Pulsed Ultraviolet Laser Irradiation Produces Endothelium-Independent Relaxation of Vascular Smooth Muscle

P. Gabriel Steg, MD, Anthony J. Rongione, BA, Dov Gal, DVM, Stephen T. DeJesus, BA, Richard H. Clarke, PhD, and Jeffrey M. Isner, MD

Recent studies have shown that continuous wave laser irradiation induces contraction of vascular smooth muscle, except at powers far below the threshold for tissue ablation. To determine the corresponding effects of pulsed laser irradiation on vascular smooth muscle tone, vascular rings of rabbit thoracic aorta were mounted isometrically with 1 g tension in Krebs-bicarbonate buffer and irradiated with 308 or 351 nm from an excimer laser through a 400-μm optical fiber. A total of 250 exposures were performed with 1–6.5 mJ/pulse (fluence=0.8–5.5 J/cm²), 10–50 Hz, and cumulative exposures of 10–120 seconds. Excimer laser irradiation in combinations of pulse energy (PE), repetition rate (RR), and cumulative exposure below, at, or above threshold for tissue ablation consistently produced relaxation unassociated with contraction in each of the 250 exposures. For the total 250 exposures, the magnitude of relaxation (reduction in recorded tension, R_max) was 55±4% (mean±SEM) of maximum vasomotor reactivity recorded in the specimen in response to administration of serotonin. R_max varied directly with both PE and RR. When PE was increased from 1 to 5 mJ/pulse (n=13), R_max increased from 57±19% to 80±19% (p<0.0001); when RR was increased from 10 to 50 Hz (n=10), R_max increased from 27±8 to 46±8 (p<0.0001). R_max varied independently of endothelial integrity (assessed anatomically and pharmacologically) and wavelength (308 vs. 351 nm). Simultaneously recorded tissue-temperature profiles disclosed that during pulsed laser irradiation, tissue temperature rise did not exceed 5°C. Thus, in contrast to continuous wave laser ablation, pulsed laser irradiation does not cause contraction of vascular smooth muscle but instead induces a relaxation response. This appears to represent the net result of photoillumination, unaccompanied by significant tissue heating. The fact that excimer laser irradiation does not produce contraction of vascular smooth muscle could represent an important advantage for systems designed to accomplish vascular recanalization with laser irradiation. *(Circulation* 1989;79:189–197)

Previous in vitro experiments in our laboratory have shown that continuous wave argon (457–514 nm) laser irradiation of isolated segments of rabbit aorta at powers exceeding 1.0 W consistently produces contraction of vascular smooth muscle. Simultaneous recordings of tissue-temperature profiles indicated that such increases in vascular tone were consistently accompanied by a significant rise in tissue temperature. In these same experiments, however, continuous wave laser irradiation at lower powers (less than 0.1 W) was unaccompanied by a significant increase in temperature and consistently produced vascular smooth muscle relaxation. Similar light-induced relaxation of vascular smooth muscle was observed more than 30 years earlier by Furchgott et al., who used a monochromator to study the effects of selected wavelengths from 240 to 675 nm generated by a xenon arc lamp; maximum relaxation was observed in the ultraviolet portion of the spectrum.

Excimer lasers, which generate pulsed ultraviolet light, have been investigated for a variety of biomedical applications, including laser angioplasty. Although the intensity of light generated by pulsed ultraviolet lasers greatly exceeds that which can be generated from a filtered xenon arc lamp, such irradiance is nevertheless unaccom-
panied by significant tissue heating.\textsuperscript{10,11} Accordingly, we investigated the hypothesis that excimer laser irradiation would produce relaxation of vascular smooth muscle.

**Methods**

The descending thoracic aorta of New Zealand White rabbits (weighing 3–3.5 kg) was excised, trimmed free of adherent connective tissue, and divided into three to five 3-mm-long vascular rings free of side branches. Prepared in this fashion, such rings are typically between 3.0 and 3.5 mm in diameter and between 0.20 and 0.25 mm in wall thickness. A total of 33 rings were harvested in this fashion from a total of 12 rabbits. Each ring was then placed individually in a 50-ml water-jacketed glass bath containing 37\(^\circ\)C Krebs-bicarbonate buffer that was continuously gassed with 95\% O\(_2\)-5\% CO\(_2\) to ensure a pH of 7.4 and a Po\(_2\) greater than 500 mm Hg. The buffer was prepared daily and had the following composition (mM): NaCl 118, KCl 4.8, CaCl\(_2\) 2.5, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 24, glucose 11, and Na\(_2\)-EDTA 0.03. Each ring was then mounted isometrically by placing two 0.22-in. metal hooks through the lumen and closing them in a coat-hanger shape; the lower one was attached to the bottom of the bath, and the upper one was attached to a Grass FT03 force transducer (Quincy, Massachusetts) (Figure 1). The transducers were connected to a Grass 7P122D preamplifier, DC amplifier, and Model 5 polygraph to allow continuous recording of isometric tension. Throughout the mounting procedure, the samples were kept wet with buffer. For those ring segments intended for study with an intact endothelium, special care was taken to avoid traumatizing the ring’s endothelial surface. Conversely, to assess the potential role of the endothelium in mediating vasomotor reactivity resulting from excimer laser irradiation, a cotton swab was used to denude portions of the endothelium from selected aortic rings.

All samples were allowed to equilibrate for 1 hour while resting tension was adjusted to 1.0 g. The rings were then precontracted with \(10^{-7}-10^{-4}\) M 5-hydroxytryptamine (5-HT) and tested with \(10^{-8}-10^{-6}\) M acetylcholine (ACh) to confirm the presence or absence of functional endothelium. The samples were then rinsed with buffer and allowed to return to baseline and equilibrate for at least 30 minutes. Samples were irradiated with or without prior pharmacologic precontraction with \(10^{-7}-10^{-4}\) M 5-HT. When drugs were added to the bath (5-HT creatinine sulfate complex and ACh chloride; Sigma Chemical, St. Louis, Missouri), the additional volume was always less than 1 ml.

To confirm the pharmacologic assessment of endothelial function, aortic rings, which had been intentionally denuded, were placed in 0.1 M cacodylate-buffered 3% glutaraldehyde (pH 7.3) at the end of the experiment and then processed for examination by scanning electron microscopy as previously described.\textsuperscript{12} Briefly, each specimen was postfixed in 0.1 M cacodylate-buffered 1% osmium tetroxide (pH 7.3), washed, and dehydrated. After critical point drying, the specimen was coated with gold palladium (40:60) and examined with an Amray-1000 scanning electron microscope. Aortic rings with intact endothelium, which were adjacent to those irradiated with laser light, were also submitted for similar processing at the time of excision from the donor rabbit to confirm that harvesting of the rings had not resulted in significant endothelial disruption.

Those samples that were not processed for ultrastructural examination were fixed in 10% buffered formalin and processed for light microscopy to assess morphologic consequences of laser irradiation (specifically signs of thermal injury).\textsuperscript{13}

Laser irradiation was performed with two different excimer lasers. A Lambda Physik (Acton, Massachusetts) Model EMG 52 MSC excimer laser with a pulse duration of 12 nsec was filled with a mixture of XeF and helium gas to achieve an output of 351 nm.

**FIGURE 1.** Diagrammatic representation of fiberoptic delivery of laser light to the aortic ring segment in the bath chamber and probe positioning for simultaneous temperature recordings. The fiberoptic, attached to a delivery arm, is positioned in the lumen of the vascular ring. The temperature probe is positioned on the adventitial surface of the ring, on the same wall and opposite the luminal position of the fiberoptic.
A Summit Technology (Watertown, Massachusetts) UV-200 Excimer excimer laser with a pulse duration varying between 5 and 10 nsec was filled with a mixture of XeCl and helium gas to achieve an output at 308 nm. Repetition rate was varied between 10 and 120 Hz. The output beam was coupled to a fused silica optical fiber of 400-μm core diameter (Ensign-Bickford, Avon, Connecticut). The fiber was taped to an L-shaped delivery arm, which was lowered into the bath; alignment of the distal fiber tip and the vessel axis was thus made possible. The fiber was then advanced into the aortic ring (Figure 1). The orientation of the fiber tip with respect to the vessel axis was found to be critical to the development of a vasomotor response. When the impact of the beam exiting from the distal fiber tip was directed (without direct contact) toward the intimal surface, consistent vasomotor reactivity was observed. In contrast, coaxial alignment produced no vasomotor reactivity. Placement of the distal fiber tip just off the intimal surface of the aortic ring resulted in a spot size that was equivalent to that of the fiber core. The angle between the intimal surface and the distal fiber tip varied between 25 and 40°. Energy per pulse (calculated according to the average power delivered from the distal fiber tip) was measured with a Coherent power meter (Palo Alto, California) and was varied to achieve calculated pulse energies from 1.0 to 6.5 mJ/pulse. (Previous in vivo experiments in our laboratory confirmed that this range of pulse energies includes and exceeds the threshold for percutaneous in vivo, excimer laser ablation of atherosclerotic plaque.) Between laser exposures, samples were allowed to rest until the tension returned to a stable baseline value. Alterations in vasoreactivity observed in response to variations in pharmacologic precontraction, wavelength, repetition rate, and pulse energy were statistically analyzed with Student’s t test.

Analysis of Tissue-Temperature Profile

To investigate the extent to which the vector and magnitude of laser-induced vasomotion were related to temperature alterations in the laser-irradiated vascular ring segments, tissue temperature was monitored simultaneously with tension in selected specimens with a custom-designed insulated thermistor probe (Yellow Springs Instruments, Yellow Springs, Ohio) with a temperature range of −80° to +150 °C, a sensitivity of ±0.2 °C in the 0−70 °C range, and a response time of 2 seconds. The probe was precalibrated with a standard mercury thermometer; baseline was set at the 37 °C water bath temperature. The proximal end of the probe was interfaced with the Grass polygraph recorder. With regard to positioning of the distal end of the probe, pilot experiments performed in our laboratory indicated that temperatures recorded from the adventitial aspect of the vascular ring were similar to, and directionally tracked measurements made from the intimal surface. Furthermore, positioning of both the probe and optical fiber within the vascular ring was found to occasionally affect the signal recorded from the probe because of interference from fiber-mediated irradiation upon the probe tip. Thus, to accommodate these two issues, the distal end of the probe was placed in apposition with the adventitial aspect of the vascular ring, opposite from the intimal location of the fiber tip (Figure 1).

Results

A total of 250 exposures were preformed with 1.0−6.5 mJ/pulse (fluence = 0.8−5.5 J/cm²), 10−50 Hz, and cumulative exposures of 10−120 seconds. In each of the 250 exposures, excimer laser irradiation produced vascular smooth muscle relaxation. For the total 250 exposures, the magnitude of excimer-induced relaxation was 55±4% (mean±SEM) of maximum vasomotor reactivity recorded in the specimen in response to administration of 5-HT. The onset of relaxation was typically delayed for 2−10 seconds after the onset of laser irradiation. Relaxation then increased as a first order process and reached a plateau after approximately 20−30 seconds (Figure 2). After discontinuation of irradiation, relaxation decreased in an exponential fashion and returned to the baseline within several minutes.

The magnitude of relaxation varied directly with the repetition rate (Figures 3 and 4). To evaluate this parameter, a total of 30 exposures were performed at the same site, in the same specimen, and at a constant exposure (20 seconds) while varying the repetition rate incrementally at each of four different pulse energies (1,2,3, and 4 mJ/pulse). When repetition rate was increased from 10 to 30 Hz (n=12), magnitude of relaxation (as percentage of maximum tension developed in response to 5-HT) increased from 43±16 to 58±19 (p=0.0065). When repetition rate was increased from 10 to 50 Hz (n=10), relaxation increased from 27±8 to 46±8 (p<0.0001). Finally, the repetition rate was progressively increased from 10 to 50 Hz at 10-Hz increments (n=8): From 10 to 20 Hz, relaxation increased
from 16±3 to 24±3 (p=0.0051); from 20 to 30 Hz, relaxation increased from 24±3 to 29±4 (p=0.0511); from 30 to 40 Hz, relaxation increased from 29±4 to 33±4 (p=0.0213); and from 40 to 50 Hz, relaxation increased from 33±4 to 37±5 (p=0.0193).

The magnitude of relaxation also varied directly with the pulse energy (Figures 5 and 6). To evaluate this parameter, a total of 50 exposures were performed at the same site, in the same specimen, at a constant exposure (20 seconds), and at a constant repetition rate (10 Hz) while varying the pulse energy from 1 to 5 mJ/pulse. When pulse energy was increased from 1 to 2 mJ/pulse (n=17), the magnitude of relaxation (as percentage of maximum tension developed in response to 5-HT) increased from 39±10 to 47±10 (p=0.0027). When pulse energy was increased from 1 to 3 mJ/pulse (n=20), relaxation increased from 29±5 to 33±6 (p<0.0001). When pulse energy was increased from 1 to 5 mJ/pulse, relaxation increased from 57±19 to 80±19 (p<0.0001).

Functional and anatomic endothelial integrity of the harvested vascular rings was determined by the response to ACh (Figure 7) and by examination with scanning electron microscopy (Figure 8), respectively. Among the 33 rings, endothelial integrity was maintained in 24, whereas in the remaining nine specimens, the endothelium was manually denuded. Relaxation was consistently observed in each of the 33 vascular rings, regardless of the status of the endothelium. Thus, excimer laser-induced relaxation of vascular smooth muscle is an endothelium-independent phenomenon.

Relaxation was also observed whether or not the aortic specimen was pharmacologically precontracted before laser irradiation. Among 88 exposures at the same wavelength (351 nm) and repetition rate (10 Hz) throughout a range of pulse energies from 1 to 4 mJ/pulse, the magnitude of relaxation observed in 22 nonprecontracted exposures was not significantly different from that observed in 66 precontracted exposures (55±1 vs. 59±7; mean±SEM as percentage of maximum vasomotor reactivity induced in ring by 5-HT prelaser or postlaser irradiation; p=0.7395).
The magnitude of relaxation also did not differ significantly as a function of the wavelength. To evaluate this issue, 308 and 351 nm were used to perform 54 and 95 exposures, respectively, at a constant repetition rate throughout a range of pulse energy (10 Hz). The observed magnitude of relaxation (as percentage of maximum tension induced by 5-HT) measured 53±9 for 308 and 58±6 for 351 (p=0.1485).

Temperature measurements, which were performed simultaneously with excimer laser irradiation of the vascular ring segments, established that the rise in tissue temperature above the baseline bath temperature of 37°C ranged from 0°C to 5°C (mean±SEM=3±0.75) (Figure 9). The increase in tissue temperature followed a first order kinetic with an initially steep slope, followed by a diminished rate of rise. The higher the irradiance (achieved by increasing the energy per pulse or repetition rate or both), the higher was the temperature recorded; maximum increase in recorded tissue temperature (5°C) was achieved as a result of a combined increase in repetition rate (to 50 Hz) and pulse energy (5 ml). However, even among specimens subjected to such serial increases in repetition rate and pulse energy, the corresponding increases in tissue temperature were still insufficient to prevent incremental degrees of vascular smooth muscle relaxation. Furthermore, examination by light microscopy documented that in no case,
including those specimens irradiated with combined increments of pulse energy and repetition rate, did excimer laser irradiation result in thermal injury\(^\text{13}\) of the irradiated vascular rings.

**Discussion**

This study is the first to investigate the effects of pulsed ultraviolet laser irradiation on vascular smooth muscle reactivity. Excimer laser irradiation at two different wavelengths consistently produced relaxation of vascular smooth muscle. Furthermore, both the magnitude and vector of this response were independent of the presence or absence of a functionally and anatomically intact endothelium. These findings are consistent with the results of previous observations\(^\text{15–18}\) made with nonlaser sources to illuminate vascular smooth muscle. Experiments performed in the early part of this century demonstrated that the interaction of light and smooth muscle could result in diminished muscular tone. Furchgott and coworkers,\(^\text{2–4}\) however, were the first to show that such relaxation was not dependent on an aerobic environment, was not associated with irreversible injury to normal muscle function, and

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**Figure 8.** Scanning electron photomicrographs of endothelium from two different aortic ring segments. Independence of photorelaxation response of vascular smooth muscle to endothelial integrity was confirmed by assessment of anatomic status of the endothelium. Top panel: Excimer laser irradiation produced relaxation (lower pair of recordings in Figure 7) even though ultrastructural examination disclosed loss of anatomic endothelial integrity (original magnification, \(\times1,500\)). Bottom panel: Ultrastructural appearance of anatomically intact endothelium for comparison (original magnification, \(\times1,500\)).
most importantly, did not require the use of exogenous photosensitizing agents.

Like Furchgott et al.,2–4 we observed consistent vasomotor relaxation in rabbit aortic smooth muscle that was precontracted with a variety of pharmacologic agents. In contrast to the results reported by Furchgott et al., we observed significant vasomotor relaxation (up to 560 g tension or 33% of the maximum response to 5-HT) even when no pharmacologic precontraction was used. The basis for these differing outcomes is most likely the light source. Furchgott et al. performed their experiments with a xenon arc lamp; a grating monochromator was used to select out individual wavelengths throughout a range of 240–675 micrometers. Because the xenon arc lamp constitutes a relatively weak source of illumination in comparison with the laser and because the intensity is further diminished by filtering through the monochromator, the resultant intensities achieved were calculated to range from 1.0×10^-7 to only 3.5×10^-8 W/cm^2. In contrast, the fluences generated by the excimer lasers in the present series of experiments ranged from 6.6×10^7 to 4.0×10^8 W/cm^2/pulse. Such increased intensity of light appears to be responsible for relaxation observed in nonpharmacologically precontracted rings; further increases in the number of photons delivered to the tissue specimen, which were accomplished by increasing pulse energy or repetition rate, augmented relaxation in an incremental fashion. Variations in baseline relaxation were presumed to result from two factors: inherent differences in the robustness of vascular smooth muscle of the respective rings and slight variations in fibertip-vessel intimal geometry from one ring to another.

The intensity of tissue irradiation with the excimer laser in the present series of experiments was generated without a physiologically significant increase in tissue temperature. Previous investigations by Welch et al.19 have shown that heat generated as a result of each pulse of laser irradiation is absorbed by the intima and transferred to the adventitia by conduction. Temperature measurements in the present experiments were recorded at the adventitial surface and represent steady-state temperature due to accumulated laser pulses. This temperature would be expected to be slightly lower than the local transient temperature elevations at the irradiated intimal surface. This assumption is based on comparisons of intimal and adventitial temperature measurements recorded during laser irradiation with a metal-capped optical fiber.19 Nevertheless, the low temperatures recorded, together with the relatively long measurement intervals, suggest that the rise in average temperature at the adventitia was much lower for pulsed laser irradiation than was the rise in adventitial temperature recorded previously (up to 38° C) during continuous wave laser irradiation. This is because the energy profile of the excimer laser is pulsed; the number of pulses per second is sufficiently low, and the interval between pulses is sufficiently long to permit delivery of peak powers in the megawatt range without exceeding the thermal relaxation time of the irradiated tissue. Consequently, although both the pulse energies and repetition rates were above, at, and below the threshold for tissue ablation, the increase in steady-state adventitial temperature in no case exceeded 5° C.

These contrasting effects of pulsed and continuous wave laser irradiation on tissue temperature appear to constitute the principal determinant for the differing vasomotor responses observed after pulsed and continuous wave energy delivery. Whereas pulsed laser irradiation consistently produced vascular smooth muscle relaxation, continuous wave laser irradiation produced photorelaxation only at extremely low powers (<0.1 W), which were unassociated with a significant rise in tissue temperature.1 At powers greater than 1.0 W, the greater magnitude of tissue temperature rise resulting from continuous wave laser irradiation consistently overwhelmed light-induced relaxation; the net result was reproducible contraction of the vascular ring. The effect of temperature augmentation on vasomotor response has also been previously studied by Harkins and Henry20 with vascular ring preparations from rabbit aorta as well as human coronary arteries. Incubation in Krebs buffer heated to 48–50° C consistently produced strong, transient contraction; maintaining this temperature for 20 minutes resulted in irreversible abolition of vasomotor responsiveness (contraction as well as endothelium-dependent relaxation).

Previous investigations from our laboratory with native beam (rather than fiber-mediated) irradiation
of cardiovascular tissues showed that even with pulsed lasers, such as the excimer laser, pulse energy or repetition rate or both can be increased to a point at which aggregate irradiance exceeds the thermal relaxation time of the irradiated tissue; the increased tissue temperature resulting from such irradiation may be sufficient to produce classic thermal injury. In the present series of experiments, however, limits imposed by fiberoptic transmission of short-pulsed excimer laser light prevented investigation of such excessive irradiance. The use of alternative excimer laser-fiber systems designed to transmit at higher frequencies or pulse energies or both could conceivably increase tissue temperature sufficiently to produce thermal injury or vasoconstriction or both.

The mechanism responsible for light-induced relaxation of vascular smooth muscle remains uncertain. Furchgott et al. suggested that the aortic strip must contain some endogenous photosensitive material which is activated by the radiation, and this material in the activated state somehow leads to inhibition of some reaction necessary for the production of active contraction and actually compared the relaxing effect of light on vascular smooth muscle with "relaxation produced by the chemical relaxing agent NaNO_2." A reasonable candidate for such a putative endogenous photosensitive material would be endothelium-derived relaxing factor, the existence of which was first shown by Furchgott and Zawadzki 20 years later and the identity of which was subsequently established by Palmer et al. to be nitric oxide. In the present series of experiments, however, the fact that anatomically and functionally confirmed purposeful endothelial denudation failed to alter either the vector or vigor of the vasomotor response to laser light mitigates against a participatory role for endothelium-derived relaxing factor.

Several alternative proposals to explain the phenomenon of photorelaxation of vascular smooth muscle are intriguing but as yet have not been fully investigated. Because Ehrereich and Furchgott observed that light may alter the electrical properties of smooth muscle, they suggested that light may alter the permeability properties of the cell membrane and lead to diminished ability of the muscle to maintain tone. Vanhoutte proposed that photorelaxation of vascular smooth muscle may be related to the disulfide-sulfhydryl configuration of the cell membrane because ultraviolet light acts upon proteins to both cleave disulfide bridges and initiate sulfhydryl-disulfide exchanges. Finally, photorelaxation may involve direct conformational alteration in contractile proteins that is induced by the absorbed photons of the laser beam. Furchgott et al. did not favor this theory because of the latent period observed between the onset of laser irradiation and the onset of relaxation. The duration of this latent period, including that observed in the present study, may not be inconsistent with this theory, however, because intracellular protein dynamics may involve multiple interdependent events.

Light-induced photorelaxation does appear, however, to be a wavelength-dependent function. Findings in the present study with an excimer laser, together with previously reported findings at very low powers of argon laser light, confirm Furchgott's observation that the action spectrum for photorelaxation extends from the ultraviolet into the visible portions of the electromagnetic spectrum. More recent experiments carried out in our laboratory with an Nd:YAG (neodymium:yttrium, aluminum, garnet) laser at its fundamental wavelength of 1,060 nm and a holmium laser (2.1 nm wavelength) have indicated that this action spectrum does not extend into the near or far infrared wavelengths.

The findings of these in vitro experiments have potentially important clinical implications. Laser-induced contraction of vascular smooth muscle has indeed been observed with alternative systems in vitro and in vivo, both experimentally and as a complication of continuous wave laser irradiation for human peripheral and coronary laser angioplasty. Indeed, recent in vivo experiments by Barbieri et al. have confirmed the results of previous experiments performed in vitro indicating that the probe temperature of metal-tipped optical fibers used for laser angioplasty may exceed 350°C. Results of percutaneous, in vivo laser angioplasty carried out in our laboratory with atherosclerotic rabbits and microsine also confirm these in vitro observations: whereas angiographic evidence of spasm was nearly always observed during attempts to perform continuous wave laser angioplasty, excimer laser angioplasty consistently produced no angiographic signs of arterial spasm. Thus, the absence of increased arterial tone (in fact, the development of diminished arterial tone) accompanying laser irradiation of the vascular wall may constitute a distinct advantage of pulsed laser light for laser recanalization procedures.

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References


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