Ultrasonic Energy
Effects on Vascular Function and Integrity

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Background. Ultrasonic energy transmitted via flexible wire probes provides a new means of ablating atherosclerotic plaque. We studied the effects of ultrasonic energy (20 kHz) delivered via a ball-tipped wire probe on arterial vasomotor behavior in rabbit thoracic aortas in a perfused whole-vessel model.

Methods and Results. After preconstriction with phenylephrine (10⁻⁵ M) or KCl (60 mM), the effects of ultrasonic energy (0.7–5.5 W×60 seconds, 42–330 J) on arterial vasomotor behavior were measured using long-axis ultrasonic vessel imaging of the proximal (ultrasound-treated) and distal (untreated) control segments. The efficacy of plaque ablation at these same probe-tip power outputs was evaluated in atherosclerotic, human cadaver iliofemoral arteries. Ultrasonic energy caused dose (energy)-dependent relaxation in rabbit aortas after preconstriction with phenylephrine in arteries with endothelium (n=8) and without endothelium (n=8) (p<0.001 versus ultrasound treated at power outputs of 2.9 and 5.5 W). There was no difference in the relaxation dose responses between endothelialized and endothelially denuded segments (p=NS). Ultrasonic energy also caused significant relaxation (67±8%) after voltage-dependent preconstriction with 60 mM KCl. Temperature measurements revealed less than 1°C warming of the vessel wall during as long as 2 minutes of treatment at a power output of 5.5 W. Pathological examination showed no smooth muscle injury at (moderate) power outputs that caused arterial relaxation. At probe-tip power outputs of 2.9–5.5 W, ultrasonic energy recanalized two of two totally occluded cadaveric iliofemoral vessel segments. The ultrasonic ablation catheter was also demonstrated to cause arterial relaxation in a recanalized canine femoral artery in vivo.

Conclusions. Ultrasonic energy delivered via a flexible-wire probe produces dose-dependent, endothelium-independent smooth muscle relaxation capable of reversing both receptor-mediated and voltage-dependent vasoconstriction in vitro. At moderate power outputs, this relaxation response does not appear to be due to thermal effects or irreversible smooth muscle cell injury. This vasorelaxant effect of ultrasonic energy is also apparent in vivo, at doses that effectively ablate atherosclerotic plaque, and may improve the safety of arterial recanalization using ultrasonic energy. (Circulation 1991;84:1783–1795)

Ultrasonic energy delivered via a flexible probe is a new and potentially useful means of ablating atherosclerotic plaque.¹,² The clinical application of this technology to treat peripheral artery disease has recently been reported with encouraging preliminary results.³,⁴ In particular, this technique appears to be a promising method for the treatment of chronic total or subtotal occlusions.⁵ One of the important features of any device or technique used to treat obstructive arterial disease is the effect that the intervention has on arterial vasomotor behavior. Some devices, such as the “hot-tip” laser, the Rotablator, and even balloon angioplasty catheters, may induce arterial spasm, which can result in ischemia or infarction and theoretically may promote thrombus formation.⁶–⁸ Unlike these devices, ultrasonic energy appears to be capable of causing arterial relaxation. One prior report has suggested that this relaxation is endothelium dependent and presumably mediated by release of endothelium-derived relaxing factor (EDRF).⁹ If this ultrasound-mediated arterial relaxation can be further documented and characterized, it may enhance the acceptance and applicability of arterial recanalization using ultrasonic energy.

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The purpose of the present study was to evaluate in a detailed manner this phenomenon of ultrasonic energy-induced arterial relaxation, using a previously established\textsuperscript{10,11} perfused whole-vessel (ex vivo) model. The specific goals were to determine the dose-response characteristics (time and power output dependence) of arterial relaxation induced by ultrasonic energy, to determine whether this phenomenon is endothelium dependent, to determine whether this relaxation occurs at doses that are capable of plaque ablation, and to try to characterize the mechanism of this relaxation (e.g., whether it is secondary to thermal effects or smooth muscle cell disruption).

Methods

Ultrasound Device

These experiments were performed using a prototype device (Baxter Laboratories, Irvine, Calif.), with ultrasonic frequency of 20 kHz. The energy was transmitted to vascular target sites by a ball-tipped titanium wire probe (1.5F diameter wire with 2.0-mm ball tip, 30-cm length; Figure 1). Experiments were performed at probe-tip power outputs of 0.7, 2.9, 4.0, or 5.5 W. These probe-tip power outputs were measured (in pulsed mode) using a motor-generator test setup that was calibrated using a calorimetric reference standard. The ultrasonic power that was actually delivered to the blood vessel wall was not directly measured. For all of the reported experiments, the device was operated in a pulsed mode (50% duty cycle, 30-msec pulses). Unless otherwise stated, the ultrasonic energy was delivered for 60 seconds in each of the experiments, corresponding to energies delivered at the probe tip of 42, 174, 240, and 330 J at power outputs of 0.7, 2.9, 4.0, and 5.5 W, respectively. The g forces at the probe tip ranged from 0g to 82,000g, varying as a direct function of power output in watts.\textsuperscript{11}

Tissue Collection and Preparation

The thoracic aortas from 32 female New Zealand White rabbits (weight, 1–3 kg) were used for the series of experiments described below, with all studies carried out in compliance with the Stanford University Panel on Animal Laboratory Care guidelines. The rabbits were killed by cerebral concussion, and their thoracic aortas, approximately 4 cm in length, were dissected free. The arteries were mounted in the muscle bath by tying the proximal and distal ends of the vessel to the tapered and adjustable vessel attachment pieces using 2-0 silk suture, as shown in Figure 2. The arteries were suspended to approximate their original in vivo length. The vessels were bathed in physiological saline solution (PSS) with following composition of (mM) NaCl 118, KCl 4, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2, dextrose 5, NaHCO\textsubscript{3} 24, and Na\textsubscript{2}HPO\textsubscript{4} 1.2. pH was kept constant at 7.40–7.45, and the muscle bath solution was warmed to 36–37°C and aerated with 95% O\textsubscript{2}–5% CO\textsubscript{2}. All perfusates were heated to 36–37°C by passage through a capillary tubing network within the outer heating bath and then delivered to the proximal vertical port via polyvinyl chloride intravenous tubing.

To permit perfusion at physiological pressures and flow rates without side branch leakage, all of the small intercostal arteries arising from the posterior aspect of the rabbit aortas were cauterized using a miniature, battery-powered surgical cautery device (Accu-Temp, Concept, Inc., Clearwater, Fla.). The cautery is performed at a minimum distance of 0.5 mm from the lumbar artery takeoff to ensure that there is no thermal damage to the thoracic aorta. A previously described ultrasonic imaging technique\textsuperscript{10,11} was used to assess the segmental diameters of the rabbit aortas. This technique uses a high-frequency (10 MHz) ultrasound probe (Diasonics 200 RF) that is positioned approximately 1 cm above the artery, in contact with the muscle bath solution. The transducer head is aligned along the vessel so as to optimize the long-axis image of the upper and lower vessel walls. This system provides a uniform, sharp vessel wall image of at least 3 cm of vessel length at short focal distances (less than 1 cm). All ultrasonic images are recorded using a ¾-in. videocassette tape recorder (Sony U-matic, VO-5800). The long-axis ultrasound images are then analyzed using computerized edge-detection image processing to measure segmental internal vessel diameters. The resolution of this imaging and image processing system has been calculated to be ±0.22 mm.

Effects of Endothelium on Ultrasonic Energy–Related Relaxation

To determine whether ultrasonic energy–induced arterial relaxation was endothelium dependent, as suggested by Chokshi et al.,\textsuperscript{9} we compared ultrasonic energy relaxation dose responses in aortas with intact endothelium with those of aortas that had mechanical endothelial denudation. Mechanical endothelial denudation was performed by cannulating the isolated
Schematic of experimental apparatus. Vessel is mounted between proximal and distal vessel connectors. Ultrasonic probe is introduced into rabbit aorta via proximal central port.

Aortas with a small glass rod (2 mm o.d.) and gently rolling the vessel on moistened filter paper. This technique has previously been shown to have no effect on vasoconstrictor responsiveness. Aortas with intact (n=8) or denuded (n=8) endothelium were mounted in the muscle bath, perfused with PSS at 50–60 ml/min (perfusion pressure, 70 mm Hg), and allowed to equilibrate. The aortas were precontracted with phenylephrine (10⁻⁵ M). After precontraction, the ultrasound probe (catheter) was advanced into the aorta via the connector port. The proximal 5 mm of each vessel was treated with one 60-second ultrasound exposure at a probe-tip power output of 2.9 W. The diameter of this segment was recorded before and immediately after the ultrasound catheter treatment. This 5-mm-long segment was removed for histological studies that included light microscopy, transmission electron microscopy to assess smooth muscle morphology, and scanning electron microscopy to assess endothelial integrity. After remounting and reequilibration with phenylephrine, an ultrasonic energy, relaxation dose–response curve (1-minute exposures at probe-tip power outputs 0.7, 2.9, and 5.5 W) was obtained in the endothelium-intact and endothelially denuded vessels. For all of the experiments described, percent relaxation for a given arterial segment at any time (t) was defined as percent relaxation (t)=100× [measured diameter (t)–precontracted diameter]/[relaxed (baseline) diameter–precontracted diameter]. To determine whether the vasoconstrictor or vasodilator responsiveness was impaired by a moderate dose of ultrasound (power tip output, 2.9 W; 1-minute treatment), a separate set of experiments was performed in six rabbit aortas with intact endothelium. After precontraction with phenylephrine (10⁻⁵ M) and relaxation with ultrasound treatment, we examined the ability of ultrasound-treated (versus control) segments to recontract with phenylephrine (10⁻⁵ M, 1-hour perfusion) and then relax with nitroglycerin (10⁻⁴ M, 15-minute perfusion).

Relaxation After KCl-Induced Contraction

The ability of ultrasonic energy to reverse voltage-dependent vasoconstriction induced by KCl was assessed in four rabbit aortas with denuded endothelium. In these experiments, the aortas were mechanically denuded of endothelium (as above) and then precontracted with 60 mM KCl added to PSS. After equilibration contraction with KCl, the proximal vessel segment was treated with 1 minute of...
ultrasound at a probe-tip power output of 5.5 W (330 J). The distal (untreated) segment served as the control segment.

**Time Dependence of Relaxation**

An additional six rabbit aortas were used to study the time dependence of the relaxation responses at a constant power output. These aortas were precontracted with phenylephrine (10⁻⁵ M) and then treated with an increasing duration of ultrasound treatment (15, 30, or 60 seconds) at a probe-tip power output of 4.0 W (in pulsed mode), with reequilibration after each timed exposure. Internal vessel diameters in the treated segment were determined as described for the dose–response experiments.

**Vessel and Perfusate Temperature Measurements**

To evaluate whether the relaxation responses to ultrasonic energy might be due to thermal effects, temperature measurements of the perfusate and vessel wall were made during treated segments at the highest probe-tip power output. The perfusate and vessel wall temperatures were measured using a digital temperature probe (Dual Probe-Model 52, John Fluke Manufacturing Co., Everett, Wash.) with temperatures recorded every 15 seconds during 2 minutes of treatment at a probe-tip power output of 5.5 W (n=4).

**Histological Examination**

Scanning electron microscopy was used to assess endothelial cell integrity, and transmission electron microscopy and light microscopy were used to assess smooth muscle cell injury. For all of the histological studies, aortic ring segments were fixed in 1% glutaraldehyde in 0.067 M cacodylate buffer. As described above, a series of experiments was carried out using light microscopy and scanning and transmission electron microscopy to examine the effects of a 1-minute treatment at a probe-tip power output of 2.9 W in eight aortas with endothelium and in eight aortas after endothelial denudation. The presence or absence of endothelium in these segments was qualitatively graded (scanning electron microscopy) in a blinded fashion with a scale from 0 to 4+ (4+ representing complete endothelial denudation, and 0 representing a completely intact endothelial monolayer; see Table 1). Additional light and transmission electron microscopy (×1,000–28,000) was performed in aortic ring segments obtained from four vessels with and four vessels without endothelium after 1-minute ultrasound treatment at 5.5 W (two ring segments each from the control and ultrasound-treated segments). The integrity of the smooth muscle cells in the control segments and in the segments treated for 1 minute at probe-tip power outputs of 2.9 or 5.5 W was qualitatively graded using light microscopy in a blinded fashion as described in Table 2.

**In Vitro Recanalization Experiments**

Two atherosclerotic occluded (fibrocalcific) human iliofemoral arterial segments were obtained from two individuals at postmortem study. The arteries were dissected free within 24 hours of death and stored in normal saline at 3°C. The ultrasound probe was applied to the occluded lumen of the segments using a pulsed mode with a 50% duty cycle of 30 msec. Three different probe-tip acoustic power outputs were tested (0.7, 2.9, and 5.5 W). The probe was applied for 15 seconds at a time, during which 5 ml of normal saline was infused through a 7F guide catheter (20 ml/min) to prevent

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**Table 1. Scanning Electron Microscopy Results Demonstrating Endothelial Cell Integrity**

<table>
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<th>Subgroup</th>
<th>Ring segments examined (n)</th>
<th>Endothelial cell injury score</th>
</tr>
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<tr>
<td>Control segment, nondenuded</td>
<td>16</td>
<td>0/16</td>
</tr>
<tr>
<td>Ultrasound treated, nondenuded</td>
<td>16</td>
<td>0/16</td>
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<tr>
<td>Control segment, denuded</td>
<td>16</td>
<td>0/16</td>
</tr>
<tr>
<td>Ultrasound treated, denuded</td>
<td>16</td>
<td>0/16</td>
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Qualitative endothelial cell injury score: 0, intact endothelial cell monolayer; 1+, isolated endothelial cell loss (less than 5% of cells); 2+, patchy/focal areas of endothelial denudation (~5–25% of cells lost); 3+, large areas of endothelial cell denudation (~26–75% of cells lost); 4+, diffuse endothelial cell denudation (~76–100% of cells lost).

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**Table 2. Light Microscopy Results Demonstrating Smooth Muscle Cell Integrity**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Ring segments examined (n)</th>
<th>Smooth muscle cell injury score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control segment; output, 2.9 W</td>
<td>32</td>
<td>0/32</td>
</tr>
<tr>
<td>Ultrasound treated; output, 2.9 W</td>
<td>32</td>
<td>0/32</td>
</tr>
<tr>
<td>Control segment; output, 5.5 W</td>
<td>16</td>
<td>0/16</td>
</tr>
<tr>
<td>Ultrasound treated; output, 5.5 W</td>
<td>16</td>
<td>0/16</td>
</tr>
</tbody>
</table>

Qualitative smooth muscle cell injury score: 0, no evidence of any smooth muscle cell injury; 1+, isolated smooth muscle cell loss (less than 5% of cells); 2+, patchy/focal areas of smooth muscle cell lysis (~5–25% of cells lost); 3+, large areas of smooth muscle cell lysis (~26–75% of cells lost); 4+, diffuse smooth muscle cell injury (~76–100% of cells lost).
heating of the probe during its use. In this semiflexible system, the range of applied force was approximately 60±20g as determined by a dynamometer (PK Neuses Inc., Arlington, Ill.).

In Vivo Testing

A chronic fibrocellular stenosis was induced in a dog's superficial femoral artery by the following technique. First, a 3-ml specimen of autologous subcutaneous fat was removed from the femoral triangle. A pellet was created by wrapping the fat in a strip of gel foam 0.25 cm thick and 2 cm long. This pellet was then embolized to the canine femoral artery through a 7F angiography catheter. A baseline angiogram documented a femoral arterial obstruction. Twelve months after initial embolization, a repeat angiogram demonstrated a complete superficial femoral arterial obstruction. The ultrasound probe ensheathed in a 7F catheter was passed percutaneously through an 8F introducer to the femoral arterial obstruction under angiographic guidance.

Drugs

L-Phenylephrine hydrochloride was obtained from Sigma Chemical Co., St. Louis, Mo. All drugs were prepared fresh on the day of the experiments. Phenylephrine was dissolved initially in distilled water and then added in precalculated volumes (0.4 ml) to PSS to achieve the desired drug concentration in each perfusate.

Statistical Analysis

Analysis of variance (repeated measures, Fisher’s PLSD test) was used to compare the diameters of the control and ultrasound-treated vessel segments in rabbit aortas subjected to increasing doses of ultrasound energy and to compare the relaxation responses in the ultrasound-treated segment with varying durations of ultrasound exposure. A Student’s paired t test was used to compare the relaxation observed in the ultrasound-treated versus that in the control segment after precontraction with KCl (60 mM). Unless otherwise stated, data are presented as the mean±SEM.

Results

Endothelium Independence of Ultrasonic Energy–Induced Arterial Relaxation

Ultrasonic energy was found to cause dose (energy)-dependent arterial relaxation in aortic segments with and without endothelium (Figure 3). There was no significant difference in the relaxation dose–response effects of ultrasonic energy in vessels with endothelium compared with those without endothelium, with 4±2%, 31±3%, and 51±5% relaxation in the segments with endothelium and 11±5%, 33±3%, and 57±7% relaxation in segments without endothelium after 1 minute of treatment with probe-tip power outputs of 0.7, 2.9, and 5.5 W, respectively. The untreated control segment showed no relaxation at any of the outputs, suggesting that there is little longitudinal transmission of ultrasonic energy beyond the probe tip. The relaxation in the ultrasound-treated segment was significantly greater than that observed in the control segment at outputs of 2.9 and 5.5 W (see p values, Figure 3). Scanning electron microscopy demonstrated that the endothelium was intact in the nondenuded segments and confirmed the effectiveness of the mechanical endothelial denudation in the intentionally denuded segments (see Figure 4 and Table 1). An example of ultrasound-mediated arterial relaxation in an endothelially denuded segment is presented in Figure 5.

Effects of Ultrasonic Energy on Voltage-Mediated Vasosconstriction

Ultrasonic energy was also demonstrated to cause significant arterial relaxation in rabbit aortas con-
stricted by high concentration KCl (i.e., voltage-dependent vasoconstriction). The results of these experiments are summarized in Figure 6. After the introduction of 60 mM KCl into the perfusate, the aortas exhibited vasoconstriction, followed by significant relaxation after a 1-minute treatment with ultrasonic energy at a probe-tip power output of 5.5 W. Again, no relaxation was observed in the untreated control segment distal to the ultrasound probe tip.

**Time Dependence of Ultrasonic Energy–Mediated Relaxation**

Figure 7 summarizes the time (exposure) dependence of ultrasound-mediated arterial relaxation. At a constant ultrasound power output of 4.0 W, maximal relaxation was observed after 30 seconds of ultrasound treatment.

**Effects of Ultrasonic Energy on Vessel Wall and Perfusate Temperature**

The effects of ultrasonic energy on vessel wall and perfusate temperature are depicted in Figure 8. For these experiments, the ultrasonic energy was delivered at the highest power output (5.5 W) for time intervals ranging from 15 to 120 seconds. At perfusate flow rates of 50–60 ml/min, there was a less than 1°F increase in either the vessel wall or perfusate temperature during exposure to ultrasound.

**Effects of Ultrasonic Energy on Smooth Muscle Cell Integrity**

Both light and transmission electron microscopy were used to assess the effects, if any, of 1-minute exposures of ultrasound at probe-tip power outputs of 2.9 W (n=16) and 5.5 W (n=8) in nondiseased rabbit aortas. The histological findings are summarized in Table 2. In general, there was virtually no smooth muscle injury identified in the segments treated with 1-minute exposures at a power output of 2.9 W. In contrast, seven of the 16 ring segments (44%) treated with an output of 5.5 W for 1 minute showed patchy areas of medial smooth muscle cell necrosis. Representative findings from transmission electron microscopy are shown in Figure 9. To further evaluate whether the arterial relaxation observed after ultrasound treatment was due to irreversible smooth muscle cell injury, we also examined the ability of rabbit aortas to recontract with phenylephrine (10⁻⁵ M) and to relax after treatment with nitroglycerin (10⁻⁴ M) after a 1-minute exposure to ultrasonic energy at a probe-tip power output of 2.9 W. These results (n=6) are shown in Figure 10. The ultrasound-treated segments recontracted significantly after the ultrasound treatment, although contractile responses were mildly impaired compared with the control segment (p<0.05). However, the contractile response to phenylephrine after ultra-

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**Figure 4.** Scanning electron micrographs (original magnification, ×700) from nondenuded and denuded rabbit aortas. Panel A: Intimal surface from rabbit aorta treated with 1-minute exposure to ultrasound at a probe-tip power output of 2.9 W, demonstrating an intact endothelial cell monolayer (arrows point to intact endothelial cell nuclei). Panel B: Intimal surface of a rabbit aorta subjected to mechanical endothelial cell denudation, demonstrating complete loss of endothelial cells.
sound treatment was not significantly changed from the response before ultrasound treatment (p=NS by analysis of variance). The ultrasound-treated segment demonstrated significant relaxation to nitroglycerin, which did not differ from the responses seen in the control segment.

**In Vitro Recanalization in Cadaver Segments**

The application of the ultrasound probe at 0.7 W (probe tip) power output for as long as 60 seconds did not provide arterial recanalization or significant penetration of the surface of the first occluded cadaver arterial segment. This 1-cm occlusion was then successfully crossed after four 15-second applications of the ultrasound probe at a power output of 2.9 W. The second occlusion was recanalized in less than 15 seconds using ultrasound delivered at 5.5 W power output.

**In Vivo Testing**

After 90 seconds of ultrasound application, the arterial occlusion in the canine femoral artery was recanalized with pulsed wave ultrasound at 18 W power output from the acoustic horn or 5.5 W at the probe tip (Figures 11A–11C). After the vessel was recanalized, the angiogram revealed distal arterial spasm without evidence of embolization (Figure 11C). Subsequently, the ultrasound at the same power output settings (~5.5 W) was applied for 30 seconds in this constricted segment with a resultant improvement in the luminal diameter, suggestive of ultrasound-mediated arterial vasodilation (Figure 11D). Histology of this arterial segment demonstrated a patent lumen with minimal residual stenosis at the site of the fibrocellular lesion. There was evidence of mild fibrocellular intimal thickening in the distal segment that appeared to vasodilate after treatment with ultrasound. There was no evidence of perforation, thermal damage, blast injury, thrombosis in the recanalized segment, or any medial smooth muscle damage in the distal (treated) segment of femoral artery.

**Discussion**

Results of the present study demonstrate that ultrasonic energy at 20 kHz, delivered via a flexible wire probe, causes dose (energy)-dependent vasodilatation capable of reversing both receptor-mediated and voltage-dependent vasoconstriction. This vasorelaxant effect is observed at power outputs that were shown to effectively ablate atheromatous plaque.

It is interesting to speculate regarding the potential mechanisms of ultrasonic energy-mediated smooth muscle relaxation. The possibility that this phenomenon is mediated by the release of endothelium-derived vasorelaxant factor(s) was an initially appealing hypothesis because some forms of mechanical stimulation (e.g., air bubble stimulation) have been shown to cause endothelium-dependent arterial relaxation. However, in contrast to the findings of a previous study of this phenomenon using ring segments from rabbit aortas, the ultrasound-mediated smooth muscle relaxation in our model was not dependent on the presence of endothelium (Figures 4–6). Furthermore, the data presented demonstrating no difference in the ultrasound-mediated relaxation dose responses between vessels with and without endothelium suggest that ultrasonic energy at 20 kHz does not significantly promote the release of EDRF or other endothelium-derived vasorelaxant substances (e.g., prostacyclin). Ultrasonic energy at
the frequency and energies used in these studies appears to cause arterial relaxation via a direct effect on vascular smooth muscle.

One potential simplistic explanation of the reversal of vasoconstriction after ultrasonic energy administration is that ultrasonic energy severely injures or

**Figure 6.** Plot demonstrating ultrasonic energy–mediated relaxation of voltage-dependent vasoconstriction after precontraction of rabbit aortas with KCl. Application of ultrasonic energy (5.5 W for 1 minute) caused significant relaxation in ultrasound-treated segment without any effects in more distal untreated control segment.

**Figure 7.** Plot demonstrating time dependence of arterial relaxation due to ultrasound. In this context, exposure time refers to actual time that device was turned on in pulsed mode. Maximal relaxation was observed after approximately 30 seconds (power output, 4.0 W; pulsed mode). No further relaxation was observed with a treatment time of 60 seconds (n=6, p values as shown).
destroys the smooth muscle cells themselves. Relatively high-frequency (750 kHz) ultrasonic energy has been shown to cause vascular injury.\textsuperscript{14} For this reason, we carefully documented the histopathological effects of ultrasonic energy on smooth muscle (including transmission electron microscopy) and examined the ability of the arteries to reconstruct after ultrasound exposure. After 1-minute exposures at an output of 2.9 W, which caused significant arterial relaxation, there essentially was no histopathological evidence of smooth muscle injury or any statistically significant impairment of vasoconstrictor or vasodilator responsiveness (see Figure 3). These observations confirm that at least at moderate power outputs, ultrasound-mediated smooth muscle relaxation is not attributable to irreversible smooth muscle cell injury. At a higher power output (i.e., 5.5 W), a minority of the ultrasound-treated aortic segments demonstrated patchy smooth muscle cell lysis and impaired vasoconstrictor responses that could have contributed to the observed "vasorelaxant" effect. At even higher power outputs, smooth muscle injury or cell lysis could become the primary mechanism of arterial "relaxation" after exposure to ultrasonic energy.

The thermal effects of ultrasonic energy delivery could be another potential cause of altered vasomotor behavior. The effects of temperature on smooth muscle contractile function are complex. Cooling of arteries to temperatures significantly below 37°C will typically result in vasoconstriction, followed by relaxation when the artery is rewarmed to physiological temperature (37–38°C). There is some evidence that in certain vascular beds, further warming to 41°C can result in smooth muscle contraction.\textsuperscript{15} In our ex vivo model, we have observed no significant relaxation or contraction in rabbit aortas when the muscle bath temperature is increased from 37°C to 42°C (unpublished data). The temperature measurements made in the present study demonstrated that when the perfusate flow is maintained in a physiological range (\~60 ml/min), there is minimal perfusate or vessel wall warming during even prolonged exposure to ultrasonic energy at a power output of 5.5 W. These observations suggest that the ultrasound-mediated smooth muscle relaxation observed in our model cannot be reasonably attributed to thermal effects.

Smooth muscle relaxation has been previously described after the delivery of other modes of external energy, such as light. The ability of laser energy at wavelengths in the 310–520-nm range to cause smooth muscle relaxation has been described in several studies.\textsuperscript{16–18} This photorelaxation effect appears to be caused by a light-mediated activation of smooth muscle guanylate cyclase, with the heme moiety of soluble guanylate cyclase serving as a chromophobe and absorbing the laser light.\textsuperscript{17,18} The resultant increases in smooth muscle cyclic GMP then lead to vasodilatation via mechanisms similar to those seen with nitrovasodilators or EDRF.\textsuperscript{19,20} It is unlikely that the vasorelaxation observed with ultrasonic energy can be attributed to this specific mechanism (i.e., photorelaxation). However, we cannot exclude the possibility that ultrasonic energy may activate guanylate cyclase via a different mechanism.

Ultrasound-mediated smooth muscle relaxation may be caused by direct effects on the contractile proteins (actin–myosin complex). It is reasonable to speculate that the ultrasonic energy disrupts or
FIGURE 9. Transmission electron microscopic examination of varying effects of ultrasonic energy in rabbit aorta (original magnification, ×4,000). Panel A: Uninjured (control) segment with normal-appearing smooth muscle cells (Sm). Nu, cell nucleus. Panel B: Segment treated with 1-minute exposure to ultrasonic energy at a power output of 2.9 W, demonstrating normal-appearing Sm as well as intact endothelial cells on luminal surface. En, endothelial cell. Panel C: Segment treated with 1-minute exposure to ultrasound at a power output of 5.5 W, again demonstrating intact Sm. Panel D: Area of severe Sm injury with Sm lysis (arrows) in rabbit aorta treated with 1-minute exposure at 5.5 W. Cy, cytoplasmic fragment; pNu, pyknotic Sm nucleus.
fragments the relatively weak actin (polymer) filaments in the actin–myosin contractile apparatus.\(^{21,22}\) This hypothesis is supported by the fact that ultrasonic energy ("sonication") at a frequency of 20 kHz (same frequency as ultrasound ablation catheter) is a standard laboratory technique to reversibly fragment actin filaments.\(^{23-25}\) It is known to cell biologists familiar with this technique of sonication of actin filaments that the energy required to disrupt actin–actin bonds is substantially lower than the energy needed to disrupt cell membranes (personal communication, Dr. James Spudich), an observation that is compatible with the dose responses to ultrasonic energy that we observed in intact vessels. In addition, the actin filaments fractured by sonication have been shown to spontaneously reassemble (reanneal) into functional filaments, which could explain the ability of these vessels to recontract, albeit with some impairment of contractile responses, after a brief period of recovery (see Figure 3).\(^{23-25}\) It is less likely that the ultrasonic energy delivered would disrupt the relatively strong and continuously (rapidly) cycling, actin–myosin covalent bonds.\(^{26,27}\) Although actin filament disruption is an attractive hypothesis to explain the observations of the present study, we cannot exclude the possibility that the ultrasonic energy could disrupt other structural components of the smooth muscle cell’s contractile apparatus. As already mentioned, at high power outputs, ultrasound-mediated "relaxation" may be caused in large part by smooth muscle cell lysis.

We have also demonstrated that ultrasonic energy can cause vasodilation in vivo in a canine model of chronic femoral artery occlusion (Figure 3). In this case, there was evidence of femoral artery spasm in a mildly diseased segment distal to the site of recanalization, which was then reversed after a 1-minute exposure to ultrasonic energy delivered by a flexible probe/catheter. Although we have not performed a series of these experiments, the findings from this case suggest that the ex vivo observations of ultrasound-mediated relaxation in nondiseased (elastic) rabbit aortas may also occur in mildly diseased muscular arteries in vivo. The results of these experiments, however, do not allow us to determine with any certainty whether this relaxation response will occur in severely (atherosclerotic) diseased human coronary arteries.
Clinical Implications

The vasomotor consequences of any catheter-based revascularization technique may affect the short- and long-term efficacy and safety of the procedure. A number of the newer devices for arterial revascularization such as the hot-tip laser and the Rotablator have been reported to have a relatively high incidence of catheter-induced arterial spasm. Arterial spasm has also been observed after the application of continuous wave laser but is less frequent after the use of pulsed laser delivery. Moderate vasoconstriction is also commonly observed in the dilated arterial segment after balloon angioplasty. In all of these instances in which a revascularization device promotes vasoconstriction or spasm, there is the potential for acute complications secondary to vessel closure. It is possible that such vasoconstriction may result in turbulent blood flow and high shear rates, which may promote platelet aggregation and thrombus formation. Ultimately, the combination of vasoconstriction and enhanced thrombus formation could adversely affect short- and long-term results.

Ultrasonic ablation has been demonstrated to be a reasonably safe and effective means to treat occluded arterial segments in patients with peripheral vascular disease. The ability of ultrasonic ablation to safely recanalize totally occluded segments may be attributable, in part, to the relative tissue selectivity of this technique. That is, the mechanical ablation effect is much more pronounced in noncompliant (diseased) arterial segments than in normal, compliant arterial tissue. Although speculative, the observation that ultrasonic ablation may also be accompanied by arterial relaxation rather than vasoconstriction could further enhance the safety and efficacy of this technique. This may be of particular importance when the device is ultimately used in the less-forgiving coronary circulation. It is also possible that this vasorelaxant effect of ultrasonic energy could be useful in the treatment of severe vasospasm that is refractory to pharmacological interventions. It should be emphasized, however, that these studies were performed primarily in nondiseased peripheral arteries. Clinical studies will ultimately be required to assess the effects of ultrasonic energy on vasomotor behavior in diseased human coronary arteries in
vivo and to determine the importance, if any, of this ultrasound-mediated vasorelaxation effect.

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