Chemoreflexes

An Experimental Study

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Hypothesis: Transmyocardial laser revascularization (TMLR) will not denervate the heart, because it does not destroy all of the afferents. This study was designed to determine if stimulation of cardiac sympathetic and vagal afferents from an area of the left ventricle treated with TMLR could evoke reflex effects, and thus whether TMLR would denervate the heart.

Methods: The effect of TMLR on reflexes evoked by chemically stimulating cardiac afferents was examined in 9 dogs. Bradykinin and capsaicin were applied topically or injected into the left anterior descending coronary artery before and after TMLR and after bilateral vagotomy and sympathectomy. Aortic (AoP) and left ventricular pressures (LVP) and electrocardiography were monitored. The first derivatives of LVP (dP/dt) were calculated.

Results: Topical bradykinin elicited variable hemodynamic responses. Topical capsaicin evoked pressor responses, increasing mean (± SEM) AoP (105±9 to 115±9 mm Hg; P<.001) and positive dP/dt (+dP/dt) (1032±81 to 1159±10 mm Hg/s; P<.01) before TMLR. Intracoronary capsaicin evoked a depressor response before TMLR. Pressor responses remained intact after TMLR with increases in mean AoP and +dP/dt (115±6 to 128±5 mm Hg and 1039±98 to 1136±100 mm Hg/s, respectively; P<.01). Depressor responses also remained intact after TMLR (91±10 vs 101±11 mm Hg [P<.02], and 865±104 vs 931±104 mm Hg/s [P<.05], respectively). Hemodynamic responses were diminished after bilateral vagotomy and abolished after bilateral sympathectomy.

Conclusion: Since activation of cardiac afferent nerves and reflex responses remained intact after TMLR, but changed after vagotomy or sympathectomy, TMLR does not denervate the heart sufficiently to be the cause of improved angina after TMLR.

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Transmyocardial laser revascularization (TMLR) is an alternative treatment for patients with refractory angina who are not candidates for angioplasty or bypass surgery.1 In clinical trials, patients treated with TMLR showed improvements in New York Heart Association angina class at up to a 12-month follow-up.2 Although there is extensive experience using TMLR as sole therapy or in conjunction with bypass surgery,3 the mechanism by which TMLR provides angina relief is not clear. It has been postulated that the TMLR channels provide a passage for blood to flow from the ventricle into the myocardium.4,5 However, significant data suggest that TMLR channels do not remain patent, and this hypothesis is falling out of favor.6,7 Laser injury accompanied by angiogenesis and increased collateral perfusion has been proposed as an alternative mechanism to explain the improvement in perfusion after TMLR.8,9 However, the question of why patients are relieved of their symptoms immediately after surgery has not been answered. Another possible explanation for angina relief immediately after TMLR is denervation of the myocardium. Although evidence has been presented that afferent cardiac responses are inhibited by laser treatment,10-12 other investigators have reported that TMLR does not denervate the heart.13

The heart is innervated by sympathetic and vagus nerves, and cardiac pain is known to travel via sympathetic afferents,14,15 which have multiple endings.16 Thus, unless TMLR ablates major proximal nerve branches, it is unlikely that TMLR would denervate the heart sufficiently to prevent transmission of sensory information. The purpose of our study was to determine, in a canine model, if stimulation of cardiac sympathetic and vagal afferents from an area of the left ventricle treated with TMLR could evoke re-
MATERIALS AND METHODS

Experiments were performed on 9 mongrel dogs (body weight, 20-23 kg). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The Institutional Animal Care and Use Committee reviewed and approved this study.

Animals were anesthetized with intravenous thiopental sodium (Pentothal) (15 mg/kg) and intubated, and their lungs were ventilated with an oxygen mixture by a veterinary anesthesia ventilator (Model 2000; Hallwell, Inc, Pittsfield, Mass). Anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium (4mg/kg per hour). Electrocardiography (ECG) (lead II) was monitored. The left carotid artery was isolated, and a catheter transducer (Mikro-tip; Millar Instrument Inc, Houston, Tex) was inserted into the aorta to measure aortic pressure (AoP). The right femoral artery was isolated, and another catheter (Millar Instrument Inc) was inserted into the left ventricle to measure the left ventricular pressure (LVP). Both cervical vagal nerves were isolated. The left chest was entered by a left lateral thoracotomy incision through the fifth intercostal space in 6 animals. In the other 3 animals, a midsternotomy was performed to allow for exposure of the stellate ganglia and sympathetic chains. In 4 animals, a catheter (Angio cath 20; Becton Dickinson, Sandy, Utah) was inserted into the left anterior descending coronary artery (LAD), proximal to the second diagonal artery. The LVP, AoP, and ECG were recorded using a thermal array recorder (TA11; Gould Instrument Systems Inc, Valley View, Ohio) and simultaneously using a computerized data acquisition system (Labtech, Andover, Mass).

We stimulated chemically sensitive cardiac afferents before and after TMLR and after interrupting vagal and sympathetic pathways. Bradykinin and capsaicin (Sigma-Aldrich Corp, St Louis, Mo) were used to stimulate cardiac sensory (vagal and sympathetic) afferents by topical application and by injection into the LAD. For topical application, each test solution (with different concentrations) was selected randomly, and applied to the anterior wall of the left ventricle with a piece of filter paper (2 × 2 cm²), and then removed. We continued the protocol with the chemical that gave the more prominent cardiac response. After each application, the epicardium was rinsed with isotonic sodium chloride solution. Bradykinin was applied to all animals, and capsaicin was applied to 6 animals. In 4 animals, doses of capsaicin or bradykinin were also injected into the LAD to elicit cardiac reflexes. The interval between testings was 15 minutes.

Laser transmyocardial channels (average, 24) were made in the anterior wall of the left ventricle in the LAD distribution at a density of 1/cm². The animals were then allowed to stabilize for 30 to 60 minutes before applying chemical agents. Topical application and LAD injection of bradykinin or capsaicin were repeated after TMLR. The cervical vagal nerves were then cut, and topical applications or LAD injections of bradykinin or capsaicin were repeated. In midsternal-approach animals, the stellate ganglia and sympathetic chains (T1-T4) were transected bilaterally, and epicardial stimulation with capsaicin or bradykinin was repeated. Animals were killed humanely with pentobarbital and potassium chloride after all protocols were completed.

During each application or injection of bradykinin and capsaicin, LVP, AoP, and ECG data were collected continuously for 2 minutes (pre-TMLR). Using software developed in Visual Basic for Excel (Microsoft Excel 7.0; Microsoft Inc, Redwood, Wash), we extracted hemodynamic variables from the stored data file. Mean AoP, heart rate, and the first derivatives (positive and negative) of LVP (+dP/dt and −dP/dt) were calculated. Data were reported as mean±SE. A paired t test was used to compare baseline distribution at a density of 1/cm². The animals were then removed. We continued the protocol with the chemical that evoked cardiac reflexes. The interval between testings was 15 minutes.

The TMLR procedure did not change the hemodynamic responses to topical bradykinin or to topical and intracoronary capsaicin. Topical application of bradykinin elicited depressor responses in 3 dogs, pressor responses in 2, and no changes in the remaining 4. The hemodynamic responses induced by topical application of bradykinin in the animals that had a response are shown in the Table. Topical application of capsaicin evoked pressor responses in 6 of 6 dogs, increasing mean AoP from 105±9 to 115±9 mm Hg (a 10%±1% increase; P<.001) and +dP/dt from 1032±81 to 1159±10 mm Hg/s (a 12%±2% increase; P<.001). On average, the heart rate did not change significantly from control (83±3 to 84±4 beats/min). After TMLR, the pressor response remained intact. Topical application of capsaicin also increased mean AoP (12%±4%; P<.01) and +dP/dt (11%±3%; P<.02). However, the pressor response to capsaicin was significantly decreased after bilateral vagotony and completely abolished after bilateral sympathectomy and vagotomy (Figure 1 and Figure 2).

In 3 of 4 dogs that had injection of capsaicin into the LAD, mean AoP decreased from 101±11 to 91±10 mm Hg (−10%±2%; P<.02), +dP/dt decreased from 931±104 to 865±104 mm Hg/s (−7%±2%; P<.05), and −dP/dt increased from −846±55 to −735±74 mm Hg/s (13%±3%; P<.02). In these dogs, mean heart rate (84±6 beats/min) did not change significantly after capsaicin in-
The depressor response was similar before and after TMLR (an 11%±1% decrease of mean AoP, and a 6%±3% decrease of +dP/dt). However, after bilateral vagotomy, the depressor response to capsaicin was abolished in all 3 dogs. In the fourth dog, in whom capsaicin elicited a pressor response, the response still occurred after bilateral vagotomy.

Our results demonstrated there were no statistically significant differences in the responses to bradykinin or capsaicin between studies performed before and after TMLR.

### Responses Evoked by Epicardial Application of Bradykinin*

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### Figure 1.
Pressor response evoked by epicardial application of capsaicin (n=6). TMLR indicates transmyocardial laser revascularization; Vx, bilateral cervical vagotomy; Sx, bilateral section of the stellate ganglia and sympathetic chains (T1-T4); +dP/dt, positive and negative first derivatives of left ventricular pressure; asterisk, P<.05 compared with baseline; and dagger, P<.05 compared with control (pre-TMLR) response. Bars indicate percentage of changes in mean aortic pressure and +dP/dt from control (mean±SEM). The TMLR did not significantly alter the response to epicardial application of capsaicin, but mean aortic pressure and positive +dP/dt were decreased by bilateral vagotomy and were abolished by bilateral sympathectomy (n=3).

### Figure 2.
Pressor response abolished by bilateral sympathectomy. Abbreviations are given in the legend to Figure 1. This tracing represents the response of one animal and shows a typical response to capsaicin after bilateral vagotomy and sympathectomy. The pressor response was completely abolished after bilateral sympathectomy.

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**COMMENT**

Our results demonstrated there were no statistically significant differences in the responses to bradykinin or capsaicin between studies performed before and after TMLR. We found variable responses to topical application of bradykinin, but topical capsaicin consistently evoked pressor responses. Since the pressor and depressor responses remained intact after TMLR, there did not appear to be an effect on vagal or sympathetic responses.
We found that the pressor responses evoked by topical application of capsaicin were abolished after bilateral sympathectomy. The depressor responses induced by intracoronary injection of capsaicin were diminished after bilateral vagotomy. These results suggest that the pressor or depressor responses were reflex in nature and were mediated by sympathetic or vagal afferents rather than by direct drug actions on the heart or vessels. In our study, TMLR did not prevent cardiac afferent receptors from evoking reflex responses.

Cardiac reflexes are quite complicated and show large individual variation. A number of reflexes participate in the control of coronary vascular resistance through activation of the sympathetic or parasympathetic nervous system. It is well known that minute amounts of certain chemical substances introduced into the distribution of the left coronary artery or epicardium can initiate a powerful cardiac reflex. Stimulation of ventricular receptors can cause reflex hypotension and bradycardia (mediated by vagal afferents) or, alternatively, tachycardia and hypertension (mediated by sympathetic afferents). Responses also may result from simultaneous stimulation of vagal and sympathetic afferents.

The application of capsaicin to the epicardium of the left ventricle consistently results in increases in blood pressure and heart rate. Studies have shown that there are pressor and depressor responses to topical or intracoronary administration of these chemical agents. Felder and Thames reported that topical application of bradykinin elicited inhibitory, excitatory, and biphasic responses. Excitatory and inhibitory reflexes were eliminated by cardiac sympathetic afferent denervation. Injection of capsaicin into the left circumflex coronary artery caused systemic hypotension and bradycardia, a pressor response associated with tachycardia, or a biphasic effect. The reflex hypotension and bradycardia were reversed to increases in blood pressure and heart rate after bilateral vagotomy. Our results support these findings, with the exception that changes in heart rate were minimal, probably as a result of the barbiturate anesthesia. Anesthesia in general can inhibit reflex responses, and barbiturates have been found to attenuate neurally mediated changes in blood pressure and heart rate. Although the changes in heart rate and blood pressure that we report are relatively small, they are qualitatively comparable to what others have reported. In some cardiac reflex studies, α-chloralose, which may accentuate certain reflexes, has been used. With chloralose, however, the level of anesthesia may be light, making it necessary to use other anesthetics and drugs for muscular relaxation. Thus, there may be additional confounding variables. In a pilot study, we used isoflurane, but we were not able to evoke cardiac chemoreflexes consistently. However, with a continuous infusion of pentobarbital, animals were well anesthetized, and the reflex changes in blood pressure and dP/dt were consistent; only the heart rate responses were variable. This could result from anesthesia, or perhaps from competing reflex responses.

Different results have been reported. In the study by Kwong et al., 2 weeks after TMLR treatment, hemodynamic responses to topical bradykinin were not seen after stimulation of laser- or phenol-treated areas, but a depressor response was seen after stimulation of untreated areas in the same animals. These results are different from what we observed acutely with a lower concentration of bradykinin and might be explained by the direct effects of bradykinin on the vasculature or by the different TMLR treatment and recovery process. Arora et al., however, reported that sympathetic and parasympathetic cardiac afferent neurons that were activated electrically or chemically by epicardial application of bradykinin or veratridine induced similar ventricular augmentation before and after TMLR. Since TMLR did not appear to alter afferent or efferent axonal function in the treated ventricle, they concluded that the efficacy of TMLR could not be ascribed to local denervation. Our results support this concept and, in addition, show that activation of chemosensitive vagal and sympathetic afferent pathways by capsaicin or bradykinin persists after TMLR.

Studies have shown clearly that vagal and sympathetic nerves are close to the surface of the heart in the region of the atrioventricular groove. Vagal fibers then go deeper into the myocardium as they follow the distribution of the coronary arteries. Sympathetic fibers are found close to the myocardial surface in their distribution to the ventricle and may be more accessible to topical stimulation. This may explain the sympathetically mediated response to capsaicin that was prevalent in our experiments. In our study, whether the responses were evoked by bradykinin or capsaicin, and whether the chemicals stimulated the epicardial or intracoronary receptors, pressor or depressor responses remained intact after TMLR treatment.

The thermal and structural damage to the myocardium during TMLR is proportional to the laser wavelength, and a relatively large increase in damage imposed by the holmium (Ho):YAG laser has been reported. We used a Ho:YAG laser in our experiments with a density of 1 laser channel per square centimeter of myocardial surface; however, cardiac reflexes remained intact after TMLR. This result does not imply that laser treatment would not damage nerve endings. However, because of multiple endings of sympathetic afferent fibers, creation of laser channels in a relatively small region of the left ventricle is unlikely to cause complete denervation. From our experiments, we conclude that the myocardium is not denervated after TMLR, because cardiac chemoreflexes (vagal and sympathetic) remained intact. Therefore, our evidence supports the notion that improvement of symptoms of angina after TMLR is not likely caused by the denervation of the heart.

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REFERENCES


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Thomas H. Cogbill, MD, La Crosse, Wis: Transmyocardial laser revascularization is emerging as a potential treatment op-