Enhancement of the Force–Frequency Effect on Myocardial Contractility by Adrenergic Stimulation in Conscious Dogs

Masashi Kambayashi, MD; Toshiro Miura, MD; Byung-Hee Oh, MD; Howard A. Rockman, MD; Kazuya Murata, MD; and John Ross Jr., MD

**Background.** The influence of changes in heart rate on myocardial contractility (the force–frequency effect) differs under various experimental conditions, including the anesthetized versus the conscious state.

**Methods and Results.** To assess the influence of β-adrenergic stimulation on force–frequency effects on myocardial contraction and relaxation, seven instrumented conscious dogs were studied in which heart rate could be controlled by atrial pacing after the intrinsic rate was slowed with a bradycardiac agent (UL-FS 49 0.5–0.75 mg/kg). Left ventricular (LV) pressure was measured with a micromanometer under resting conditions and during dobutamine infusion at low, intermediate, and high doses (2.7, 5.4, and 10.7 μg/kg/min). At each dose, heart rate was progressively increased from 100 to 210 beats per minute. In the absence of dobutamine (control), no significant positive force–frequency effect was detected on LV dP/dt max; this was probably due to the known effect of the observed decrease in preload to reduce LV dP/dt max thereby offsetting an effect of the force–frequency response to increased dP/dt. However, during dobutamine infusions, the force–frequency effect was observed to increase significantly in a dose-dependent manner with increases in heart rate. An increase in heart rate from 100 to 210 beats per minute increased LV dP/dt max by 12.4±12.5% with low-dose, 22.7±13.1% with intermediate-dose, and 27.5±8.9% with high-dose dobutamine. Changes in preload and aortic pressure were within the same ranges under control conditions and at each of the three dobutamine doses. The time constant of LV pressure fall (τ) was significantly shorter with increases in heart rate during control, but only the highest dobutamine dose caused further significant shortening in τ with increased heart rate.

**Conclusions.** These data indicate that there is a pronounced dose-dependent action of β-adrenergic stimulation to enhance force–frequency–induced contractile responses in normal conscious dogs. (Circulation 1992;86:572–580)

**KEY WORDS** ● dobutamine ● myocardial contractility ● ventricular relaxation ● UL-FS 49 ● left ventricular function ● heart rate

The effect of increased heart rate to augment myocardial contractility, known as the force–frequency effect, has been well demonstrated in isolated mammalian heart muscle,1 in the intact hearts of anesthetized animals under controlled hemodynamic conditions,2–6 and in some studies in conscious animals.7 However, only recently has there been interest in the influence of stress or changes in adrenergic stimulation on the force–frequency effect.7–9 Because increases in both heart rate and inotropic state occur due to enhanced adrenergic stimulation during exercise, it is difficult to assess the role of the force–frequency effect per se. In the accompanying study, we provide evidence that the force–frequency effect importantly influences left ventricular contractility during exercise,10 suggesting and that this effect may be enhanced by increased adrenergic stimulation. Accordingly, the present study was designed to investigate the effects of direct infusion of a β-adrenergic agonist in varying doses on left ventricular contractility and relaxation in resting, conscious dogs subjected to a wide range of heart rates.

**Methods**

The animals in this study were handled according to the animal welfare regulations of the University of California San Diego. These regulations are in accordance with the animal use principles of the American Physiological Society and the American Heart Associa-
tion. The protocol was approved by the animal use committee of this institution.

**Animal Preparation**

Mongrel dogs of either sex weighing between 26.5 and 35.8 kg were trained to lie on a table before surgical instrumentation. On the day of surgery, dogs were tranquilized with morphine (1.0 mg/kg i.m.) and anesthetized with sodium thiopental (25 mg/kg i.v.). After endotracheal intubation, anesthesia was maintained with isoflurane (1–2%). Arterial blood gases were measured repeatedly throughout surgery, and ventilatory adjustment was made as necessary to keep PO₂ above 150 mm Hg and PCO₂ and pH within the physiological range. The heart was exposed through a left lateral thoracotomy in the fifth intercostal space, and the pericardium was opened. A high-fidelity micromanometer (Koningsberg FT7) and a Tygon fluid-filled catheter (1.27-mm i.d.) were inserted through a stab wound in the apex to measure left ventricular pressure. The micromanometer was calibrated by matching it to the ventricular pressure through the fluid-filled catheter (Statham P-23Db). Zero pressure reference was taken at the estimated level of the right atrium. Silicon rubber catheters were inserted into the upper descending aorta for measuring arterial pressure. A pair of pacing electrodes was sutured on the left atrial appendage.

For measuring left ventricular wall thickness, pairs of ultrasonic crystals were implanted in the anterior and posterior walls by standard techniques. The pericardium was left open, and all wires and the catheters were passed subcutaneously to the back of the dog and brought through the skin between the scapulae. The thorax was evacuated through a chest tube in the sixth intercostal space. Cefazolin (500 mg/kg i.m.) was administered 1 hour before surgery and every 3 hours throughout the procedure for a total of three doses.

**Protocols**

Experiments were conducted 1 week or later after surgery, when the animals had recovered fully. All data were obtained with the animal lying quietly on the table.

Our goal was to obtain experiments in which complete data could be obtained over a full range of heart rates (100–210 beats per minute) both under control resting conditions and during dobutamine infusions at different doses. In the first protocol, which was used in seven dogs, the bradycardic agent UL-FS 49 (1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[2-(3,4-dimethoxyphenyl)ethyl][methylimino][propyl]2H-3-benzazepin-2-on hydrochloride) (0.5–0.75 mg/kg diluted in 10 mL of saline) was injected through the left atrial catheter, and control recordings were obtained 15 minutes later at the intrinsic heart rate and paced heart rates of 90 (when possible), 100, 120, 150, 180, and 210 beats per minute. Atropine (0.1-mg boluses) was used as necessary to prevent atrioventricular block. Data were collected during a steady state at least 30 seconds after the start of each new pacing rate. Dobutamine (low dose, 1.8–3.2 μg/kg/min; mean, 2.7) was then administered continuously, and after a hemodynamic steady state was obtained, recordings were repeated at the intrinsic heart rate and paced heart rates of 90 (when possible), 100, 120, 150, 180, and 210 beats per minute. The same procedures were repeated with dobutamine infusion at intermediate dose (3.5–6.3 μg/kg/min; mean, 5.4) and high dose (6.8–12.6 μg/kg/min; mean, 10.7). In five of the seven dogs, we could not obtain heart rates lower than 120 beats per minute with the higher dobutamine doses; therefore, only two animals in which this protocol was used were included in the final analysis.

A second protocol was used in six dogs in order to obtain slower heart rates during dobutamine infusion. Once a hemodynamic steady state was achieved (approximately 15 minutes after injection of UL-FS 49), control data were obtained at the intrinsic heart rate and with left atrial pacing at the rate of 90 (when possible), 100, and 120 beats per minute; no atropine was administered. Dobutamine at low dose was then infused continuously through the left atrial catheter, and after a hemodynamic steady state was obtained (approximately 5 minutes after the start of dobutamine infusion), data were collected at the intrinsic heart rate and at paced heart rate of 90 (when possible), 100, and 120 beats per minute. Data collection was repeated with dobutamine infusions at intermediate and high doses. After an interval of at least 30 minutes to allow recovery from the effects of dobutamine, when hemodynamic conditions had returned to the initial control state, atropine sulfate (0.1 mg) was administered through the left ventricular catheter to prevent atrioventricular block, and data collections were repeated at higher heart rates of 150, 180, and 210 beats per minute. Additional atropine was given at a dose of 0.1 mg when necessary. Data were then obtained at the same heart rates during low-, intermediate-, and high-dose dobutamine infusions. Hemodynamic conditions did not return to the initial control state in one of the six dogs, and the remaining five dogs were used in the final analysis.

Except for the availability of lower heart rates in the second protocol, the responses of the heart showed no differences between the two protocols. Therefore, the data from the two protocols were pooled, two dogs from the first and five from the second protocol, giving seven dogs with a full range of heart rates for use in the final analysis.

In pilot studies, we examined the potential influence of UL-FS 49 on hemodynamic variables under resting conditions. This agent has previously been shown to have no direct negative inotropic effects. In the pilot studies, peak left ventricular (LV) pressure, left ventricular end-diastolic pressure (LVEDP), and LV dP/dṫmax were examined before and after UL-FS 49 in five conscious, resting dogs over a paced range of heart rates from 120 to 210 beats per minute. There were no statistically significant differences in any of these hemodynamic variables in presence or absence of the drug.

**Data Acquisition and Analysis**

Data at heart rates of 100–210 beats per minute were available for all seven dogs for all parameters except aortic pressure; aortic pressure data were available for six dogs. Data at a heart rate of 90 beats per minute are shown in the tables and figures to show the trend of the data but were not used for statistical analysis because of the lack of sufficient numbers. The number of dogs in each dobutamine subgroup included control, seven; dobutamine low dose, six; middle dose, five; and high dose, four.
Data were recorded on an eight-channel chart recorder (Gould, Brush) and on 1.3-cm magnetic tape (Hewlett-Packard, HP 3955D). Digitized data (250 Hz) were simultaneously recorded on a microcomputer for beat averaging. At least 10 consecutive beats were used for each observation; in five dogs, beats were averaged over 10 seconds. Measurements of myocardial wall thickness were made with a sonomicrometer (Triton Technologies, San Diego, Calif.).

The measured parameters were LV peak systolic pressure, LVEDP, the maximum (positive) and minimum (negative) first derivative of LV pressure (LV dP/dt max, LV dP/dt min), mean and minimal aortic pressures, end-diastolic wall thickness (WT), and systolic wall thickening presented as the percent change from the end-diastolic thickness (%WT). End diastole (ED) was defined as the time coincident with the rapid rise of LV dP/dt max. End systole (ES) was defined as the point of maximal systolic wall thickness within 20 msec before LV dP/dt min. Percent systolic wall thickening was calculated as (WTES−WTED)×100/WTED. Either the anterior or the posterior wall thickness crystals were used, the pair showing the largest percent thickening being selected. Four pairs of anterior crystals and three pairs of posterior crystals were used in the analysis.

The ratio of LV dP/dt developed to LV pressure (DP) at a developed LV pressure of 40 mm Hg [(dP/dt)/D40] was also calculated.

The time constant (τ) of isovolumic pressure fall of the LV pressure was determined using data from individual beats from LV dP/dt min to the time LV pressure had fallen to 10 mm Hg or to an LVEDP equal to that of the subsequent beat, whichever was larger. These pressure and time data were used to calculate τ with monoexponential fitting. The correlation coefficients (r) were generally between 0.993 and 0.995, and the lowest r value was 0.991. Residual sums of square (RSS) of curve fitting were mostly within 0.015 (mm Hg)², and the maximal RSS was 0.018 (mm Hg)².

**Statistical Analysis**

Data were analyzed using a two-way, repeated-measures ANOVA with post hoc tests based on t tests for simple effects.12 Comparison with normalized values that have no variance was done using a one-sample t test. A value of p<0.05 was considered significant. For each studied variable, statistical significance was examined in two ways: significances between different heart rates and significances between control and three dobutamine doses. Data are presented as mean±SD except in the figures in which mean±SEM is used.

**Results**

**Hemodynamic Variables and Wall Thickness**

LVEDP was significantly lower than control at higher heart rates, an effect also seen with dobutamine infusions, but LVEDP values at each heart rate during dobutamine infusion were not significantly different from control (Table 1). LV peak systolic pressure showed no significant changes between heart rates but was increased during high-dose dobutamine and at some middle doses. LV pressure at LV dP/dt max did not change significantly either between heart rate levels or between dobutamine doses, and it was always lower than the minimal aortic pressure (AOP min), although the AOP min decreased at higher dobutamine (Table 1).

WTED showed no significant change between dobutamine doses but was significantly greater at higher heart rates. Percent systolic wall thickening (%WT) was significantly reduced at higher heart rates compared with control, and it was significantly higher at the higher dobutamine doses.

**Ventricular Contractility Indexes**

LV dP/dt max was not affected by increasing heart rate from 90 to 210 beats per minute under control conditions (Figure 1A). However, during dobutamine infusions, LV dP/dt max was progressively higher with increasing cardiac frequency, and the positive force–frequency effect was dose dependent (Figure 1A); that is, the increase in LV dP/dt max caused by augmented heart rate was more pronounced as adrenergic stimulation increased.

Because LV dP/dt is influenced by preload, the possible influence of LVEDP was analyzed. The ranges of LVEDP were nearly identical for control and each dobutamine dose (Figure 1B).

To further evaluate the influence of dobutamine on the force–frequency effect, LV dP/dt max was expressed as a percentage of the LV dP/dt max at a heart rate of 100 beats per minute for each dobutamine dose (normalized dP/dt%). Normalized LV dP/dt max did not change during control and was significantly greater at the higher heart rates and the higher dobutamine doses (Figure 2).

LV (dP/dt)/DP40 was also studied as an index of contractility, because it has reduced afterload and preload dependency. LV (dP/dt)/DP40 during dobutamine infusions was significantly increased at the higher heart rates in a dose-dependent manner (Table 2). Normalized (dP/dt)/DP40 expressed as percent of that at 100 beats per minute also was significantly increased.

**Ventricular Relaxation**

LV dP/dt min was significantly greater at higher heart rates compared with the control rate, as well as at the higher dobutamine doses (Table 2). The time constant of LV pressure fall (τ) was significantly reduced as heart rate was increased under control conditions (Figure 3A), and the effect became larger at higher dobutamine doses and was significantly augmented at the highest dobutamine dose (Figure 3A).

To further evaluate the influence of the force–frequency effect on relaxation during dobutamine infusion, τ was normalized as percent of that at 100 beats per minute. Normalized τ was significantly reduced at the higher dobutamine doses at higher heart rates, but it was significantly reduced compared with lower doses only at heart rates of 180 and 210 beats per minute during high-dose dobutamine (Figure 3B).

**Discussion**

**Force–Frequency Effects on Myocardial Contractility and Relaxation**

The present study demonstrates that whereas a force–frequency effect on myocardial contractility assessed by LV dP/dt max was not apparent over a wide range of heart rates under control resting conditions, it was enhanced by dobutamine in a dose-dependent manner. This indi-
The lack of sensitivity of LV end-diastolic pressure; LVEDP, pressure; LV end-diastolic wall thickness; %WT, percent wall thickening. Normalized values are expressed as percent (100% at PR 100).

Values are mean±SD; n=7 (n=6 for AOP) except pacing rate (PR) 90. Values at PR 90 were not used for statistical analysis because of lack of sufficient numbers.

Cont, control; Low, dobutamine low dose; Mid, dobutamine middle dose; High, dobutamine high dose; LVP, left ventricular pressure; LVEDP, end-diastolic LVP; LVPmax, peak systolic LVP; LVP at dp/dtmax, LVP at LV maximum positive dp/dt; AOPmin, minimum aortic pressure; WTED, end-diastolic wall thickness; %WT, percent wall thickening.

The increase in basal inotropic state induced by adrenergic stimulation can change the manifestation of the force–frequency effect. The lack of a demonstrable force–frequency effect under basal conditions in the present study undoubtedly reflects the well-known sensitivity of LV dP/dt to ventricular preload; because LV end-diastolic pressure fell as heart rate increased (Figure 1B), this effect would tend to reduce dP/dt and hence could offset an increase in LV dP/dtmax caused by the force–frequency effect observed in studies when preload was held constant, or load-independent contractility measures were used, as discussed further below.

Based on our findings, it may be expected that when basal inotropic state is elevated by anxiety or exercise, the force–frequency effect will be enhanced. The Ca2+ transient has been shown to increase with augmented frequency of contraction in isolated cardiac muscle.
**β-Adrenergic stimulation probably enhances the force-frequency relation by further altering Ca\(^{2+}\) availability at the myofilaments, perhaps through cyclic AMP-mediated phosphorylation effects on the Ca\(^{2+}\) channel**\(^{14}\) and/or enhanced sarcoplasmic reticular Ca\(^{2+}\) release,\(^{15}\) but the precise mechanism of the effect of β-adrenergic stimulation to enhance the force-frequency effect remains to be determined.

Data in a recent report on ventriculoventricular coupling by Freeman and Colston\(^7\) in anesthetized dogs with ganglionic blockade are in general agreement with our observations. In that study, high-dose dobutamine infusion (10 μg/kg/min) at two cardiac frequencies (160 and 200 beats per minute) appeared to cause a larger increase in the slope of the end-systolic pressure–volume relations (ESPVR) between the two frequencies than under control conditions; at matched left ventricular end-diastolic volumes, there was no significant increase in LV dP/dt\(_{max}\) between 160 and 200 beats per minute under control conditions, but during high-dose dobutamine, this change was larger and statistically significant. Although these studies were not in conscious animals and examined only a single change in cardiac frequency with a single drug dose, they provide evidence that an additive interaction occurs between adrenergic stimulation and the force-frequency effect. Moreover, the effect observed in that study was evident in the absence of UL-FS 49, suggesting that there was not a direct influence of that agent in our study.

Early investigations in the left ventricles of anesthetized dogs with a right heart bypass preparation in which hemodynamic conditions could be controlled showed a positive force-frequency effect on myocardial contractility.\(^4\) More recently, Suga et al\(^16\) used the end-systolic pressure–volume relation (ESPVR) to evaluate contractility under controlled conditions in anesthetized animals and reported that an increase in heart rate over the range from 100 to 160 beats per minute had minimal effects on the ESPVR, whereas Maughan et al\(^17\) demonstrated in a similar animal preparation that the ESPVR slope increased over a frequency range from 60 to 120 beats per minute but showed little change in the range between 120 and 180 beats per minute.

In conscious animals, poststimulation potentiation has been demonstrated,\(^18\) but Noble et al\(^19\) reported that myocardial contractility was unaffected by a change in heart rate over the range of 90–190 beats per minute. Arentzen et al\(^20\) also reported that steady-state changes in contraction frequency did not significantly alter LV dP/dt over a heart rate range from 100 to 200 beats per minute. In studies by Higgins et al,\(^21\) LV dP/dt increased only slightly (14%) over a heart rate range from 94 to 220 beats per minute when filling pressure was main-
<table>
<thead>
<tr>
<th>Pacing rate (beats per minute)</th>
<th>90</th>
<th>100</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LV dP/dt\textsubscript{min} (mm Hg/sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>2,327±240</td>
<td>2,403±219</td>
<td>2,450±288</td>
<td>2,557±342</td>
<td>2,509±308</td>
<td>2,483±338</td>
</tr>
<tr>
<td>Low</td>
<td>2,362±354</td>
<td>2,503±374</td>
<td>2,585±419</td>
<td>2,676±612*</td>
<td>2,710±579*</td>
<td>2,686±533*</td>
</tr>
<tr>
<td>Mid</td>
<td>2,434±496</td>
<td>2,501±505</td>
<td>2,644±549</td>
<td>2,706±675*</td>
<td>2,766±598*</td>
<td>2,830±647*</td>
</tr>
<tr>
<td>High</td>
<td>2,307±281</td>
<td>2,614±529</td>
<td>2,731±545*</td>
<td>2,804±766*</td>
<td>2,890±725*</td>
<td>2,995±765*</td>
</tr>
<tr>
<td><strong>LV dP/dt\textsubscript{max} (mm Hg/sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>2,854±249</td>
<td>2,955±403</td>
<td>3,014±420</td>
<td>3,015±381</td>
<td>3,031±325</td>
<td>2,922±293</td>
</tr>
<tr>
<td>Low</td>
<td>3,072±381</td>
<td>3,144±301</td>
<td>3,288±375</td>
<td>3,513±329*</td>
<td>3,602±382*</td>
<td>3,521±414*</td>
</tr>
<tr>
<td>Mid</td>
<td>3,092±247</td>
<td>3,243±288*</td>
<td>3,550±498*</td>
<td>3,825±534*</td>
<td>3,930±532*</td>
<td>3,981±578*</td>
</tr>
<tr>
<td>High</td>
<td>3,231±151</td>
<td>3,576±542*</td>
<td>3,935±629*</td>
<td>4,280±792*</td>
<td>4,477±775*</td>
<td>4,569±810*</td>
</tr>
<tr>
<td><strong>Normalized dP/dt (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>100.0±0.0</td>
<td>102.0±3.1</td>
<td>102.3±5.8</td>
<td>103.1±7.6</td>
<td>99.4±5.7</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>100.0±0.0</td>
<td>104.5±4.5*</td>
<td>112.0±8.1*</td>
<td>114.9±10.1*</td>
<td>112.4±12.5*</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>100.0±0.0</td>
<td>109.2±8.2**</td>
<td>117.7±8.8**</td>
<td>121.0±9.6**</td>
<td>122.7±13.1**</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>100.0±0.0</td>
<td>110.0±4.3*</td>
<td>119.2±7.1**</td>
<td>124.9±7.6**</td>
<td>127.5±8.9**</td>
<td></td>
</tr>
<tr>
<td><strong>(dP/dt)/DP\textsubscript{ao} (1/sec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>59.1±8.1</td>
<td>62.6±8.7</td>
<td>64.3±7.8</td>
<td>62.7±5.2</td>
<td>63.2±4.4</td>
<td>58.8±4.4</td>
</tr>
<tr>
<td>Low</td>
<td>65.2±7.2</td>
<td>67.1±5.1</td>
<td>69.0±6.7</td>
<td>75.7±3.7*</td>
<td>76.0±5.3*</td>
<td>70.2±13.9*</td>
</tr>
<tr>
<td>Mid</td>
<td>65.0±3.1</td>
<td>69.4±5.1*</td>
<td>76.1±8.3*</td>
<td>83.7±7.0**</td>
<td>84.2±7.5**</td>
<td>82.9±16.2**</td>
</tr>
<tr>
<td>High</td>
<td>67.6±9.8</td>
<td>73.8±13.4*</td>
<td>85.6±10.4*</td>
<td>93.9±9.9*</td>
<td>99.1±11.9*</td>
<td>97.7±19.8*</td>
</tr>
<tr>
<td><strong>Normalized (dP/dt)/DP\textsubscript{ao} (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>100.0±0.0</td>
<td>103.1±5.0</td>
<td>101.2±10.2</td>
<td>103.0±18.5</td>
<td>95.9±16.9</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>100.0±0.0</td>
<td>102.8±4.7</td>
<td>113.3±9.8*</td>
<td>114.1±14.4*</td>
<td>105.8±25.3</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>100.0±0.0</td>
<td>109.9±11.2</td>
<td>121.2±13.7*</td>
<td>122.1±16.4*</td>
<td>121.1±30.8*</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>100.0±0.0</td>
<td>118.0±16.7*</td>
<td>131.1±31.1*</td>
<td>139.3±39.1*</td>
<td>139.1±51.5*</td>
<td></td>
</tr>
<tr>
<td><strong>τ (msec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>25.2±1.8</td>
<td>23.4±1.6</td>
<td>21.7±1.5*</td>
<td>20.8±2.5*</td>
<td>20.9±2.3*</td>
<td>20.8±2.0*</td>
</tr>
<tr>
<td>Low</td>
<td>23.6±1.3</td>
<td>22.4±2.0</td>
<td>21.0±2.3*</td>
<td>19.7±1.6*</td>
<td>19.2±1.4*</td>
<td>19.4±2.7*</td>
</tr>
<tr>
<td>Mid</td>
<td>23.7±0.6</td>
<td>21.7±2.1*</td>
<td>20.6±1.0*</td>
<td>18.8±2.3*</td>
<td>18.0±1.7**</td>
<td>18.1±2.5**</td>
</tr>
<tr>
<td>High</td>
<td>23.1±1.9</td>
<td>21.6±1.1*</td>
<td>19.3±1.1*</td>
<td>18.0±1.9**</td>
<td>16.7±1.5*</td>
<td>16.4±2.2*</td>
</tr>
<tr>
<td><strong>RSS (mm Hg²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>0.010±0.009</td>
<td>0.012±0.008</td>
<td>0.014±0.008</td>
<td>0.013±0.007</td>
<td>0.011±0.008</td>
<td>0.011±0.008</td>
</tr>
<tr>
<td>Low</td>
<td>0.013±0.007</td>
<td>0.012±0.009</td>
<td>0.013±0.008</td>
<td>0.013±0.007</td>
<td>0.010±0.007</td>
<td>0.010±0.007</td>
</tr>
<tr>
<td>Mid</td>
<td>0.009±0.008</td>
<td>0.011±0.009</td>
<td>0.013±0.008</td>
<td>0.014±0.008</td>
<td>0.012±0.005</td>
<td>0.013±0.009</td>
</tr>
<tr>
<td>High</td>
<td>0.010±0.013</td>
<td>0.010±0.011</td>
<td>0.011±0.008</td>
<td>0.015±0.008</td>
<td>0.016±0.006</td>
<td>0.018±0.010</td>
</tr>
<tr>
<td><strong>Normalized τ (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>100.0±0.0</td>
<td>92.7±2.9*</td>
<td>88.6±7.9*</td>
<td>89.3±8.7*</td>
<td>88.8±7.6*</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>100.0±0.0</td>
<td>93.8±5.3*</td>
<td>88.2±6.0*</td>
<td>86.2±6.9*</td>
<td>86.9±12.6*</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>100.0±0.0</td>
<td>95.7±11.0</td>
<td>87.0±11.3*</td>
<td>83.6±9.1*</td>
<td>83.7±12.4*</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>100.0±0.0</td>
<td>89.1±3.6*</td>
<td>83.0±6.3*</td>
<td>77.2±4.9*</td>
<td>75.9±7.6*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD; n=7 except pacing rate (PR) 90. Values at PR 90 were not used for statistical analysis because of lack of numbers. Cont, control; Low, dobutamine low dose; Mid, dobutamine middle dose; High, dobutamine high dose; LV dP/dt\textsubscript{min}, left ventricular (LV) minimum negative dP/dt; LV dP/dt\textsubscript{max}, LV maximum positive dP/dt; (dP/dt)/DP\textsubscript{ao}, LV dP/dt at developed LV pressure of 40 mm Hg; τ, time constant of LV pressure fall; RSS, residual sum of squares for the curve fitting of τ. No statistical analysis was done for RSS. Normalized values are expressed as percent (100% at PR 100).

*p<0.05 vs. PR 100.

*p<0.05 vs. Cont.

*p<0.05 vs. PR 100 and PR 120.

*p<0.05 vs. PR 100, PR 120, and PR 150.

*p<0.05 vs. Low.

*p<0.05 vs. Cont, Low, and Mid.

*p<0.05 vs. PR 100, PR 120, PR 150, and PR 180.

Freeman et al.\textsuperscript{22} who reported that E\textsubscript{es} increased by 228% when heart rate was increased from 100 to 200 beats per minute. In those studies in which ventricular end-diastolic volumes were matched at different heart rates, LV dP/dt\textsubscript{max} also increased significantly as heart
rate was augmented, as might be expected, but to a lesser degree than $E_{\text{sv}}$; these investigators also studied two animals treated with propranolol and atropine and found a significant force–frequency effect in the autonomically blocked dogs by $E_{\text{sv}}$ and dP/dt analysis.\textsuperscript{22} Whether the force–frequency effect was significantly less marked than without blockade because of the mild negative inotropic action of $\beta$-blockade was not analyzed. Spratt et al\textsuperscript{23} reported earlier that changes in heart rate had minimal effects on $E_{\text{sv}}$. There are studies in human subjects showing either a positive force–frequency effect\textsuperscript{24} or little change.\textsuperscript{25}

These widely differing results may be influenced by the differences in the experimental setting, in which preload varied or was controlled, and the basal inotropic state undoubtedly differed as well. Also, various indexes were used to evaluate changes in contractility. It seems likely in the present studies in conscious, resting dogs that the lack of a significant force–frequency effect over a wide heart rate range under control conditions was observed for two reasons: First, the index of contractility used is preload sensitive, and LVEDP progressively fell with increased heart rates; second, the basal inotropic state was not enhanced under these experimental conditions in conscious, resting dogs.

LV dP/dt$_{\text{max}}$ as a Contractility Index

As an index of contractility of the whole heart, isovolumic phase measures (mainly LV dP/dt$_{\text{max}}$) have frequently been used, although recently an end-systolic index (the slope of the end-systolic pressure–volume relation) has been used because of its load independence. The latter approach has some disadvantages in conscious animals because it is technically complicated to use; reflex effects can occur with changes in loading, and accurate measurement of ventricular volume measurement may be difficult over a wide range of loading conditions. Moreover, end-systolic measures are somewhat less sensitive to changes in inotropic state than LV dP/dt$_{\text{max}}$.\textsuperscript{26} On the other hand, LV dP/dt$_{\text{max}}$, although highly sensitive to acute changes in inotropic state and easy to measure accurately with a micromanometer,\textsuperscript{26–29} is known to be dependent on changes in preload.\textsuperscript{27,29,30}

In this study, LVEDP progressively decreased with increases in heart rate, and the effect of LVEDP alone would be to diminish LV dP/dt$_{\text{max}}$, with underestimation of any force–frequency effect in this setting. Thus, under control conditions, there appeared to be no force–frequency effect on contractility; presumably, it was masked by the reduced LVEDP and LV end-diastolic volume. Such a response might explain the lack of a force–frequency effect in some studies in which preload was not held constant, whereas in a previous study in this laboratory, a potentiating effect (poststimulation potentiation) in conscious dogs was demonstrated by studying LV dP/dt$_{\text{max}}$ in the first beats after cessation of the heart rate increase, when LVEDP had increased to the prepaing value.\textsuperscript{18} Another possible limitation of the use of LV dP/dt$_{\text{max}}$ as a contractility index is the influence of minimal aortic pressure on dP/dt$_{\text{max}}$; when minimal aortic pressure is low, dP/dt$_{\text{max}}$ may be underestimated, because dP/dt$_{\text{max}}$ cannot reach a maximum before aortic valve opening.\textsuperscript{31,32} However, LV dP/dt$_{\text{max}}$ is largely independent of changes in afterload, provided dP/dt$_{\text{max}}$ occurs before aortic valve opening.\textsuperscript{28,33} In our study, the LV pressure at LV dP/dt$_{\text{max}}$ was always lower.
than the AoP\textsubscript{min}. Also, LV (dP/dt)/DP\textsubscript{aw} occurs before aortic valve opening and is less sensitive to changes in preload and afterload, and this index showed almost the same results as LV dP/dt\textsubscript{max}. Therefore, in the present study, it was possible to use LV dP/dt\textsubscript{max} to evaluate the \(\beta\)-adrenergic effect on the force–frequency relation because the preload changes for control and three dobutamine doses were identical (Figure 1B), and afterload effects were insignificant.

It has been shown that dobutamine infusion can increase the slope of the relation between LV dP/dt\textsubscript{max} and LV end-diastolic volume during vena caval occlusion, such that dP/dt\textsubscript{max} is increased more by dobutamine at higher than at lower preloads.\textsuperscript{30} However, our conclusion that a dose-dependent dobutamine influence on the force–frequency effect exists would not be affected by such a phenomenon, because a larger preload existed at lower heart rates, when the smallest force–frequency effect on LV dP/dt\textsubscript{max} was observed both at control and during dobutamine infusions (Figures 1A and 1B), and vice versa. Thus, the effect of dobutamine on the force–frequency effect was probably underestimated by the methods used.

**Force–Frequency Effect and Relaxation**

We observed a consistently more rapid relaxation with increasing cardiac frequency under control conditions, as reported previously,\textsuperscript{22} which was further enhanced with the highest dobutamine dose. The effect of heart rate and inotropic agents on myocardial relaxation has been studied by many investigators. Weiss et al\textsuperscript{34} reported only a slight influence of heart rate on the time constant \(\tau\) of left ventricular pressure fall, but it has been shown in subsequent studies to be shortened by higher heart rate,\textsuperscript{35–37} and catecholamines abbreviate \(\tau\) both in animal models and in human subjects.\textsuperscript{34–36} \(\tau\) also has been shown to be lengthened with volume loading\textsuperscript{38} and with increased afterload\textsuperscript{39} and was shown to decrease during exercise in humans\textsuperscript{40} and in normal experimental animals.\textsuperscript{41} However, the effect of catecholamine infusion on the force–frequency effect in terms of relaxation has received little attention.

The time constant \(\tau\) of LV pressure fall is a standard index of LV isovolumic relaxation, but debate exists about the methods of assessing \(\tau\), including approaches to curve fitting. Conventionally, the LV pressure fall has been fitted to a monoexponential equation with a fixed asymptote of 0 mm Hg \(P=\text{Ae}^{-t}\).\textsuperscript{34} However, Thompson et al\textsuperscript{42} reported that monoexponential fitting with variable asymptote \(P=\text{Ae}^{-t}+B\) yields better results. Yellin et al\textsuperscript{43} examined these two approaches, assessing the LV pressure fall in nonfilling ventricles in which the true time constant could be measured, and concluded that when \(\tau\) is calculated using the conventional monoexponential formulation assuming zero pressure asymptote, there was no significant difference from the true value and that the conventional method yielded a better estimate of true \(\tau\) than the variable asymptote. Another debate has concerned which portion of the LV pressure curve should be assessed. By definition, the pressure curve during isovolumic relaxation should be used, i.e., from closure of the aortic valve to the opening of the mitral valve. Usually, analysis begins at or after LV dP/dt\textsubscript{min}, and we used this approach. However, there is no general agreement as to when the analysis should end, and often, it is not possible to determine the time of mitral valve opening. The end of isovolumic relaxation generally has been assumed to occur at the LVEDP of the subsequent beat or a somewhat higher pressure; in this study, large changes in heart rate produced a wide range of LVEDP, and we used 10 mm Hg or the LVEDP of the subsequent beat, whichever was higher.

The validity of monoeponential fitting can be estimated from the correlation coefficient \(r\) and RSS of the curve fitting. Thompson et al\textsuperscript{45} suggested that the \(r\) value offers little guidance to the validity of the estimate of \(\tau\), because the predicted course could deviate from a monoeponential despite a good value \((r=-0.98)\), but Freeman et al,\textsuperscript{22} in their study on the influence of heart rate, which used monoeponential analysis with a variable asymptote, used RSS. In the present study, both the \(r\) values (the smallest \(r\) value was 0.991) and RSS (the maximal RSS was 0.018 [mm Hg]) were within acceptable ranges, suggesting that our estimate of \(\tau\) was satisfactory.

The mechanism of the observed further enhancement of relaxation by force–frequency effect in the presence of \(\beta\)-adrenergic stimulation is not clear but, as mentioned, the calcium transient is not only increased but shortened with augmented contraction frequency.\textsuperscript{13} \(\beta\)-Adrenergic stimulation is known to increase the rate of sarcoplasmic reticular Ca\textsuperscript{2+} reuptake\textsuperscript{15} and appears to complement the enhanced relaxation caused by the force–frequency effect alone. It is also possible that \(\tau\) might have been affected by enhanced restoring forces secondary to the augmented ventricular emptying effect generally produced by dobutamine.

**Limitations**

Certain potential experimental limitations must be considered in interpreting our results. The specific bradycardic agent UL-FS 49 was used to lower the sinus node rate and is considered to have little or no effect on other cardiovascular parameters, including inotropic state.\textsuperscript{44} In our laboratory, both in dogs and pigs, a direct negative inotropic effect has not been demonstrated.\textsuperscript{45,46} As reviewed in detail in association with the accompanying study from this laboratory,\textsuperscript{10} because this drug has a relatively specific effect on the sinoatrial node, it also seems unlikely that UL-FS 49 per se had an inotropic influence on the ventricles with increasing heart rates during adrenergic stimulation. Atropine was used in some of the studies, but in our second protocol, atropine was not given in studying the influence of dobutamine on force–frequency relations at lower heart rates (90–120 beats per minute), and these responses did not differ from those in the initial protocol in which atropine was used. Finally, it should be noted that the findings in this study were obtained from normal hearts, and the effects of adrenergic stimulation on the force–frequency relation remain to be evaluated under pathological conditions.

**Acknowledgments**

We thank Margaret R. Hill, Denice J. Brannigan, and Farid Abdel-Wahhab for technical assistance. We also thank Elizabeth Gilpin for valuable statistical advice. In addition, we thank Peggy Ganong of Boehringer Ingelheim Pharmaceuticals, Inc., for providing UL-FS 49.
References


Downloaded from http://circ.ahajournals.org/ by guest on July 16, 2017
Enhancement of the force-frequency effect on myocardial contractility by adrenergic stimulation in conscious dogs.

M Kambayashi, T Miura, B H Oh, H A Rockman, K Murata and J Ross, Jr

_Circulation_. 1992;86:572-580
doi: 10.1161/01.CIR.86.2.572

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/86/2/572