Effect of Dysfunctional Vascular Endothelium on Myocardial Performance in Isolated Papillary Muscles

Kai Li, J.L. Rouleau, L.J. Andries, and D.L. Brutsaert

Vascular endothelium has been shown to modify the contractile characteristics of vascular smooth muscle, and endocardial endothelium has been shown to modify the contractile characteristics of adjacent myocardium. In this study, whether vascular endothelium also modifies the contractile characteristics of adjacent myocardium and whether these effects are additive to those of endocardial endothelium were investigated. Rabbit hearts (n=54) were excised and mounted in a Langendorff preparation. Vascular reactivity was verified by acetylcholine infusion. One group of these hearts had Triton X-100 injected as a bolus into the coronaries to render the vascular endothelium dysfunctional. The other portion served as control hearts. Triton X-100 bolus injection resulted in little or no pathological changes on morphological examination; however, the vasodilatory response to acetylcholine in these hearts was abolished, suggesting vascular endothelial dysfunction. Vascular smooth muscle reactivity was verified in Triton X-100-injected hearts by nitroprusside infusion. In the control Langendorff-perfused hearts, there was little evidence of vascular endothelial dysfunction, with the coronary perfusion rate increasing from 8.9±0.4 to 11.0±0.3 ml/g per minute (p<0.01) in response to acetylcholine. All hearts were then removed, and right ventricular papillary muscles were excised for myocardial mechanical studies. Control Langendorff-perfused hearts had myocardial mechanical characteristics similar to those of muscles from 18 other control hearts without Langendorff perfusion, indicating that the Langendorff perfusion itself had little effect on myocardial mechanics. The muscles from the Triton X-100-injected Langendorff hearts had marked changes: a shortening of twitch duration (3.63±1.16 versus 4.49±3.9 msec, p<0.01) and decreases in total tension (2.17±0.2 versus 2.49±0.2 g/mm², p<0.01), dT/dt (9±1 versus 12±1 g/mm² per second, p<0.05), and maximum velocity of unloaded muscle shortening (V_m) (0.89±0.06 versus 1.14±0.07 length at which maximum developed tension occurred [L_mmax/sec, p<0.05]). Endocardial endothelial removal of the papillary muscles in the two control groups (with and without Langendorff perfusion) by Triton X-100 caused the same changes in twitch characteristics as occurred in muscles from the Langendorff-perfused hearts injected with Triton X-100 but with intact endocardial endothelium, suggesting that vascular endothelial dysfunction had similar effects on contractile characteristics as endocardial endothelial removal. Endocardial endothelial removal of the papillary muscles from Langendorff-perfused hearts that had a bolus injection of Triton X-100 caused further shortening of twitch duration and a further decrease in total tension (1.7±0.1 versus 1.0±1.7 g/mm², p<0.05), dT/dt, and V_m (0.69±0.03 versus 0.89±0.01 L_mmax/sec, p<0.01), suggesting that the myocardial contractile effects of endocardial and vascular endothelium are additive. Increasing extracellular calcium concentration to 15 mM normalized dT/dt and V_m, such that these parameters were similar in all groups of muscles before and after vascular and endocardial endothelial dysfunction. Total tension normalized completely only at higher extracellular calcium concentrations (20 and 25 mM). The addition of 10⁻⁵ M phenylephrine to muscles with and without vascular endothelial dysfunction in a bath with 15 mM calcium normalized tension, also suggesting that the changes documented in this study were not due to myocardial damage but to endothelial dysfunction. These results suggest that both endocardial and vascular endothelium modulate the contractile characteristics of their adjacent myocardium and that their effects are additive.

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KEY WORDS • vascular endothelium • endocardium • contractility • myocardium

The endocardial endothelium, the internal lining of the cardiac chamber, has been shown to modulate the contraction of adjacent myocardium. Removal of this endocardial endothelial layer modifies twitch configuration in a typical manner, i.e., decreasing time to peak tension and thus decreasing tension development. This effect of endocardial endothelium on adjacent myocardium has been confirmed by others in isolated papillary muscles from various species.

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such as cat, dog, rabbit, and ferret. In addition, the presence or absence of this endocardial endothelial layer has been shown to greatly modify the effects of platelets, serotonin, eosinophils, vasopressin, endothelin, and other circulating substances on the contractile characteristics of isolated papillary muscles. Vascular endothelium modulates vascular tone by the release of varying contractile and vasodilating substances. Several of these vasoactive substances, such as endothelium-dependent relaxation of various vessels, have also been shown to have direct myocardial effects. The exact mediator or mediators that lead to the modulatory effects of endocardial endothelium on its adjacent myocardium have not been conclusively identified; however, they appear to enhance myocardial performance by increasing myofilament calcium responsiveness. One recent study suggests that contractile proteins are also regulated by substances produced by blood vessels; however, it is unknown to what extent coronary vascular endothelium modulates the contractile characteristics of the immediate adjacent myocardium, particularly in the microvascular bed. Hence, as vascular endothelium and endocardial endothelium constitute one continuous stretch of tissue, it would be of interest to examine whether and to what extent vascular endothelium modulates the contractile characteristics of the adjacent myocardium and to what extent these effects are additive to those of endocardial endothelium.

Therefore, we examined the cardiac performance of isolated papillary muscles from Langendorff-perfused rabbit hearts in which the vascular endothelium had been experimentally made dysfunctional by intracoronary Triton X-100 bolus injection, and we compared the performance of these muscles with muscles from Langendorff-perfused rabbit hearts in which no Triton X-100 bolus injection had been given. The results obtained from papillary muscles of the Langendorff-perfused hearts were then compared with those obtained from hearts in which no Langendorff perfusion was done. The effects of damaging the endocardial endothelium by brief immersion of the papillary muscles in Triton X-100 were then evaluated in all muscle groups. Our findings suggest that the effects of damage to either the vascular or endocardial endothelium by Triton X-100 result in a similar modulation of the contractile characteristics of adjacent myocardium: abbreviation of twitch duration and a decrease in developed tension. Our results also suggest that the effects of damage to both of these endothelial layers are additive.

Materials and Methods

Fifty-four rabbits of either sex weighing 2.5±0.3 kg were used in the study. The rabbits were anesthetized with pentobarbital (25–40 mg/kg) and anticoagulated with heparin (3,000 units/kg). The chest was opened and the heart was excised and used either for immediate excision of a right ventricular papillary muscle (group 1, 24 hearts) or for the Langendorff preparation (groups 2 and 3, 30 hearts).

Langendorff Preparation

Once excised, the hearts were mounted in a Langendorff preparation with a perfusion pressure of 60 mm Hg. The hearts were perfused with Krebs-Henseleit solution containing (mM) NaCl 118, KCl 3.5, MgSO4 2.43, CaCl2 1.25, KH2PO4 1.2, NaHCO3 24.9, and dextrose 5.0. The solution was kept at a temperature of 37°C and bubbled with 95% O2–5% CO2 gas mixture at pH 7.4. The superficial veins and coronary sinus were then perforated, and the right atria were removed to minimize penetration of the bolus injection of Triton X-100 (Scientillar Mallinkrodt Inc., Paris, Ky.) into the right ventricle via venous return.

Protocol A. The hearts were permitted to stabilize for 25 minutes, at which time the coronary perfusion rate was assessed by accumulating perfusate over a 2-minute period and then dividing by 2 (Figure 1). An acetylcholine (Sigma Chemical Co., St. Louis, Mo.) infusion was then started to assess endothelium-dependent relaxation of the coronaries. Acetylcholine was delivered by a pump (Harvard Apparatus, South Natick, Mass.) and mixed with the perfusate just above the aortic valve at a rate calculated to deliver a final concentration of 5×10−7 M to the coronaries. This final concentration was obtained by adjusting the infusion rate of the 10−3 M stock solution of acetylcholine to the coronary perfusion. Coronary perfusion was then calculated by collecting coronary effluent. In two hearts, acetylcholine did not cause an increase in coronary effluent. These two hearts were discarded because of the risk of endothelial dysfunction.

Protocol B. Hearts were then allocated randomly to either saline (group 2) or Triton X-100 (group 3) bolus injections (Figure 1). Pilot experiments using Triton X-100 in concentrations of 1:1000, 1:500, 1:200, and 1:100 were studied both in rat and rabbit hearts to establish the minimal concentration of Triton X-100 necessary to abolish the coronary vasodilatory response to acetylcholine. A 1:200 concentration injected over a 1-second period was found to be the minimal effective concentration. The amount injected was 1% of the coronary perfusion rate per minute. This was injected into the aorta just above the coronary arteries. In those hearts randomized to saline, an equivalent amount of saline was injected in the same manner. Twenty-five minutes later, the effects of the Triton X-100 bolus on

![Figure 1. Diagram summarizing the protocol. Protocol A: Effect of acetylcholine (Ach) on coronary flow before the bolus injection of Triton X-100 or saline. Protocol B: Effect of Ach on coronary flow once saline (group 2) or Triton X-100 (group 3) had been injected. Protocol C: Mechanical studies of papillary muscles from rabbit hearts never having had Langendorff perfusion (group 1) or having had Langendorff perfusion and intracoronary saline (group 2) or Triton X-100 (group 3). Protocol D: Papillary muscle mechanical studies after endocardial endothelial removal by Triton X-100.](http://circres.ahajournals.org/doi/abs/10.1161/01.CIR.82.3.769)
coronary perfusion rate was evaluated by collecting the coronary effluent. The effects of acetylcholine on coronary perfusion rate were then reevaluated 5 minutes later in both saline-treated and Triton X-100-treated hearts to assess the ability of the vascular endothelium to produce coronary vasodilatation.

The ability of coronary vascular smooth muscle to vasodilate after the Triton X-100 injection was verified by nitroprusside infusion before and after Triton X-100 in six Langendorff-perfused hearts. Nitroprusside was delivered by Harvard pump and mixed with the perfusate just above the aortic valve at a rate calculated to deliver a final concentration of 3 × 10⁻⁹ M to the coronary. This final concentration was obtained by adjusting the infusion rate of 1.7 × 10⁻⁴ M stock solution of nitroprusside to the coronary perfusion. Coronary perfusion was then calculated by collecting coronary effluent.

**Isolated Papillary Muscle Studies**

Once the 17 hearts not used for the Langendorff studies (group 1) were excised from the chest and the 28 hearts used for the Langendorff and papillary muscle studies (groups 2 and 3) were removed from the Langendorff preparation, the right ventricle was opened, and a right ventricular papillary muscle was excised. The papillary muscle was then mounted in a bath with Krebs-Henseleit solution similar to the one used in the Langendorff preparation, but in this experimental situation the bath was kept at 29°C. The base of the muscle was held by a stainless-steel clamp, and the other end was tied to a lever identical to that described in detail by Brutsaert et al. The muscles were stimulated at 10% above threshold at 12 stimuli per minute with a model S-88 stimulator (Grass Instrument Co., Quincy, Mass.) through platinum field electrodes. The preload was adjusted so that the muscle length was at the length at which maximum developed tension occurred (Lₘₐₓ).

**Protocol C.** The muscles were stabilized at Lₘₐₓ for 2 hours, and then isometric and isometric contractions were recorded at a speed of 100 mm/sec on a model 2400 s recorder (Gould Inc., Cleveland, Ohio) (Figure 1). After the elastic damping of the force-length-velocity lever feedback system was adjusted to compensate for electromechanical transients, the maximum velocity of unloaded muscle shortening (Vₘₐₓ) was obtained by abruptly decreasing the load on the muscle at the time of activation (zero load clamp). The output of the amplifier of the Gould 2400 s recorder was connected to an electronic differentiator, and both of these were fed into an analog-to-digital converter (model DT 2821-F-801, Data Translation Inc., Marlborough, Mass.), which in turn was connected to a microcomputer (model 286, COMPAQ Computer Corp., Houston, Tex.). Analysis of force-length characteristics was performed by custom-made software running under MS-DOS. Once basal values at 1.25 mM calcium (physiological) were recorded, extracellular calcium was increased to 15 mM by adding CaCl₂ from a stock solution (high calcium), and repeat isometric, isometric, and unloaded contractions were recorded.

**Protocol D.** Once the basal values for groups 1–3 muscles were obtained, the endothelial layer of the endocardium was removed by immersing the papillary muscle in 1% Triton X-100 dissolved in Krebs-Henseleit solution at 29°C for 1 second and then washing abundantly (Figure 1). This technique has been shown to destroy the endothelial layer of the endocardium without damaging myocardial cells. Muscles were then permitted to restabilize for 2 hours, and repeat isometric, isometric, and unloaded contractions were recorded at 1.25 mM calcium concentration from the papillary muscles of all three groups. This was done to assess whether removing the layer of endothelial cells covering the endocardium altered twitch characteristics similarly in papillary muscles in which vascular endothelium had been rendered dysfunctional or not. To assess whether the changes in contractile characteristics caused by altering endothelial function were reversible by increasing extracellular calcium concentration, repeat isometric, isometric, and unloaded contractions were recorded at 15 mM extracellular calcium concentrations in all three groups of muscles (groups 1–3). As the total tension of muscles with vascular or endocardial endothelial dysfunction did not completely normalize at extracellular calcium concentrations of 15 mM, two further sets of experiments were conducted. In the first set of experiments, 10⁻³ M phenylephrine in the presence of 10⁻⁵ M propranolol (Sigma) was added to a bath with 15 mM extracellular calcium for eight muscles with vascular endothelial dysfunction (group 3) and eight control muscles (group 1) with intact and functional endocardial and vascular endothelium, and isometric, isometric, and unloaded contractions were then recorded. In a second set of experiments, a dose–response curve from 1.25 to 25 mM extracellular calcium was performed before and after endocardial endothelial removal in six muscles to assess whether maximal calcium activated force at saturating bath calcium was changed by the removal of endocardium. Muscles had excessive ectopic contractions or went into contracture at extracellular calcium concentrations higher than 25 mM.

The muscle cross section was measured by assuming a cylindrical shape of the muscles and dividing muscle weight by length. The average cross section of the rabbit papillary muscles was 0.51 ± 0.03 mm².

**Morphological Evaluation**

Scanning electron microscopy, light microscopy, and confocal laser-scanning light microscopy were used to detect morphological changes in Langendorff-perfused hearts. The viability of the endothelial cells was verified by confocal laser-scanning light microscopy using propidium iodide before fixation to stain the nuclei of dead cells. After fixation, F-actin in endocardial endothelial and myocardial cells was stained with Bodipy-phallacidin.

For scanning electron microscopy, three control and six Triton X-100–treated rabbit hearts were perfusion-fixed with 2% glutaraldehyde in 0.1 M Millonig buffer at the end of a Langendorff experiment. Endocardial and myocardial tissue blocks were further processed as previously described. Specimens were examined with a Cambridge Stereoscan scanning electron microscope operating at 20 kV.

For confocal laser-scanning light microscopy, one control and four Triton X-100–treated rabbit hearts were perfused for 10 minutes with a Krebs-Henseleit solution containing 0.1 mM propidium iodide before fixation. The hearts were then perfusion-fixed with 4% formaldehyde in 0.1 M Millonig buffer for 5 minutes.
Results

Langendorff Perfusion and Coronary Vasodilatation

Protocol A. Before the injection of Triton X-100, the infusion of acetylcholine caused a similar increase in coronary perfusion rate in both groups of Langendorff-perfused hearts (Figure 2, Table 1).

Protocol B. The injection of Triton X-100 (group 3) led to a significant decrease in coronary perfusion rate (Table 1). The bolus injection of Triton X-100 also abolished the vasodilatory effects of acetylcholine but did not modify the vasodilatory effects of nitroprusside (Sigma) (Figure 2, Table 1). The vasodilatory response to acetylcholine was not modified by saline injection (group 2).

Isolated Right Ventricular Papillary Muscle Studies

Protocol C. Before endocardial endothelial removal, the contractile characteristics of papillary muscles obtained from hearts that did not undergo Langendorff perfusion (group 1) and right ventricular papillary muscles from hearts that underwent Langendorff perfusion and had a bolus injection of saline (group 2) were similar (Table 2). However, the contractile characteristics of papillary muscles from hearts that underwent Langendorff perfusion and had a bolus injection of Triton X-100 (group 3) had considerably different contractile characteristics (Figure 3, Table 2). Peak twitch tension, maximum rate of tension development (dT/dt), time to peak tension, time to half tension decline, and V<sub>max</sub> decreased significantly, indicating an abbreviation of twitch duration and a slight decrease in contractility.

Protocol D. The removal of endocardial endothelium in right ventricular papillary muscles from hearts without vascular endothelial dysfunction (groups 1 and 2) showed characteristics similar to those described previously for this intervention. Twitch duration decreased, and there was a mild decrease in peak twitch tension and dT/dt and a smaller decrease in V<sub>max</sub> (Figure 3, Table 2). These changes were very similar to those caused by creating vascular endothelial dysfunction alone by bolus injection of Triton X-100 into the coronary arteries (group 3) (Figure 3, Table 2). Endocardial endothelial removal of the right ventricular papillary muscle from the Langendorff-perfused hearts in which a bolus injection of Triton X-100 was given and vascular endothelial dysfunction was created (group 3) resulted in further shortening of twitch duration and a further decrease in peak twitch tension, dT/dt, and V<sub>max</sub> (Figure 3, Table 2).

Table 1. Bolus Triton X-100 Injection and the Effects of Acetylcholine on Coronary Flow in Langendorff-Perfused Rabbit Hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline 1 (ml/g per minute)</th>
<th>Ach 1</th>
<th>Baseline 2 (ml/g per minute)</th>
<th>After Triton (ml/g per minute)</th>
<th>Ach 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8</td>
<td>8.6±0.3</td>
<td>12.1±0.5</td>
<td>8.9±0.4</td>
<td></td>
<td>11.0±0.3</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>9.0±0.3</td>
<td>12.4±0.6</td>
<td>9.7±0.4</td>
<td>7.1±0.5*</td>
<td>7.0±0.5*</td>
</tr>
</tbody>
</table>

Baseline 1, values before acetylcholine (Ach) injection; Ach 1, response after Ach injection; baseline 2, response before injection of Triton X-100; after Triton, response to bolus injection of Triton X-100; Ach 2, reevaluation of effects of Ach after Triton X-100 injection; group 2, saline-injected hearts with Langendorff perfusion; group 3, Triton X-100-injected hearts with Langendorff perfusion. Values are mean±SEM.

*p<0.05 vs. corresponding baseline value.

†p<0.01 vs. corresponding value for group 2.
TABLE 2. Effect of Endothelial Dysfunction on the Contractile Characteristics of Right Ventricular Papillary Muscles of Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>TT (g/mm²)</th>
<th>dT/dt (g/mm² per second)</th>
<th>TTTP (msec)</th>
<th>RT² (msec)</th>
<th>Vmax (Lm,,/sec)</th>
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</thead>
<tbody>
<tr>
<td>1.25 mM Ca²⁺</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control (n=18)</td>
<td>2.9±0.2</td>
<td>11.6±0.7</td>
<td>279±6</td>
<td>449±9</td>
<td>1.14±0.07</td>
</tr>
<tr>
<td>-EE (n=11)</td>
<td>2.2±0.2*</td>
<td>9.2±0.9†</td>
<td>231±10*</td>
<td>363±16*</td>
<td>0.89±0.06†</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>2.7±0.2</td>
<td>11.9±0.7</td>
<td>271±7</td>
<td>441±19</td>
<td>1.01±0.05</td>
</tr>
<tr>
<td>-EE (n=8)</td>
<td>2.1±0.2*</td>
<td>8.5±0.4†</td>
<td>232±6*</td>
<td>355±9*</td>
<td>0.84±0.04*</td>
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<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-VE (n=14)</td>
<td>2.1±0.1*</td>
<td>9.7±0.6†</td>
<td>216±5*</td>
<td>345±11*</td>
<td>0.89±0.01*</td>
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<tr>
<td>-VE, -EE (n=14)</td>
<td>1.7±0.2*‡</td>
<td>7.2±0.5*‡</td>
<td>191±7*§</td>
<td>285±10*§</td>
<td>0.69±0.03*§</td>
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<tr>
<td>15 mM Ca²⁺ + PHE</td>
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<tr>
<td>Group 1</td>
<td></td>
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<tr>
<td>Control (n=18)</td>
<td>7.1±0.2</td>
<td>47.1±2.4</td>
<td>235±7</td>
<td>523±13</td>
<td>3.15±0.09</td>
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<tr>
<td>-EE (n=11)</td>
<td>6.7±0.2</td>
<td>45.4±3.3</td>
<td>206±4*</td>
<td>455±8*</td>
<td>2.97±0.14</td>
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<td>Group 2</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>7.0±0.4</td>
<td>46.4±4.1</td>
<td>240±5</td>
<td>498±12</td>
<td>2.9±0.23</td>
</tr>
<tr>
<td>-EE (n=8)</td>
<td>6.6±0.4</td>
<td>43.2±4.2</td>
<td>211±3†</td>
<td>449±10*</td>
<td>2.81±0.19</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>-VE (n=14)</td>
<td>6.3±0.2†</td>
<td>42.0±2.6</td>
<td>216±6†</td>
<td>440±7*</td>
<td>2.95±0.11</td>
</tr>
<tr>
<td>-VE, -EE (n=8)</td>
<td>5.8±0.3*</td>
<td>41.1±3.7</td>
<td>203±9†</td>
<td>415±9*‡</td>
<td>2.94±0.15</td>
</tr>
<tr>
<td>15 mM Ca²⁺</td>
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<td></td>
<td></td>
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<tr>
<td>Group 1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>9.0±0.2</td>
<td>71.2±3.4</td>
<td>223±8</td>
<td>586±23</td>
<td>4.17±0.18</td>
</tr>
<tr>
<td>Group 3</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>-VE (n=8)</td>
<td>8.7±0.2</td>
<td>65.4±2.5</td>
<td>222±6</td>
<td>516±14t</td>
<td>3.76±0.19</td>
</tr>
</tbody>
</table>

TT, total twitch tension; dT/dt, maximum rate of tension development; TTTP, time to peak tension; RT², time from the onset of the twitch to half relaxation; Vmax, maximum shortening velocity of an unloaded (zero load) contraction; group 1, control muscles from non-Langendorff-perfused hearts; -EE, endocardial endothelial removal; group 2, control muscles from saline-injected Langendorff-perfused hearts; group 3, muscles from Triton X-100-injected Langendorff-perfused hearts; -VE, vascular endothelial dysfunction; PHE, phenylephrine.

* p<0.01 and †p<0.05 vs. baseline.
‡p<0.05 and §p<0.01 vs. -VE.

Mechanical studies done at 15 mM calcium showed that the depression in peak twitch tension, dT/dt, and Vmax observed at physiological concentrations of calcium (1.25 mM) were nearly completely reversible, with dT/dt and Vmax being similar in all groups (groups 1–3) before and after endocardial endothelial removal (Table 2). Nevertheless, the use of 15 mM calcium concentrations did not totally abolish the changes in twitch configura-

FIGURE 3. Representative curves of twitches from papillary muscles with functional endocardial and vascular endothelium (control), with dysfunctional vascular endothelium (-VE), with the endocardial endothelium removed (-EE), and with dysfunctional vascular endothelium after endocardial endothelial removal (-VE-EE). At physiological calcium concentration (1.25 mM), endothelial removal or dysfunction caused a decrease in contractility and abbreviation of twitch duration. At high calcium concentrations, twitch duration remained shorter and total tension was lower, but other indexes of contractility had normalized. When 10⁻³ M phenylephrine (PHE) in the presence of 10⁻³ M propranolol was added to high extracellular calcium concentrations, total tension normalized in papillary muscles with dysfunctional vascular endothelium (-VE), but contraction duration remained abbreviated.
Twitch duration documented at lower calcium concentrations. Twitch duration remained shorter in all groups in which endocardial or vascular endothelium was removed (Table 2). Also, a mild decrease in total tension persisted, a decrease that can be explained by the shortened time to peak tension in those groups (Table 1). However, the addition of $10^{-7}$ M phenylephrine to 15 mM calcium concentration resulted in complete normalization of the twitch tension, time to peak tension, $dT/dt$, and $V_{\text{max}}$ of papillary muscles with intact endocardium but dysfunctional vascular endothelium (Figure 3, Table 2). Nevertheless, twitch duration remained shorter. Finally, increasing extracellular calcium concentrations to saturating bath calcium (25 mM) eliminated all differences in total tension between papillary muscles with and without endocardial endothelium (Figure 4).

**Morphology**

Scanning electron microscopy of the endothelial layer of the large coronary vessels and their smaller branches was normal in both saline-injected (group 2) and Triton X-100–injected (group 3) Langendorff-perfused hearts (Figures 5A and 5B). The viability of these endothelial cells was documented by lack of propidium iodide staining of the nuclei and no change in the pattern of actin filament staining in both groups (Figure 6A). In arterioles and capillaries, there was occasional damage of endothelial cells in several vessel segments of group 3 hearts; however, large areas were observed without propidium iodide staining of nuclei, and the smooth muscle cells of these vessel segments and their surrounding myocytes were intact (Figure 6B). The changes in the microvascular bed were more marked than those in the macrovascular bed (Figure 6C), with only occasional damage of endothelial and myocardial cells being found in group 3 hearts, with no association occurring between damage of the two cellular groups. In group 2 hearts, no changes in the vascular bed were found; however, as occurred in group 3 hearts, occasional damage to myocardial cells was found. Interstitial edema was detected in both group 2 and group 3 hearts.

Scanning electron microscopy of the endocardial endothelium of the left and right ventricles revealed many transcellular holes along the periphery of endocardial endothelial cells in both groups of hearts, indicating that this was not due to Triton X-100. However, the endocardial endothelium of the papillary muscles from the right ventricle usually showed less or no transcellular holes than elsewhere in the heart, with most cells having well-developed intercellular borders (Figure 5C). With confocal laser-scanning light microscopy, very few endocardial endothelial cells were found with propidium iodide–stained nuclei, indicating cellular viability (Figure 6D). However, at the periphery of the endocardial endothelial cells, circular structures outlined with F-actin staining distorting the organization of peripheral actin bands were found (Figure 6D). Endocardial endothelial damage was similar in both saline-injected and Triton X-100–injected hearts.

**Discussion**

By creating vascular endothelial dysfunction with a bolus injection of Triton X-100 in Langendorff-perfused hearts, the myocardial performance of subsequently isolated papillary muscles from the same hearts was modified in a manner typical of that created by endocardial endothelial dysfunction, i.e., a decrease in twitch duration with a concomitant decrease in peak twitch tension with little or no change in $V_{\text{max}}$. Endocardial endothelial removal had the same effects on papillary muscle characteristics whether vascular endothelium was dysfunctional or not. These findings suggest that coronary vascular endothelium directly modulates the contractile characteristics of adjacent myocardium in a manner similar to that of endocardial endothelium and that these effects appear to be additive.

In this study, vascular endothelial dysfunction was created by a bolus injection of Triton X-100 into the aorta, just above the coronary arteries. This resulted in little or no change in the morphological characteristics of both macrovascular and microvascular endothelial layers or of the adjacent myocardium. Lack of cell damage was verified by staining with propidium iodide. In view of this apparently largely intact vascular endothelium, the functional integrity of the vascular endothelium was verified before isolation of the papillary muscles by examining coronary perfusion rate responsiveness to acetylcholine in the Langendorff heart preparation. Before the bolus injection of Triton X-100, acetylcholine caused relaxation of adjacent vascular smooth muscles, suggesting the presence of intact functioning vascular endothelium. After the bolus injection of Triton X-100, the lack of change in coronary perfusion rate in response to acetylcholine suggests that the Triton X-100 injection resulted in endothelial dysfunction. That coronary perfusion increased similarly
in response to nitroprusside before and after the Triton X-100 injection supports an endothelium-mediated defect in vasodilatation after Triton X-100 injection and suggests that the vasodilatory capacity of coronary vascular smooth muscle was not altered.

In addition to abolishing the vasodilator response to acetylcholine, the bolus injection of Triton X-100 led to a 27% decrease in basal coronary flow. This decrease could have been due to suppressed basal release of coronary vasodilating substances such as endothelium-derived relaxing factor, much as has been shown to occur with the use of another detergent (CHAPS) or nitro-L-arginine in the guinea pig isolated perfused heart. An alternative possibility that cannot fully be excluded at present is a compressive effect of the coronary vascular bed caused by perivascular edema. However, because some perivascular edema was present in both Triton X-100–injected and saline-injected hearts, this second possibility is less probable.

The Langendorff perfusion itself did not appear to significantly alter myocardial performance of subsequently isolated papillary muscles, nor did it modulate endothelium-dependent contractile characteristics. The bolus injection of Triton X-100 in the coronary vasculature resulted in a change in contractile characteristics similar to those found after endocardial endothelial removal. This is compatible with a direct effect of the vascular endothelium on the contractile characteristics of adjacent myocardium, much as has been shown for the endocardial endothelium and its adjacent myocardium. Endocardial endothelial removal in this study had a similar effect on the contractile characteristics of all groups of muscles, whether intracoronary Triton X-100 bolus injection had been given or not, suggesting that functionally intact endocardial endothelium was present in all preparations. It would thus appear, from these observations, that vascular endothelium modulates myocardial performance, regardless of the functional status of the endocardial endothelium, and that this occurs independently and to a similar degree in both endothelial layers. It would also seem that the modulatory effects of both systems on myocardial contraction are complementary. Finally, because the effects of endocardial endothelium on myocardial contractile characteristics occurred despite some breaks in the endocardial endothelial layer and the effects of vascular endothelium could be inhibited despite a largely intact layer of cells, it would appear that the effects of endothelium on myocardial contractile characteristics are not dependent on an intact cellular barrier. Nevertheless, it is also possible that complete removal of the
vascular endothelium would have resulted in an even more marked alteration of contractile characteristics.

In this study, intensive morphological investigation allowed us to verify the cellular integrity of the whole of the coronary vascular tree. The endothelial layer was essentially intact with only occasional evidence of damaged endothelial cells. Experiments with acetylcholine permitted us to also verify the functional integrity of the macrovascular system.\textsuperscript{11,12} To what extent the acetylcholine experiments provided information about the functional integrity at the microvascular level remains open to question. However, because damage to vascular endothelial cells in Triton X-100–injected hearts appeared to be greater in the microvascular tree, there is reason to believe that vascular endothelium in the microvascular tree was also dysfunctional and that this contributed to the contractile changes documented in the papillary muscles of the Triton X-100–injected hearts. That vascular endothelium in the microvascular tree is in direct opposition to myocardium, much as endocardial endothelium is, would also appear to favor the view that changes in contractile characteristics caused by vascular endothelial dysfunction are at least, in part, the result of microvascular endothelial dysfunction. Finally, although the presence of a well-developed glycocalyx on vascular endothelial cells may diminish the penetration and the effects of detergents on membranes, detergents such as Triton X-100 (the nonionic

**Figure 6.** Confocal scanning light microscopy of perfused rabbit heart treated with Triton X-100. Panel A: En face view of the endothelial layer of a coronary artery. Vascular endothelial cells were viable, as indicated by the lack of staining of the nuclei with propidium iodide and the normal (yellow color) actin filament system. Bar, 25 μm. Panel B: Optical section through myocardium of the left ventricle from a perfused heart that was fixed with glutaraldehyde. Part of the microvascular tree showed highly elongated and aligned nuclei of damaged endothelial cells in arterioles (arrows) and more rounded capillary nuclei (arrowheads) stained with propidium iodide. A large part of the tissue did not contain propidium iodide–stained nuclei. No damaged myocytes were observed in this area. Bar, 100 μm. Panel C: Optical section through myocardium showing myocytes and an arteriole. The striated pattern of actin filaments (yellow color) and the absence of propidium iodide–stained nuclei indicate that the myocytes were viable at the time of staining. The tangentially sectioned arteriole, in the middle of the figure, showed filaments of concentrically arranged smooth muscle cells (arrowhead). The elongated blue-colored nuclei in the arteriole are nuclei of damaged endothelial cells, since they are oriented parallel to the blood flow. Again, the absence of propidium iodide–stained nuclei indicates the viability of vascular smooth muscle cells. Bar, 50 μm. Panel D: Confocal laser scanning light microscopy of an en face preparation of endocardial endothelium from right ventricular papillary muscles of hearts receiving intracoronary Triton X-100 (group 3) and stained with propidium iodide. Only two dead cells with a propidium iodide–stained nucleus (blue color) were found, attesting to the viability of the endocardial endothelial cells. Peripheral actin bands (arrowheads) outlined the intercellular borders of endocardial endothelial cells. Bar, 10 μm.
detergent polyoxyethylene ether) seem to bind to cell membranes even at very low concentrations and may indeed, without lysis, change the functional properties of these membranes.  

The results of studies done at extracellular calcium concentrations of 15 mM suggest that the changes in total twitch tension, dT/dt, and V_max caused by endothelial removal were largely reversible and not due to myocyte damage. At 15 mM calcium, the dT/dt and V_max values of all groups were similar, indicating complete reversibility of these abnormalities. As previously described, the twitch configuration remained unchanged, a finding that can explain the slightly lower total tension once endocardial or vascular endothelial dysfunction occurred. Also, morphological studies with propidium iodide found a similar but rare incidence of damaged myocardial cells in both saline-injected and Triton X-100-injected hearts, suggesting that any myocardial damage was the result of the Langendorff perfusion rather than the result of the Triton X-100 injection. Taken together, these findings suggest that the changes in contractile characteristics that resulted from the various interventions done in this study were the results of endothelial rather than myocardial dysfunction.

Nevertheless, as opposed to previous studies in myocardium from other species, increasing extracellular calcium concentration to 15 mM was insufficient to normalize either total twitch tension or time to peak tension. Only at greater extracellular calcium concentrations of 20 and 25 mM was the difference in total twitch tension abolished between normal muscles and muscles with the endocardial endothelium removed. This apparent discrepancy with the literature is in fact compatible with species differences in contractile characteristics and with recent concepts regarding the mechanism by which endothelium modulates myocardial contractile characteristics. As compared with other species in which the myocardial contractile effects of endocardial endothelium have been evaluated, at physiological calcium concentrations, rabbit myocardium has a relatively poor performance. It responds rather well to increases in extracellular calcium concentrations, but as opposed to other species such as the cat, even at 15 mM extracellular calcium concentration, rabbit myocardium is far from reaching maximal performance. A further increase in extracellular calcium concentration to at least 25 mM is necessary to reach maximal performance. Alternatively, a further intervention such as the addition of phenylephrine or endothelin is required to reach its full potential. It would thus appear that, in rabbit papillary muscles, increasing the extracellular calcium concentration to at least 25 mM or increasing the myofibrillar affinity for calcium at 15 mM calcium concentration is necessary for the myocardium to reach maximal performance, with additional interventions not necessary in the cat or ferret.

Recently, Wang and Morgan and McClellan et al have demonstrated that both endocardial and vascular endothelium appear to modify the contractile characteristics of adjacent myocardium by increasing myofilament calcium responsiveness or affinity. In this study done in rabbit myocardium, the addition of phenylephrine to 15 mM extracellular calcium concentrations was necessary to completely normalize total twitch tension and time to peak total tension in papillary muscles with dysfunctional vascular endothelium. Our findings are thus in full agreement with current knowledge and reinforce the findings linking endothelial effects on myocardium to changes in myocardial myofibrillar calcium affinity. Nevertheless, as occurred with endocardial endothelium, when vascular endothelium is removed, normalizing twitch tension is not accompanied by a normalizing of twitch duration, underscoring the probable multifactorial nature of the relation between endothelium and its adjacent myocardium.

That modulation of the performance of adjacent myocardium by endocardial and by vascular endothelium is complementary is not surprising. In early embryonic cardiogenesis, there is no coronary circulation, and exchange of substances between the luminal blood and adjacent myocardium—and perhaps modulation of its performance—is accomplished exclusively through the endocardial endothelium. The function of the endocardial endothelium is subsequently complemented and partially replaced by the endothelium of the coronary circulation, the development of which appears to accompany the formation of compact myocardium in the outer portion of the ventricular wall. A reminiscence of this developmental feature is present in fish and reptiles, in which capillaries were observed in the epicardial and outer compact regions of the ventricular wall only. In higher animals including, although not always unequivocally, humans, the intertrabecular spaces regress as the coronary circulation develops. The spaces are either reduced to strands of endothelium without lumen or may give rise to capillaries in the central and inner portions of the myocardium with persistence of ventricular communication through the thebesian and arteriosinusoidal vessels.

In conclusion, it would appear from this study that coronary vascular endothelium modulates the contractile characteristics of adjacent myocardium in a manner similar to that of endocardial endothelium and that these effects are additive. Thus, it would appear that the “cross talk” between different cell types in the heart is more widespread than previously thought.

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