Myocardial Inotropic Responses to Aggregating Platelets and Modulation by the Endocardium

Ajay M. Shah, MB, MRCP, Ann L. Meulemans, DRs, and Dirk L. Brutsaert, MD, PhD

Ventricular mural thrombi complicate many cardiac diseases. The endocardial endothelium can modulate the mechanical performance of subjacent myocardium and mediate responses to certain physiopharmacologic agents. We studied the effects of aggregating platelets on the contractile performance of isolated cardiac muscle. The role of the endocardium was investigated by selectively damaging it by very brief (1 second) exposure to 1% Triton X-100 in some muscle preparations before experiments. Cat papillary muscles (n = 54) were attached to an electromagnetic length-tension transducer in organ baths containing Krebs-Ringer solution (1.25 mM Ca++, 35°C), and stimulated electrically at 0.2 Hz. Homologous washed platelets (final concentration 3 × 10^11/l) aggregated spontaneously on addition to baths. Mechanical performance increased significantly more in muscles with damaged endocardium than in intact muscles (p < 0.05); total peak isometric twitch tension increased by 31.8 ± 7.8% (with damaged endocardium) and 11.8 ± 2.6% (with intact endocardium), and peak isotonic twitch shortening increased by 36.7 ± 7.8% (with damaged endocardium) and 9.6 ± 2.0% (with intact endocardium). Increases in maximum velocity of unloaded shortening were similar in both muscle groups. Time to half isometric twitch tension declined in intact muscles (3.6 ± 1.0%) but increased in Triton-treated muscles (2.5 ± 1.3%, p = 0.003 for difference between groups).

The inotropic response to platelets in muscles with intact endocardium was unaltered by pretreatment of muscles with indomethacin (10 μM) or by stimulation of platelet aggregation with thrombin (0.1 unit/ml). ATP (≥10 μM) produced significantly larger responses in muscles without intact endocardium compared with those with (p < 0.05), at 50 μM increasing twitch tension by 14.0 ± 3.4% (with damaged endocardium) and 4.7 ± 2.8% (with intact endocardium). Responses to ADP (0.1–100 μM) were similar in the presence or absence of endocardium. 5-Hydroxytryptamine (≥10 μM) also produced significantly greater inotropic effects in Triton-treated muscles (p < 0.01), at 30 μM increasing twitch tension by 107.3 ± 19.5% (with damaged endocardium) compared with 37.3 ± 8.6% (with intact endocardium). 5-Hydroxytryptamine delayed isometric relaxation in Triton-treated muscles but induced an earlier onset in intact muscles. These results show that an intact endocardium diminishes and modulates myocardial contractile responses to aggregating platelets. This action was not dependent upon the production of prostacyclin. Inotropic responses to ATP and 5-hydroxytryptamine (but not ADP) were also reduced in the presence of an intact endocardium. In the intact heart, these responses could contribute to nonuniformity of contractile performance and therefore impair cardiac hemodynamic output. (Circulation 1989;79:1315–1323)
intensive investigation in recent years,\textsuperscript{10-14} the physiologic function of the endocardium of the heart is nearly unexplored. We have recently shown that the endocardial endothelium can modulate the peak contractile performance and relaxation of subjacent myocardium in isolated mammalian cardiac muscle.\textsuperscript{15} The presence of an intact endothelium results in a characteristic modulation of twitch contractions—a delayed onset of isometric tension decline and a concomitant increase in peak isometric tension but without any significant change in maximum velocity of unloaded shortening ($V_{\text{max}}$)—particularly at a physiologic extracellular calcium concentration (1.25 mM) and physiologic temperatures (35–37° C). This typical inotropic response differs from most other inotropic responses that also affect $V_{\text{max}}$ and that, in general, shorten twitch duration. The endocardium can also mediate inotropic responses to substances such as the atrial natriuretic peptides. In isolated cardiac muscle, atrial natriuretic peptide has no effect in the presence of a damaged endocardium but induces an earlier isometric tension decline in muscles with intact endocardium.\textsuperscript{16}

We have therefore investigated the effects of homologous aggregating platelets on the mechanical performance of isolated feline cardiac muscle. The role of the endocardial endothelium in this interaction was examined by selectively damaging it in some preparations before experiments. The effect of this selective endocardial damage on inotropic responses to 5-HT and adenine nucleotides was also studied.

**Methods**

**Cardiac Muscle Preparations**

Right ventricular papillary muscles isolated from cats anesthetized with pentobarbital were mounted vertically in Plexiglas organ baths containing Krebs-Ringer solution (mM): 118 NaCl, 4.7 KCl, 1.2 MgSO\textsubscript{4}·7H\textsubscript{2}O, 24 NaHCO\textsubscript{3}, 1.1 KH\textsubscript{2}PO\textsubscript{4}, 4.5 glucose, 1.25 or 2.5 CaCl\textsubscript{2}·2H\textsubscript{2}O bubbled with 95% O\textsubscript{2}·5% CO\textsubscript{2}. The lower end of the muscle was held by a phosphor-bronze clip, and the upper tendinous end was attached to an electromagnetic length-tension measuring and controlling transducer.\textsuperscript{15} Muscles were stimulated electrically at 12 beats/min and by a voltage approximately 10% above threshold by rectangular pulses of 5-msec duration through two longitudinally arranged platinum electrodes. After stabilization for 2–3 hours at 29° C and with 2.5 mM Ca\textsuperscript{2+}, experiments were performed at $L_{\text{max}}$ (the muscle length at which active force development was maximal) in Ringer's solution containing 1.25 mM ionized Ca\textsuperscript{2+} at 35° C.

**Removal of Functional Endocardium**

Endocardial endothelium was selectively damaged by a 1-second immersion of muscles in 1% Triton X-100 (Sigma Chemical, St. Louis, Missouri) while in their working position, immediately fol-

lowed by an abundant wash with warmed Ringer’s solution.\textsuperscript{15} Muscles were subsequently allowed to stabilize for at least 30 minutes. This procedure results in characteristic endocardial damage (Figure 1) but does not damage subjacent myocardium either morphologically (determined by light microscopy and transmission electron microscopy) or functionally as assessed by maximal contractile performance at high extracellular calcium concentrations.\textsuperscript{15} Similar changes in twitch performance are produced when the endocardium is damaged by gentle mechanical abrasion.\textsuperscript{15} The integrity of the myocardium after either of these procedures is also indicated by an unaltered maximum velocity of unloaded shortening. The integrity of the endocardium in muscles not treated with Triton was confirmed by scanning electron microscopy.

**Isolation of Platelets**

Washed cat platelets were prepared by a modification of the method described by Coene et al.\textsuperscript{17} Blood was collected into 0.075 volume 77 mM ethylenediaminetetraacetic acid (EDTA) by cardiac puncture, taking care not to cause hemolysis. Platelet-rich plasma was prepared by centrifugation (250g, 10 minutes), and a platelet pellet was obtained by further centrifugation (900g, 15 minutes). Platelets were resuspended and washed in isotonic Tris-buffered saline with 0.2% gelatin (900g, 15 minutes). No prostacyclin was used. The final suspension was prepared in calcium-free Krebs-Ringer solution with 0.2% gelatin (otherwise of identical composition to the bathing solution), was placed in a 95% O\textsubscript{2}·5% CO\textsubscript{2} atmosphere at room temperature, and was used within 2 hours. Platelet viability was confirmed by observing aggregation of an aliquot with 0.1 unit/ml bovine thrombin (bioMerieux, France). Suspensions were diluted and warmed to 35° C before addition to 10 ml organ baths to obtain a final concentration of 3×10\textsuperscript{11} platelets/l. Some intact muscle preparations were treated with the cyclooxygenase inhibitor indomethacin (10 μM) for 1 hour before washing in fresh Ringer’s solution to remove excess indomethacin and after which the platelet suspension was added.

**Measurement of Contractile Parameters**

The length-tension transducer\textsuperscript{15} was modified after Brutsaert and Claes\textsuperscript{16} and consisted of an aluminum lever (30 mm long and 1 mm thick) firmly attached to a coil suspended in a strong magnetic field. The equivalent moving mass of the whole system was 225 mg. The torque on the lever and, hence, the loading on the preparation was proportional to the current through the coil. This current was generated by a current source calibrated for step changes in loads of 0.1, 1, 10, and 100 mN to a total of 150 mN.

Displacement of the lever was measured by an optico-electronic system involving modulation of a miniature infrared light-emitting diode (TIL32, Texas Instruments, Dallas, Texas) by the lever acting as a
Shah et al  Aggregating Platelets Increase Myocardial Contractility 1317

FIGURE 1. Scanning electron micrographs of the endothelial surface of feline papillary muscles. Scale bars: A, 25 μm; B, C, D, 5 μm. Panels A and B: Intact (untreated) muscle showing normal endocardial endothelium with a continuous sheet of cells with bulging nuclei (arrowheads) and clear intercellular borders (arrows). At higher magnification (Panel B), small microvilli and knoblike protrusions are present on the endothelial surface. Panels C and D: Muscles exposed to 1% Triton X-100 for 1 second. The endothelial cells have damaged cell membranes with the basal lamina visible where there are intercellular gaps (arrows, Panel C). In Panel D, there is nearly complete extraction of the cell surface membrane but without changes in cell shape. Distinct intercellular borders are still present and cell nuclei are visible (arrowheads).

shutter. The light was captured by a photodiode (Philips BPW41, Eindhoven, Holland), and the resulting signal was amplified. The linear range was 2.5 mm.

A unidirectional force-sensing feedback circuit enabled measurement of force. The unfiltered displacement signal was compared with a preset reference level representing the position of an “electronic stop.” If the signal was smaller than this level, no feedback occurred, and the preparation had to bear the imposed load. When the preparation was lengthened beyond the reference point, feedback action held the lever at the reference position and current through the coil was proportionally decreased and represented the load carried by the preparation. By adjusting the reference level, resting length and preload could be altered. The force signal was filtered with a low-pass, third-order filter thereby minimizing noise on the derivative signal.

After any intervention, an isotonic preloaded twitch and an isometric twitch at $I_{\text{max}}$ were recorded after muscle stabilization to enable measurement of peak isotonic twitch shortening (PS), total peak isometric twitch tension (TT), time to half isometric tension decline ($RT_{1/2}$), and the peak rates of change of length and tension. The maximum velocity of unloaded shortening ($V_{\text{max}}$) was obtained by abruptly unloading the stimulated muscle to zero load after the latency period. All test twitches were separated by at least eight isotonic preloaded...
twitches to eliminate the muscle’s memory for length and load. Only muscles with resting tension less than 15% of total developed tension at 2.5 mM Ca$^{2+}$ were studied.

Results were normalized for muscle cross-sectional area (MCSA) and length at $L_{\text{max}}$. Absolute values for the above parameters (except for $V_{\text{max}}$) changed significantly after Triton treatment at 1.25 mM Ca$^{2+}$ as described previously. Therefore, mean±SEM percent changes (relative to baseline values before addition of platelets or drugs) were used for comparisons between effects on muscles with intact endocardium and muscles with damaged endocardium. Statistical analysis was by two-tailed paired $t$ test, Wilcoxon’s signed-ranks test, or Mann-Whitney $U$ test as appropriate for comparisons between and within groups.

Interventions Studied

A total of 54 muscle preparations were used in the study. Only one intervention was studied in any single preparation. Homologous unstimulated platelets were added to seven intact muscles, six Triton-treated muscles, and four intact muscles pre-treated with indomethacin. Thrombin-stimulated platelets were added to four intact muscles. The responses to 5-hydroxytryptamine creatine sulphate (5-HT) were measured in six intact muscles, six Triton-treated muscles, and in another five preparations in the presence of tramulypromine. Responses to ATP (five muscles), ADP (seven muscles), or the thromboxane analogue U46619 (four muscles) were measured before and after Triton treatment of preparations.

Drugs

Fifty microliter aliquots of freshly prepared solutions of drug were added to muscles in 50-ml organ baths. U46619 was a gift from Upjohn, Kalamazoo, Michigan. All other drugs were purchased from Sigma Chemical, St. Louis, Missouri. Indomethacin was dissolved in dimethylsulfoxide, and U46619 was dissolved in absolute alcohol; all other drugs were dissolved in distilled water. Dimethylsulfoxide and absolute alcohol in the concentrations used had no effect on muscle performance.

Results

Effects of Platelets on Isolated Cat Papillary Muscle

The baseline characteristics of a group of muscles with intact endocardial endothelium and a separate group in which the endocardium was selectively damaged with 1% Triton before platelet addition are shown in Table 1. Addition of a washed cat platelet suspension to the papillary muscle organ bath resulted in spontaneous platelet aggregation as indicated by aggregates observed by scanning electron microscopy, visible clumping, and gradual clearing of the initially cloudy solution. Addition of an equal volume of supernatant, obtained by centrifuging the platelet suspension (900g, 15 minutes), had no significant effect on muscle performance. Platelet aggregation resulted in significant positive inotropic effects (Figure 2 and 3). In the muscles with intact endocardium, there was a significant increase in total peak isometric twitch tension (11.8±2.6%, $p<0.002$) and peak isotonic twitch shortening (9.6±2.0%, $p<0.002$), and there was a reduced time to TT (-4.8±1.1%, $p=0.008$). Much larger changes in contractile performance were observed after platelet aggregation in muscles with a damaged endocardium. In the latter group, TT increased by 31.8±7.8% ($p=0.021$) and PS by 36.7±7.8% ($p<0.002$) compared with values before platelet addition (Figure 2). The mean absolute increases in TT and PS were also greater in the muscles with damaged endocardium. Thus, an intact endocardial endothelium markedly attenuated the myocardial inotropic response to aggregating platelets. $V_{\text{max}}$ increased by 26.6±1.8% ($p=0.003$) in intact muscles and by 22.7±7.5% ($p<0.04$) in muscles with damaged endocardium. In addition to the differences in magnitude of response, the patterns of isometric twitch profile were altered in opposite directions in the two groups of muscles (Figure 3). Platelet aggregation resulted in delayed isometric relaxation in muscles with damaged endocardium but earlier isometric relaxation in intact muscles. Thus, $RT_{1/2}$ increased by 2.5±1.3% in the former group but decreased by 3.6±1.0% in the latter ($p=0.003$ for difference between groups).

Pretreatment with 10 $\mu$M indomethacin in four muscles with intact endocardium ($L_{\text{max}} 5.8±0.8$ mm; MCSA, 0.55±0.14 mm$^2$; TT, 48.0±11.8 mN/mm$^2$; and PS, 0.14±0.02 $L_{\text{max}}$ at 35°C and 1.25 mM Ca$^{2+}$) failed to alter (i.e., further increase) the magnitude of inotropic response to aggregating platelets. TT increased by 6.5±1.6% and PS by 6.7±1.4%, whereas $tTT$ decreased by 5.0±1.9% and $RT_{1/2}$ by 1.8±1.4%. In an additional four experiments, platelet aggregation was induced with 0.1

### Table 1. Baseline Characteristics of Muscles Used To Study Effects of Aggregating Platelets

<table>
<thead>
<tr>
<th></th>
<th>Intact endocardium</th>
<th>Damaged endocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscles (n)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>$L_{\text{max}}$ (mm)</td>
<td>5.3±0.2</td>
<td>6.2±0.6</td>
</tr>
<tr>
<td>MCSA (mm$^2$)</td>
<td>0.95±0.06</td>
<td>0.82±0.14</td>
</tr>
<tr>
<td>RT/TT (%)</td>
<td>11.8±1.1</td>
<td>9.7±1.3</td>
</tr>
<tr>
<td>Before Triton</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (mN/mm$^2$)</td>
<td>24.9±2.3</td>
<td>27.5±2.8</td>
</tr>
<tr>
<td>PS (L$_{\text{max}}$)</td>
<td>0.11±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>tTT (msec)</td>
<td>201.9±4.7</td>
<td>242.3±24.9</td>
</tr>
<tr>
<td>RT$_{1/2}$ (msec)</td>
<td>324.6±7.5</td>
<td>386.7±36.1</td>
</tr>
<tr>
<td>After Triton</td>
<td></td>
<td></td>
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</table>
| Values are mean±SEM at 35°C, 1.25 mM Ca$^{2+}$. $L_{\text{max}}$, length for maximal active tension development; MCSA, muscle cross-sectional area; RT/TT, ratio of resting tension to total tension at 2.5 mM Ca$^{2+}$; TT, total peak isometric tension; PS, peak isotonic shortening; tTT, time to peak isometric tension; $RT_{1/2}$, time to half isometric tension decline.
FIGURE 2. Bar graphs of effects of platelet aggregation on contractile performance of intact cardiac muscles (n=7, hatched columns) and muscles with damaged endocardium (n=6, solid columns). All results are mean±SEM% of baseline values before platelet addition. *p<0.05, **p<0.01, ***p<0.005 compared with baseline values. TT, total peak isometric twitch tension; RT1/2, time to half isometric twitch tension decline; PS, peak isotonic twitch shortening; V_max, maximum velocity of unloaded shortening.

Effects of Adenine Nucleotides

The responses to ATP were examined in a group of five muscles. The baseline parameters of these muscles at 1.25 mM Ca^2+ and 35°C were L_max, 6.8±0.27 mm; MCSA, 0.89±0.20 mm^2; TT, 57.5±9.8 mN/mm^2; PS, 0.16±0.01 L_max; and RT1/2, 476±40 msec. Dose responses were obtained in each muscle before and after selective endocardial damage with Triton. Contractile parameters returned to baseline values when the bathing solution was replaced with fresh drug-free Krebs-Ringer solution. ATP concentrations 10 μM or higher produced significantly larger percent increases (p<0.05) in TT and PS in muscles where the endocardium had been damaged (Figure 4). There was a small but significant decrease in RT1/2 in intact muscles at ATP concentrations 10 μM or lower (−1.9±0.6% at 10 μM, p=0.036). At higher concentrations, there was a tendency toward slightly earlier isometric relaxation in intact muscles and delayed relaxation in muscles with damaged endocardium although this tendency was not significant. Percent increases in V_max were of similar magnitude before and after endocardial damage at all ATP concentrations (data not shown).

ADP altered contractile performance significantly only at relatively high concentrations. At concentrations above 10 μM, it typically caused a biphasic response with an initial rapid but transient decrease in TT, PS, and V_max followed by a gradual recovery to slightly higher values than baseline. This response was similar in the presence or absence of an intact endocardium. The mean increases in TT produced by 100 μM ADP were 4.4±1.4% (with an intact endocardium) and 3.4±1.8% (with damaged endocardium), n=7.

Effects of 5-Hydroxytryptamine

Experiments with 5-HT were performed in a group of muscles with intact endocardium and a separate group with damaged endocardium. Table 2 shows the baseline characteristics of these muscles. Concentrations of 5-HT greater than 50 μM resulted in frequent arrhythmias and were therefore not studied. Concentrations of 5-HT 10 μM or higher produced larger percent increases in TT and PS in muscles with damaged endocardium than in intact muscles (Figure 5). At a 5-HT concentration of 30 μM, TT increased by 37.3±8.6% in intact muscles and by 107.3±19.5% in muscles with damaged endocardium (p=0.008 for difference between groups). Isometric relaxation occurred much earlier after 5-HT administration in intact muscles but was
significantly delayed in muscles treated with Triton (Figure 5). This pattern of twitch modulation was therefore similar to that occurring after platelet aggregation (Figure 3). These differences in response to 5-HT between muscles with intact and damaged endothelium persisted in experiments (n=5) performed in the presence of the monoamine oxidase inhibitor tranylcypromine (1 μM).

The thromboxane A₂ analogue U46619 (1 μM) had no effect on any contractile parameter regardless of endocardial integrity (n=4, data not shown).

**Discussion**

The present study shows for the first time that blood platelets can directly affect myocardial contractile function. Aggregating platelets had a small but significant positive inotropic effect on intact papillary muscles. After selective endocardial damage, however, much larger increases in inotropic response were noted. Not only was the magnitude of the myocardial positive inotropic response to platelets markedly diminished in the presence of an intact endocardium, but the pattern of myocardial contraction and relaxation was altered in a quite different manner in intact muscles compared with Triton-treated preparations (Figure 3). Of note, however, the maximum velocity of unloaded shortening (V<sub>max</sub>) increased by similar proportions in both muscle groups after platelet addition. V<sub>max</sub> is a measure of the force-velocity relation of muscle; it has been shown to be proportional to myosin ATPase activity and, hence, to represent maximal turnover.

**FIGURE 4.** Plot of effects of ATP on isometric tension (TT) and peak shortening (PS). Cumulative dose-responses were studied in five intact muscles (•) and then repeated after washing the muscle and selectively damaging the EE endocardium (○). Results are mean±SEM% of baseline values before drug addition. *p<0.05, **p<0.01 compared with baseline values. Right: Representative tracings of isometric twitches show effects of 50 μM ATP in the presence of intact endocardium (+EE) and damaged endocardium (−EE). Baseline twitches are denoted by "a."

**FIGURE 5.** Plot of effects of 5-hydroxytryptamine on isometric tension (TT) and time to half tension decline (RT₁/₂). Results are mean±SEM% of baseline values for six intact muscles (•) and six muscles with damaged endocardium (○). SEM bars are omitted where values are too small to plot on the graphs. *p<0.05, **p<0.01, ***p<0.001 compared with baseline values. Differences in TT increases between the groups were significant at 30 μM 5-HT and in RT₁/₂ change at all concentrations above and including 0.1 μM 5-HT. Right: Representative tracings of isometric twitches show effects of 30 μM 5-HT on a muscle with intact endocardium (+EE) and on a muscle with damaged endocardium (−EE). Baseline twitches are denoted by "a."

**TABLE 2. Baseline Characteristics of Muscles Used for 5-Hydroxytryptamine Experiments**

<table>
<thead>
<tr>
<th></th>
<th>Intact endocardium</th>
<th>Damaged endocardium</th>
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<tbody>
<tr>
<td>Muscles (n)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>L&lt;sub&gt;max&lt;/sub&gt; (mm)</td>
<td>5.6±0.6</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>MCSA (mm²)</td>
<td>1.06±0.17</td>
<td>0.8±0.11</td>
</tr>
<tr>
<td>RT/TT (%)</td>
<td>12.2±0.7</td>
<td>12.0±1.6</td>
</tr>
</tbody>
</table>

**Before Triton** After Triton

| TT (mN/mm²) | 34.8±7.4 | 29.6±3.8 | 15.4±1.5 |
| PS (L<sub>max</sub>) | 0.15±0.1 | 0.13±0.02 | 0.08±0.01 |
| RT₁/₂ (msec) | 434.4±18.3 | 419.0±27.7 | 342.8±18.1 |

Values are mean±SEM at 35° C, 1.25 mM Ca²⁺. L<sub>max</sub> length for maximal active tension development; MCSA, muscle cross-sectional area; RT/TT, ratio of resting tension to total tension at 2.5 mM Ca²⁺; TT, total peak isometric tension; PS, peak isometric shortening; TTT, time to peak isometric tension; RT₁/₂, time to half isometric tension decline.
rate of a single crossbridge between actin and myosin filaments within the muscle.\textsuperscript{19} Because brief Triton X-100 treatment causes only endocardial damage while leaving subjacent myocardium undamaged,\textsuperscript{15} it is not surprising that increases in $V_{\text{max}}$ were similar in intact and Triton-treated muscles. This finding also provides circumstantial evidence against the possibility that the differences in inotropic response between the two groups are simply a nonselective manifestation of tissue damage. Furthermore, previous studies from this laboratory have shown that Triton-treated muscles develop inotropic responses in magnitude and character similar to intact muscles after administration of substances such as dibutyryl cGMP,\textsuperscript{16} nitroprusside,\textsuperscript{16} and angiotensin I and II,\textsuperscript{20} which presumably do not act through the endocardium. Thus, the endocardial endothelium does appear to play a true modulatory role in the platelet-myocardial cell interaction.

The modulatory role of the endocardium in this study was not explained by the synthesis of arachidonic acid metabolites such as prostacyclin\textsuperscript{10,21,22} that may inhibit platelet aggregation because cyclooxygenase inhibition with indomethacin failed to alter the inhibitory effects of an intact endocardium. The results with thrombin-stimulated platelet aggregation would also argue against significant anti-aggregatory activity due to other secreted substances\textsuperscript{22-24} being the main explanation for the effect of endocardium. However, qualitative assessment of platelet aggregation was not the primary aim of this study, and no comment can therefore be made about the role and extent of anti-aggregatory activity under other experimental conditions or in vivo.

Platelet aggregation results in the release of several substances including 5-HT, ADP, ATP, calcium, and thromboxane $A_2$.\textsuperscript{25} In vascular preparations, 5-HT and adenine nucleotides play a major role in mediating endothelium-dependent responses to aggregating platelets.\textsuperscript{8,9} In this study, inotropic responses to 5-HT and to ATP (but not ADP) were found to be significantly greater in the absence of an intact endocardium. In the case of 5-HT, onset of isometric relaxation was modulated by endocardium in the same way observed after addition of platelet suspension. The content of biogenic amines\textsuperscript{26} and adenine nucleotides\textsuperscript{27} in cat platelets is sufficient to allow concentrations of the order of 10 $\mu$M 5-HT, 1 $\mu$M ADP, and 10 $\mu$M ATP to accumulate after platelet aggregation. However, the precise role of these substances in mediating myocardial responses to aggregating platelets requires further study. Amplification by 5-HT of responses to ATP or other released substances\textsuperscript{28,29} and possible changes in calcium concentration, catecholamines, and pH may also play some part in the myocardial response.

The possible mechanisms involved in the modulatory role of the endocardial endothelium in the platelet-myocardial cell interaction remain speculative. The endocardium may inhibit penetration of platelet products into underlying muscle,\textsuperscript{30} perhaps by metabolizing 5-HT by endocardial monoamine oxidase or hydrolyzing extracellular adenine nucleotides by means of ectonucleotidases as occurs in vascular endothelium.\textsuperscript{31} However, the results with tranylcypromine argue against monoamine oxidase activity as an explanation for the effect of endocardium. Such mechanisms would also fail to explain the quite different pattern of isometric twitch modulation in muscles with damaged endocardium and the discrepancy between increases in $V_{\text{max}}$ and those in total isometric tension after selective endocardial damage. Hence, it is improbable that the endocardial endothelium acts merely as a physical barrier. As a physiologic barrier with variable permeability, the endocardium may control the homeostasis of the microenvironment of the interstitial fluid surrounding adjacent myocytes,\textsuperscript{15} perhaps by means of a transendothelial electrochemical potential.\textsuperscript{32} Alternatively, endocardial modulation of myocardial contraction may be analogous to endothelial modulation of vascular tone\textsuperscript{12,13,33} where an endothelium-mediated relaxant action sums up with direct constrictor actions on smooth muscle to produce net effects smaller in vessels with endothelium compared with those without. In particular, vascular endothelium releases endothelium-derived relaxing factor, EDRF,\textsuperscript{34} which acts by stimulating soluble guanylate cyclase to increase intracellular cGMP levels\textsuperscript{35,36} and thereby exerts potent vasodilator actions. EDRF release is stimulated by arterial flow\textsuperscript{37} or by specific stimulants such as 5-HT, acetylcholine, noradrenaline, ADP, ATP, bradykinin, and others.\textsuperscript{8,9,12,33,38} With respect to vascular responses to aggregating platelets, both 5-HT and adenine nucleotides induce EDRF release, hence producing smaller contractions in isolated coronary vessels with endothelium compared with denuded vessels\textsuperscript{8,9,38} or vessels with regenerated endothelium.\textsuperscript{39} They also produce relaxation of intact precontracted vessels. A postulated endocardial mediator would decrease isometric tension and cause premature relaxation of myocardial cells,\textsuperscript{15,40} thereby opposing the direct myocardial effects of aggregating platelets. There is some circumstantial evidence supporting the existence of such an agent. Premature isometric relaxation and decreased peak tension are produced by cGMP analogues, nitroprusside (which like EDRF stimulates soluble guanylate cyclase), and atrial natriuretic peptides (which increase cGMP content by stimulating particulate guanylate cyclase). This action of atrial natriuretic peptides is only observed in the presence of an intact endocardium.\textsuperscript{16,40} In addition, modulation of the release by intact endocardial endothelium of another mediator that promoted myocardial contraction\textsuperscript{14,40} (a postulated myocardial contracting factor), thereby prolonging isometric twitch duration and augmenting peak twitch tension, should also be taken into account when interpreting the present data.
In the absence of relevant in vivo data, extrapolation of these results to the intact human heart must be cautious. The possibility that the endocardium may affect the contractile performance of the relatively large mass of subjacent myocardium may cause intuitive reservations. However, the effects of endocardial removal in isolated cardiac muscle are completely independent of muscle thickness. Even if myocardial mass was a limiting factor, the endocardium may play a modulatory role in the right ventricle where the ratio of endocardium to myocardium is much smaller than in the left ventricle.

Endocardial damage in the intact heart is a frequent occurrence in conditions such as ischemic heart disease and cardiomyopathies (particularly endomyocardial fibrosis) and is often associated with thrombus formation. Increases in myocardial contractility and delayed relaxation localized to areas with damaged overlying endocardium and active thrombus formation would result in increased nonuniformity of muscle performance and therefore have a deleterious effect on overall cardiac performance as a pump. Furthermore, the risks of thrombus embolization may also be higher in regions with nonuniform contractile behavior. Therefore, changes in contractile performance resulting from platelet aggregation may play a role in the pathophysiology of ventricular dysfunction in diseases where endocardial damage and mural thrombi occur.

Acknowledgments

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