1-T5 eliminated the main cardiac spinal (sympathetic) afferents from the heart to the central nervous system. Ventral roots, which contain the sympathetic efferent outflow, remained intact (59). All rats were allowed to recover for 2 wk. Upon completion of the studies, the site of the spinal deafferentation was confirmed by autopsy.

Experimental Procedures

Ventricular arrhythmia threshold. Conscious, unrestrained rats were studied in their home cages for all experiments. Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. After the stabilization period, beat by beat, steady-state preocclusion hemodynamic variables were recorded over 10 to 15 s. Subsequently, the left main coronary artery was temporarily occluded by use of the prolene suture. Specifically, acute coronary artery occlusion was performed by pulling up on the suture that was around the left main coronary artery (37). In control rats, rapid changes in the ECG (peaked T wave followed by ST segment elevation) as well as an increase in arterial pressure and heart rate occur within seconds of pulling on the suture, documenting coronary artery occlusion (36–38, 40–43). In contrast, arterial pressure and heart rate decrease during coronary artery occlusion in rats with
cardiac (sympathetic) spinal deafferentation, and there is a markedly lower elevation in the ST segment (Figs. 1 and 2). The reduction in arterial pressure is likely the result of the reduced cardiac output that occurs during coronary artery occlusion (39, 42); and the reduced heart rate and lower elevation in the ST segment are likely the result of disruption of the excitatory cardiac-cardiovascular reflex responses (35, 45, 49). The coronary artery occlusion was maintained until the onset of sustained ventricular tachycardia but no longer than 10 min to prevent permanent myocardial damage (52). The ventricular arrhythmia threshold (VAT) was defined as the time from coronary artery occlusion to sustained ventricular tachycardia resulting in a reduction in arterial pressure. If the time to sustained ventricular tachycardia exceeded 10 min, the occlusion was stopped and 10 min was used as the VAT. Ventricular tachycardia was defined as sustained ventricular rate (absence of P wave, wide bizarre QRS complex) > 1,000 beats/min, with a reduction in arterial pressure to ~40 mmHg. Normal sinus rhythm appeared upon termination of the occlusion by gently compressing the thorax. Without compressing the thorax, the sustained ventricular tachycardia progresses to VF. VF was defined as a ventricular rhythm without recognizable QRS complex in which signal morphology changed from cycle to cycle and for which it was impossible to estimate heart rate. In the event that the animal did not resume normal sinus rhythm, cardioversion was achieved (after the rat lost consciousness) with the use of one shock (10 J) of DC current.

Data analysis. All recordings were sampled at 2 kHz and the data were expressed as means ± SE. All data were the average of every beat during the last 10–15 s of the period.

A Student’s unpaired t-test was used to compare the VAT (Fig. 3) between the deafferentation and control groups. In addition, a two-factor ANOVA with repeated measures on one factor, was used to compare mean arterial blood pressure, heart rate, ST segment elevation, and rate-pressure product immediately before the occlusion (rest) and during the first 3 min of occlusion between the two groups (Fig. 4). The first 3 min of occlusion were chosen (as the standardized time points) to compare identical time points between groups. This was necessary because the VAT was different between the deafferentation and control groups. Importantly, no animal in either group experienced sustained ventricular tachycardia before 3 min of occlusion.

Finally, a Student’s unpaired t-test was used to compare mean arterial blood pressure, heart rate, ST segment elevation, and rate-pressure product immediately before the onset of ventricular tachycardia between the deafferentation and control groups (Fig. 5).

The ECGs were analyzed off-line to measure the ST segment elevation (voltage difference between the baseline and J point) using the ECG analysis software for Chart (ADInstruments (68)). The rate-pressure product, an index of myocardial oxygen demand, was calculated as systolic blood pressure × heart rate/1,000 (30).

RESULTS

Figure 3 presents the VAT in the two groups of rats (control and deafferentation). The VAT was significantly longer in the deafferentation group compared with the control group (7.0 ± 0.7 vs. 4.3 ± 0.3 min, respectively). Importantly, in the deafferentation group, the VAT exceeded the 10-min occlusion limit in one animal; thus 10 min was used as the VAT. This animal experienced sustained ventricular tachycardia upon reperfusion. No animals in the control group exceeded the 10-min occlusion limit.

Mean arterial blood pressure, heart rate, ST segment elevation, and rate-pressure product immediately before the occlusion (rest) and during the first 3 min of occlusion in the two
groups are presented in Fig. 4. The first 3 min of occlusion were chosen (as the standardized time points) to compare identical time points between groups. This was necessary because the VAT was different between the two groups. All values were significantly lower in the deafferentation group (significant group effect).

Mean arterial blood pressure, heart rate, ST segment elevation, and rate-pressure product immediately before the onset of sustained ventricular tachycardia in the control and deafferentation groups are presented in Fig. 5. Mean arterial pressure and ST segment elevation were not different between the two groups. In contrast, heart rate and double product were significantly lower in the deafferenation group.

**DISCUSSION**

In this study, we tested the hypothesis that cardiac spinal deafferentation reduces the susceptibility to sustained ventricular tachycardia, initiated by occlusion of the left main coronary artery. To test this hypothesis, we recorded the VAT induced by myocardial ischemia in control and deafferented conscious rats. Results document a significantly higher VAT in the deafferented group (Fig. 3). The reduced susceptibility to ventricular tachyarrhythmias was associated with a reduced cardiac metabolic demand during ischemia (lower mean arterial pressure, heart rate, rate-pressure product, and ST segment elevation, Fig. 4). The higher VAT in the deafferented group extends previous reports that disruption of the excitatory cardio-cardiac sympathetic reflex is protective against ventricular arrhythmias (19, 58, 59, 62, 63, 65, 67, 75).

The present study also extends the pioneering work of Schwartz et al. (59), which documented that C8-T5 dorsal root section in anesthetized and vagotomized dogs and cats decreased the absolute number of ectopic beats elicited by multiple brief (5–90 s) occlusions and reperfusions (60 s) of the circumflex and/or the anterior descending coronary artery. The authors concluded that dorsal root section reduced the number of ectopic beats associated with short-lasting coronary artery occlusions by interruption of the cardio-cardiac sympathetic reflex with afferent fibers running through the dorsal roots. It is important to note, however, that surrogate endpoints for VF, such as the absolute number of ectopic beats, may be misleading because spontaneous ventricular ectopy can be suppressed by drugs without subsequent suppression of VF (2). Thus all arrhythmia scaling systems should be approached with caution. The critical determinant should be whether VF had or had not occurred, because in the clinic, VF seldom (if ever) spontaneously reverts to a sinus rhythm. In addition, although the design employed by Schwartz et al. (59) allowed internal control analysis instead of group comparison as performed in the present study; it is well documented (50) that brief periods of
ischemia interspaced with reperfusion alters the response to subsequent ischemic events. Therefore, the findings of a higher VAT in the deafferented group, reported in the present study, extends the pioneering work of Schwartz et al. (59) to the conscious animal in which clinically relevant ventricular tachyarrhythmias can be induced.

Activation of spinal afferents by a reduction in perfusion to active skeletal muscle in man and rat elicits a pressor and tachycardiac reflex commonly called the muscle metaboreflex (3, 18). Similarly, myocardial ischemia elicited a metaboreflex-mediated increase in arterial pressure, heart rate, ST segment elevation, and double product (Fig. 4) in the present study. Dorsal root section significantly reduced these hemodynamic responses to myocardial ischemia (Fig. 4). These results contrast sharply with the results from Schwartz et al. (59) who documented that C8-T5 dorsal root section produced only minor changes in heart rate and blood pressure. It is likely that the multiple short-lasting (5–90 s) occlusions of either the left anterior descending or the circumflex coronary artery in the previous study (59) compared with the up to 10 min of occlusion of the left main coronary artery in the present study as well as the confounding effects of anesthetic agents and surgical trauma accounts for this difference.

Myocardial ischemia produces ventricular arrhythmias that are reduced by surgical ablation of cardiac sympathetic nerves in a variety of experimental and clinical models (19, 58, 62, 63, 65, 67, 75). Results from these studies and others (35, 45, 49) document that ischemia-induced ventricular arrhythmias are mediated, in part, by an excitatory cardio-cardiac reflex that increases cardiac sympathetic efferent activity. For example, Schwartz and colleagues (47, 59) demonstrated that myocardial ischemia provokes a powerful increase in cardiac sympathetic efferent activity that directly promotes ventricular tachycardia. Furthermore, sympathetic nerve activity increases before the onset of ventricular tachyarrhythmias in conscious dogs (77). Taken together, data document that cardiac sympathetic afferents are excited by ischemia and elicit a cardio-cardiac sympathetic reflex (10, 46), which plays a major role in the genesis of ventricular arrhythmias (59). The cardio-cardiac sympathetic reflex, in addition to mediating cardiac sympatho-excitation, inhibits cardiac vagal efferent activity (low vagal activity is associated with ventricular arrhythmic events) (13, 61).

Limitations

The dorsal root ganglia contain cell bodies of fibers that transmit sensory information from somatic and visceral receptive fields. Specifically, the dorsal roots from T1-T5 contain spinal (sympathetic) afferent fibers with cardiac endings as well as cutaneous and muscle endings (46). Dorsal root excision therefore interrupts both somatic and visceral input. Schwartz et al. (59) documented that interruption of the somatic input does not account for the decrease in the number of

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**Fig. 5.** Mean AP (A), heart rate (B), ST segment elevation (C), and double product (D) immediately before the onset of sustained ventricular tachycardia in control rats and rats that had their T1-T5 dorsal root ganglia bilaterally excised (Afferent-X). Although mean AP and ST segment elevation were not different between groups, heart rate and double product were significantly lower in the Afferent-X group. *P < 0.05, control vs. Afferent-X.
ectopic beats observed after dorsal root section. Specifically, the investigators documented that removal of the ribs, muscles, and skin from the left side (a procedure that destroys cutaneous and muscle nerves that travel through the dorsal roots) in cats did not alter the number of ectopic beats elicited by coronary artery occlusion. Thus, the reduced susceptibility to sustained ventricular tachycardia likely resulted from sectioning input from the heart.

In addition to the dorsal roots, the ventral roots contain a significant number of unmyelinated fibers with receptive fields in the viscera that transmit sensory information (14–16). Thus complete cardiac deafferentation is not possible.

A concern is that excision of the T1-T5 dorsal root ganglia produces an anesthetic or masking effect of pain during anginal episodes (20, 21, 57). Despite this concern, left stellate ganglionectomy (removal of cardiac afferent and efferent fibers) is effective for individuals with intractable angina (57). Studies in patients with angina documented that left stellate ganglionectomy prevented anginal attacks and improved exercise tolerance (9, 34, 57, 74). Schwartz (57) suggested that the cardioprotective effect of left stellate ganglionectomy was the elimination of the excitatory cardio-cardiac sympathetic reflex which initiates angina. In support of this idea, Leriche and Fontaine (32) stated that the left stellate ganglion had a central role in the reflexes that initiate angina.

Clinical Perspective

SCD causes more than 350,000 deaths annually in the United States (1, 76, 78) and is most commonly caused by ischemia-induced ventricular tachyarrhythmias that culminate in VF (6, 28). Importantly, implantable cardioverter-defibrillators (ICD) reduce mortality in high-risk patients; however, ICDs treat ventricular arrhythmias without preventing them and can be proarrhythmic if patients sustain recurrent ICD shocks. ICDs are also expensive and are associated with morbidity from implantation complications as well as potential mechanical dysfunction. Antiarrhythmic drugs are often used for reducing SCD; however, the pharmacological approach to antiarrhythmic therapy has serious problems. Most antiarrhythmic drugs are less effective and far more dangerous than once believed (11, 12, 23, 54, 73) and are often poorly tolerated and have serious side effects.

The proarrhythmic effects of ICDs and most currently available antiarrhythmic drugs, combined with the enormity of the problem make it essential to investigate novel strategies for the prevention of SCD. In this context, the present data strengthens and supports the extensive clinical and preclinical evidence that disruption of cardiac innervation either pharmacologically with local anesthetics or antiadrenergic interventions such as beta blockers or with surgical ablation of cardiac nerves are therapeutic alternatives for the management of life-threatening ventricular arrhythmias (4, 7, 8, 19, 29, 33, 44, 48, 51, 56, 60, 62–64, 66, 67, 72, 75). Accordingly, cardiac spinal deafferentation may become an additional therapeutic option. For example, deafferentation by several specific procedures including dorsal root entry zone lesions and thermocoagulation and dorsal rhizotomy are currently in use for a variety of conditions associated with intractable pain as well as over activity of skeletal and smooth muscle associated with spinal cord injury. Specifically, sacral deafferentation is effective to control autonomic dysreflexia and spasticity in individuals with spinal cord injury (31). Deafferentation was also effective for bilateral intercostal neuralgia that developed following breast augmentation since the pain was completely relieved by T3-T5 dorsal rhizotomies (22). Thus, disruption of spinal afferent fibers is a well-tolerated, effective procedure with low complications for several conditions. It is possible that similar procedures could be used to reduce ventricular arrhythmias and SCD in high-risk patients; however, additional studies are required to further characterize the physiological responses to this procedure and to determine whether this new approach is safe and efficacious for the treatment of conditions associated with excess sympathetic activity.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


