Myocardial contractile depression from high-frequency vibration is not due to increased cross-bridge breakage

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Campbell, Kenneth B., Yiming Wu, Robert D. Kirkpatrick, and Bryan K. Slinker. Myocardial contractile depression from high-frequency vibration is not due to increased cross-bridge breakage. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1141–H1151, 1998.—Experiments were conducted in 10 isolated rabbit hearts at 25°C to test the hypothesis that vibration-induced depression of myocardial contractile function was the result of increased cross-bridge breakage. Small-amplitude sinusoidal changes in left ventricular volume were administered at frequencies of 25, 50, and 76.9 Hz. The resulting pressure response consisted of a depressive response [\( \Delta P_p(t) \)], a sustained decrease in pressure that was not at the perturbation frequency and an infr frequence response [\( \Delta P_p(t) \), that part at the perturbation frequency]. \( \Delta P_p(t) \) represented the effects of contractile depression. A cross-bridge model was applied to \( \Delta P_p(t) \) to estimate cross-bridge cycling parameters. Responses were obtained during \( \text{Ca}^{2+} \) activation and during \( \text{Sr}^{2+} \) activation when the time course of pressure development was slowed by a factor of 3. \( \Delta P_p(t) \) was strongly affected by whether the responses were activated by \( \text{Ca}^{2+} \) or by \( \text{Sr}^{2+} \). In the \( \text{Sr}^{2+} \)-activated state, \( \Delta P_p(t) \) declined while pressure was rising and relaxation rate decreased. During \( \text{Ca}^{2+} \) and \( \text{Sr}^{2+} \) activation, velocity of myofilament sliding was insignificant as a predictor of \( \Delta P_p(t) \) or, when it was significant, participated by reducing \( \Delta P_p(t) \) rather than contributing to its magnitude. Furthermore, there was no difference in cross-bridge cycling rate constants when the \( \text{Ca}^{2+} \)-activated state was compared with the \( \text{Sr}^{2+} \)-activated state. An increase in cross-bridge detachment rate constant with volume-induced change in cross-bridge distor- tion could not be detected. Finally, processes responsible for \( \Delta P_p(t) \) occurred at slower frequencies than those of cross-bridge detachment. Collectively, these results argue against a cross-bridge detachment basis for vibration-induced myocardial depression.

Methods and Procedures

Many aspects of these experimental methods have been reported previously (4, 5, 13). They are briefly repeated here for the sake of completeness.

Experimental preparation. Hearts were isolated from 10 adult male rabbits (avg wt = 3.26 ± 0.20 kg). Procedures for isolating the heart and attaching it to a volume-servo device have been described in detail elsewhere (4, 13). Briefly, the brachiocephalic artery was cannulated, and perfusion was begun with oxygenated relaxing solution (in mM: 121.4 Na+, 35.0 K+, 137.4 Cl−, 0.1 Ca2+, 1.1 Mg2+, 21.0 HCO3−, 0.36 PO43−, and 11.1 glucose and 2.5 U/l insulin) to arrest the heart before it was isolated from the rabbit. The perfusate was oxygenated by vigorous bubbling with 95% O2-5% CO2.

The heart was transferred to a perfusion support system consisting of a gas-exchange chamber, a roller pump, a constant-pressure chamber, and an environmental chamber. The heart was placed within an environmental chamber, where the coronary arteries were perfused at 90 mmHg. Temperature was kept constant at 25°C. The heart was submerged in perfusate at all times by allowing the coronary effluent to accumulate in the environmental chamber until it reached the chamber overflow at the level of the base of the heart. The perfusate was not recirculated.

Depression of Myocardial contractile function in response to high-frequency vibration is a well-known phenomenon. This depression is characterized not only by a loss of force-generating capacity (16, 17, 20, 26), but also by an increased efficiency of force production (20) and a shortening of the relaxation period (11, 12, 24).

A commonly held hypothesis, put forth originally by Vukas et al. (27) and accepted by most workers since then, is that vibration induces an increased rate of cross-bridge breakage, such that fewer cross bridges remain in the attached force-bearing state at any one time. A decrease in force-bearing cross bridges results in a loss of force-generating capacity. This is a logical hypothesis, because vibration was thought to cause cross-bridge strain to deviate from the strain found in isotonic conditions, and such deviation has long been taken as a primary means of increasing cross-bridge detachment rates (9).

We tested this hypothesis in a series of experiments in which left ventricular (LV) volume was perturbed sinusoidally using frequencies from 25 to 76.9 Hz. We saw a characteristic depressive response that was similar to those reported by other workers using various high-frequency vibrations. We asked three questions: 1) Is the depressive response during \( \text{Ca}^{2+} \) activation different from that during \( \text{Sr}^{2+} \) activation in a manner that is consistent with sinusoid-induced cross-bridge breakage, or is the difference in depressive response related to the activation process? 2) Is the dependence of the depressive response on frequency, amplitude, and velocity of perturbation consistent with what would be expected from increased cross-bridge breakage? 3) Can increased cross-bridge breakage with volume sinusoids be detected? The answers to these three questions lead us to conclude that contractile depression during high-frequency vibration is not due to increased cross-bridge breakage. Instead, we propose that some feature of the activation process is likely affected by vibrational perturbations such that a depressive response ensues.
A thin latex balloon, secured to the piston cylinder of a volume-servo system, was drawn into the LV chamber, such that its tip was anchored through a puncture in the apex. The puncture in the apex served as a vent for any fluids between the balloon and chamber wall. A draw-string suture in the mitral annulus was tightened around the obturator of a piston-cylinder device and secured the balloon in the LV chamber. The balloon was filled with degassed distilled water until passive chamber pressures reached 5 mmHg. Balloons were sized to fill the ventricle with neither excessive folding nor contribution to pressure at the volumes encountered in these ventricles. Thus balloons did not contribute to measured pressure.

The perfusing solution was changed from the relaxing solution to one that allowed spontaneous beating (in mM: 148.4 Na⁺, 7.4 K⁺, 139.1 Cl⁻, 1.24 Ca²⁺, 11.43 Mg²⁺, 21.0 HCO₃⁻, 0.36 PO₄³⁻, and 11.1 glucose and 2.5 U/l insulin). Spontaneous beating always occurred with a period ∼1 s, such that the heart, suspended in the spent perfusate, was paced at 1 Hz with field stimulation using 5-ms pulses of 15 mV and 250 mA heart, suspended in the spent perfusate, was paced at 1 Hz and estimated VBL at which experiments were to be performed. VBL was chosen as the volume equal to 80% of average LV free wall-plus-septum weight and VBL among these 10 hearts were 5.25 ± 0.39 g and 1.76 ± 0.15 ml, respectively. This protocol was also used to establish the passive pressure-volume relationship. A monoeponential equation was fit to points over the range of end-diastolic pressure and volume values generated in this protocol. Thus the contribution to pressure by parallel passive structures at any volume was estimated and removed from all ensuing data records so that we could focus only on active contractile properties.

After VBL was established, a high-frequency volume perturbation protocol was conducted as follows. Nine records, consisting of pressure and volume signals, were taken. Each record consisted of an unperturbed beat that took place isovolumically at VBL, as had the steady-state train of beats that preceded it, and a single volume-perturbed beat. During the volume-perturbed beat, the linear motor was commanded to deliver a sinusoidal volume change \[\Delta V(t) = \Delta V_c \cdot \sin(2\pi f t)\] at one of three frequencies (f = 76.9, 50, or 25 Hz corresponding to periods of 13, 20, or 40 ms) and one of three commanded amplitudes (\(\Delta V_c = 0.75, 1.0,\) or 1.25% of VBL). As a result of the dynamic responsiveness of the volume-servo system (damping ratio = 0.5, damped natural frequency = 80 Hz), volume change measured using the LVDT signal from the linear motor was slightly different from the commanded volume change. Therefore, the measured signal was fit with the function \(\Delta V(t) = \Delta V_c \cdot \sin(2\pi f t + \phi)\), and the fitted \(\Delta V(t)\) and estimated \(\Delta V\), rather than the commanded values, were used in all analyses (5). Repeated records were taken until all nine combinations of frequencies and amplitudes were recorded. Pressure responses to the volume perturbation are the subject of analysis.

Following the high-frequency volume perturbation protocol, a second single-beat Frank-Starling protocol was conducted to generate a Frank-Starling curve that could be compared with that collected at the onset of the experiment. This allowed detection of any deterioration in the preparation during the course of the high-frequency protocol. No detectable deterioration occurred.

The protocol was run during an initial period, in which beating took place with Ca²⁺ as the activator substance, and it was run once again after the perfusate had been changed to Sr²⁺ as the activator substance (in mM: 148.4 Na⁺, 7.4 K⁺, 140.8 Cl⁻, 0.10 Ca²⁺, 2.0 Sr²⁺, 1.1 Mg²⁺, 21.0 HCO₃⁻, 0.36 PO₄³⁻, and 11.1 glucose and 2.5 U/l insulin). Approximately 20 min elapsed before steady-state beating was obtained after switching to the Sr²⁺ perfusate. Data were taken only after steady state had been reached. Differences in responses between the Ca²⁺- and Sr²⁺-activated states were fundamental to testing the hypothesis that sinusoid-induced depression of contraction was due to increased cross-bridge breakage.

Data analysis. The pressure response \(\Delta P(t)\) to sinusoidal volume perturbation \(\Delta V(t)\) was defined as the difference between the pressure of the reference isovolumic beat \(P_{iso}(t)\), i.e., the pressure that would have developed had no volume perturbation been administered] and the pressure of the perturbed beat \(P(t)\)

\[
\Delta P(t) = P(t) - P_{iso}(t)
\]  

Representative \(P_{iso}(t)\), \(P(t)\), and \(\Delta P(t)\) are shown in Fig. 1 [frequency of vibration (f) = 50 Hz, \(\Delta V_c = 1\% VBL\)]. All responses clearly contained two components: a depressive response \(\Delta P_c(t)\), called “depressive” because it represented a sustained decrease in pressure below \(P_{iso}(t)\) that was not at the perturbation frequency and an in-frequency response \(\Delta P_f(t)\), i.e., that part of the response at the perturbation

\[
\Delta P(t) = P(t) - P_{iso}(t)
\]
The topic of this report is $\Delta P_d(t)$. We sought to determine whether $\Delta P_d(t)$ was due to increased cross-bridge breakage. To that end, $\Delta P(t)$ was analyzed to obtain cross-bridge detachment information to establish whether cross-bridge mechanisms are responsible for $\Delta P_d(t)$. Detailed description and interpretation of $\Delta P_d(t)$ analysis have been published elsewhere (5). $\Delta P_d(t)$ was quantified by its value at the time of peak $P(t)$ ($\Delta P_{pt}$), by its maximum value ($\Delta P_{dmax}$), and by its average value over the entire heart period ($\Delta P_{dav}$). The dependence of these quantities on $f$ and amplitude of the volume perturbation was established by regression procedures described below. In addition, differences in these quantities between the Ca$^{2+}$- and Sr$^{2+}$-activated state were also established. Finally, qualitative features of $\Delta P_d(t)$ were noted and compared between Ca$^{2+}$- and Sr$^{2+}$-activation, including time in the contraction cycle when $\Delta P_{dmax}$ was reached ($T_{P_dmax}$). Mean values of the various quantities between Ca$^{2+}$- and Sr$^{2+}$-activation were compared by paired $t$-test.

Dependence of depressive response on amplitude, frequency, and velocity of perturbation. If, during sinusoidal perturbation, cross bridges are caused to detach more rapidly, then cross-bridge-generated force and, consequently, LV pressure will be depressed. Current cross-bridge theory dictates that cross bridges detach more rapidly when the velocity with which myofilaments slide past one another increases and/or when cross-bridge strain is caused to deviate from the strain achieved during isometric contraction (6, 9, 10, 21, 22, 25). Because myofilament sliding velocity and cross-bridge strain are causally linked (see Eqs. 10, 11, and 17), these are not independent factors causing enhanced cross-bridge detachment. In this analysis, we dealt with each factor using separate approaches: myofilament sliding velocity was treated empirically using regression analysis, whereas cross-bridge strain was evaluated theoretically using a cross-bridge model. In the presence of small-amplitude sinusoidal volume perturbation, the root-mean-square velocity of linear motion within the muscular LV wall is proportional to $f \cdot \Delta V$ (see APPENDIX in Ref. 5). For this reason, $f \cdot \Delta V$ may also be taken as a measure of myofilament sliding velocity. Thus it is expected that increased cross-bridge detachment due to sinusoidal-induced velocity of myofilament sliding would yield a significant dependence of measures of the depressive response on the frequency. Thus

$$\Delta P(t) = \Delta P_d(t) + \Delta P_r(t)$$

(2)

$\Delta P_d(t)$ and $\Delta P_r(t)$ were individually identified as follows. $P(t)$ was considered to be composed of $\Delta P_r(t)$ and a pressure around which $\Delta P_r(t)$ occurred [$P_r(t)$]. $P_r(t)$ was extracted from $P(t)$ by filtering $P(t)$ to remove $\Delta P_r(t)$ and leave a signal [$P_r(t)$] without frequency content at the perturbation frequency. Filtering to obtain $P_r(t)$ was done by assuming a Fourier series representation of $P(t)$ and then truncating that series after the 15th harmonic. Then the truncated series that did not contain the harmonic of vibration was fit to $P(t)$ using a heuristic minimization procedure (Levenberg-Marquardt algorithm) to adjust the parameters of the Fourier series so as to minimize the sum of squares errors between the Fourier series and $P(t)$. The result was a time series, $P_r(t)$, that contained virtually all the signal content of $P(t)$ minus the component at the frequency of vibration. This is given as

$$P_r(t) = B_0 + \sum_{n=1}^{15} B_n \sin\left(\frac{2\pi}{T} t + \theta_n\right)$$

(3)

where $n$ is the harmonic number, $B_n$, and $\theta_n$ are harmonic amplitude and phase, respectively, and $T$ is the beat period. Because the shortest beat period used in these studies was 1 s, the 15th harmonic (15 Hz) was well below the lowest frequency used in the perturbation signal, i.e., 25 Hz. The amplitude and phase parameters ($B_n$ and $\theta_n$) had no particular significance other than to give a wave shape to $P_r(t)$ that did not include components of the in-frequency response. Once $P_r(t)$ was identified by fitting with Eq. 3, it was subtracted from $P(t)$ to yield $\Delta P_r(t)$

$$\Delta P_r(t) = P(t) - P_r(t)$$

(4)

Subtraction of $P_{iso}(t)$ from $P_r(t)$ generated $\Delta P_d(t)$

$$\Delta P_d(t) = P_r(t) - P_{iso}(t)$$

(5)

The entire process by which signals for analysis were extracted from measured $P(t)$ and $P_{iso}(t)$ is illustrated in Fig. 2.
f·ΔV term in the regression analysis. Failure of that term to be included as a significant predictor variable would mean that velocity (and, consequently, increased cross-bridge break- 
age due to velocity) makes no significant contribution to \( \Delta P_{d_t} \).

To determine the dependence of the depressive response on \( f \), \( \Delta V \), and \( f·ΔV \), quantitative measures of that response (\( \Delta P_{dT} \) and \( \Delta P_{d_{max}} \)) were regressed against each variable. Stepwise multiple regression techniques (Minitab, release 9 for Windows) were used to facilitate predictor variable selection. The regression equations were of the form

\[
\Delta P_{dT} = a_0 + a_1 f + a_2 \Delta V + a_3 f·\Delta V + a_4 f^2 + a_5 \Delta V^2 \quad (6a)
\]

\[
\Delta P_{d_{max}} = b_0 + b_1 f + b_2 \Delta V + b_3 f·\Delta V + b_4 f^2 + b_5 \Delta V^2 \quad (6b)
\]

where \( a_i \) and \( b_i \) are regression coefficients and \( f·\Delta V, f^2, \) and \( \Delta V^2 \) allow for nonlinear dependencies. The stepwise regression procedure incorporated a test of whether the individual \( a_i \) and \( b_i \) were significantly different from zero. If not different from zero, the coefficient and its associated candidate predictor variable were not included in the final regression equation. Dummy variables and effects coding were used to account for between-subjects differences, and the subject dummy variables were forced into the stepwise regression (7).

A candidate predictor variable was considered significant when \( P \) for its inclusion was <0.05.

Assessment of cross-bridge detachment dynamics. To evaluate differences in \( \Delta P_d(t) \) between \( Sr^{2+} \) and \( Ca^{2+} \) activation, it was desirable to determine whether there was any difference in the rate constants of cross-bridge detachment during these two activation conditions. To do this, the component of the response that was in-frequency with the vibration, \( \Delta P_f(t) \), was analyzed using a cross-bridge model. Rationale for the analysis and the model are given elsewhere (5). Briefly, cross bridges were viewed as force generators that, as a result of their actions, also generated pressure. Small perturbations and an assumption of homogenous myocardium allowed a linear transformation between force-length relationships of the myocardium and pressure-volume relationships of the LV chamber (5). A linear transformation allows myocardial force and LV pressure to be treated as analogous variables and myocardial fiber length and LV chamber volume to also be treated as analogous variables. Therefore, inasmuch as cross-bridge dynamics can be observed in myocardial force-length behavior, these dynamics can also be observed in LV pressure-volume behavior.

Cross bridges, as parallel elastic force generators, generate myocardial force equal to the stiffness of the entire parallel population times the average distortion among these cross bridges. By analogy, LV pressure equals chamber elastance times average volumetric distortion. Cross bridges exist in two stiffness-possessing (attached) states: a prepower stroke attached state (ep) and a postpower stroke attached state (ap). These states differ, in that the prepower stroke state does not contribute to force generation during isometric contraction, whereas the postpower stroke state, having been mechanically distorted by the mechanochemical energy transduction event of the power stroke, is solely responsible for isometric force. However, during changes in length, as in a vibration, because pre- and postpower stroke states are attached, cross bridges in both states are subject to induced distortion and contribute to the pressure. If our analogy for small perturbations is pursued further, cross-bridge stiffness is analogous to chamber elastance and cross-bridge distortion is analogous to volumetric distortion of elastance elements. Thus pressure is given by

\[
P(t) = E_{ep}(t)X_{ep}(t) + E_{ap}(t)X_{ap}(t) \quad (7)
\]

where \( E_{ep}(t) \) and \( X_{ep}(t) \) are the respective elastance and...
volumetric distortion associated with the prepower stroke state and \(E_{ep}(t)\) and \(X_{ep}(t)\) are the respective elastance and volumetric distortion associated with the postpower stroke state.

During volume vibration around an otherwise isovolumic condition

\[
E_{0i}(t) = E_{00}^{iso}(t) + \Delta E_{0i}(t) \quad (8)
\]
\[
E_{pi}(t) = E_{00}^{iso}(t) + \Delta E_{pi}(t) \quad (9)
\]
\[
X_{0i}(t) = X_{00}^{iso}(t) + \Delta X_{0i}(t) \quad (10)
\]
\[
X_{pi}(t) = X_{00}^{iso}(t) + \Delta X_{pi}(t) \quad (11)
\]

where \(iso\) indicates a value during the isovolumic condition and \(\Delta\) indicates vibration-induced changes in the respective variable. By the manner in which we defined pre- and postpower stroke isometric distortion, \(X_{00}^{iso} = 0\) whereas \(X_{00}^{iso} + X_{0i}^{iso}\) and is a constant. Therefore

\[
P(t) = E_{00}^{iso}(t)X_{00}^{iso} + \Delta E_{0i}(t)X_{0i}^{iso}
+ E_{00}^{iso}(t)\Delta X_{0i}(t) + E_{pi}(t)\Delta X_{pi}(t) \quad (12)
\]

By use of an important assumption that is discussed below, the various components on the right-hand side of Eq. 12 can be assigned as follows

\[
P_{iso}(t) = E_{00}^{iso}(t)X_{00}^{iso} \quad (13)
\]
\[
\Delta P_{e}(t) = \Delta E_{0i}(t)X_{0i}^{iso} \quad (14)
\]
\[
\Delta P_{r}(t) = E_{00}^{iso}(t)\Delta X_{0i}(t) + E_{pi}(t)\Delta X_{pi}(t) \quad (15)
\]

which, when substituted back into Eq. 12, relate definitions of the various parts of the pressure response given in Eqs. 1, 2, 4, and 5 to their elastance and distortion origins.

The assumption allowing Eqs. 14 and 15 was based on the notion that the time scale of vibration-induced changes in \(E_{0i}(t)\) was slow relative to the time scales of vibration-induced changes in \(X_{0i}(t)\) and \(X_{pi}(t)\). The slowness of \(\Delta E_{0i}(t)\) was such that very little change in this variable occurred during the period of a single vibrational cycle of 25–77 Hz. In contrast, the speed of change in \(\Delta X_{0i}(t)\) and \(\Delta X_{pi}(t)\) was such that changes in these variables were clearly expressed in those same cycle periods. Thus \(\Delta P_{e}(t)\) was made up entirely of vibration-induced distortion changes, \(\Delta P_{r}(t)\) was made up entirely of vibration-induced elastance changes, and the time variation in elastance due to background activation changes served to amplitude modulate these responses. Defense of this assumption rests on the notion that variation in elastance is the result of recruitment and derecruitment of actively cycling cross bridges and that the process responsible for recruitment/derecruitment, i.e., activation, is slow relative to the process governing the distortion of force-bearing cross bridges. These issues are discussed at length elsewhere \(5(\).

A model of transitions between pre- and postpower stroke cross-bridge states is given in Fig. 3. Transitions between and away from these states invoke the following rate constants: \(g\) (the constant governing the detachment of the postpower stroke state), \(h\) (the constant governing the power stroke), and \(d\) (the constant governing the backreaction of the prepower stroke state to detached states). By use of this model, it has been shown \(5(\) that elastances may be calculated from

\[
\frac{dE_{0i}(t)}{dt} = \frac{E_{00}^{iso}(t)}{E_{00}^{iso}} + h + \Delta E_{0i}(t) + \Delta V(t) \quad (16)
\]
\[
\frac{dX_{0i}(t)}{dt} = \frac{E_{00}^{iso}(t)}{E_{00}^{iso}} + g + \Delta X_{0i}(t) + \Delta V(t) \quad (17)
\]

Equation 17 is derived in Ref. 5 by relating \(E_{0i}(t)\) and \(X_{0i}(t)\) to the cross-bridge states \(N_{0i}\) and \(N_{pi}\) in Fig. 3 and then substituting Eq. 16 into the differential equation describing the kinetics of \(N_{0i}\). Further considerations in Ref. 5 allow differential equations describing the time rate of change of distortion to be written as

\[
\frac{dX_{0i}(t)}{dt} = \frac{E_{00}^{iso}(t)}{E_{00}^{iso}} + h + \Delta E_{0i}(t) + \Delta V(t) \quad (18)
\]
\[
\frac{dX_{pi}(t)}{dt} = \frac{E_{00}^{iso}(t)}{E_{00}^{iso}} + g + \Delta X_{0i}(t) + \Delta V(t) \quad (19)
\]

where a dot over a variable indicates its first time derivative. These equations demonstrate that distortions are dynamically driven by the derivative of \(\Delta V(t)\), and these distortions recover from a volume disturbance at a rate that depends on the respective elastances and the rate constants governing disappearance of the state. Equations 16–19 constitute the cross-bridge model.

By use of measured values of \(\Delta V(t)\), estimated values of \(g, h, d,\) and \(d\), and calculated values of \(E_{0i}(t)\) and \(E_{0pi}(t)\) per Eqs. 16 and 17, the differential Eqs. 18 and 19 were integrated numerically by fourth-order Runge-Kutta methods (integration step size = 0.5 ms) to obtain predicted values of \(X_{0i}(t)\) and \(X_{0pi}(t)\). These values, together with the calculated elastances, were then inserted into Eq. 15 to predict \(\Delta P_{r}(t)\). By use of this procedure, the model was fit to the collective set of nine records (3 amplitudes and 3 frequencies) of in-frequency
Equations describing the model with distortion-dependent degradation or improvement in the representation of the are given in Ref. 5.

The second use of the model was to determine whether sinusoidal volume perturbation increased cross-bridge detachment. The cross-bridge detachment rate constant $g$ was examined for evidence of distortion dependence. A functional form of distortion-dependent $g$ in accord with cross-bridge theory may be given by

$$g = g_0 + g_1 \left[ \frac{\Delta X_{ep}(t)^2}{X_{iso}^2} \right]$$

where $g_0$ is the value of $g$ during isovolumic beating and $g_1$ is a coefficient representing the strength of induced-distortion influence on $g$. In Eq. 20, $g > g_0$ for positive and negative values of $\Delta X_{ep}(t)$, i.e., reduction, as occurs during shortening, and enhancement, as occurs during stretch, of baseline distortion increase $g$ and the rate of detachment. This distortion-dependent $g$ was then incorporated into the model. Equations describing the model with distortion-dependent $g$ are given in Ref. 5.

Two tests were used to determine whether there was degradation or improvement in the representation of the signal when fitting with the model with constant $g$ rather than the model with distortion-dependent $g$. The first of these tests used the Akaike information criterion (AIC) and the Schwartz criterion (SC) (19). These were calculated, from model fits with and without the distortion-dependent term in Eq. 20 included, according to

$$AIC = N \ln (RSS) + 2K$$

$$SC = N \ln (RSS) + K \ln (N)$$

where $N$ is the number of sampled data points (2,000 points/record $\times 9$ records fit simultaneously $= 18,000$ points), $RSS$ is the residual sum of squares, and $K$ is the number of parameters ($K = 4$ with constant $g$; $K = 5$ with distortion-dependent $g$). In considering two competing model formulations, the better formulation is the one with the smaller AIC and SC. The second test to determine whether significant reduction in the RSS occurred with incorporation of distortion-dependent detachment was an incremental F test (7).

**RESULTS**

The time course of isovolumic pressure was very different, depending on whether the response was activated by Ca$^{2+}$ or Sr$^{2+}$ (Fig. 4), with a much slower pressure time course during Sr$^{2+}$- than during Ca$^{2+}$ activation. For instance, time to peak isovolumic pressure was three times greater in the Sr$^{2+}$-activated state (0.85 $\pm$ 0.081 s) than in the Ca$^{2+}$-activated state (0.29 $\pm$ 0.013 s, $P < 0.0001$). Despite differences in time course, magnitudes of peak isovolumic pressure ($P_{iso(max)}$) during Sr$^{2+}$ and Ca$^{2+}$ activations were not different: 144.1 $\pm$ 18.9 mmHg during Ca$^{2+}$ activation and 150.3 $\pm$ 14.4 mmHg during Sr$^{2+}$ activation ($P = 0.14$). The extended time of contraction during Sr$^{2+}$ activation, especially the extended time during which pressure rose to its peak value, presented more opportunity for sinusoidal volume perturbations to induce cross-bridge breakage while pressure was rising and maintained than was the case during Ca$^{2+}$ activation.

Depressive response during Sr$^{2+}$ activation is qualitatively and quantitatively different from that during Ca$^{2+}$ activation. Qualitative differences in $\Delta P_d(t)$ between the Ca$^{2+}$- and Sr$^{2+}$-activated states are shown in Fig. 5 ($f = 50$ Hz, $\Delta V = 1\%$ of baseline volume). Quantitative measures of these differences were as follows: 1) the Ca$^{2+}$-activated state, $T_{P_{d,max}}$, occurred during late contraction, always on the descending limb of isovolumic pressure; in the Sr$^{2+}$-activated state, it occurred during early contraction, mostly on the ascending limb and never after time of peak pressure (Table 1). It is particularly relevant that the depressive response is...
Table 1. TP_{d,max}, relative to time of peak pressure (ΔV_c = 1% V_{BL})

<table>
<thead>
<tr>
<th></th>
<th>25 Hz</th>
<th>50 Hz</th>
<th>76.9 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^{2+}</td>
<td>1.83 ± 0.49</td>
<td>1.95 ± 0.45</td>
<td>2.09 ± 0.17</td>
</tr>
<tr>
<td>Sr^{2+}</td>
<td>1.01 ± 0.32</td>
<td>0.71 ± 0.32</td>
<td>0.57 ± 0.22</td>
</tr>
<tr>
<td>P (Ca^{2+} vs. Sr^{2+})</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD of 10 hearts. TP_{d,max}, time of peak depressive response; ΔV_c, command amplitude; V_{BL}, baseline volume.

Table 2. T_{75–25} (ΔV_c = 1% V_{BL})

<table>
<thead>
<tr>
<th></th>
<th>25 Hz</th>
<th>50 Hz</th>
<th>76.9 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^{2+}</td>
<td>0.20 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>P (nonperturbed vs. perturbed)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sr^{2+}</td>
<td>0.33 ± 0.04</td>
<td>0.35 ± 0.05</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>P (nonperturbed vs. perturbed)</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SD of 10 hearts, expressed in s. T_{75–25}, time required for pressure to fall to 75 to 25% of peak isovolumic pressure.

The depressive response depends on the manner in which the myofilament system is activated.

Table 3. These differences establish that the nature of the Ca^{2+}-activated state; ΔV appeared only in all equations, whereas P appeared only during Ca^{2+} activation and f - ΔV appeared only in Eq. 22a. In all cases, ΔV was the first variable entered in the stepwise regression. This was because ΔV alone accounted for >90% of all the variation in ΔP_d in every case. Neither f nor ΔV appeared as significant variables in determining either measure of the depressive response (ΔP_d and ΔP_{d,max}) in the Sr^{2+}-activated state. To determine the relative importance of ΔV, f, and f - ΔV in Eq. 22a, a and b, we considered how a change in each around some reference values (ΔV_0 and f_0) contributed to a change in ΔP_d. Thus we write

\[ d(ΔP_d) = \frac{∂(ΔP_d)}{∂ΔV} dΔV + \frac{∂(ΔP_d)}{∂f} df + \frac{∂(ΔP_d)}{∂(f - ΔV)} d(f - ΔV) \]  

where d represents the differential change in the variable, and the partial derivatives were the values of the corresponding coefficients in the regression equation. Around a reference ΔV_0 of 1% V_{BL} (−0.02 ml) and a reference f_0 of 50 Hz, Eq. 22a predicts a ΔP_d of −4.53 mmHg. A 50% increase in ΔV causes a further increment in the depressive response of −3.74 mmHg for a total ΔP_d of −8.27 mmHg, i.e., an 82% increase in ΔP_d magnitude. This compares with a 50% increase in f, which takes away 1.11 mmHg from the depressive response for a total ΔP_d of −3.42 mmHg, a 24% decrease as pressure is rising during Sr^{2+} activation, which is inconsistent with continued vibration-induced breakage of cross bridges as more cross bridges form during increasing activation. 2) ΔP_d(t) during late relaxation was strongly negative in the Ca^{2+}-activated state, indicating that relaxation is speeded in the perturbed beat relative to the nonperturbed beat; this in contrast to Sr^{2+} activation, where ΔP_d(t) was often positive, indicating that relaxation was slowed in the perturbed beat relative to the nonperturbed beat. This effect of volume perturbation on relaxation is confirmed by evaluation of the time required for pressure to fall from 75 to 25% of P_{d,max} (T_{75–25}). The T_{75–25} of perturbed beats was smaller than that of nonperturbed beats in the Ca^{2+}-activated state, indicating that relaxation was speeded by the volume sinusoid. However, in the Sr^{2+}-activated state, T_{75–25} of perturbed beats was longer than that of nonperturbed beats, indicating that relaxation had been slowed by the volume sinusoid (Table 2). 3) ΔP_d(t) was greater in the Ca^{2+}- than in the Sr^{2+}-activated state; ΔP_{d,max} and ΔP_{d,avg} are compared in Table 3. These differences establish that the nature of the depressive response depends on the manner in which the myofilament system is activated.

Depressive response depends mostly on sinusoidal amplitude with little dependence on frequency or velocity. An example of a family of nine ΔP_d(t) responses obtained in one heart at the three f and three ΔV_c used in this study is shown in Fig. 6. Clearly, ΔP_d(t) increased with increasing ΔV_c. Apparently, ΔP_d(t) also increased with increasing f. However, because of the underdamped character of the volume-servo system, the actual ΔV increased with increasing f, such that the apparent increase in ΔP_d(t) with f may have been secondary to the concordant increase in ΔV, despite the constant ΔV_c. This effect is accounted for in the regression analysis, where measured ΔV is used rather than ΔV_c.

These apparent trends in Fig. 6 were quantitatively evaluated using data from all 10 hearts, with stepwise multiple regression analysis of ΔP_d(t) and ΔP_{d,max} and Eq. 6, a and b. According to our criteria for acceptance of predictor variables (and not reporting coefficients for between-subjects variability), the simplest best regression equations for predicting ΔP_d(t) and ΔP_{d,max} were

\[ ΔP_d(t) = -0.293 - 314.1ΔV + 0.0784f + 1.019(f · ΔV) \]  
\[ R^2(adj) = 0.956 \]  
\[ ΔP_{d,max} = -5.34 - 706.4ΔV + 0.249f - 0.0019f^2 \]  
\[ R^2(adj) = 0.943 \]  

for Ca^{2+} activation and

\[ ΔP_d(t) = 0.911 - 377.5ΔV + 3.148ΔV^2 \]  
\[ R^2(adj) = 0.946 \]  
\[ ΔP_{d,max} = 0.398 - 316.8ΔV \]  
\[ R^2(adj) = 0.950 \]  

for Sr^{2+} activation. ΔV appeared in all equations, whereas f appeared only during Ca^{2+} activation and f · ΔV appeared only in Eq. 22a. In all cases, ΔV was the first variable entered in the stepwise regression. This was because ΔV alone accounted for >90% of all the variation in ΔP_d in every case. Neither f nor ΔV appeared as significant variables in determining either measure of the depressive response (ΔP_d and ΔP_{d,max}) in the Sr^{2+}-activated state. To determine the relative importance of ΔV, f, and f · ΔV in Eq. 22a, a and b, we considered how a change in each around some reference values (ΔV_0 and f_0) contributed to a change in ΔP_d. Thus we write

\[ d(ΔP_d) = \frac{∂(ΔP_d)}{∂ΔV} dΔV + \frac{∂(ΔP_d)}{∂f} df + \frac{∂(ΔP_d)}{∂(f · ΔV)} d(f · ΔV) \]  

where d represents the differential change in the variable, and the partial derivatives were the values of the corresponding coefficients in the regression equation. Around a reference ΔV_0 of 1% V_{BL} (−0.02 ml) and a reference f_0 of 50 Hz, Eq. 22a predicts a ΔP_d of −4.53 mmHg. A 50% increase in ΔV causes a further increment in the depressive response of −3.74 mmHg for a total ΔP_d of −8.27 mmHg, i.e., an 82% increase in ΔP_d magnitude. This compares with a 50% increase in f, which takes away 1.11 mmHg from the depressive response for a total ΔP_d of −3.42 mmHg, a 24%
Table 3. Magnitude of depressive response (ΔV = 1% VBL)

<table>
<thead>
<tr>
<th></th>
<th>ΔP_dmax</th>
<th>ΔP_davg</th>
</tr>
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<tbody>
<tr>
<td>Ca²⁺</td>
<td>-9.18 ± 3.32</td>
<td>-3.38 ± 1.99</td>
</tr>
<tr>
<td>Sr²⁺</td>
<td>-4.07 ± 0.685</td>
<td>-0.71 ± 0.47</td>
</tr>
<tr>
<td>P (Ca²⁺ vs. Sr²⁺)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD of 10 hearts, expressed in mmHg. ΔP_dmax, maximum depressive response; ΔP_davg, average depressive response over entire heart.

decrease in ΔP_dT magnitude. Furthermore, a 50% increase in f · ΔV takes away 0.51 mmHg from the depressive response for a total ΔP_dT of 4.02 mmHg. These calculations show that, rather than contributing to the depressive response, f and f · ΔV actually reduce that response. Continuing these calculations with Eq. 22b for ΔP_dmax, a ΔV of 1% VBL (~0.02 ml) and an fQ of 50 Hz predict a ΔP_dmax of 7.02 mmHg. A 50% increase in ΔV brings about a 100% increase in the magnitude of ΔP_dmax, whereas a 50% increase in f takes away 90% of ΔP_dmax.

To summarize these results, ΔV was always a significant predictor of the depressive response during Ca²⁺ and Sr²⁺ activation and, by itself, accounted for >90% of the variation in ΔP_dT and ΔP_dmax. Of less importance, and of significance only during Ca²⁺ activation and not during Sr²⁺ activation, was the effect of f. Instead of increasing the depressive response, f decreased it; larger f resulted in less magnitude of ΔP_dT and ΔP_dmax. This decrease in the depressive response with f has consequence with regard to the character of underlying dynamic processes and is discussed relative to the dynamics of f-induced changes in cross-bridge strain in the Discussion. Importantly, f · ΔV (analogous to the velocity of filament sliding) was not a significant predictor of ΔP_dmax during Ca²⁺ activation, nor was it a significant predictor of ΔP_dT or ΔP_dmax during Sr²⁺ activation. During Ca²⁺ activation, f · ΔV was found to be a significant predictor of the depressive response only for ΔP_dT. In that one case, it was the last variable entered in the stepwise regression, indicating that its incremental contribution in accounting for variation in ΔP_dT was least among all the candidate variables. Furthermore, rather than contributing to the depressive response, f · ΔV actually reduced that response, an effect that is contrary to the hypothesis that increased cross-bridge breakage due to increased velocity of filament sliding is contributing to the depressive response.

Cross-bridge cycling in the Sr²⁺-activated state was not different from that in the Ca²⁺-activated state. As we found in our earlier study (5), ΔP_d (t) was well fit with the cross-bridge-based model, i.e., Eqs. 16–19. Fit was to all nine responses simultaneously. The median R² of fit in Ca²⁺- and Sr²⁺-activated states were 0.974 and 0.981, respectively. Very little of the total variation in ΔP_d (t), which was due not only to the sinusoidal changes in V but also to the changes in f and ΔV among the nine separate responses that were fit, remained unaccounted for by the model. Importantly, cross-bridge cycling parameters g, h, and d were estimated to be the same during Ca²⁺ and Sr²⁺ activation (Table 4); some caution should be used in the interpretation of the

Fig. 6. Families of depressive responses (ΔP_d(t)) obtained during sinusoidal perturbations at 3 frequencies (25, 50, and 76.9 Hz) and 3 amplitudes (0.75, 1.0, and 1.25% of baseline volume). Top: Ca²⁺-activated state; bottom: Sr²⁺-activated state. Time of peak isovolumic pressure. Increasing magnitude of depressive response in any 1 panel is associated with increasing amplitude of perturbation.
value of d, as discussed in Ref. 5). Despite in the depressive response between the Ca2+- and the Sr2+-activated states, there were no differences in the underlying dynamics of cross-bridge cycling between Ca2+ and Sr2+ activation.

Cross-bridge detachment rate does not increase with sinusoidal volume perturbation. We could find no evidence for increased cross-bridge detachment during sinusoidal volume perturbation due to perurbation-induced changes in cross-bridge distortion. There was no improvement in accounting for variation in the data when distortion-dependent attachment was incorporated into the cross-bridge model: median $R^2$ was the same for the models with and without distortion-dependent detachment in the Ca2+- (0.9768 with and without distortion-dependent detachment) and the Sr2+-activated state (0.9810 with and without distortion-dependent detachment). Furthermore, during Ca2+ activation, incorporating distortion-dependent detachment into the model resulted in a lower AIC in only 3 of 10 hearts and resulted in a lower SC in only 1 of 10 hearts. Furthermore, a significant reduction in RSS as determined by the incremental F test was achieved in only 1 of 10 hearts. This compares with Sr2+ activation, during which there was a reduction in AIC and SC in only 1 of 10 hearts and a significant incremental F test also in only 1 of 10 hearts. Finally, the values of $g$, $h$, $d$, and $X_0$ were virtually unaffected by whether or not distortion-dependent detachment was part of the model. These results, showing no detectable improvement in the model with the addition of distortion-dependent detachment (i.e., $g_t$ in Eq. 20), are completely in accord with earlier results from an identical test conducted on $\Delta P_r(t)$ data from 14 other Ca2+-activated hearts at 30°C and reported in Ref. 5. Thus, accounting for distortion-dependent detachment did not improve the model representation of the data. These results are consistent with no increased cross-bridge breakage in the perturbed beat over and above that during the isovolumic beat as a consequence of the sinusoidal perturbation that produced the depressive response.

**DISCUSSION**

We present six lines of evidence to indicate that small-amplitude volume sinusoids do not depress cardiac contraction through increased breakage of cross bridges. 1) The depressive response was strongly affected by whether the myocardium was activated by Ca2+ or by Sr2+ (Fig. 4), indicating that the depressive response was dependent on activation as opposed to cross-bridge cycling. 2) During prolonged contraction, as in the Sr2+-activated state, rather than increased depression as pressure rose on the ascending limb of the pressure waveform, the depressive response actually declined (Fig. 5, Table 3), despite the extended opportunity for volume sinusoids to cumulatively break more cross bridges and cause greater depression. 3) Although relaxation rate was increased with volume sinusoids during Ca2+ activation, as is consistent with volume-induced increase in cross-bridge detachment, the relaxation rate actually decreased with volume sinusoids during Sr2+ activation (Table 2), which is counter to an increase in cross-bridge detachment rate. 4) $f \cdot \Delta V$, as an analog of velocity of myofilament sliding, which is assumed to enhance cross-bridge breaking, was insignificant as a predictor of the depressive response or, when significant, participated by diminishing the depressive response rather than contributing to its magnitude (Eqs. 22 and 23). 5) Despite the differences in the depressive response between the Ca2+- and the Sr2+-activated state, we found no difference in cross-bridge detachment rate constant or in any other cross-bridge cycling parameters (Table 4). 6) We were unable to detect any increase in the cross-bridge detachment rate constant due to volume-induced change in cross-bridge strain, as would be predicted from current cross-bridge theory and as one must assume in a rationale to support a hypothesis for vibration-induced increase in cross-bridge breakage. Collectively, these results argue against a cross-bridge detachment-based cause for a sinusoidally induced myocardial depressive response.

Depressive response during Ca2+ activation in these experiments is identical to vibration-induced contractile depression reported by others. One difference between our method and the methods of most other workers for mechanically inducing contractile depression is that we used a sinusoidal LV volume perturbation, whereas others have used external vibrators on the LV wall (15–17, 20, 24, 26). These other workers have found that vibrations imposed on the LV anterior wall travel to the posterior wall and throughout the myocardium (14, 20) to induce a global myocardial effect. Although none of these previously reported LV depressions were analyzed in the same quantitative manner as we have done here, certain similarities can be seen in the contractile depression obtained in our experiments and that obtained by others. In the only other studies in which vibrations were applied over the full cycle duration of an isovolumically beating heart (16, 20), the qualitative features of the depression in those blood-perfused dog hearts at 37°C appeared to be the same as those in our Ca2+-activated rabbit heart at 25°C. In studies where vibration was imposed on ejecting hearts (16, 17, 26), the depressive response involved decrements in pressure and in ejection, which makes comparison with the current results in isovolumic hearts difficult.

Of particular interest is the comparison to data obtained when vibration was confined to the relaxation and diastolic phases of the cycle (12, 24). These authors found, as we did in the Ca2+-activated heart (Table 2), that mechanical perturbations during this phase of the
cycle caused a speeding of relaxation. That this effect originated from the contractile apparatus rather than from some other feature of the intact heart was confirmed by studies in isolated rat papillary muscle where length vibration speeded relaxation (11). Of particular note in these papillary muscle studies was that the vibrational effect that speeded relaxation did not depend on frequency of vibration for frequencies > 30 Hz, whereas this effect was strongly dependent on vibrational amplitude at these frequencies. This is consistent with our findings in these isolated heart studies that the depressive response was not dependent on frequency of 25–76.9 Hz but strongly dependent on amplitude at all frequencies.

Therefore, despite the differences in method of mechanical perturbation, whether it is given as a sinusoidal change in LV volume, a myocardial vibration by an external oscillator on the LV anterior wall, or a sinusoidal change in papillary muscle length, the net result to depress contractile function is the same.

Dynamics underlying depressive response are different from those associated with cross-bridge detachment. Features of dynamic processes are revealed by the manner in which the response variable changes with $f$. For instance, in simple first-order systems, the response variable will change strongly with $f$ if the characteristic frequency of the process is in the vicinity of $f$ but will change only weakly with $f$ if $f$ is far greater than the characteristic frequency. Furthermore, the response will decrease with $f$ if the process is driven directly by the input but will increase with $f$ if it is driven by the derivative of the input (e.g., the frequency dependence of a 1st-order low-pass filter vs. that of a 1st-order high-pass filter). We found that the depressive response did not change significantly with $f$, or, in the one instance in which $f$ was significant, changed by decreasing magnitude with increasing $f$. Furthermore, decreasing magnitude of the response variable with increasing $f$ was sufficient to prove that $V(t)$, analogous to $f \cdot \Delta V$, was not the effective driving function for the depressive response as it is for the in-frequencies response (5). As argued in the following paragraph, our findings that the depressive response does not depend on $f$ for decreased with $f$ argue against accepting perturbation-induced cross-bridge breakage as being responsible for the depressive response.

We reasoned that if contractile depression depends on increased cross-bridge breakage due to changes in cross-bridge distortion, as currently hypothesized (5), then the depressive response will change with $f$ and $\Delta V$ in the same manner as cross-bridge distortion. Previously (5), we showed that, to an approximation, frequency-dependent changes in distortion of the force-bearing state, $\Delta X(f)$, with sinusoidal volume changes, $\Delta V(f)$, are given by

$$\Delta X(f) = \left[ \frac{2 \pi f}{\sqrt{(2\pi f)^2 + g^2 \theta(f)^2}} \right] \Delta V(f)$$

where $j = \sqrt{-1}$ and $\theta(f)$ is the phase difference between the distortional response and input volume sinusoid. From Eq. 25, a root-mean-square distortion ($\Delta X_{\text{rms}}$) at any $f$ and $\Delta V$ may be calculated as

$$\Delta X_{\text{rms}} = \frac{\sqrt{2\pi} f \cdot \Delta V}{\sqrt{(2\pi f)^2 + g^2 \theta(f)^2}}$$

Equation 26 defines the manner in which an average distortion varies with $f$ and $\Delta V$ during sinusoidal perturbations: for all $2\pi f$ within some range around $g$, $\Delta X_{\text{rms}}$ increases with $f$ and $\Delta V$. For $2\pi f$ far above $g$, $\Delta X_{\text{rms}}$ retains a strong dependence on $\Delta V$ and continues to increase with $f$ but only weakly. For instance, a $g$ of $50$ s$^{-1}$, as we estimated with these data, yields a characteristic frequency of $\approx 8$ Hz. This means that increasing $f$ from 25 to 75 Hz would cause a 5% increase in distortion. Such an increase in distortion would be expected to cause an increase in the depressive response if this response was due to increased cross-bridge breakage secondary to increased distortion. However, this is opposite to what was observed when increased $f$ was associated with no change or a decrease in the depressive response. Thus the depressive response and cross-bridge distortion do not change similarly with $f$, and different dynamic processes must be responsible for each. Furthermore, these data support the hypothesis that the characteristic frequency of the process underlying the depressive response is much lower (a slower process) than that responsible for cross-bridge detachment.

Possible mechanism involves activation, perhaps the cooperative feedback between force-bearing cross bridges and activation. Previous studies have attempted to discriminate between $Ca^{2+}$ handling associated with excitation-contraction coupling and cross-bridge kinetics as competing alternative mechanisms for vibrational depression of contraction. Considerable evidence has now accumulated to eliminate $Ca^{2+}$ handling associated with excitation-contraction coupling as a possible mechanism (11, 20). By default then, explanations were sought for the effects of vibration on cross-bridge kinetics. This was reasonable, because vibration-induced myofilament sliding or vibration-induced variation in cross-bridge strain would, according to current cross-bridge theory, enhance cross-bridge breakage and reduce force (6, 9, 10, 21, 22, 25). In fact, mathematical models incorporating vibrational effects in cross-bridge kinetics do reproduce, at least qualitatively, some aspects of contractile depression (8). However, the two above alternatives do not constitute a complete list of possible mechanisms, for it is now known that other factors can modulate force development in the presence of fixed $Ca^{2+}$ without changing cross-bridge kinetics (18, 23). Among these other factors are 1) changes in the force developed by individual cross bridges, 2) changes in the binding affinity of $Ca^{2+}$ for troponin C, and 3) changes in the cooperative mechanisms by which force-bearing cross bridges feed back to enhance their own activation.
The results reported here demonstrate that vibration does not change cross-bridge kinetics. Thus we must reject changes in cross-bridge kinetics as the basis for vibration-induced depression of contraction. Furthermore, changes in force by an individual cross bridge are associated with changes in the isometric strain of these cross bridges (18). It has been shown that the X^2 parameter in our cross-bridge model is related to cross-bridge isometric strain (2). Because X^2 did not change, even when the depressive response was markedly different, as it was during Ca^2+ vs. Sr^2+ activation (Table 4), we reject the possibility that vibrational depression of contraction is due to a decrease in force developed by individual cross bridges. Thus we are left with choosing between a vibration-induced decrease in the binding affinity of Ca^2+ for troponin C and a vibration-induced decrease in the cooperative feedback between force-bearing cross bridges and cross-bridge activation. We favor the latter of these two possibilities, because we found that the dynamics of the processes underlying contractile depression were slower than the dynamics of cross-bridge cycling. We recently demonstrated (1) that cooperativity in activation will cause force transients to become much slower than would be predicted on the basis of the speed of cross-bridge cycling. The consequence is that the characteristic frequency of the activation process is much lower than that of the cross-bridge cycle. This suggests that our finding of an apparently slow process underlying the depressive response is consistent with activation being that underlying process. That we observed such different depressive responses relative to pressure development with Ca^2+ and Sr^2+ activation argues further that the underlying mechanism is based on activation.

In summary, we conclude that contractile depression due to small-amplitude perturbation at frequencies in the 25- to 76.9-Hz range is not due to increased cross-bridge breakage but, more likely, involves a change in the manner in which thin filaments are activated, perhaps in the cooperative feedback between force-bearing cross bridges and activation.

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