Transmyocardial Laser Revascularization Remodels the Intrinsic Cardiac Nervous System in a Chronic Setting

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Background—Prospective trials have demonstrated that transmyocardial laser revascularization (TMLR) imparts symptomatic relief to patients with refractory angina. Because peak clinical effectiveness of TMLR is usually delayed by several months, it has been proposed that ventricular denervation is one mechanism whereby TMLR imparts symptomatic relief. We have demonstrated that TMLR does not denervate the heart in the acute setting, nor does it modify the intrinsic cardiac nervous system (ICNS) in the acute setting. However, the long-term effects of TMLR on the ICNS remain unknown.

Methods and Results—A holmium:yttrium-aluminum-garnet laser created 20 channels through the anterolateral left ventricular free wall of 10 dogs. Four weeks later, the function of cardiac sensory inputs to the ICNS was studied by applying veratridine (7.5 μmol/L) to ventricular sensory fields. Chronotropic and inotropic responses elicited by cardiac sympathetic or parasympathetic efferent neurons stimulated electrically (10 Hz, 4 V, 4 ms) or chemically (nicotine 5 to 20 μg/kg IV) were also assessed. Chemical activation of epicardial sensory neurites with veratridine elicited expected ICNS excitatory responses. Electrical stimulation of sympathetic and parasympathetic efferent neurons induced expected altered cardiac responses. In contrast, the responsiveness of the ICNS to systemically administered nicotine was obtunded.

Conclusions—Although chronic TMLR does not affect cardiac afferent or extracardiac efferent neuronal function, it does “remodel” the ICNS so that its responsiveness to a known potent chemical agonist (ie, nicotine) becomes obtunded. Remodeling of the ICNS may account in part for the delayed symptomatic relief that TMLR imparts to patients with refractory angina. (Circulation. 2001;104[suppl I]:I-115-I-120.)

Key Words: nervous system, autonomic □ angiotensin □ conduction □ contractility

Patients with anatomically diffuse, end-stage coronary artery disease not amenable to standard revascularization procedures typically suffer from intractable angina (Canadian Cardiology Society [CCS] functional class 3 to 4) despite maximal medical therapy. Several prospective studies have demonstrated that the creation of multiple transmural channels in the heart by means of a laser (transmyocardial laser revascularization, TMLR) provides symptomatic relief of refractory angina in such patients. Channels produced by TMLR in the wall of an ischemic ventricle were initially thought to remain patent after the procedure, thereby permitting blood from the chamber to reach underperfused cardiomyocytes by forming direct communications between the chamber and ventricular blood vessels. Recently, this theory has been challenged with the demonstration that such channels are only transiently patent. An alternate hypothesis proposed is that nonspecific laser-induced myocardial damage may involve local neuronal tissue. It has been proposed that such injury damages local ventricular sensory neurites and autonomic efferent axons, thereby producing local cardiac denervation and thus anginal relief. However, we have shown that TMLR does not destroy cardiac afferent or efferent neuronal function in an acute setting.

TMLR imparts symptomatic relief of ≥2 CCS anginal classes in 25% to 76% of patients 1 year after TMLR. Such symptomatic relief usually is evidenced by a reduction in nitrate usage, increased exercise tolerance and higher quality-of-life scores. Although TMLR does not modify the cardiac nervous system on a short-term basis, it remains to be established whether TMLR induces delayed effects on this system. This is particularly relevant with respect to its effects on the intrinsic cardiac nervous system (ICNS).

Intrinsic cardiac neurons are situated in gangliated plexuses located in discrete atrial and ventricular regions. In the canine and human models, these ganglionated plexuses contain afferent, efferent, and local circuit neurons that allow for “fine tuning” of cardiac function on a beat-to-beat basis. As such, the ICNS acts as the final common regulator of regional cardiac function.

To determine whether TMLR imparts delayed effects on the function of the cardiac nervous system, the effects of...
TMLR were studied in a chronic, nonischemic canine model. The nonischemic model was investigated to avoid the confounding effects that ischemia has on the ICNS.\textsuperscript{16,17} This was done by (1) activating local sensory neurites associated with intrinsic cardiac afferent neurons with the topically applied ion-modifying agent veratridine; (2) electrical activation of sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; (3) chemically activating sympathetic and parasympathetic efferent neurons via systemically administered nicotine; (4) chemically activating sympathetic efferent neurons via systemically administered angiotensin II; and (5) testing the capacity of laser-treated sympathetic efferent neurons via systemically administered nicotine; (4) chemically activating sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; (3) chemically activating sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; (2) electrical activation of sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; (1) activating local sensory neurites associated with intrinsic cardiac afferent neurons with the topically applied ion-modifying agent veratridine.

In this manner, we assessed the long-term effects that TMLR exerts on not only local ventricular cardiomyocyte function but also the cardiac nervous system.

Methods

Animal Preparation

Adult mongrel dogs (n = 14) of either sex, weighing between 25 and 30 kg, were used in this study. All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and were approved by the institutional animal care and use committee of Dalhousie University. The dogs were sedated with a mixture of morphine (0.5 mg/kg IM), atropine (0.2 mg/kg IM), and acepromazine (0.1 mg/kg IM). After endotracheal intubation, general anesthesia was maintained to effect with halothane.

These channels were created in the distribution of the terminal left cervical vagosympathetic trunks. The cervical vagi were divided into transmural channels sites, when required, to achieve homeostasis. If ineffective, a figure-of-8 suture was placed around the epicardial opening. The creation of transmural channels required to traverse the left ventricular wall. Digital pressure was applied to the epicardial openings of transmural channels sites, when required, to achieve homeostasis. If ineffective, a figure-of-8 suture was placed around the epicardial opening. The creation of transmural channels was noted intraoperatively by the change in the pitch of the laser as it entered the left ventricular cavity and was confirmed on examination of myocardial sections at the completion of the chronic physiological experiments described below. Subsequently, the thoracotomy was closed, pneumothorax was reduced and the animal was recovered. Appropriate postoperative pain management was achieved with a combination of long- and short-acting narcotics (morphine sulfate 0.5 to 1 mg/kg and bupinorphone 0.02 mg/kg) and nonsteroidal anti-inflammatory medication (ketoprofen 1 to 2 mg/kg). All animals received a dose of cefazolin preoperatively and postoperatively (20 mg/kg).

Physiological Studies

Four to five weeks after creation of these transmural channels, the animals underwent functional studies. The animals were sedated with sodium pentothal (15 to 20 mg/kg IV) and then anesthetized with sodium pentothal (~5 mg/kg IV to effect for 5 to 10 minutes). After initiation of anesthesia, the animals were intubated and respiration was maintained with a Bird Mark 7 positive-pressure respirator using 100% O\textsubscript{2}. After completion of surgery, anesthesia was maintained with an initial bolus infusion of α-chloralose (50 mg/kg IV). Thereafter, supplemental doses of α-chloralose were administered throughout the experiments every 30 minutes or less. The adequacy of anesthesia was assessed throughout the experiments by checking jaw tone and squeezing a paw while monitoring any change in heart rate.

A lead-II ECG was recorded on an Astomed MT9500 8 channel rectilinear chart recorder. Left ventricular chamber and aortic pressures were measured with Bentley Trantec model 800 transducers connected to a Cordis No. 7 pigtail catheter into the left ventricular chamber and a Cordis No. 6 catheter inserted into the ascending aorta via a femoral arteries. Two miniature solid-state pressure transducers (Konigsberg Instruments, model P190; 5 mm in diameter, 1.5 mm thick) were inserted in the left ventricular ventral wall to record regional intramyocardial pressures. One transducer was placed in the region that underwent the laser treatment; the other, in an adjacent untreated region. All data, including neuronal activity, were recorded on an Astro-Med, Inc model MT 9500 8-channel rectilinear recorder. The outputs of the optical recording were fed to an A.R. Vetter Co model 820 videocassette recorder.

A midline incision was made in the neck to expose the right and left cervical vagosympathetic trunks. The cervical vagi were divided so that the distal ends of each could be stimulated electrically without producing afferent axonal-induced cardiovascular reflexes. After a bilateral thoracotomy to expose the heart, the ventral pericardium was incised to expose the ventral right atrial deposit of fat that contains the ventral component of the right atrial ganglionated plexus. Neurons in the right atrial ganglionated plexus are representative of those found in the various intrinsic cardiac ganglionated plexuses. To minimize epicardial motion during each cardiac cycle, a ring of heavy-gauge wire was gently placed around the circumference of the epicardial fat on the ventral surface of the right atrium. The right atrial ganglionated plexus was explored with a single tungsten microelectrode mounted on a micromanipulator and placed over the epicardial fat so that the assembly could be slowly advanced into the fat to search for neuronal activity at depths ranging from the surface of the fat to regions adjacent to cardiac musculature. The recording microelectrode (Frederick Haer 25-10-3) had a 250-μm shank diameter, an exposed tip of 10 μm, and impedances of 9 to 11 MΩ at 1000 Hz. Proximity to cardiac musculature was indicated by increases in the amplitude of the ECG artifact. The indifferent electrode was attached to the adjacent mediastinum.

Signals generated by atrial neurons were differentially amplified by a Princeton Applied Research model 113 amplifier that had bandpass filters set at 300 Hz to 10 kHz and amplification ranges of 100× to 5000×. The output of this device, further amplified (50× to 200×) and filtered (band width, 100 Hz to 2 kHz) by means of optically isolated amplifiers (Applied Microelectronics Institute), was led to a Nicolet model 207 oscilloscope and to a Grass AM8 Audio Monitor. Activity generated by individual neurons was identified by the amplitude and shape of recorded action potentials. Separate loci were identified from which action potentials with signal-to-noise ratios >3:1 were recorded; individual units were identified by the amplitude and configuration of their action potentials. With these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage but rather records action potentials generated by cell bodies and/or dendrites.

Electrical Stimulation of Autonomic Efferent Neurons

Stellate ganglia were decentralized and bipolar electrodes were placed around them so that they could be stimulated electrically later in the experiments. Acutely decentralized right and left stellate ganglia were stimulated individually (10 Hz, 5 ms, 4 V) for 20 seconds. The distal ends of the sectioned right or left cervical vagi were stimulated individually for 10 seconds (20 Hz, 5 ms, 4 V). The bipolar stimulating electrodes (electrode tips 5 mm apart) were connected to a Grass SD-9 square wave stimulator, the output of which was monitored on a Telequipment D-54 oscilloscope.
Administration of Pharmacological Agents

Veratridine 7.5 μmol/L was applied for 30 seconds to epicardial loci overlying the affected left ventricular TMLR zone with the use of 2×2-cm gauze squares soaked with 2 mL of the chemical. After removal, the epicardial locus was washed with normal saline for 30 seconds (~2 mL/s). At least 10 minutes was allowed to elapse before the next intervention. Gauze squares soaked with room-temperature normal saline were applied to the same epicardial sensory fields to determine whether neuronal responses elicited by epicardial chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

The following 3 chemicals were then administered individually into the systemic circulation: (1) nicotine (5 to 20 μg/kg IV), a chemical that binds to cholinergic nicotinic receptors, thereby activating sympathetic and parasympathetic efferent postganglionic neurons; (2) angiotensin II (0.01 to 5 μg/kg IV), a chemical that binds to AT1 receptors associated with intrinsic cardiac neurons; and (3) the β-adrenoceptor agonist isoproterenol (5 μg IV), which acts directly on the myocyte. These agents were then readministered to ensure reproducibility of effects. At least 5 minutes was allowed to elapse between these interventions for cardiovascular variables to return to baseline values. Heart rate, left ventricular chamber systolic pressure, and intramyocardial systolic pressure in the normal and TMLR-treated zones of the left ventricle.

Echocardiographic Analysis

Echocardiographic assessment of left ventricular wall motion was performed in 2 animals before and during stellate ganglion stimulation with an Ultramark 8 Ultrasound Systems echocardiographic apparatus (model UM-8-OPO1, Advanced Technology Laboratory). The cross-sectional midapical view of the left ventricle was obtained via an Access C ultrasound probe placed on the free wall of the left ventricle.

Data Analysis

Heart rate, left ventricular chamber systolic pressure, and intramyocardial systolic pressure in the normal and TMLR-treated zones of the left ventricular free wall were monitored for 1 minute before and after each of the interventions described above. The activity generated by identified right atrial neurons was analyzed for 1-minute periods before and during each intervention. Cardiac, vascular, and neuronal data obtained before and during each intervention are presented as mean ± SEM. One-way ANOVA and paired t test with Bonferroni’s correction for multiple tests were used for statistical analysis. Significance values of P<0.05 or P<0.01 were used for these determinations.

Results

Laser-Treated Animals

Left ventricular dynamics responded in a normal fashion to systemically administered isoproterenol, including the zone that had undergone TMLR. Echocardiographic analysis performed in 2 dogs demonstrated normal left ventricular transverse diameters in diastole and systole during control states. These dimensions were reduced during stellate ganglion stimulation in a manner consistent with that found in normal dogs.19 The ventricular free wall zone that had undergone TMLR did not display any detectable wall motion abnormality.

However, the heart did not appear to respond to stress in a normal fashion. Indeed, the left ventricular systolic pressure progressively diminished throughout the experiment (during exposure of the heart and its instrumentation, left ventricular systolic pressure fell from 132±2 to 96±8 mm Hg; P<0.01). Additionally, dysrhythmias developed in 2 animals. The heart of 1 dog fibrillated during the dissection of pericardial adhesions to expose the ventricular epicardium. After this heart was defibrillated, baseline hemodynamic variables returned to control values. In another animal, atrial pacing was required because of the development of a third-degree heart block. In this animal, only responses to stellate ganglion stimulation and systemic administration of the chemicals were studied.

The sodium channel modifier veratridine, when applied to the epicardium over the laser-treated zone, modified the activity generated by identified right atrial neurons (increase in activity in 7 animals, decreased in 2 animals; delta neuronal change=19.0±3.1 impulses per minute; P<0.01). Reaplication of veratridine to the same epicardial region induced similar neuronal responses.

Electrical stimulation of acutely decentralized stellate ganglia enhanced all monitored cardiac indices in a normal fashion (Table). Specifically, both the lasered and nonlasered regions responded in a similar fashion to this intervention (Figure 1 and Table). Electrical stimulation of parasympathetic efferent neurons suppressed intramyocardial systolic pressure in both regions of the left ventricle to similar degrees (Table).

On the other hand, nicotine, which normally activates the ICNS, failed to alter recorded neuronal activity (Figure 2). Neuronal activity was unaffected even when supranormal doses of nicotine were administered (Figure 3). Minor alterations in recorded cardiovascular variables occurred only
when the highest dose of nicotine (20 μg/kg IV) was studied. Angiotensin II, when administered in doses that normally induce intrinsic cardiac neuronal and cardiac responses in normal hearts (ie, 0.01 to 5 μg/kg IV), failed to elicit neuronal responses overall. When the largest dose of angiotensin II was studied (5 μg/kg), cardiac indexes increased a little (Table). Isoproterenol not only enhanced both inotropic and chronotropic responses but also increased ICNS activity. In this chronic model, isoproterenol enhanced recorded cardiac indexes, including left ventricular intramyocardial systolic pressures in the TMLR zone; it failed to enhance intrinsic cardiac neuronal activity (Table).

Phenol destroys epicardial nerves. To demonstrate that ventricular myocyte responses elicited by autonomic efferent neurons can be eliminated, phenol was applied in 2 animals to the epicardium surrounding the intramyocardial pressure sensor located in the treated (lasered) ventricular zone. After epicardial application of phenol, stimulation of either stellate ganglion failed to produce regional inotropic responses that were previously inducible (Figure 4A, laser). The unaffected ventricular region (not surrounded by epicardial phenol) responded to stellate ganglion stimulation in a fashion that was similar to that induced before phenol application (Figure 4A, control). To test myocyte viability independent of the ICNS, isoproterenol was exogenously administered. Both the phenol-treated and untreated ventricular regions responded similarly to this intervention (Figure 4B).

**Sham-Operated Animals**

Unlike the laser treated animals, the performance of the thoracotomy did not alter hemodynamics in the 4 sham animal. Inotropic and chronotropic changes elicited during stellate ganglia stimulation were similar to those in the chronic TMLR animals (right stellate ganglion: left ventricular intramyocardial pressure, control versus stimulation, 50±11.3 versus 134±22.5 mm Hg; heart rate, control versus stimulation, 140±7.1 versus 163.7±6.6 bpm [mean±SEM]). In contrast to laser-treated animals, neuronal responses elicited with nicotine (5 μg/kg IV: delta neuronal change = 67.5±35.0) and angiotensin II (0.01 μg/kg IV: delta neuronal change = 27.5±11.1) were similar to those previously reported for normal controls.12,16,20,21

**Discussion**

The results of the present study indicate that TMLR remodels the ICNS over time, thereby affecting its capacity to influence regional cardiac function. This occurs despite the fact that TMLR does not affect the function of cardiac sensory neurons or alter the capacity of sympathetic and parasympathetic efferent neurons to modulate regional ventricular dynamics.

Prospective studies indicate that TMLR relieves angina of cardiac origin.1–3 The mechanisms whereby this occurs remain unknown. Although up 85% of patients attain significant symptomatic improvement after TMLR, peak anginal relief is usually delayed 3 to 6 months after TMLR, regardless of the type of laser used.1–3 It has been suggested that cardiac denervation may be one mechanism whereby TMLR exerts its salutary effects.10 Kwong et al,10 by applying high doses of bradykinin to the ventricular epicardium before and after TMLR, concluded that TMLR renders sensory neurites in the affected myocardium nonfunctional. However, direct assessment of cardiac afferent neuronal function before and after acute TMLR indicated that this is not the case.12 The cellular sodium channel modifier veratridine modifies ventricular sensory neurites associated with cardiac afferent neurons in a consistent fashion.22 The effects of veratridine on ventricular sensory inputs to intrinsic cardiac neurons after chronic TMLR were similar to those induced in normal prepara-
tions. These data indicate that the function integrity of intrinsic cardiac afferent neurons is preserved in this model of chronic TMLR.

It has been suggested that cardiac tyrosine hydroxylase immunoreactivity, an indirect measure of sympathetic efferent postganglionic axonal density, becomes reduced in ventricular regions subjected to TMLR, which equates with functional sympathetic efferent neuronal denervation. However, direct assessment of the function of cardiac sympathetic efferent neurons demonstrated that TMLR does not affect their capacity to influence ventricular tissues in an acute, nonischemic canine model. Neither did TMLR modify the capacity of cardiac adrenergic efferent neurons to enhance regional ventricular dynamics in a chronic setting when activated electrically (Figure 1 and Table). Similarly, electrical stimulation of parasympathetic efferent preganglionic axons suppressed regional intramyocardial systolic pressures in treated and untreated regions of the left ventricular free wall to comparable degrees (Table). These data demonstrate that TMLR does not obtund the influence of extracardiac autonomic efferent neurons (with intracardiac axonal projections) on ventricular function in a chronic setting.

In contrast, regional denervation occurs after the topical application of phenol, which negates the ability of electrical activation of sympathetic efferent postganglionic neurons to enhance regional ventricular contractility. In confirmation of that, ventricular regions treated with phenol failed to respond to electrical activation of sympathetic efferent neurons even though adjacent untreated ventricular regions elicited normal responses to the stellate ganglion stimulation (Figure 4A). On the other hand, systemic administration of isoproterenol enhanced intramyocardial systolic pressure in both regions similarly (Figure 4B). These data indicate that one can functionally destroy the sympathetic efferent innervation to a region of the heart without causing detectable cardiomyocyte injury.

Despite the normalcy of cardiac afferent and extracardiac efferent neurons, intrinsic cardiac neuronal function was not normal after chronic TMLR. As previously stated, the ICNS is an important regulator of regional cardiac function. In extensive experience with the canine model in our laboratory, the canine preparation used was a robust model, capable of tolerating manipulation without preparation compromise. However, 4 to 5 weeks after the TMLR procedure, it was apparent that the animals did not tolerate surgical stress in the usual fashion. Two animals suffered significant dysrythmias on exposing of the heart. No dysrythmias or hemodynamic instability was seen in the sham animals. Additionally, the ICNS proved to be nonresponsive to the potent chemical agonists nicotine and angiotensin II.

Nicotine activates both parasympathetic and sympathetic efferent postganglionic neurons (cell bodies) of the ICNS, thereby inducing bradycardia (parasympathetic efferent neuronal effects on atrial tissues), followed by enhancement of
cardiac indices (via activating sympathetic efferent neurons). In other words, both populations of autonomic efferent neurons on the heart become activated after systemic administration of nicotine,23. Nicotine continued to enhance cardiac indices immediately after TMLR was performed in a canine model12 and in sham-operated animals in this study. In the chronic TMLR model, however, nicotine not only failed to enhance intramyocardial pressures in the treated zone but also failed to alter the other monitored cardiac indices monitored (Figure 2). This occurred despite the fact that the maximum dose of nicotine studied was 4 times that required to elicit a maximal response in normal preparations.12,23 Angiotensin II activates AT1 receptors associated with the ICNS. Normally, angiotensin II activates this neuronal system to enhance cardiac indexes.20,21 A lack of effect occurred when angiotensin II was administered to this chronic TMLR model (Table). Taken together, these data indicate that the global functioning of intrinsic cardiac efferent neurons becomes obtunded within a month after TMLR. This correlates with the animals’ inability to tolerate the usual manipulation required during the course of experimentation.

On the other hand, TMLR did not affect the capacity of ventricular myocytes to respond to the exogenously administered β-adrenergic agonist isoproterenol. This agent enhanced intramyocardial systolic pressure by 47% in the treated region and by 42% in the untreated area (Table). That the treated zone retained its normal contractile responsiveness to such a challenge was confirmed in 2 animals by echocardiography (data not shown).

In conclusion, data derived from this study indicate that the function of the ICNS is substantively altered within a few weeks of TMLR. TMLR does not denervate cardiac afferent or extracardiac efferent neuronal function in ventricular regions undergoing the procedure in a chronic setting. This is in accordance with the lack of TMLR effects on a short-term basis.12 Thus, the beneficial effects that TMLR affords patients with ischemic heart disease cannot be ascribed to local ventricular denervation. Rather, alteration or “remodeling” of the ICNS, the final common regulator of regional cardiac function, may occur within a month of TMLR. Remodeling of the ICNS has been shown to occur in autotransplanted dogs,24 chronically decentralized dogs,25 and dogs undergoing spinal cord stimulation.16 It therefore appears that some surgical procedures and therapeutic interventions have the ability to alter the functioning of this important neuronal system. As such, remodeling observed chronically after TMLR may represent a novel mechanism whereby such therapy imparts symptomatic relief. However, remodeling the ICNS in this manner may exert deleterious effects on cardiac function.

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