Alteration of Left Ventricular Endocardial Function by Intracavitary High-Power Ultrasound Interacts With Volume, Inotropic State, and α1-Adrenergic Stimulation

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Background. High-power intracavitary ultrasound abbreviates left ventricular (LV) ejection duration, thereby decreasing mechanical LV performance, presumably by selective impairment of endocardial endothelial function.

Methods and Results. Effects of ultrasound were evaluated in the ejecting LV of anesthetized, open-chest dogs under different conditions of LV volume and contractile state and after mild selective α1-adrenergic stimulation. LV pressures, left atrial pressures, and regional segment lengths were measured in anterior and posterior midwall. A cylindrical ultrasound probe (0.9 MHz, 25 W) mounted on a catheter was inserted into the LV cavity through the apex and was activated for 4 minutes in each condition. In protocol A (n=7), LV volume was altered with caval vein occlusion and intravenous dextran infusion. The ultrasound probe was activated at low (4.1±0.9 mm Hg), mid (10.6±1.5 mm Hg), and high (17.9±1.8 mm Hg) LV end-diastolic pressure (EDP). Effects of ultrasound were less pronounced at higher EDP. For example, the time interval from end-diastole to peak (-)dP/dt decreased by 7.5±2.3% at low, 4.4±2.2% at mid, and 1.9±1.6% at high LVEDP (p<0.001). In protocol B (n=7), LV inotropic state was altered by slow intravenous infusion of low-dose calcium. The ultrasound probe was activated before and after calcium. Effects of ultrasound were less pronounced after calcium. Time from end-diastole to peak (-)dP/dt decreased by 8.4±3.1% at baseline and by 3.5±2.1% after calcium (p<0.001). In protocol C (n=7), activation of the ultrasound probe was performed at baseline and after mild selective α1-adrenergic stimulation (propranolol plus phenylephrine). Effects of ultrasound were similar at baseline and after propranolol but increased after phenylephrine. Time from end-diastole to peak (-)dP/dt decreased by 5.2±2.4% at baseline, by 5.3±1.9% after propranolol, and by 8.9±3.2% after phenylephrine (p<0.05).

Conclusions. Effects of intracavitary ultrasound, which are presumably mediated through modulation of endocardial endothelial function, were more important at low volume, lower calcium, and under mild selective α1-adrenergic stimulation. (Circulation 1995;97:1275-1285)

Key Words • cardiac function • diastole • endocardium • endothelium • contractility • phenylephrine

Endocardial endothelium (EE) modulates myocardial performance. In isolated cardiac muscle, selective damage of EE results in an immediate and irreversible abbreviation of isometric twitch duration with a slight concomitant decrease in peak twitch force, but with no significant change in maximal unloaded velocity of shortening (V_Tmax).1,2 Of the various physical, chemical, and pharmacological methods used to damage EE, irradiation with high-power, high-frequency, continuous-wave ultrasound (0.9 MHz, 25 W) seemed highly selective and most appropriate for in vivo application.2 It was subsequently shown that intracavitary ultrasound irradiation indeed affected in vivo ventricular function by shortening left ventricular (LV) ejection duration, decreasing systolic LV performance, and slightly accelerating LV pressure fall.3 The present study was designed to evaluate in anesthetized, open-chest dogs whether effects of ultrasound on EE modulation of LV performance interacted with modulation of LV performance by volume, inotropic state, and some aspects of humoral control. Therefore, we analyzed the magnitude of ultrasound effects at different LV volumes, after intravenous administration of calcium at low dose, and under mild selective α1-adrenergic stimulation with phenylephrine. Effects of ultrasound on LV performance, presumably mediated through EE, seemed more pronounced at low volume, lower calcium, and after low-dose phenylephrine.

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Methods

Experimental Preparation

The study was performed in adult mongrel dogs of either sex (n=30). The animals were premedicated with an injection of morphine sulfate (5 mg/kg s.c.) and anesthetized with a slow intravenous injection of a solution containing 5,5-diallylbarbituric acid (50 mg/kg), urethane (200 mg/kg), and 2-imidazolidolone (200 mg/kg). No additional anesthetics were required during the surgical procedure and experimental protocol. The trachea was intubated and artificial ventilation instituted, delivering oxygen-enriched air. Respiratory rate and tidal volume were adjusted to maintain arterial oxygen tension and pH within physiological limits throughout the experiment. A 0.9% saline solution was administered through the right femoral vein to compensate for perioperative fluid losses.

A left thoracotomy was performed in the sixth intercostal space. The pericardium was widely excised and the heart suspended in a pericardial cradle. A catheter including a high-fidelity micromanometer (Gaeltec, Dunvegan, Scotland) and a fluid-filled lumen connected to a Statham P231a transducer was advanced retrogradely through the left femoral artery into the LV cavity. The manometers were calibrated against a mercury column before the experiment. The zero was set at the level of the vertebral column. To correct for zero-line shifting, diastolic pressures of the micromanometer were matched with diastolic pressures of the fluid-filled catheter. Left atrial pressures were measured through a polyvinyl tube inserted into the left atrium through an incision in the left atrial appendage and connected to a Statham P231a transducer. A limb lead ECG was recorded throughout.

The right atrium was paced. A small sucking cup was fixed to the right atrial appendage. Two small rods in the wall of the cup were connected to an electrical stimulator (Grass S4, Quincy, Mass.) for bipolar stimulation of the appendage. Regional ventricular function was assessed with ultrasonic segment-length gauges, which were implanted in the anterior and posterior midventricle. Since we observed in a previous study that the anterior wall exhibited a nonuniform response to ultrasound from base to apex (i.e., a more pronounced response at the base than at the apex), we evaluated in the present study whether ultrasound also induced a nonuniform response of anterior and posterior midventricle. Each segment gauge was composed of two 5-MHz piezoelectric crystals with a diameter of 2 mm. Crystals were implanted approximately 1 cm apart through epicardial stab wounds and plunged into midwall. They were oriented in a circumferential direction, parallel to midwall fiber direction. The segment gauges were connected to a sonomicrometer amplifier system that was calibrated with time signals of known duration (Triton Technology Inc., San Diego, Calif.).

The EE was damaged with a custom-made ultrasound probe described previously.3 6 This system transformed a 25-W electrical signal into 0.9-MHz continuous-wave ultrasound. The probe was inserted into the LV cavity through a stab wound in the LV apex and secured in place with an epicardial purse-string suture. This suture fixed the coaxial cable at a 20-mm distance from the crystal so that the probe was positioned between the mitral papillary muscles below the insertion of the chordae tendineae. The positions of the probe and segment-length gauges were verified by autopsy at the end of the experiment.

Effects of Intracavitary Ultrasound Irradiation

Irradiation of the endocardial surface with a source of high-power (25-W), high-frequency (900-kHz), continuous-wave ultrasound has been shown to induce selective morphological damage of the EE. This selective damage of the EE resulted in an altered myocardial function both in isolated cardiac muscle and in the intact ejecting ventricle.3 In isolated cardiac muscle, selective damage of EE by ultrasound immediately and irreversibly abbreviated isometric twitch duration, with concomitant slightly decreased peak twitch tension. These effects were quantitatively and qualitatively similar to the effects of previously used methods of EE damage. With all these techniques, the EE was selectively damaged without damage to the subjacent myocardium. Light microscopy, transmission electron microscopy, and lucifer yellow studies revealed damaged EE with a morphologically intact myocardium.2 The myocardium was also functionally intact, as was evidenced by the absence of change in Vmax and by the preservation of a normal and unaltered inotropic response to high calcium.

In the ejecting ventricle, ultrasound induced changes that were quite analogous to the in vitro findings, i.e., decreased LV function induced by a selective decrease in LV ejection duration with no change in initial rate of pressure rise.3 These in vivo effects were shown to be transient and reproducible. Microscopic analysis of the LV revealed an intact myocardium. In a small area near the ultrasound probe, EE cells were clearly damaged. At a distance from the probe, dispersed granulocytes were attached to large areas of the EE, a phenomenon suggestive of EE dysfunction. The transient nature of the in vivo observations could be attributed to a transient inactivation of normal EE function by ultrasound irradiation. Interruption of the irradiation would allow rapid functional recovery, e.g., by remodeling of the EE layer secondary to a rapid resealing or spreading of EE cells over the small wounded areas. In addition, deposition of granulocytes and platelets might also have contributed to the rapid functional recovery. With higher acoustic power, effects on hemodynamics were more pronounced and not fully reversible (unpublished data). With these higher acoustic powers, irreversible microscopic damage was widespread throughout the LV, clearly establishing the relation between ultrasound-induced hemodynamic changes and morphological, selective destruction of EE.3 Changes in ventricular function with ultrasound were shown to be distinct from changes observed when the temperature of the heart was raised by extracorporeal warming, so that the observed ultrasound effects were not likely to have been induced by changes in temperature with ultrasound.3

These in vitro and in vivo data provided converging evidence that application of high-power, high-frequency, continuous-wave ultrasound induced transient and selective damage of normal EE function.
without morphological or functional damage to the myocardium.

**Experimental Protocol**

Effects of intracavitary ultrasound on LV performance were evaluated under different experimental conditions. Each intervention consisted of a 4-minute activation of the intracavitary ultrasound probe and was preceded by a stabilization period of at least 30 minutes at each condition. Measurements were obtained at baseline, at the first end expiration after the intervention, and after a recovery period of more than 10 minutes. After recovery, it was carefully checked that all measurements had returned to baseline.

Reproducibility and reversibility of ultrasound effects were demonstrated in a previous study and were further confirmed in three placebo dogs with an experimental protocol consisting of four consecutive and identical interventions. It appeared from these experiments that the intervention could be performed repeatedly, at least four times, with similar global and regional functional effects after each intervention. For example, the time interval from end diastole to peak (−)dP/dt decreased similarly with ultrasound in each intervention (from 210±15 to 195±19 msec for intervention 1, from 209±14 to 194±18 msec for intervention 2, from 210±15 to 195±20 msec for intervention 3, and from 210±17 to 195±19 msec for intervention 4).

Protocol A (n=7) evaluated the effects of intracavitary ultrasound irradiation at different LV volumes. Paced heart rate was elevated (mean, 140 beats per minute; range, 126–150 beats per minute) to suppress spontaneous heart rate at low LV end-diastolic pressure (EDP) and was kept unchanged throughout the experiment. LV volumes were altered by inferior caval vein occlusion and intravenous dextran infusion (Pfeiffer and Lange, Dormagen, Germany). Different LV volumes were referred to by their LVEDP. The preparation was allowed to stabilize at each LV volume, thereby allowing for time-dependent changes in LV contractility. Interventions were performed at low (4.1±0.3 mm Hg), mid (10.6±1.5 mm Hg), and high (17.9±1.8 mm Hg) LVEDP. In three additional dogs, interventions were performed in opposite sequence to exclude biasing of the results by protocol sequence. The interventions were performed at high (14.6±2.8 mm Hg), mid (8.8±1.2 mm Hg), and low (5.6±0.7 mm Hg) LVEDP. LV volumes were lowered by inferior caval vein occlusion and slow removal of blood.

Protocol B (n=7) evaluated effects of intracavitary ultrasound before and after intravenous injection of calcium chloride (CaCl₂). Heart rate was 125 beats per minute (range, 118–135 beats per minute). Interventions were performed in baseline conditions (LVEDP, 5.4±1.2 mm Hg) and after CaCl₂ (LVEDP, 5.5±1.3 mm Hg). CaCl₂ was administered in a slow intravenous infusion over 5 minutes in a dose titrated to reach a steady-state increase of peak (+)dP/dt of about 10%. The effective dose ranged from 5 to 10 mg/kg.

Protocol C (n=7) evaluated the effects of intracavitary ultrasound in the presence of slight selective α₁-adrenergic

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**Figure 1.** Tracings showing effects of intracavitary ultrasound (25 W, 0.9 MHz) on canine left ventricular performance at three loading conditions (protocol A). Left ventricular pressure (LVP, mm Hg), derivative of the LVP tracing (dP/dt, mm Hg/sec), and segment-length tracings (mm) in LV anterior and posterior midventricle are displayed at three loads. Low LV end-diastolic pressure (EDP) corresponded to a mean LVEDP of 4.1±0.3 mm Hg, mid LVEDP to a mean pressure of 10.6±1.5 mm Hg, and high LVEDP to a mean pressure of 17.9±1.8 mm Hg. Test tracings (dashed lines) after activation for 4 minutes of an intracavitary source of high-power, high-frequency, continuous-wave ultrasound were superimposed on baseline tracings (solid lines). After the intervention, the test tracing of LVP deviated from the baseline during ejection, and pressure fell occurred earlier. Segment length tracings shifted upward, with a decrease in systolic shortening and increase in early segment reextension, which was most apparent in the posterior wall. Minimum segment length occurred earlier in both segments. These effects were most pronounced at low LVEDP.
stimulation after β-blockade. Heart rate was 125 beats per minute (range, 103–146 beats per minute). Ultrasound activation was achieved under four consecutive experimental conditions: 1) baseline, 2) after mild β-adrenergic blockade with propranolol 0.1 mg/kg, 3) after increasing peak systolic LV pressure by about 10% through graded descending aorta constriction, and 4) during continuous intravenous administration of phenylephrine at a dose that induced a matched increase in peak systolic LV pressure of about 10%. The effective dose range was 0.20 and 0.60 µg·kg⁻¹·min⁻¹ (mean±SD, 0.40±0.16 µg·kg⁻¹·min⁻¹).

All measurements were obtained with respiration suspended at end expiration. ECG, pressure signals, and segment-length signals were recorded on an eight-channel electrostatic recorder (Gould ES1000, Gould Inc., Oxnard, Calif.) at a paper speed of 50 mm/sec and were simultaneously converted to digital data (Codas, DataQ, Akron, Ohio) at 2-msec intervals. dP/dt was calculated on digitized data with a seven-point smoothing algorithm. Except for left atrial pressures, which were measured on chart, measurements were made on digitized data graphically displayed on a screen. Data from five consecutive cycles were measured, and measurements were averaged.

End diastole was defined by the trough in the LV pressure tracing after atrial systole. If this was not readily apparent, end diastole was timed by the peak of the R wave on the ECG. Pressure at mitral valve opening was measured as the peak v-wave from the left atrial pressure tracing. Timing of mitral valve opening was determined by transposing the corresponding pressure onto the LV pressure tracing. Time intervals were measured from end diastole to peak (−)dP/dt, to mitral valve opening, and to minimum segment lengths.

Rate of isovolumic relaxation was assessed with peak (−)dP/dt, time interval from peak (−)dP/dt to mitral valve opening, and time constant τ. τ was calculated as the inverse slope of the linear relation of ln(pressure) versus time, assuming the asymptote of LV pressure to be 0 mm Hg and using LV pressures from peak (−)dP/dt to 5 mm Hg above mitral valve opening pressure.³⁵ Sample correlation coefficient yielded values of r>0.98.

Segment lengths were measured at end diastole and at mitral valve opening. Minimum systolic length was measured as the minimum length preceding or coinciding with peak (−)dP/dt. Fractional shortening was calculated as the percent segment length change from end diastole to minimum segment length. Early segment reextension was calculated as the change from minimum segment length to segment length at mitral valve opening and was expressed as percent of end-diastolic length. Negative values indicated segment

![Figure 2](http://circ.ahajournals.org/content/87/4/1278/F2.large.jpg)
shortening, and positive values indicated segment lengthening.

All data were analyzed by ANOVA for repeated measurements to determine effects of the different experimental conditions and effects of intracavitary ultrasound activation. Trial interaction between ultrasound and the different conditions was used to assess the influence of the different conditions on the magnitude of the effect induced by ultrasound activation. Statistical significance was accepted at a value of \( p<0.05 \). Data are expressed as mean±SD.

**Results**

**Protocol A**

Effects of intracavitary ultrasound were evaluated at three different LV volumes (low, mid, and high LVEDP) and are illustrated in Figure 1, which displays LV pressure, dP/dt, and segment length tracings of anterior and posterior midventricle. With volume loading and with increased LV diastolic pressures (baseline tracings, solid lines), rate of LV pressure rise increased, systolic LV pressure was higher, and LV pressure fall was delayed. Regional segment lengths were larger during diastole and systole, extent of shortening increased, and segment reextension occurred later. With ultrasound (test tracings, dashed lines), LV pressure fall occurred prematurely with negligible changes in peak systolic LV pressure. Regional segment lengths were larger during diastole and systole, extent of shortening decreased, and segment reextension before mitral valve opening was more pronounced. These effects of ultrasound were observed at all LV volumes but were more pronounced when LVEDP was lower.

Hemodynamic data are shown in Figure 2. Volume loading increased diastolic and systolic LV pressures, time from end diastole to peak \((-\)dP/dt, peak LV \((+)\)dP/dt, and peak \((-\)dP/dt. Ultrasound increased LVEDP at low volume but not at mid and high volume. LV systolic pressures did not change with ultrasound. Ultrasound decreased peak \((+)\)dP/dt at low volume. Duration of systole was measured as the time interval from end diastole to peak \((-\)dP/dt and decreased with ultrasound \((p<0.001)\). This decrease was more pronounced at lower EDP (trial interaction, \( p<0.001)\). Time interval from end diastole to peak \((-\)dP/dt decreased by 7.8±2.3% at low LVEDP (from 188±23 to 174±22 msec), 4.4±2.2% at mid (from 211±22 to 201±23 msec), and 1.9±1.6% at high LVEDP (from 220±29 to 217±29 msec). Tau decreased by 13.4±5.7% at low LVEDP (from 24±5 to 20±6 msec), 10.5±1.8% at mid (from 28±5 to 25±4 msec), and 0.3±1.7% at high LVEDP (from 34±6 to 33±7 msec) (Figure 3).

Volume loading increased anterior and posterior segment lengths throughout the cardiac cycle. Baseline fractional shortening was significantly greater in the anterior than in the posterior segment. Volume loading increased fractional shortening in both segments. Ultrasound increased minimum systolic segment lengths and slightly increased end diastolic segment lengths. Ultrasound decreased fractional shortening, especially at lower LVEDP (trial interaction, \( p<0.001\)). An increased early segment reextension was frequently observed with ultrasound, especially at lower LVEDP (trial interaction, \( p<0.05\)). There was no different regional response to ultrasound between anterior and posterior midventricle.

Effects of ultrasound on contractility were assessed with end-systolic pressure–length computations. Since peak LV pressure did not change with ultrasound, the observed increase in minimum systolic segment length with ultrasound represented a shift to the right of the
systolic pressure–segment length relation at that given pressure (Figure 4). This was assessed by the end-systolic pressure–length ratio. The change in this ratio with ultrasound was most pronounced at low LVEDP, suggesting a more pronounced effect on myocardial function of ultrasound at low LVEDP. Ultrasound decreased end-systolic pressure–segment length ratio for the anterior segment by 7.9±0.7% (from 6.3±0.5 to 5.8±0.4 mm Hg/mm) at low LVEDP, by 2.4±0.3% (from 8.2±0.3 to 8.0±0.2 mm Hg/mm) at mid LVEDP, and by 1.0±0.4% (from 9.9±0.7 to 9.8±0.9 mm Hg/mm) at high LVEDP. Similarly, ultrasound decreased end-systolic pressure–segment length ratio for the posterior segment by 8.6±0.7% (from 7.6±0.5 to 7.0±0.6 mm Hg/mm) at low LVEDP, by 3.0±0.5% (from 10.1±0.6 to 9.8±0.5 mm Hg/mm) at mid LVEDP, and by 0.8±0.4% (from 12.4±0.6 to 12.3±0.5 mm Hg/mm) at high LVEDP. Changes in end-systolic pressure–segment length ratio were significantly more pronounced at low LVEDP (trial interaction, p<0.01).

To exclude biasing of the results by protocol sequence, interventions were performed in reversed loading sequence, starting from high to low LVEDP, in three additional dogs. These experiments resulted in similar findings, and the hemodynamic effects of ultrasound were again most pronounced at low LVEDP. For example, the time interval from end diastole to peak (−dP/dt) decreased with ultrasound from 26±4 to 259±21 msec at high LVEDP, from 242±7 to 232±8 msec at mid LVEDP, and from 220±14 to 206±11 msec at low LVEDP. Hence, the more pronounced effect of ultrasound was not an artifact of the sequence of the experimental protocol.

Protocol B

This protocol evaluated effects of ultrasound at two inotropic states and is illustrated in Figure 5, which displays LV pressure, dP/dt, and segment-length tracings of anterior and posterior wall at baseline (left panels) and after CaCl2 administration (right panels).

![Figure 4](image)

**Figure 4.** Tracings showing pressure–length loops of the anterior and posterior midventricle at low, mid, and high left ventricular end-diastolic pressure (LVEDP) (protocol A). In each panel, two tracings are superimposed, representing the tracings at baseline (solid lines) compared with the ones observed after ultrasound (dashed lines). With higher volumes, the pressure–length loops shifted to the right with increased end-diastolic and minimum systolic lengths and an increased fractional shortening in both segments. LV pressures (LVP) also increased with volume load. Ultrasound increased segment lengths and decreased fractional shortening but did not alter LV pressures. Therefore, with ultrasound, the end-systolic pressure–length point shifted to the right. This effect is most pronounced at low volume.

![Figure 5](image)

**Figure 5.** Tracings showing effects of intracavitary ultrasound at two different inotropic states (protocol B). Left ventricular pressure (LVP, mm Hg), derivative of the LVP tracing (dP/dt, mm Hg/sec), and segment-length tracings (mm) in LV anterior and posterior midventricle are displayed at two inotropic states, baseline and after calcium administration. Test tracings (dashed lines) after the activation for 4 minutes of an intracavitary ultrasound of high-power, high-frequency, continuous-wave ultrasound are superimposed on baseline tracings (solid lines). The intervention at baseline conditions resulted in changes similar to those for protocol A with a decrease in ejection duration, an increase in LV pressure fall, an upward shift of the segment wall tracings with a decreased systolic shortening, and an increase in early segment reextension. These effects were significantly less after calcium administration.
CaCl₂ induced a slight increase in peak (+)dP/dt with no changes in peak LV pressure (baseline tracings, solid lines); end-diastolic and minimum systolic lengths decreased slightly. The ultrasound effects (test tracings, dashed lines) were similar to those observed in protocol A: LV pressure fall occurred earlier with no significant changes in LV pressure rise or in LV diastolic and systolic pressures. Anterior and posterior segment lengths increased during diastole and systole, fractional shortening decreased, and segment reextension before mitral valve opening was more pronounced. After CaCl₂, these effects were also observed, but they were all less pronounced.

Hemodynamic data are shown in Figure 2. CaCl₂ increased peak (+)dP/dt from 1,879±158 to 2,096±167 mm Hg/sec. Peak (-)dP/dt and LV pressures were hardly affected by the amount of CaCl₂ administered. Likewise, none of these LV hemodynamic data changed with ultrasound. In the dose used in this protocol, CaCl₂ did not alter time intervals or rate of LV pressure fall. Of note, time from end diastole to peak (-)dP/dt could be mildly shortened by CaCl₂, but this was not observed systematically, given the slight dose of CaCl₂ administered.

With ultrasound, time intervals decreased in both conditions, and this decrease was more pronounced at baseline than after CaCl₂ (trial interaction, p<0.01). The time interval from end diastole to peak (-)dP/dt decreased by 8.4±3.1% at baseline (from 226±12 to 207±10 msec) and by 3.5±2.1% after CaCl₂ (from 216±11 to 208±10 msec). Tau decreased by 11.1±4.8% at baseline (from 32±7 to 28±7 msec) and by 5.9±3.8% after CaCl₂ (from 31±7 to 29±6 msec) (Figure 3).

Protocol C
This protocol compared the effects of ultrasound at baseline, after slight β-blockade, under aortic constriction, and under mild selective α₁-adrenergic stimulation with phenylephrine. A representative example of the effects of ultrasound in the different conditions is illustrated in Figure 6. Ultrasound decreased time to peak (-)dP/dt. This effect was similar at baseline, after mild β-blockade with propranolol, and after aortic constriction but was more pronounced under phenylephrine infusion.

Hemodynamic data are shown in Figure 2. Propranolol (0.1 mg/kg) increased the time interval from end diastole to peak (-)dP/dt (p<0.01). LV diastolic and...
systolic pressures and peak dP/dt did not change significantly. Aortic constriction and phenylephrine infusion induced an additional increase in time to peak (−) dP/dt and increased peak LV pressure with 10%. This increase was matched for both conditions. The change of LV systolic pressure waveform also was similar in both conditions. Isovolumic relaxation time and time constant tau increased with propranolol and increased further with aortic constriction and phenylephrine (p < 0.05). The time interval from end diastole to peak (−) dP/dt decreased with ultrasound (p < 0.01). This effect was comparable at baseline (5.2±3.4%, from 210±13 to 199±10 msec), after propranolol (5.3±1.9%, from 225±10 to 213±11 msec), and under aortic constriction (5.1±2.4%, from 231±14 to 218±18 msec) but was significantly higher under phenylephrine (8.9±3.2%, 234±11 to 216±15 msec) (trial interaction, p < 0.05). Tau decreased by 9.8±3.4% at baseline (from 25±4 to 21±3 msec), 10.0±3.6% under propranolol (from 30±6 to 27±5 msec), and 9.6±4.2% under aortic constriction (from 35±5 to 32±6 msec) but by 15.3±3.8% in the presence of phenylephrine (from 35±6 to 30±6 msec).

**Discussion**

Previous in vitro and in vivo studies demonstrated that direct irradiation with high-power, high-frequency, continuous-wave ultrasound induced a selective morphological damage of the EE without morphological damage of subjacent myocardium. It was hypothesized that the observed changes in myocardial function with ultrasound were due to EE damage, and hence, that EE had a functional role in the regulation of myocardial performance. The transient nature of the myocardial effects with ultrasound observed in the in vivo studies was similar to what had been observed in isolated muscle when the endocardium was only partially damaged. Therefore, it was suggested that suppression of normal EE function in vivo was only partial. EE has been proposed to be capable of rapid rescaling with spreading of cells over small wounded areas of the EE. Deposition of platelets and granulocytes and other circulating substances in the blood could also be involved in the rapid functional endocardial regeneration in the in vivo experiments. The exact mechanisms by which ultrasound can influence myocardial function through its effects on EE are still unknown. The experiments still do not allow distinction between transient endocardial functional damage, changes in release of endocardial substances with myocardial effects, and altered endocardial receptor function. In addition, it cannot be excluded that vascular endothelium was damaged as well and also released substances with inotropic effects.

The results from the present study suggest that modulation of myocardial function by ultrasound interacts with heterometric and homeometric autoregulation of LV function and with α1-adrenergic agonism with phenylephrine.

The effects of ultrasound were significantly more pronounced at low volume. This finding indicated that the EE-mediated effects of ultrasound on LV performance were more important at low volume, and hence, that myocardial function could be particularly dependent on EE integrity when LV volume is decreased. Since the exact mechanisms by which EE modulates LV performance are still unknown, it is as yet speculative to explain why modulation of myocardial function by EE can be altered by LV volume. In vitro experiments, it appeared that changes in twitch pattern induced by EE destruction (i.e., decreased twitch duration with minor changes in the rate of force development) were similar to changes induced by acute beat-to-beat reduction of initial fiber length at each calcium. This observation suggested a common underlying mechanism. It could be that, as for length modulation of myocardial function, this endocar-
Calcium dependence of the effects of ultrasound is supported by the present data, because the magnitude of the effects of EE damage by ultrasound on LV function was highest at the lower calcium. The physiological basis of this protective action of high calcium against EE-mediated changes in myocardial function remains to be established. It has been shown that calcium may help to preserve functional and structural integrity of cellular membranes as well as of intercellular connections. Sealing processes also appear to depend largely on calcium, suggesting that the protective action of calcium might also be based on its membrane-stabilizing properties. Whether this is the only effect of calcium or whether some interaction between modulation by EE and calcium metabolism downstream to the EE cells is involved still is under investigation. It could be postulated that ultrasound might possibly cause unloading of calcium from the sarcoplasmic reticulum, an effect that would be overcome by higher calcium levels. However, the present in vivo data corroborate the results obtained in vitro on isolated papillary muscles: physiological effects of EE removal also depended on the amount of extracellular calcium. This phenomenon was also apparent with other methods of in vitro EE damage (such as brief immersion in Triton X-100 [a detergent] or rubbing of the endocardial surface) that could not have directly affected sarcoplasmic function. These data indicate that calcium modulation of endocardial function was not dependent on the ultrasound technique.

The effects of ultrasound on LV performance were significantly more pronounced under phenylephrine infusion. The causes for this phenomenon remain to be established. It has to be ruled out that the observed effects were caused by increased peripheral resistance and systolic pressures with phenylephrine. The effects under phenylephrine were therefore compared with the effects after similar changes in LV systolic pressure obtained by slight constriction of the descending aorta. As is apparent from Figure 6, both aortic constriction and phenylephrine infusion induced similar changes in systolic LV pressure values and systolic LV pressure waveform. Changes in time intervals, rate of LV pressure fall, and segment length values were not different from the ones observed under phenylephrine. Yet, the effect of ultrasound was significantly higher under phenylephrine than under aortic constriction. This difference suggested that the greater effects of ultrasound under phenylephrine were not likely to be caused by the effects of ultrasound on systemic hemodynamics but rather by an interaction of the EE-mediated effects of ultrasound and the effects of phenylephrine. This suggested that at least part of the inotropic activity of phenylephrine might be mediated through EE. Comparison of pressure increases induced by aortic constriction and phenylephrine has its limitations. However, the similar LV response to ultrasound before and after aortic constriction indicated that changes in systolic load did not affect magnitude of endocardial modulation significantly. Subtle differences between aortic constriction and administration of phenylephrine with regard to reflected waves and systolic LV wall stress, therefore, are not expected to explain a different response to ultrasound. In addition, these in vivo results corroborate the in vitro data, which demonstrated that part of the inotropic effects of phenylephrine were mediated through EE. Another issue is that phenylephrine...
may also exert an effect on coronary circulation that might influence the response to ultrasound. However, the similarity of the present in vivo findings to the in vitro results provides circumstantial evidence that the magnitude of the effects of ultrasound depends on phenylephrine and not on the effects of ultrasound on coronary circulation. In addition, phenylephrine-induced changes in coronary flow might be expected to alter regional myocardial function, a phenomenon that was not apparent in our observations.

The mechanisms by which α₁-agonists induce their positive inotropic response at the myocardial level are still not fully elucidated. A number of possible mechanisms have been suggested, such as an increase in the amplitude of the intracellular calcium transient, 20 probably by an increase in calcium influx 21, 22 but also by an increase in the responsiveness of the myocardial contractile proteins to calcium. 23 Facilitation of the phosphoinositide turnover, 24-26 resulting in release of inositol 1,4,5-triphosphate and activation of protein kinase C, have also been suggested. 27 How these suggested mechanisms of positive inotropism at the level of the myocytes could be part of a cascade of EE-dependent events triggered by low concentrations of phenylephrine remains to be elucidated.

It is of interest to note that, except at relatively high concentrations of phenylephrine, no inotropic response has been found in isolated ventricular preparations of normal humans. 28 This lack of responsiveness was also reported in isolated cardiac muscle from patients with chronic heart failure. 29, 30 In view of the results of the present study, it is to be considered that this absence of findings could be the mere reflection of some EE damage secondary to the isolation procedure of the preparations or to EE functional impairment in congestive heart failure.

In conclusion, this study evaluated the effects of high-power, high-frequency, continuous-wave ultrasound at different LV volumes after low-dose calcium and in the presence of mild selective α₁-adrenergic stimulation. It appeared that effects of ultrasound on LV performance were significantly higher at low volume, in the presence of lower calcium, and under α₁-adrenergic stimulation with low-dose phenylephrine. Previous in vitro and in vivo studies linked these effects of ultrasound to a specific transient alteration of normal EE function. The present study, therefore, suggested that this EE-mediated effect of ultrasound might interact with regulation of myocardial function by volume, by calcium, and by low-dose phenylephrine.

It is essential to keep in mind that the intracavitary ultrasound technique used in the present experiments is significantly different from the technique used in the clinical setting for ultrasound imaging of the heart. The present ultrasound wave emits at 900 kHz in continuous-wave mode and has an electrical power density of 5 W/cm². Commercially available transducers use pulsed waves with frequencies between 2 and 10 MHz and spatial peak temporal average intensities <100 mW/ cm², the intensity below which no significant biological effects have been observed in mammalian tissues. 21 This peak intensity, however, is applied only for a fraction (e.g., 0.1%) of total time.

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References


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