Relaxation effect of CGP-48506, EMD-57033, and dobutamine in ejecting and isovolumically beating rabbit hearts

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We have shown previously (21) that, in addition to slowing relaxation by increasing left ventricular (LV) wall stress, $2 \times 10^{-6}$ M EMD-57033 also has a stress-independent effect to slow relaxation in the isolated rabbit heart (buffer perfused at 30°C). Higashiya et al. (11) also showed that EMD-57033 significantly prolonged relaxation in the isolated rabbit heart (blood perfused at 37°C). Furthermore, they noted a trend toward a stronger inotropic effect in ejecting versus isovolumic beats. However, they did not evaluate whether ejection influenced the effect of EMD-57033 on relaxation. Accordingly, the first purpose of our study was to develop a broader analysis of stress-dependent relaxation, which included ejecting beats, to evaluate whether the effects of positive inotropic interventions on relaxation were different in ejecting versus isovolumic beats.

We then applied this analysis to compare the lusitropic effects of EMD-57033 and another recently introduced Ca$^{2+}$-sensitizer, the novel 1,5-benzodiazocine derivative BA-41899 (5-methyl-6-phenyl-1,3,5,6-tetrahydro-3,6-methano-1,5-benzodiazocine-2,4-dione) (8, 9). Like EMD-57033, BA-41888 possesses enantiospecific effects: the Ca$^{2+}$-sensitizing activity resides in the positive enantiomer CGP-48506 (8), which is devoid of PDE I-IV activity at concentrations $\leq 3 \times 10^{-4}$ M in both human (17) and guinea pig (27) myocardium, making it the most selective Ca$^{2+}$-sensitizer introduced to date (8).

Although in vitro studies have suggested that CGP-48506 slows relaxation (8, 9, 17, 18, 24, 27), some of these data also suggested that, unlike EMD-57033, CGP-48506 does not adversely affect resting cell length (24) or force (18). On the basis of these and other observations, it has been suggested both that the site of action of CGP-48506 is different from that of EMD-57033 and that CGP-48506 might have fewer adverse effects on diastolic function than EMD-57033 (18, 24).

Accordingly, the second purpose of our study was to test the hypothesis that CGP-48506 would have less effect than EMD-57033 on relaxation in the isolated buffer-perfused rabbit heart. This analysis of stress-dependent relaxation in both ejecting and isovolumic beats readily differentiates between the negative lusitropic effects of $2 \times 10^{-6}$ M EMD-57033, the negligible lusitropic effect of 6 M CGP-48506 and 6 M EMD-57033, and the positive lusitropic effect of $1.25 \times 10^{-6}$ M dobutamine. Furthermore, comparison of the effect of the two Ca$^{2+}$-sensitizing drugs in ejecting versus isovolumic contractions shows that CGP-48506 affects relaxation differently in ejecting contractions than it does in isovolumic contractions, whereas EMD-57033 affects relaxation similarly in both ejecting and isovolumic contractions.

Calcium sensitizing drugs increase the force of myocardial contraction by increasing the responsiveness of myofilaments to Ca$^{2+}$ rather than by increasing the amount of Ca$^{2+}$ released to activate myofilaments (15). Many Ca$^{2+}$ sensitizers also possess some degree of phosphodiesterase (PDE) III-inhibiting activity. For the thia diazizine derivative EMD-53998 [5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydrochinoxalin-6-yl]-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one], these two actions are enantiospecific, with the positive enantiomer, EMD-57033, acting predominately as a Ca$^{2+}$ sensitizer and the negative enantiomer, EMD-57439, acting predominately as a PDE III inhibitor (5,16, 23).

The isolation of very specific Ca$^{2+}$-sensitizing action, as with EMD-57033, makes it important to understand the effects of these drugs on ventricular relaxation; although Ca$^{2+}$-sensitizing compounds have generated considerable enthusiasm, there are concerns about their ultimate usefulness because a specific Ca$^{2+}$-sensitizing effect might also prolong relaxation (3, 15, 22) or increase diastolic tone (5, 7, 19).

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METHODS
Isolated Heart Preparation
This study was approved by the Institutional Animal Care and Use Committee. Experiments were performed using hearts isolated from 24 adult male New Zealand White rabbits (median weight 3.1 kg; range 2.8–3.5 kg). Details of the procedure for removing the heart from an anesthetized
rabbit and mounting it on a volume-servo system for pressure- and volume-control experiments have been described previously (2, 14). Briefly, rabbits were anesthetized by intramuscular injection of ketamine, xylazine, and atropine (35, 7.5, and 0.02 mg/kg, respectively). After a tracheostomy was performed, surgical anesthesia was maintained with 1–2% isoflurane via positive-pressure ventilation. A midsternal thoracotomy was performed, the brachiocephalic artery was cannulated, and 1,000 U heparin were administered via the cannula. A modified Tyrode solution that contained 35 mM K+ was vigorously bubbled with 95% O2-5% CO2 and delivered via the brachiocephalic cannula to arrest the heart. This relaxing solution was composed of (in mM) 121 Na+, 35 K+, 138 Cl−, 1.24 Ca2+, 1.08 Mg2+, 21 HCO3−, 0.36 PO43−, and 11.1 glucose, with 2.5 U/l insulin. The heart beat isovolumically, which contained a lower K+ perfusate was then changed to a modified Tyrode solution, which yielded a family of functional indexes to serve as control values. After these two protocols were completed, perfusion was switched from control perfusate to a perfusate containing one of the three inotropic drugs, followed by a 15-min period for a stable baseline to be established, as indicated by stable peak pressure. The FS and AL protocols were then once again conducted, and records were taken during the influence of the inotropic drug.

FS Protocol. The single-beat FS protocol has been described in detail elsewhere (2, 20, 21). Briefly, with the heart beating isovolumically at VBL, 80 ms before a selected beat the volume was commanded to be 1 of 10 volumes (50–140% of VBL in 10% increments) (Fig. 1A). This selected beat is called the volume-perturbed beat. The contraction and relaxation of the volume-perturbed beat took place isovolumically at the commanded volume. The heart period of the volume-perturbed beat was prolonged to 115% of the basal period to ensure complete relaxation during the diastolic period at the commanded volume. The ensuing beat also took place at the commanded volume, and, after this beat, volume and heart period were returned to baseline. Fifteen seconds were allowed for transients to die out, and then the procedure was repeated at one of the other commanded volumes. This sequence was repeated until records (digitally sampled at 250 Hz) had been taken for the set of 10 commanded volumes. Stability during the protocol was assessed by variation in peak isovolumic pressure of the beat before the volume-perturbed beat among the full set of records. This variation was always <3%.

As we have done previously (21), we measured fully relaxed, passive pressure (Ppass) as the lowest pressure during the prolonged pause following each volume-perturbed beat (Fig. 1B), and peak LV developed pressure (DPeak) was determined by subtracting Ppass from peak pressure (Ppeak). The duration of pressure development (Ttotal) was defined as the time from the pacing stimulus to 10% of DPpeak during the relaxation phase. The time to peak pressure (Tpeak) was defined as time from the pacing stimulus to DPpeak. The characteristic time of relaxation (T75–25) was defined as the time of pressure decay from 75 to 25% DPpeak. Finally, DPpeak was converted to peak midwall stress (σpeak) according to

\[
σ_{\text{peak}} = \frac{DP_{\text{peak}}}{\frac{V_B}{V} + 1}^{2/3} - 1
\]

where Vw is LV wall volume (LV wall mass/1.05). When plotted against σpeak, T75–25 yields the relaxation-wall stress relationship for isovolumic beats (20, 21).

AL Protocol. The AL protocol is one we have used previously to construct end-systolic pressure-volume relationships and has been described in detail elsewhere (2). Briefly, with the heart beating isovolumically at VBL, a beat was selected, called the pressure-damped beat, in which pressure was not allowed to rise above one of eight commanded pressures (100–40% of Ppeak, in 10% decrements) (Fig. 2A). This was achieved by allowing the beat to proceed isovolumically until pressure rose to the commanded value, at which point pressure was clamped. Pressure damping, which was achieved by volume withdrawal, continued until maintenance of the commanded pressure required volume infusion. At that point, volume was held constant at the end-systolic value (Vw), while pressure fell as the heart relaxed. The heart was refilled to VBL before the ensuing beat and was allowed to beat isovolumically for 15 s, and then the next record was taken. This sequence was repeated for all eight commanded pressures.
Similar to the isovolumic beats from the FS protocol, \( P_{\text{pass}} \) was determined as the minimum pressure measured before volume was returned to \( V_{\text{BL}} \) following ejection (Fig. 2B). The time of the maximal pressure-volume ratio of each pressure-clamped beat was defined as the time of end ejection (\( T_{\text{ej}} \)), and the pressure at this time was defined as the end-ejection pressure (\( P_{\text{ej}} \)). End-systolic pressure (\( P_{\text{es}} \)) was calculated as \( P_{\text{ej}} - P_{\text{pass}} \). The volume at \( T_{\text{ej}} \) was defined as end-systolic volume (\( V_{\text{es}} \)). Stroke circumference (\( C_{\text{s}} \)), defined as the change in calculated midwall (half-mass) circumference during ejection, was calculated as \( C_{\text{BL}} - C_{\text{es}} \) after \( V_{\text{BL}} \) and \( V_{\text{es}} \) were converted to their corresponding circumferences according to

\[
C_i = \left[ 6\pi^2 V_i + \frac{V_w}{2} \right]^{1/3}
\]

where the subscript denotes either BL or es for baseline or end-systolic conditions, respectively. The \( T_{75-25} \) in these ejecting beats was quantified as the time of pressure decay from 75 to 25% \( P_{\text{es}} \). \( P_{\text{es}} \) was converted to end-systolic midwall stress (\( \sigma_{\text{es}} \)) according to

\[
\sigma_{\text{es}} = \frac{P_{\text{es}}}{\frac{V_w}{V_{\text{es}}}^{2/3} + 1} - 1
\]
When plotted against $\sigma_{es}$, T_{75-25} yielded the relaxation-wall stress relationship for ejecting beats.

**Data Analysis**

Frank-Starling relationship and passive pressure-volume relationship. The effects of each treatment on the fully relaxed, pressure-volume relationship and the Frank-Starling relationship were evaluated within each treatment group. The passive pressure-volume relationships were compared visually. The Frank-Starling relationships were compared using a multiple linear regression in which $\Delta P_{peak}$ was related to volume ($V$) according to

$$\Delta P_{peak} = b_0 + b_1 V + b_2 V^2 + b_3 D + b_0 V \cdot D$$

where $D$ is a dummy variable to encode control ($D = 0$) and drug treatment ($D = 1$) conditions and the $b$ terms are regression coefficients ($b_0$, $\Delta P_{peak}$ intercept; $b_1$, initial "slope"; and $b_2$, quadratic term). The term $b_3 D$ allows for a drug-induced change in the $\Delta P_{peak}$ axis intercept, which would be seen as a parallel shift in the Frank-Starling relationship. The term $b_0 V \cdot D$ allows for a drug-induced change in the initial slope, which would be seen as a nonparallel shift in the Frank-Starling relationship.

**Wall-Stress Dependence of Relaxation.** Similarly, the difference in the T_{75-25-$\sigma_{peak}$} relationships for isovolumic beats under control conditions and during perfusion with a drug was analyzed using multiple linear regression in which T_{75-25} was related to $\sigma_{peak}$ according to

$$T_{75-25} = c_0 + c_0 \sigma_{peak} + c_2 \sigma_{peak}^2 + c_3 D + c_0 c_3 \sigma_{peak} \cdot D$$

where $D$ is a dummy variable as defined for Eq. 4 and the $c$ terms are regression coefficients that form the basis for interpreting the effects of a drug. If drug treatment did not change the relationship between T_{75-25} and $\sigma_{peak}$, i.e., the control and drug treatment data fell on the same regression line, then any effect of this agent to slow relaxation would be ascribable entirely to its effect in increasing $\sigma_{peak}$. However, if drug treatment changed the slope (i.e., $c_0$ is significant in Eq. 5) or intercept (i.e., $c_1$ is significant in Eq. 5) of the relationship between T_{75-25} and $\sigma_{peak}$, this would indicate that the drug had an additional effect on the duration of LV relaxation that occurred independently of its effect in changing $\sigma_{peak}$.

A similar analysis was done to characterize the effect of drug treatment on relaxation for ejecting beats obtained from the AL protocol. In this case, T_{75-25} is related to $\sigma_{es}$ according to

$$T_{75-25} = d_0 + d_0 \sigma_{es} + d_2 \sigma_{es}^2 + d_3 D + d_0 \sigma_{es} \cdot D$$

where the $d$ terms are regression coefficients. Although Eq. 6, which characterizes ejecting beats, was formulated analogously to Eq. 5, which characterizes isovolumic beats, doing so ignores a potentially important difference between isovolumic and ejecting beats. Relaxation in ejecting beats depends on $C_{es}$; i.e., the amount of shortening or ejection (see Fig. 3). However, in this analysis, in which only ejecting beats are considered, $C_{es}$ and $\sigma_{es}$ are so highly correlated that we can safely ignore the explicit effects of $C_{es}$. In fact, the strong correlation between $C_{es}$ and $\sigma_{es}$ requires omission of $C_{es}$ in Eq. 6 to avoid serious multicollinearity in the regression analysis (6).

Another way that characterization of relaxation in ejecting beats differs from that in isovolumic beats is that the time of onset of relaxation (i.e., $T_{ej}$) must also be considered. Hence, $T_{ej}$ was also related to $\sigma_{es}$ according to

$$T_{ej} = e_0 + e_0 \sigma_{es} + e_2 \sigma_{es}^2 + e_3 \sigma_{es}^3 + e_2 D + e_0 \sigma_{es} \cdot D$$

**Fig. 3.** T_{75-25-$\sigma$} relationship determined in 1 left ventricle during control state. Data illustrate interpretation of Eqs. 8 and 9. For isovolumic beats, stroke circumference ($C_{is}$) = 0 and regression fit follows isovolumic data points as indicated by curve A. For variability ejecting beats, $C_{es}$ = 0 and regression fit follows + data points as indicated by curve B. Note that isovolumic and ejecting curves increasingly diverge with increasing $C_{es}$ (i.e., lower $\sigma$). Regression coefficients $f_1$ and $f_2$ in Eqs. 8 and 9 quantify this effect of ejection on T_{75-25-$\sigma$} relationship.

where the $f$ terms are regression coefficients. The cubic form of Eq. 7 was suggested by results from Elzinga and Westerhof (3), who showed that the time to end ejection (defined as the time to the maximal pressure-volume ratio, $E_{max}$) was nonlinearly related to end-systolic pressure.

Finally, a global analysis of the effect of drug treatment on the characteristic time of relaxation was done using data combined from both isovolumic and ejecting beats. This analysis used a regression model that related T_{75-25} to $\sigma$ (either $\sigma_{peak}$ or $\sigma_{es}$, depending on whether the beat was from an isovolumic or ejecting beat) and $C_{ss}$, where $C_{ss} = 0$ for isovolumic beats, according to

$$T_{75-25} = f_0 + f_0 \sigma + f_2 \sigma^2 + f_3 C_{ss} + f_4 \sigma \cdot C_{ss} + f_2 D + f_0 \sigma C_{ss} \cdot D + f_2 \sigma C_{ss} \cdot D$$

where the $f$ terms are regression coefficients. In this analysis using Eq. 8, unlike when ejecting beat data were considered separately using Eq. 6, $C_{is}$ is explicitly included. Wall stress and $C_{es}$ are not as strongly correlated in the set of data that combines both ejecting beats (wall stress and $C_{es}$ highly correlated) and isovolumic beats ($C_{is} = 0$; wall stress and $C_{es}$ noncorrelated), so the separate effect of $C_{es}$ is accounted for explicitly in this equation.

The interpretation of Eq. 8 can be illustrated by the combined data from the FS and AL protocols under control conditions shown in Fig. 3. For these data, $D = 0$ and Eq. 8 reduces to

$$T_{75-25} = f_0 + f_0 \sigma + f_2 \sigma^2 + f_3 C_{ss} + f_4 \sigma \cdot C_{ss}$$

The effect of ejection to speed relaxation is shown by curve B (Fig. 3), projecting below the isovolumic relationship (curve A; Fig. 3). As one moves to lower $\sigma$ (either $\sigma_{peak}$ or $\sigma_{es}$), so the separate effect of $C_{es}$ is accounted for explicitly in this equation.

Because each heart served as its own control, dummy variables defined using effects coding (6) were included in each regression equation to account for between-subjects variation in each parameter (excluding $\sigma_{es}$ in Eq. 7). For simplicity, these dummy variables are not shown in Eqs. 4–9. Unless otherwise stated, all summary statistics are given as...
means ± SD. All statistical analyses were performed using Minitab 11.12.

RESULTS

Frank-Starling and Passive Pressure-Volume Relationships

Equation 4 fit these FS protocol data uniformly well, with the R2 ≥ 0.99 for all three groups. The regression coefficients b0, b1, and b2 for the DP peak intercept, initial “slope,” and quadratic term in Eq. 4, respectively, were similar for the control condition in all three experimental groups. Likewise, the coefficients b0 and b1, which quantify the drug-induced changes in intercept and initial slope, respectively, were significant in all three experimental groups (all P < 0.01) and were similar in magnitude (Table 1). From the fits to Eq. 4, the Vmax under control conditions was found to be similar in all three treatment groups: 2.64 ml for the CGP group, 2.40 ml for the EMD group, and 2.71 ml for the dobutamine group. The positive inotropic effect of each drug, as judged by the upward shift in the Frank-Starling relationship at approximately Vmax, was comparable in each group: the average Frank-Starling relationship at approximately Vmax, was comparable in each group: the average Frank-Starling relationship evaluated from the fit to Eq. 4 at V = 2.6 ml increased from 118 to 139 mmHg in the CGP group, from 120 to 138 mmHg in the EMD group, and from 125 to 147 mmHg in the dobutamine group. These effects are similar to those we have shown previously with EMD-57033 [for example, see Fig. 3 in Tobias et al. (21)]. Furthermore, the similarity of these fits and calculated shifts indicates that, as we expected, all three drugs induced similar nonparallel upward shifts in the Frank-Starling relationships.

As we have shown previously for EMD-57033 (21), there was little or no effect of these three inotropic drugs on the fully relaxed, passive pressure-volume relationship [data not shown; for example, see Fig. 3 in Tobias et al. (21)].

Table 1. Selected regression coefficients

<table>
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<tr>
<th>Equation 4</th>
<th>CGP-48506</th>
<th>Coefficient</th>
<th>SE</th>
<th>EMD-57033</th>
<th>Coefficient</th>
<th>SE</th>
<th>Dobutamine</th>
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<td>b0</td>
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<td></td>
<td>6.4*</td>
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<td></td>
<td>8.4*</td>
<td>0.6</td>
<td></td>
<td>4.0*</td>
<td>0.5</td>
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<td>0.0014</td>
<td>0.0071*</td>
<td>0.0018</td>
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<td>c1</td>
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<td>0.023*</td>
<td>0.001</td>
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<td>-0.021*</td>
<td>0.001</td>
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<td>Equation 8</td>
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<tr>
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<td></td>
<td>24 × 10^-5</td>
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*P < 0.05.

Timing of Contraction Events in Isovolumic Beats

Neither CGP-48506 nor EMD-57033 substantially affected Tpeak, as can be seen in the examples shown in Fig. 4 [Tpeak was 308 ± 14 ms in the control state and 300 ± 6 ms with 6 × 10^-6 M CGP-48506 (2.6% shortening; P = 0.006) and 307 ± 16 ms in the control state and 304 ± 18 ms with 2 × 10^-6 M EMD-57033 (1% shortening; P = 0.65)]. In contrast, both Ca2+ sensitizers prolonged Ttotal, as can also be seen in Fig. 4 [Ttotal was 684 ± 35 ms in the control state and 727 ± 42 ms with 6 × 10^-6 M CGP-48506 (6% lengthening; P = 0.002) and 708 ± 37 ms in the control state and 812 ± 43 ms with 2 × 10^-6 M EMD-57033 (15% lengthening; P = 0.001)].

As expected, Tpeak and Ttotal were significantly shortened by 1.25 × 10^-6 M dobutamine [Tpeak was 314 ± 13 ms in the control state and 262 ± 14 ms with dobutamine (16.6% shortening; P < 0.001) and 693 ± 53 ms in the control state and 575 ± 40 ms with 1.25 × 10^-6 M dobutamine (17% shortening; P < 0.001)].

T75–25σpeak Relationship in Isovolumic Beats

The T75–25−σpeak relationships (Fig. 5) were fit well by Eq. 5, with all R2 ≥ 0.99. For the CGP group, the coefficients associated with drug treatment, C0 and C1, were both significant (P < 0.03; Table 1). However, as shown by the average fit lines drawn in Fig. 5, these effects are physiologically meaningless, because the T75–25σpeak relationship obtained with 6 × 10^-6 M CGP-48506 coincides almost perfectly with the T75–25−σpeak relationship in the control state.

In contrast, as we have observed previously (21), 2 × 10^-6 M EMD-57033 shifted the T75–25−σpeak relationship upward such that relaxation at any given σpeak was significantly slower with EMD-57033. Both C0 and C1 were significant (P < 0.001; Table 1). Thus the upward shift caused by EMD-57033 was not parallel (i.e., both slope and intercept increased with EMD-57033).
Dobutamine (1.25 x 10^{-6} M) shifted the T_{75-25} vs \sigma_{\text{peak}} relationship downward such that relaxation at any given \sigma_{\text{peak}} was significantly shorter with dobutamine. Both \text{Cs}_{\text{d}} and \text{Cs}_{\text{ds}} were significant (P < 0.001; Table 1). Thus the downward shift caused by dobutamine was not parallel (i.e., both slope and intercept decreased with dobutamine).

Timing of Contraction Events in Ejecting Beats

The time of onset of relaxation in ejecting beats or, equivalently, T_{ej}, depended on \sigma_{\text{es}}, as shown in Fig. 6 (and can also be seen by comparing the different pressure-clamped ejecting beats in Fig. 4), in which T_{ej} increases slowly as \sigma_{\text{es}} increases above its minimum value, reaches a maximum at an intermediate value of \sigma_{\text{es}}, then falls steeply with continued increases in \sigma_{\text{es}} as the pressure-clamped beats approach isovolumic contraction. These T_{ej}-\sigma_{\text{es}} relationships were fit well by Eq. 7 in all three groups, with R^2 equal to 0.90, 0.92, and 0.93 for the CGP, EMD, and dobutamine groups, respectively.

The effect of both Ca^{2+} sensitzers on this relationship is biphasic (Fig. 6). For the lowest levels of \sigma_{\text{es}} (i.e., the largest ejections), T_{ej} is reached more quickly when the heart is perfused by either CGP-48506 or EMD-57033 than in the control. However, at intermediate levels of \sigma_{\text{es}}, this effect crosses over to an effect in which at the highest levels of \sigma_{\text{es}} (i.e., smallest ejections) T_{ej} is reached at a later time in the presence of either CGP-48506 or EMD-57033. From the regression fits to Eq. 7 in each drug group, these crossover points are estimated to be 37 mmHg for 6 x 10^{-6} M CGP-48506 and 31 mmHg for 2 x 10^{-6} M EMD-57033.

In contrast, 1.25 x 10^{-6} M dobutamine shortened T_{ej} at all values of \sigma_{\text{es}}, with the T_{ej}-\sigma_{\text{es}} relationship for baseline control and dobutamine conditions converging only at the highest \sigma_{\text{es}} (the point of convergence calculated from the fit to Eq. 7 is 106 mmHg).

T_{75-25}-\sigma_{\text{es}} Relationship in Ejecting Beats

The T_{75-25}-\sigma_{\text{es}} relationships in ejecting beats (Fig. 7) were fit well by Eq. 6 in all three groups, with all R^2 > 0.99. These high R^2 show that it is reasonable to exclude explicit consideration of C_0 \text{es} in Eq. 5.

For the CGP group, the coefficients d_{\text{d}} and d_{\text{ds}} were both significant (P < 0.001; Table 1). Thus there was a significant CGP-48506-induced increase in intercept...
and decrease in slope of the $\tau_{75-25}$-$\sigma_{e5}$ relationship, as can be seen by the average fit lines drawn in Fig. 7A. Hence, unlike the response to this drug in isovolumic contractions, relaxation was prolonged slightly by CGP-48506 at the lowest $\sigma_{e5}$ (i.e., largest ejections). This effect is opposite to the effect of this drug on $T_{ej}$, with the overall result that the $T_{total}$-$\sigma_{e5}$ relationship in ejecting beats was largely unaffected by $6 \times 10^{-6}$ M CGP-48506 (Fig. 8A).

The upward shift in the $T_{75-25}$-$\sigma_{e5}$ relationship that occurred with $2 \times 10^{-6}$ M EMD-57033 was larger than that for CGP-48506; the coefficients $d_d$ and $d_s$ were both significant ($P < 0.006$; Table 1), indicating a significant EMD-57033-induced increase in intercept and decrease in slope of the $T_{75-25}$-$\sigma_{e5}$ relationship, as can be seen by the average fit lines drawn in Fig. 7B.

Hence, relaxation was prolonged by EMD-57033 at all $\sigma_{e5}$, but the prolongation was slightly less at higher $\sigma_{e5}$. The overall result, combining the dependence of both $T_{ej}$ and $T_{75-25}$ on $\sigma_{e5}$, was that the $T_{total}$-$\sigma_{e5}$ relationship in ejecting beats was shifted upward by $2 \times 10^{-6}$ M EMD-57033. This shift was such that the slope of the $T_{total}$-$\sigma_{e5}$ relationship was increased by EMD-57033. Thus $T_{total}$ was relatively more prolonged with EMD-57033 at high $\sigma_{e5}$ compared with at low $\sigma_{e5}$ (Fig. 8B).

Dobutamine ($1.25 \times 10^{-6}$ M) shifted the $T_{75-25}$-$\sigma_{e5}$ relationship downward such that the time of relaxation at any given $\sigma_{e5}$ was significantly shorter with dobutamine. The coefficients $d_d$ and $d_s$ were both significant ($P < 0.001$; Table 1). Thus both the slope and intercept decreased with dobutamine (Fig. 7C). The overall re-
result, combining the dependence of both $T_{ej}$ and $T_{75-25}$ on $\sigma_{es}$, was that the $T_{total}$-$\sigma_{es}$ relationship in ejecting beats was shifted downward by $1.25 \times 10^{-6}$ M dobutamine. Dobutamine decreased the slope of the $T_{total}$-$\sigma_{es}$ relationship, with the result that $T_{total}$ was shortened a relatively larger amount with dobutamine at high $\sigma_{es}$ compared with at low $\sigma_{es}$ (Fig. 8C).

$T_{75-25}$-$\sigma$ Relationship in Both Isovolumic and Ejecting Beats

The foregoing analyses characterize the effects of CGP-48506, EMD-57033, and dobutamine on relaxation separately in isovolumic and ejecting beats. To determine directly whether the effect of these drugs on relaxation was different in isovolumic versus ejecting beats, we performed an analysis that fit Eq. 8 to the combined data from isovolumic and ejecting beats. The average fits computed from Eq. 8 for these $T_{75-25}$-$\sigma$ relationships are shown in Fig. 9. The $T_{75-25}$-$\sigma$ relationships were fit well by Eq. 8 in all three groups, with $R^2$ equal to 0.98, 0.99, and 0.98 for the CGP, EMD, and dobutamine groups, respectively.

The effect of ejection on relaxation could enter either directly (i.e., the term $f_{c}C_s$ in Eq. 8) or as a work-related term (i.e., $f_{c}C_s \cdot \sigma_{es}$ in Eq. 8). In a regression model this complex, it is difficult to determine unequivocally which of these two possibilities is involved. For example, in the control state, $f_{c}$ was significant for the CGP and EMD groups (P < 0.001; Table 1), whereas $f_{c}C_s$ was significant for the CGP and dobutamine groups (P <
Fig. 9. Effect of 6 × 10⁻⁶ M CGP-48506 (A), 2 × 10⁻⁶ M EMD-57033 (B), and 1.25 × 10⁻⁶ M dobutamine (C) on T_{75–25}-wall stress (σ) relationship in data combined from both isovolumic and ejecting beats (T_{75–25} in s and σ in mmHg). For clarity of presentation, individual observations from 8 hearts are not shown (between-heart variation is similar to that shown in Fig. 5). Dashed lines show regression fit to control observations using Eq. 8, ignoring dummy variables, and represent average control fit for all 8 hearts. Similarly, solid lines show regression fit to drug-treatment observations using Eq. 8, ignoring dummy variables, and represent average drug-treatment fit for all 8 hearts. See Fig. 3 for explanation of relationship between isovolumic and ejecting data; see text and Table 1 for details of regression fits to Eq. 8.

DISCUSSION

We applied a comprehensive analysis of LV relaxation that encompassed both isovolumic and ejecting beats to identify the lusitropic effects caused by different positive inotropic interventions. Our principal findings are that the two specific Ca²⁺ sensitizers, EMD-57033 and CGP-48506, have very different effects on LV relaxation and that differences in the effects of these two drugs depend on the mode of LV contraction. At the concentrations we used, neither EMD-57033 nor CGP-48506 has been shown to have significant confounding effects that might also influence relaxation. EMD-57033 does not significantly inhibit PDE III activity at concentrations <5 × 10⁻⁶ M (19), and CGP-48506 does not significantly inhibit PDE III activity at concentrations <3 × 10⁻⁴ M (17, 27). Further, at these concentrations neither drug affects Ca²⁺ binding to troponin C (19, 24). Finally, although CGP-48506 has been reported to increase L-type Ca²⁺ channel conductance, this effect was at a high concentration, 10⁻⁴ M (10). Accordingly, we assume that the differences we observed reside in differential effects of these drugs “downstream” from troponin C.

Effect of Ca²⁺ Sensitizers on Relaxation in Isovolumic Beats

As we have shown previously (20, 21), all hearts studied showed a positive relationship between T_{75–25} and σ_{peak}. This means that any positive inotropic drug will slow relaxation because it will increase wall stress. However, if a positive inotropic drug also has an effect on relaxation that is independent of its effect to increase wall stress (i.e., a so-called lusitropic effect), this independent lusitropic effect would be evident as a shift in the T_{75–25}-σ_{peak} relationship. Hence, the overall effect on relaxation of a given dose of drug will be due to the balance of its stress-dependent and stress-independent effects.

EMD-57033 shifted the T_{75–25}-σ_{peak} relationship in isovolumic beats upward. Thus, in addition to the effect
of EMD-57033 to slow relaxation because its positive inotropic effect increased $\Delta \sigma_{\text{peak}}$; it also has a stress-independent negative lusitropic effect that further slowed relaxation by increasing $T_{75-25}$ at any given $\sigma_{\text{peak}}$ (21). In contrast, CGP-48506, when administered to give a similar increase in inotropic state, has a negligible effect on relaxation; that is, it did slow relaxation, as would any positive inotropic intervention, because it increased $\sigma_{\text{peak}}$. However, this is the only effect of CGP-48506, because the $T_{75-25}-\sigma_{\text{peak}}$ relationship with and without CGP-48506 were nearly superimposed.

For comparison, and as further validation of this framework for analyzing LV relaxation, we also studied the effect of $1.25 \times 10^{-6}$ M dobutamine on the $T_{75-25}-\sigma_{\text{peak}}$ relationship. We chose a $b$-agonist because its effect via the adenosine $3',5'$-cyclic monophosphate-dependent protein kinase led us to predict that it would cause a downward shift in the $T_{75-25}-\sigma_{\text{peak}}$ relationship [consistent with its effect as shown in Zile et al. (25)]. Indeed, our analysis shows that dobutamine has a strong positive lusitropic effect.

Effect of Ca$^{2+}$-Sensitizers on Relaxation in Ejecting Beats

An important result of the present study is the extension of the stress-dependent relaxation relationship to encompass ejecting beats by relating $T_{75-25}$ to $\sigma_{\text{es}}$. In our analysis of ejecting beats, it is also apparent that the three inotropic agents studied have distinctly different lusitropic effects. The effects of both EMD-57033 and dobutamine on stress-dependent relaxation in ejecting beats are, at first glance, similar to their respective effects in isovolumic beats (compare Figs. 5 and 7); that is, at all values of wall stress, EMD-57033 slows relaxation in both isovolumic and ejecting beats and dobutamine speeds relaxation in both isovolumic and ejecting beats. The effect of CGP-48506 in ejecting beats differs slightly from its effect in isovolumic beats: in ejecting beats there is a small, nonparallel shift in the $T_{75-25}-\sigma_{\text{es}}$ relationship such that relaxation is slowed slightly at the lowest values of $\sigma_{\text{es}}$.

Furthermore, as shown in Eq. 8 and Fig. 9, by combining the measurements of $T_{75-25}$, $\sigma_{\text{peak}}$ from isovolumic beats, and $\sigma_{\text{es}}$ and $C_3$ from ejecting beats, we can determine directly whether the lusitropic effect of these positive inotropic drugs, as judged by the $T_{75-25}-\sigma_{\text{es}}$ relationship, is different in ejecting versus isovolumic beats. This analysis shows that EMD-57033 affects stress-dependent relaxation in a similar way in both ejecting and isovolumic beats, whereas both CGP-48506 and dobutamine affect stress-dependent relaxation in ejecting beats differently from how they affect it in isovolumic beats. For CGP-48506 and EMD-57033, the result of this combined analysis agrees with the interpretation made when comparing separate data for isovolumic and ejecting beats in $T_{75-25}-\sigma_{\text{peak}}$ relationship in isovolumic beats and $T_{75-25}-\sigma_{\text{es}}$ relationship in ejecting beats. However, for dobutamine the result of this combined analysis suggests that its effect was different for isovolumic versus ejecting beats, which is difficult to appreciate from a visual comparison of the separate data shown in Figs. 5C and 7C. In summary, EMD-57033 slows relaxation similarly in both ejecting and isovolumic beats. CGP-48506 affects relaxation differently in ejecting versus isovolumic beats: it does not affect relaxation in isovolumic beats but reduces the effect of ejection to speed relaxation. Unlike CGP-48506, dobutamine affects relaxation in both ejecting and isovolumic beats but, like CGP-48506, it reduces the effect of ejection to speed relaxation.

Evaluation of the lusitropic effect of such drugs in ejecting beats is more complex than in isovolumic beats because, in addition to an effect on duration of relaxation, as quantified by $T_{75-25}$, these drugs potentially affect the time of relaxation onset. Hence, we performed an additional analysis, relating $T_{\text{ej}}$ to $\sigma_{\text{es}}$ (Fig. 6) according to Eq. 7. The effect of the two Ca$^{2+}$-sensitizers is qualitatively similar in this analysis. Both drugs reduce $T_{\text{ej}}$ by a small amount at low $\sigma_{\text{es}}$ and increase $T_{\text{ej}}$ at larger $\sigma_{\text{es}}$. These two drugs differ quantitatively in that CGP-48506 reduces $T_{\text{ej}}$ over a broader range of $\sigma_{\text{es}}$ than does EMD-57033. In contrast, dobutamine reduces $T_{\text{ej}}$ over most of the range of $\sigma_{\text{es}}$ in the ejecting beats. The effect of these drugs on $T_{75-25}$ and $T_{\text{ej}}$, combined, produces an overall effect on the duration of beat. The $T_{\text{total}}-\sigma_{\text{es}}$ relationship in ejecting beats is affected by these three inotropic drugs in virtually the same manner as the $T_{75-25}-\sigma_{\text{peak}}$ relationship in isovolumic beats was affected.

The more complex nature of relaxation in ejecting beats has been highlighted previously, and the determinant of relaxation rate in ejecting beats has been suggested to be, variously, load (26) or ejection timing (12). Our approach is much like that of Zile et al. (25, 26), who related relaxation to ventricular loading and showed that the $b$-agonist isoproterenol shifted the relaxation-load relationship (25). We treated load ($\sigma$) in our study) as the determinant of relaxation rate ($T_{75-25}$) in ejecting beats because it allowed us to analyze both isovolumic and ejecting beats in a similar manner. Moreover, doing so allowed us to combine data from both isovolumic and ejecting beats to evaluate whether an intervention had a different effect on isovolumic versus ejecting beats. We treated ejection timing as another important feature of relaxation in ejecting beats but did not treat ejection timing as a determinant of relaxation rate, as did Hori et al. (12). Because both $T_{\text{ej}}$ (Fig. 6) and $T_{75-25}$ (Fig. 7) depend on $\sigma_{\text{es}}$, it is apparent that $T_{\text{ej}}$ and $T_{75-25}$ could be analyzed in such a way as to treat $T_{\text{ej}}$ as a “determinant” of $T_{75-25}$. However, it was not our intent to decouple $T_{\text{ej}}$ from $\sigma_{\text{es}}$ so as to identify which was really the determinant of $T_{75-25}$—Rather, we treated $\sigma_{\text{es}}$ as the determinant of $T_{75-25}$ because doing so allowed a consistent framework for analyzing the effects of interventions on relaxation in both isovolumic and ejecting beats.

Mechanism(s) of Action of CGP-48506 and EMD-57033

Although CGP-48506 has not been studied as widely as EMD-57033, two recent studies suggested that the mechanism of Ca$^{2+}$ sensitization of CGP-48506 was different from the mechanism of EMD-57033.
effects of both drugs have been studied in isolated adult rat cardiomyocytes (19, 24). Although $1 \times 10^{-6}$ M EMD-57033 significantly decreased resting myocyte length in these freely shortening cells, $1 \times 10^{-5}$ M CGP-48506 did not affect resting myocyte length (24). Accordingly, it was concluded that CGP-48506, unlike the thiadiazinones such as EMD-57033, would not severely impair relaxation (24). Our results from the whole heart support this interpretation. We showed that CGP-48506 has little lusitropic activity beyond its effect, which it shares with all positive inotropic drugs, to increase $T_{75–25}$ because it increased wall stress. In contrast, EMD-57033 has a negative lusitropic effect that slows relaxation more than is attributable to increased wall stress.

In a detailed study comparing EMD-57033 and CGP-48506 in both live and skinned fiber preparations, Palmer et al. (18) concluded that CGP-48506 and EMD-57033 increased the Ca$^{2+}$ sensitivity of myofilaments via different mechanisms. One conclusion from their findings was that CGP-48506 did not affect the tension cost (i.e., did not shift the ATPase-force relationship) and that, therefore, assuming the number of cross bridges and force per cross bridge were unaffected [following Brenner (1)], CGP-48506 did not affect the cross-bridge detachment rate ($g_{\text{diss}}$). On the basis of this finding and others, they proposed that CGP-48506 affected the transition from the detached to the weakly bound state. In contrast, they proposed that the site of action of EMD-57033 was at the weak-to-strong transition.

Our results from the whole heart are consistent with the interpretation that CGP-48506 does not affect $g_{\text{diss}}$. However, we do not think our results allow us to speculate further about specific differences in the site(s) of action(s) of these two drugs that would be implied by a differential effect in ejecting versus isovolumic beats. Nevertheless, our results show that the Ca$^{2+}$-sensitizing effects of CGP-48506 and EMD-57033 must be mediated by different sites of action on the myofilaments.

Using $T_{75–25}$-$\sigma_{\text{peak}}$ Relationship to Evaluate Lusitropy

Our analysis of the positive relationship between LV relaxation and wall stress is grounded in our initial whole heart study (20) and has strong support from an isolated rat trabecular study (13), which showed that, in isosarcomeric contractions, the twitch duration was a function of peak twitch force only and was independent of sarcomere length.

The implication of a positive $T_{75–25}$-$\sigma_{\text{peak}}$ relationship is that any positive inotropic drug will slow LV relaxation because it increases LV wall stress. When comparing one beat in the absence of a positive inotropic drug to another beat in the presence of drug, the net effect on relaxation will be the balance of this effect due to increased wall stress and any stress-independent lusitropic effect the drug may have. Accordingly, evaluation of a drug's effect by comparing only two beats will not allow discrimination between the negative lusitropic effect that would be present with any inotropic intervention of the same magnitude (i.e., slowed relaxation due to increased wall stress) and the stress-independent lusitropic effect of the drug.

The necessity for such an evaluation is depicted in Fig. 10, which shows a family of four hypothetical $T_{75–25}$-$\sigma_{\text{peak}}$ relationships. Three of these depict the results of this study, whereas the fourth depicts a hypothetical positive inotropic agent (Fig. 10, line C) with a smaller positive lusitropic effect than dobutamine (line D).

Assume, for argument's sake, that all four drugs result in the same positive inotropic effect, and thus the same increased wall stress, as indicated by the vertical dashed line in Fig. 10. The arrows emanating from point 1 (i.e., the hypothetical baseline state) on the control curve (Fig. 10, line B) show the summed effect of the increased wall stress plus the stress-independent effect of the drug (i.e., shift of the curve up or down) on relaxation. Of most significance for the purposes of this illustration, consider the effect of the hypothetical inotrope (Fig. 10, line C), which has a net effect to shift the LV from point 1 to point 4. The net effect of this drug is, in fact, to slow relaxation when judged only by comparing a beat at point 1 with a beat at point 4. However, the downward shift in the $T_{75–25}$-$\sigma_{\text{peak}}$ relationship indicates that the drug is truly a positive lusitrope. Thus, one would miss this very important feature of the action of such a drug and falsely conclude that the drug was a negative lusitrope if the $T_{75–25}$-$\sigma_{\text{peak}}$ relationship was ignored. This is not to say that the net effect of such a drug to slow relaxation is unimportant but rather that to declare the drug a negative lusitrope based only on observations of the characteristics of two beats at points 1 and 4 is to incorrectly appreciate the true,
stress-independent positive lusitropic action of the drug on the heart.

The different effects of CGP-48506 and dobutamine in ejecting versus isovolumic beats also emphasize the need to evaluate the effect of inotropic interventions in ejecting contractions. For example, CGP-48506 has essentially no stress-independent lusitropic effect when evaluated using isovolumic beats but does have a small stress-independent negative lusitropic effect when evaluated using ejecting beats.

Although our results provide considerable insight regarding significant differences in how these different inotropic drugs influence LV relaxation, there are limitations to our study. First, what we consider a “normal” heart is some distance removed from the physiology of the heart in the intact animal. We study a heart perfused with a buffer containing elevated K⁺ and at a reduced temperature. Furthermore, the heart rate is slow for a rabbit, and we pace from the LV apex. Because both heart rate and activation pattern may influence LV relaxation, it is possible that these factors influenced our results. However, we have observed drug-induced differences in cardiac performance at rates as high as 140 beats/min, which are consistent with the effects on relaxation reported here (unpublished observations). Also, we have determined the basic T 75-25 wall stress relationship with field, rather than punctate, stimulation (Ref. 20 and unpublished observations) and have observed no influence of the type of stimulation on the essential features of this analysis. Second, our analysis does not provide an assessment of the overall impact of these inotropic drugs on cardiac performance. Such an assessment would have to take into account the complex interplay between a drug’s effect(s) on pressure-generating capacity, amount of ejection, time of end ejection, and rate of relaxation to determine the overall stroke volume resulting from one complete filling-and-ejection cycle at a given heart rate. Third, when extrapolating from an evaluation in nondiseased hearts, care should be taken to infer the effect of a drug in diseased hearts. For example, the difference in the effects of EMD-57033 and CGP-48506 on the force-pCa curve in skinned cardiac myocytes has been shown to be more marked in myocardium isolated from patients with dilated human cardiomyopathy (New York Heart Association class IV) than in normal human myocardium (9). Thus the complete evaluation of any positive inotropic intervention must provide a clear assessment of wall stress-independent lusitropic effects and, ultimately, the integrated action of the positive inotropic effect and lusitropic effects on overall cardiac performance in both normal and diseased hearts.

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