0022-3565/97/2801-0247$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1997 by The American Society for Pharmacology and Experimental Therapeutics
JPET 280:247–254, 1997

Vol. 280, No. 1
Printed in U.S.A.

Ca^{++} Sensitizers Impair Cardiac Relaxation in Failing Human Myocardium[1]

ROGER J. HAJJAR, ULRICH SCHMIDT, PATRICK HELM and JUDITH K. GWATHMEY[2]

From the Integrated Physiology Laboratories, Boston University School of Medicine, Evans Department of Medicine and the Whitaker Cardiovascular Institute (U.S., P.H., J.K.G.), and the Heart Failure Center, Cardiac Unit, Massachusetts General Hospital, Boston, MA (R.J.H.)

Accepted for publication July 12, 1996

ABSTRACT

The present study was aimed at investigating the effect of two Ca^{++} sensitizers, EMD 57033 (without significant phosphodiesterase inhibition) and ORG 30029 (with phosphodiesterase inhibition), in myocardium from nonfailing and failing human hearts. In nonfailing myocardium both EMD 57033 and ORG 30029 increased force of contraction by 280 ± 27% and 94 ± 13%, respectively (n = 6); the time to 80% relaxation ($t_{80\%}$) by 278 ± 45% and 155 ± 21%; and diastolic force by 28 ± 8% and 12 ± 3%, respectively. In trabeculae from failing myocardium, the increase in active force was similar to that in nonfailing trabeculae (EMD, 305 ± 30%; ORG, 88 ± 12% (n = 6)). However, the increase in $t_{80\%}$ (EMD, 378 ± 56%; ORG, 230 ± 26%) and diastolic force (65 ± 12%; 24 ± 5%) was more pronounced in failing myocardium. EMD had no effect on the peak of the $[Ca^{++}]_i$ transient; however, it prolonged the time course of the $[Ca^{++}]_i$ transient in both nonfailing and failing myocardial fibers. ORG increased the peak of the Ca^{++} transient and prolonged the time course in preparations from both nonfailing and failing hearts. Both EMD and ORG shifted the $[Ca^{++}]$-force relationship toward lower $[Ca^{++}]$ (EMD > ORG).The Ca^{++} sensitizers EMD 57033 and ORG 30029 increased active force development in nonfailing and failing human myocardium, but both impaired relaxation in failing myocardium to a greater extent than in nonfailing human myocardium in a concentration-dependent fashion.

Most inotropic agents that are used for the treatment of congestive heart failure act *via* cAMP-dependent or cAMP-independent mechanisms. The efficacy of cAMP-dependent inotropic effects is reduced in human failing myocardium because of a down-regulation of *beta* adrenoceptors (Bristow *et al.*, 1982), an increase of Giα (Feldman *et al.*, 1988; Böhm *et al.*, 1990) and reduced cAMP concentrations (Feldman *et al.*, 1987; Danielsen *et al.*, 1989). Furthermore, PDE inhibitors which constitute the main group of cAMP-dependent inotropic agents have deleterious effects on survival in patients with end-stage heart failure (Packer *et al.*, 1991).

A new class of inotropic agents, Ca^{++} sensitizers, has been developed, which act at the level of the contractile proteins to increase the sensitivity of the myofilaments to Ca^{++} (Fujino *et al.*, 1988; Hajjar *et al.*, 1988; v.Meel *et al.*, 1988; Honerjäger *et al.*, 1989; Lee and Allen, 1991; Ferroni *et al.*; 1991, Ventura *et al.*, 1992; Kawabata and Endoh, 1993; Lues *et al.*, 1993; Gambassi *et al.*, 1993; Solaro *et al.*, 1993; Hgashiyama *et al.*, 1995; Neumann *et al.*, 1995). These agents have the advantage of enhancing force production without increasing energy utilization (Grandis *et al.*, 1995). However, they have the adverse effect of slowing relaxation and elevating diastolic tension (Hajjar and Gwathmey, 1991). In heart failure, relaxation is impaired because of abnormal $[Ca^{++}]_i$ handling (Gwathmey *et al.*, 1987, 1988, 1990; Gwathmey and Hajjar, 1990; Beuckelmann *et al.*, 1992); therefore, Ca^{++} sensitizers may potentially increase diastolic force further in these failing hearts. However, most of the calcium-sensitizing agents have additional cellular effects such as inhibition of PDE III which may counter the effects of Ca^{++} sensitizers on relaxation.

We undertook this study to examine the effects of Ca^{++} sensitization in the presence and absence of PDE inhibition on force generation and intracellular Ca^{++} handling in ventricular preparations from nonfailing and failing human hearts with use of two newly developed inotropic agents EMD 57033 and ORG 30029. EMD 57033, a cardiotonic thiadiazone derivative, has been reported to have potent myofilament Ca^{++}-sensitizing effects with negligible PDE inhibitory effects at low concentrations (<1 μmol/l) and weak PDE

Received for publication February 6, 1996.
[1] This work is supported in part by RO1 HL 49574 and R44 HL52249 (to J.K.G.), by the German Research Foundation (DFG) (to U.S.) and by a stipend support from CVD Inc. (to P.H.).
[2] Established Investigator of the American Heart Association.

ABBREVIATIONS: EMD 57033, (+)-5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl-6- methyl-3,6-dihydro-2H-1,3,4-thiadiazino-2-one; ORG 30029, N-hydroxy-5,6,-dimethoxy-benzo-[β]thiophene-2-carboximide hydrochloride; pCa, −log[Ca^{++}]; $t_{80\%}$, time to 80% relaxation; EGTA, ethylene glycol-bis(β-aminoethyl ether); MOPS, 3-[N-morpholino]propanesulfonic acid; TES, N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; TnC, troponin C; PDE, phosphodiesterase; SR, sarcoplasmic reticulum.

inhibitory effects at higher concentrations (Ferroni *et al.*, 1991; Lues *et al.*, 1993; Solaro *et al.*, 1993). ORG 30029 is a cardiotonic agent which increases the sensitivity of the myofilaments to Ca^{++} and selectively inhibits PDE III (Kawabata and Endoh, 1993).

Materials and Methods

Human hearts. Experiments were performed on isolated, electrically stimulated trabeculae carneae from human left ventricular myocardium. Tissue was obtained from human hearts during cardiac transplantation ($n = 7$, 1 female, 6 males; age range, 20–61 years; 3 with dilated cardiomyopathy and 4 with ischemic cardiomyopathy). All the patients suffered from heart failure clinically classified as NYHA IV with ejection fractions <25%. All patients were receiving diuretics, nitrates, angiotensin converting enzyme inhibitors and cardiac glycosides at the time of transplantation. Nonfailing human myocardium was obtained from 7 brain-dead donors (3 car accidents and 4 hemorrhagic strokes; 4 males, 3 females; age range, 19–54 years). There was no clinical and echocardiographic evidence of left ventricular dysfunction. These hearts could not be transplanted for either technical reasons or because of age.

Isolated muscle preparations. Muscle fibers running approximately parallel with the length of the preparations (diameter: 0.6–1.0 mm) of uniform size were dissected in an oxygenated bathing solution at room temperature (see below) under microscopic control with sharp scissors. The preparations were electrically stimulated by a bipolar platinum electrode located at the base. The bathing solution used was a modified Krebs-Henseleit solution containing (in mmol/l): NaCl, 120; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; dextrose, 11.5; with 95% O_2 and 5% CO_2 and maintained at 37°C, pH 7.4. Isometric force of contraction was measured with an inductive force transducer attached to a Gould recorder. The preparations were electrically paced at 1 Hz with square wave pulses of 5-msec duration (Grass stimulator SD 9), at threshold voltage. Muscles were stretched incrementally until there was no further increase in peak twitch force. Force measurements were normalized to the cross-sectional area of the muscles (g/mm^2), and $t_{80\%}$ was measured from peak twitch force. All preparations were allowed to equilibrate at least 90 min in the bathing solution until complete mechanical stabilization.

Intracellular Ca^{++} measurements. The muscles were loaded with the bioluminescent Ca^{++} indicator aequorin with a modified chemical-loading procedure described previously (Pesaturo and Gwathmey, 1990). Aequorin was introduced into the muscle *via* macroinjection through a glass micropipette. The aequorin solution contained (mmol/l): EGTA, 0.1; Na_2ATP, 5; KCl, 120; $MgCl_2$, 2; TES, 20; 0.5 mg/ml aequorin. The muscle was then placed in Ca^{++}-free standard Krebs' solution (as described above) at 20°C, and Ca^{++} was re-added to this solution at 20-min intervals in increasing concentrations: 0.025, 0.25, 1.25 and 2.5 mmol/l. The muscle was then gradually rewarmed to 37°C. Calcium and force responses from aequorin-loaded muscles were recorded simultaneously by a force transducer and a specially designed light-collecting apparatus (Blinks, 1984). Force and $[Ca^{++}]_i$ responses were digitally acquired. For each response studied 10 to 50 signals were averaged depending on the brightness of an individual muscle preparation to improve the signal-to-noise ratio. Light signals were converted to intracellular $[Ca^{++}]$ with an *in vitro* calibration curve modeled by the following equation:

$$\frac{L}{L_{max}} = \left(\frac{1 + K_R[Ca^{++}]_i}{1 + K_{TR} + K_R[Ca^{++}]_i}\right)^3$$

where L is the luminescence signal, L_{max} is the maximum light emitted after the lysis of the muscle preparation exposed to saturating $[Ca^{++}]$ with 2% Triton X-100. K_R and K_{TR} are constants. $L_{80\%}$ is the time from peak to 80% relaxation of the $[Ca^{++}]_i$ signal.

Skinned fibers preparations. Thin muscle fibers (diameter <400 μm) were dissected and handled as described above. The electrical stimulation was turned off and the bath temperature was then lowered to 22°C. The fibers were then exposed to a skinning solution composed of (mmol/l): Na_2ATP, 5; $MgCl_2$, 7; EGTA, 60; KCl, 50; MOPS, 12; phosphocreatine, 12; creatine phosphokinase, 15 U/ml; saponin, 250 μg/ml. The muscles were exposed to this solution for 30 min. After skinning, the muscles were placed in a relaxing solution (pCa > 8.0) and then activated over a full range of pCa values (8.0–4.0). The relaxation and activating solutions were calculated with the program of Fabiato (1988). The activating and relaxing solutions were prepared at 22°C and contained (mmol/l): MOPS, 50; EGTA, 10; TES, 30; adjusted for a pMg of 2.5, pMgATP of 2.5, an ionic strength of 160 mmol/l, and pH of 7.1. EMD 57033 and ORG 30029 were added at all pCa values throughout the activation cycle. Each Ca^{++}-activation experiment (with or without drugs) yielded a $[Ca^{++}]$-force relationship which was then fitted individually to the Hill equation:

$$F = F_{max}\frac{[Ca^{++}]^n}{[Ca^{++}]_{50\%}{}^n + [Ca^{++}]^n}$$

where F is force developed, $[Ca^{++}]$ is the Ca^{++} concentration in the solution, n is the Hill parameter, $[Ca^{++}]_{50\%}$ is the $[Ca^{++}]$ required for 50% activation and F_{max} is the maximal Ca^{++}-activated force. The Hill parameters (F_{max}, n and $[Ca^{++}]_{50\%}$) are then calculated by nonlinear regression analysis for that one individual experiment. The Hill parameters are then averaged for the different groups of experiments (*i.e.*, no drug, +EMD 57033, +ORG 30029), yielding a mean ± S.E.M. for the Hill parameters of each group (Moiescu and Thieleczek, 1978; Harrison and Bers, 1989). We define differences in myofilament calcium sensitivity as a change in $[Ca^{++}]_{50\%}$ as opposed to differences in myofilament calcium responsiveness which may involve differences in F_{max} and the Hill coefficient with and without changes in $[Ca^{++}]_{50\%}$.

Materials. EMD 57033 was kindly provided by E. Merck, Darmstadt, Germany and ORG 30029 was kindly provided by Dr. M. Endoh (Yamagata University School of Medicine, Yamagata, Japan). All other chemicals were obtained from Sigma Chemical Co. (St Louis, MO).

Statistics. The data shown are represented as mean ± S.E.M. Student t test was performed to test for statistical differences with $P < .05$ considered significant. $[Ca^{++}]$-force relationships were fitted individually to the Hill equation and Hill parameters were then averaged and expressed as mean ± S.E.M. Analysis of variance was then performed to test for statistical differences between pre- and postdrug effect on Hill parameters from the experimental groups.

Results

Effect of EMD 57033 on active force, diastolic force of contraction and $t_{80\%}$ in nonfailing and failing human myocardium. The peak twitch force generated by the muscles at 1 Hz were 2.17 ± 0.22 g/mm^2 (nonfailing, $n = 6$) and 2.32 ± 0.29g/mm^2 (failing, $n = 6$). Figure 1 shows the effect of 50 μmol/l EMD 57033 on the force of contraction in failing human myocardium. An increase in active force is observed along with a significant increase in diastolic force. As shown in figure 2A, increasing the concentration of EMD 57033 increases the active force of contraction in human ventricular trabeculae to a similar extent in both nonfailing and failing myocardium (280 ± 27% *vs.* 305 ± 30%, $n = 6$, $P > .2$). However, as shown in figure 2B, EMD 57033 increased the diastolic force (% control active force) in failing myocardium to a greater extent than in nonfailing myocardium (65 ± 12% *vs.* 28 ± 8%, $n = 6$, $P < .05$) in a concentration-dependent manner. Relaxation was also affected by the addition of

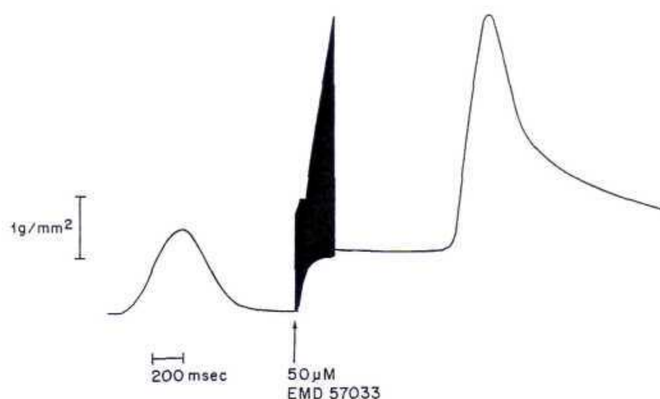


Fig. 1. The effect of adding 50 μmol/l EMD 57033 on force generation in an electrically driven muscle from a failing heart stimulated at 1 Hz with a square pulse of 5 msec at 37°C in Krebs' buffer.

EMD. The $t_{80\%}$ was increased in the presence of EMD in a concentration-dependent manner as shown in figure 2C. EMD 57033 increased $t_{80\%}$ to a greater extent in failing myocardium than in nonfailing myocardium (378 ± 56% *vs.* 278 ± 45%, $n = 6$, $P < .05$).

Effect of ORG 30029 on active force, diastolic force of contraction and $t_{80\%}$ in nonfailing and failing human myocardium. Figure 3 shows the effect of 5 mmol/l ORG 30029 on the force of contraction in failing human myocardium. As was observed with EMD, there is an increase in active force along with an increase in diastolic force and prolongation of the twitch time course. However, ORG 30029 had a smaller effect on all of these parameters when compared with the effects of EMD. As shown in figure 4A, increasing the concentration of ORG 30029 increased active force of contraction to a similar extent in both nonfailing and failing myocardium (94 ± 13% *vs.* 88 ± 2%, $n = 6$, $P > .1$). However, as shown in figure 4B, ORG 30029 increased the diastolic force (% of control active force) in failing myocardium to a greater extent than in nonfailing myocardium (24 ± 5% *vs.* 12 ± 3%, $n = 6$, $P < .05$). Relaxation was also affected by the addition of ORG. The $t_{80\%}$ was increased in the presence of ORG 30029 in a concentration-dependent fashion as shown in figure 4C. ORG 30029 increased $t_{80\%}$ to a greater degree in failing myocardium than in nonfailing myocardium (230 ± 26% *vs.* 155 ± 21%, $n = 6$, $P < .05$).

Effect of EMD 57033 and ORG 30029 on intracellular Ca^{++} transients. Intracellular Ca^{++} was measured in fibers from nonfailing and failing human hearts. As shown in table 1, at a stimulation of 1 Hz, there were no significant differences in peak intracellular $[Ca^{++}]_i$ between nonfailing and failing fibers. However, both the diastolic $[Ca^{++}]_i$ was elevated and the time course of the calcium transients was prolonged in failing myocardium as compared to nonfailing myocardium. Figure 5, A and B, demonstrates the effects of 50 μmol/l EMD 57033 and 5 mmol/l ORG 30029 on intracellular calcium transients as detected by aequorin. EMD 57033 (50 μmol/l) did not significantly change the peak of the $[Ca^{++}]_i$ transient; however, it prolonged the time course of the $[Ca^{++}]_i$ transient and increased diastolic $[Ca^{++}]_i$ in both nonfailing and failing myocardium as shown in table 1. ORG 30029 (5 mmol/l) increased the peak of the Ca^{++} transient without affecting diastolic $[Ca^{++}]_i$ in both nonfailing and failing human myocardium (fig. 5B) and prolonged the time course of the $[Ca^{++}]_i$ transient in failing myocardium as shown in table 1.

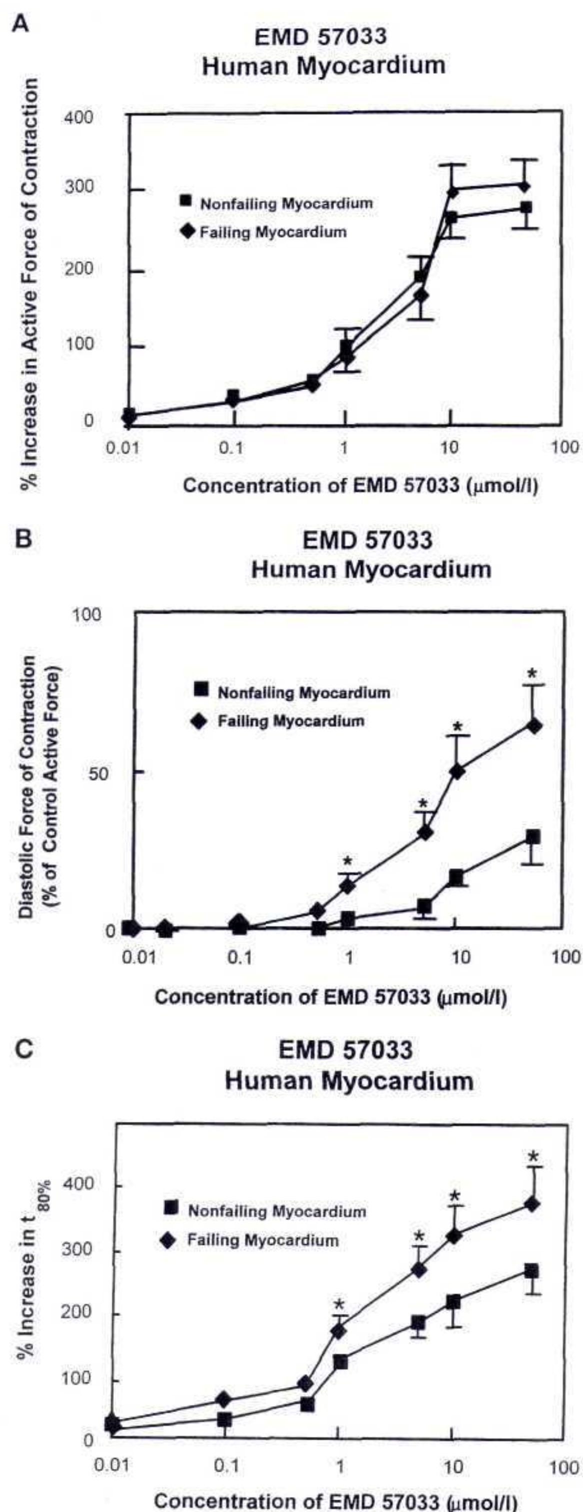


Fig. 2. Effect of increasing concentrations of EMD 57033 on active force (A), diastolic force of contraction (B) and $t_{80\%}$ (C) in failing and nonfailing human myocardium. The muscle preparations were stimulated at 1 Hz with a square pulse of 5 msec at 37°C in Krebs' buffer. * $P < .05$ compared with nonfailing myocardium.

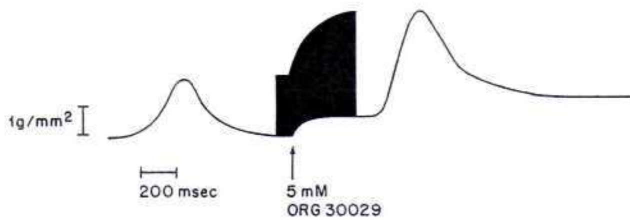


Fig. 3. The effect of adding 5 mmol/l ORG 30029 on force generation in an electrically driven muscle from a failing heart stimulated at 1 Hz with a square pulse of 5 msec at 37°C in Krebs' buffer.

Effect of EMD 57033 and ORG 30029 on force of contraction and diastolic force in the presence of isoproterenol. In isolated fibers from human myocardium, isoproterenol has been reported to increase the force of contraction and to shorten the time to relaxation by increasing intracellular cAMP and thereby increasing the rate of Ca^{++} uptake by the SR. To test whether 1 μmol/l isoproterenol would reverse the detrimental effects on relaxation by both compounds, we added isoproterenol to preparations pretreated with either EMD 57033 or ORG 30029. As seen in figure 6A, the active force of contraction remained unchanged after the addition of isoproterenol in the presence of 50 μmol/l EMD 57033; however, diastolic force decreased to pre-EMD levels. In contrast, isoproterenol further increased active force of contraction in the presence of 5 mmol/l ORG 30029 while decreasing diastolic force to pre-ORG levels (fig. 6B).

Effect of EMD 57033 and ORG 30029 on myofilament Ca^{++} responsiveness in skinned fibers preparations from human myocardium. To test whether EMD and ORG increase the sensitivity of the myofilaments to Ca^{++}, we examined the effects of these two agents in skinned fiber preparations from nonfailing and failing human myocardium. EMD 57033 and ORG 30029 both shifted the force-pCa relationship to the left (EMD 57033 ΔpCa, 0.14; ORG 30029 ΔpCa, 0.06) in nonfailing human preparations (fig. 7A and table 2). Similar effects were observed in failing human myocardium (EMD 57033 ΔpCa, 0.16; ORG 30029 ΔpCa, 0.09) (fig. 7B and table 2). As shown in table 2, ORG 30029 had no significant effect on the maximal Ca^{++}-activated force, whereas EMD 57033 increased the maximal Ca^{++}-activated force by 15 ± 10% (n = 4) and 21 ± 12% (n = 6) in nonfailing and failing myocardium, respectively.

Correlation between Ca^{++} sensitivity and increase in $t_{80\%}$ and diastolic force. To test whether Ca^{++}-sensitizing effects of EMD and ORG contribute to the increase in relaxation time and diastolic force we related the increase in $t_{80\%}$ and diastolic force to the Ca^{++}-sensitizing effects of EMD and ORG. For EMD 57033 there was a strong correlation between increase in the $[Ca^{++}]_{50\%}$ and increase in $t_{80\%}$ (R^2 = .98). ORG 30029 also demonstrated a strong correlation between the increase in the $[Ca^{++}]_{50\%}$ and the increase in $t_{80\%}$ (R^2 = .69). For EMD 57033 there was also a strong correlation between increase in the $[Ca^{++}]_{50\%}$ and diastolic force (R^2 = .98). ORG 30029 also demonstrated a strong correlation between the increase in the $[Ca^{++}]_{50\%}$ and diastolic force (R^2 =.85).

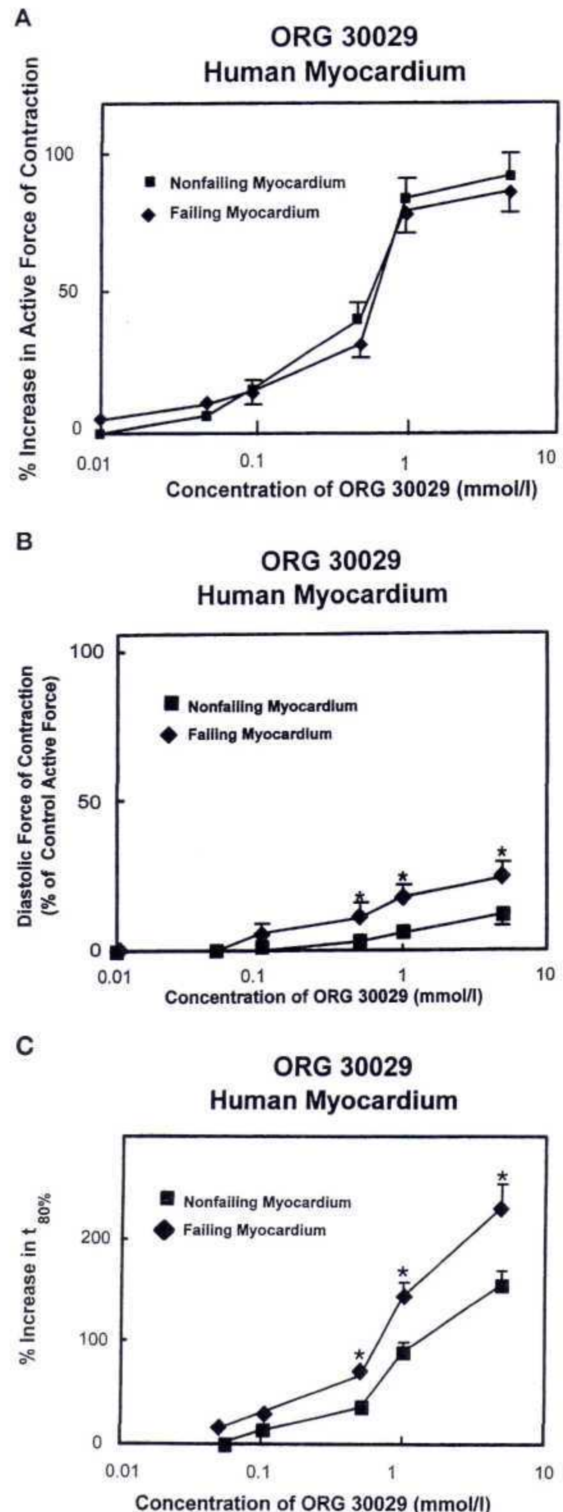


Fig. 4. Effect of increasing concentrations of ORG 30029 on active force (A), diastolic force of contraction (B) and $t_{80\%}$ (C) in failing and nonfailing human myocardium. The muscle preparations were stimulated at 1 Hz with a square pulse of 5 msec at 37°C in Krebs' buffer. * P < .05 compared with nonfailing myocardium.

Discussion

Effects of EMD 57033 and ORG 30029 on force and relaxation. The potential benefit of calcium sensitizers in the treatment of heart failure is that their actions would

TABLE 1
The effects of EMD 57033 and ORG 30029 on $[Ca^{++}]_i$[a]

	Group	Peak $[Ca^{++}]_i$	$L_{80\%}$	Diastolic $[Ca^{++}]_i$
		μmol/l	*msec*	*μmol/l*
Predrug	Nonfailing (n = 4)	1.24 ± 0.18	120 ± 10	0.19 ± 0.04
	Failing (n = 4)	1.46 ± 0.16	168 ± 16†	0.34 ± 0.05†
+50 mmol/l EMD 57033	Nonfailing (n = 4)	1.33 ± 0.15	194 ± 23*	0.40 ± 0.05*
	Failing (n = 4)	1.54 ± 0.20	249 ± 30*†	0.57 ± 0.07*†
+5 mmol/l ORG 30029	Nonfailing (n = 4)	1.80 ± 0.26*	155 ± 20	0.25 ± 0.04
	Failing (n = 4)	1.99 ± 0.23*	201 ± 18*†	0.34 ± 0.05†

[a] $L_{80\%}$, time to 80% relaxation of the $[Ca^{++}]_i$ signal; *P < .01 compared with predrug; †P < .05 compared with nonfailing myocardium; n is number of muscles studied.

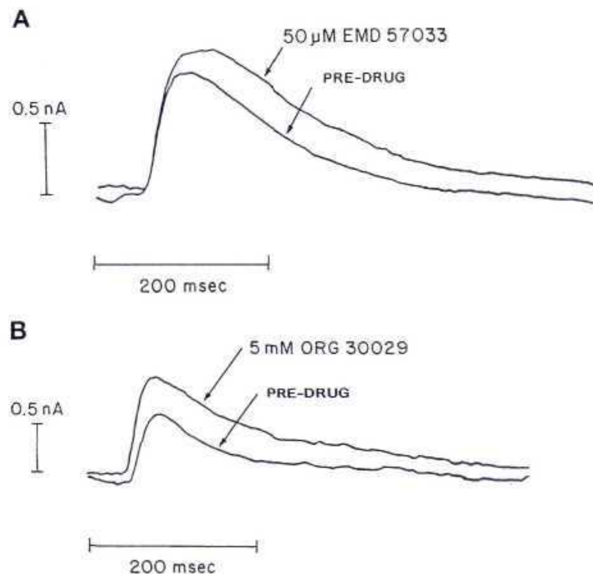


Fig. 5. Effects of 5 and 50 μmol/l EMD 57033 on the light transients in a muscle from a failing heart loaded with aequorin (A). Effect of 5 mmol/l ORG 30029 on the light transient in a muscle from a failing heart loaded with aequorin (B). Both preparations were in Krebs' buffer stimulated at 1 Hz with a square pulse of 5 msec at 37°C.

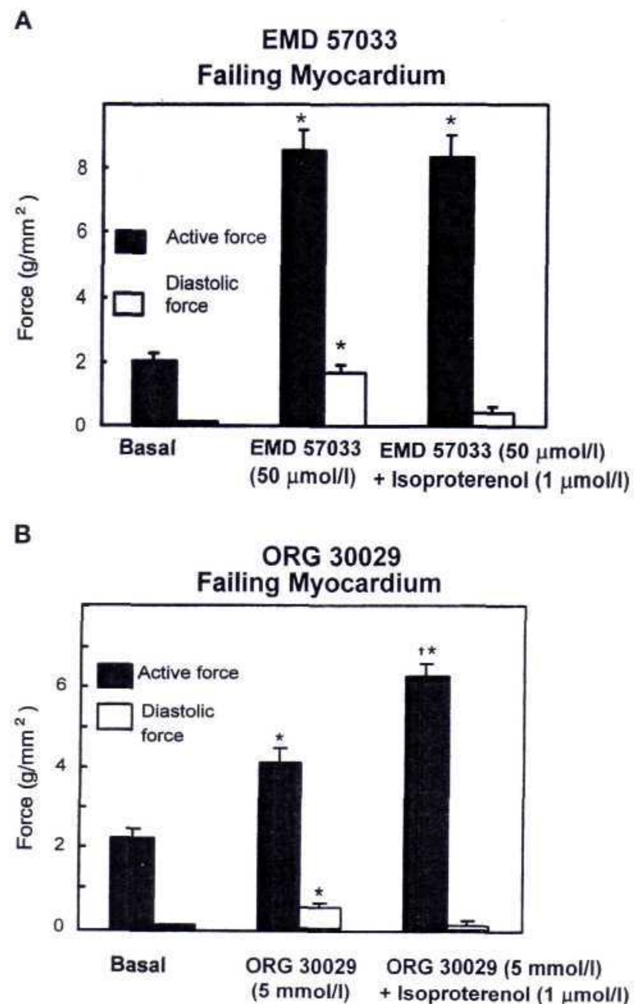


Fig. 6. Effect of EMD 57033 (A) and ORG 30029 (B) on active force and diastolic force in the presence of isoproterenol in intact muscle preparations of failing human myocardium (n = 4). The muscle preparations were stimulated at 1 Hz with a square pulse of 5 msec at 37°C in Krebs' buffer. *P < .05 compared with basal levels; †P < .05 compared with ORG 30029 (5 mmol/l).

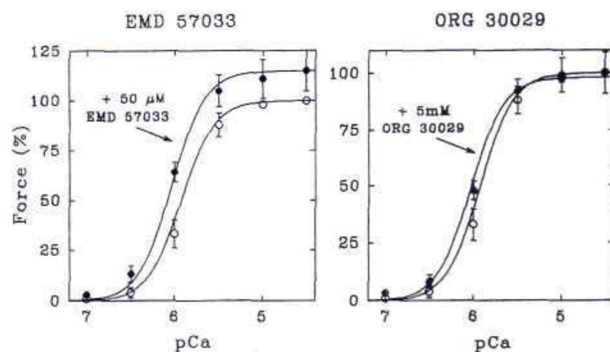
bypass defects at the level of receptor-coupled membranes in failing myocytes and target the myofilaments directly to enhance the force of contraction while having little effect on energy utilization. However, several studies have shown that agents targeted at the myofilaments may have differential effects in nonfailing *versus* failing myocardium (Hajjar *et al.*, 1988; Baudet and Ventura-Clapier, 1990; Gwathmey and Hajjar, 1992). These included DPI 201–106, which demonstrated a greater calcium sensitization in failing human myocardium (Hajjar *et al.*, 1988), and caffeine, which also demonstrated a greater calcium sensitization in pressure-overloaded hypertrophied ferret hearts (Baudet and Ventura-Clapier, 1990) and myopathic turkey hearts (Gwathmey and Hajjar, 1992).

In the present study, we found that the positive inotropic effects of the Ca^{++} sensitizers EMD 57033 and ORG 30029 do not differ between nonfailing and failing myocardium. In our skinned fiber preparations there was no evidence of differential effects of EMD 57033 and ORG 30029 on myofilament calcium sensitivity in nonfailing *versus* failing human myocardium (table 2). EMD did not alter peak $[Ca^{++}]_i$ in nonfailing or failing human myocardium, whereas ORG increased peak $[Ca^{++}]_i$ to the same extent in both nonfailing and failing human myocardium.

Although the potencies of EMD 57033 and ORG 30029 differed in human myocardium, the efficacy of both EMD 57033 and ORG 30029 to increase force of contraction was similar in nonfailing and failing myocardium. This resulted in a similar enhancement of the peak force of contraction in nonfailing and failing myocardium in the presence of both agents. These results are in accordance with those of Nankervis *et al.* (1994) who found similar increases in the presence of EMD 57033 in atrial muscles from normal and diseased human hearts.

A

Effects of EMD 57033 and ORG 30029 on Skinned Fibers in Human Nonfailing Myocardium



B

Effects of EMD 57033 and ORG 30029 on Skinned Fibers in Human Failing Myocardium

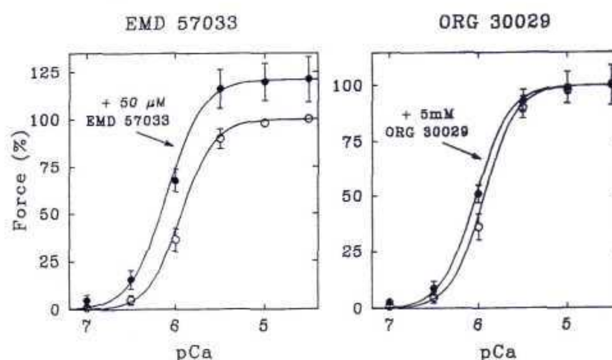


Fig. 7. *Effect of ORG 30029 and EMD 57033 on the* $[Ca^{++}]$-*force relationship in skinned fibers preparations of human nonfailing (A) and failing (B) myocardium. Each* Ca^{++}*-activation experiment (with or without drugs) was fitted individually to the Hill equation:* $F/F_{max} = [Ca^{++}]^n / [Ca^{++}]^n + [Ca^{++}]_{50\%}{}^n$; *the Hill parameters* F_{max}, n *and* $[Ca^{++}]_{50\%}$ *were then obtained by nonlinear regression analysis for that one individual experiment. The Hill parameters were then averaged for the different groups of experiments (i.e., no drug, +EMD 57033, +ORG 30029), yielding a mean ± S.E.M. for the Hill parameters of each group. The curves depicted in this figure were derived by inputting the mean of the Hill parameters from each group into the Hill equation. The data points represent the mean force generated at that pCa in each individual group.*

EMD 57033 and ORG 30029, however, increased diastolic force and prolonged relaxation in failing myocardium to a greater extent than in nonfailing myocardium. These results are explained by the findings that failing myocardium is characterized by elevated diastolic $[Ca^{++}]_i$ and abnormal $[Ca^{++}]_i$ sequestration into the SR during relaxation (Gwathmey *et al.*, 1987, 1988, 1990; Gwathmey and Hajjar, 1990; Beuckelmann *et al.*, 1992; Arai *et al.*, 1993; Hasenfuss *et al.*, 1994; O'Brien and Gwathmey, 1995). The increase in diastolic force of contraction and the prolongation of relaxation was greater in the presence of EMD 57033 than in the presence of ORG 30029 as demonstrated in figure 7, A and B. This was accompanied by a greater shift in myofilament calcium sensitivity by EMD 57033 than by ORG 30029. In addition a stronger correlation existed between the increase in the $[Ca^{++}]_{50\%}$ and increase in $t_{80\%}$ or diastolic force for EMD than ORG ($t_{80\%}$ R^2 = .98 *vs.* 0.69; diastolic force R^2 = .98 *vs.* 0.85).

ORG 30029, in addition to its calcium-sensitizing activity

TABLE 2

The effects of EMD 57033 and ORG 30029 on the Hill parameters of force-$[Ca^{++}]$ relationships in failing and nonfailing muscles[a]

	$[Ca^{++}]_{50\%}$	n	F_{max}
	μmol/l		*%*
Failing (6)	1.16 ± 0.06	2.39 ± 0.32	100
+50 μmol/l EMD 57033	0.80 ± 0.07*	2.16 ± 0.44	121 ± 12*
+5 mmol/l ORG 30029	0.94 ± 0.05*	2.28 ± 0.31	100 ± 9
Nonfailing (4)	1.19 ± 0.08	2.31 ± 0.25	100
+50 μmol/EMD 57033	0.89 ± 0.09†	2.28 ± 0.22	115 ± 10†
+5 mmol/l ORG 30029	1.00 ± 0.07†	2.41 ± 0.30	98 ± 7

[a] n is the Hill parameter; $[Ca^{++}]_{50\%}$ is the $[Ca^{++}]$ required for 50% activation; F_{max} is the maximal Ca^{++}-activated force; and the number in parentheses is the number of muscles studied. *$P < .05$ compared with failing myocardium; †$P < .05$ compared with nonfailing myocardium.

has PDE inhibitory effects (Sahid and Nicholson, 1990; Kawabata and Endoh, 1993). PDE inhibition results in an increase in cAMP which in turn phosphorylates both phospholamban and troponin I. Phosphorylation of phospholamban increases SR Ca^{++} uptake, whereas phosphorylation of troponin I leads to a decrease in the affinity of TnC for Ca^{++}, resulting in a faster dissociation of Ca^{++} from the myofilaments. Both effects would tend to enhance relaxation rate. Nevertheless, both substances (ORG and EMD) increased diastolic force and prolonged relaxation, which reflected a strong calcium-sensitizing effect by both agents, although ORG's effects on diastolic force and twitch time course were less when compared with EMD's effects on these parameters. Even though ORG 30029 has PDE inhibitory effects, the twitch was markedly prolonged in the presence of this agent, which reflected a greater effect of myofilament calcium sensitization as opposed to the effect of cAMP in the cell.

In myopathic cells, the higher level of diastolic $[Ca^{++}]_i$ and slowed Ca^{++} mobilization further contributed to the increase in diastolic force in the presence of these two calcium-sensitizing agents. Furthermore, EMD increased diastolic $[Ca^{++}]_i$ in both nonfailing and failing myocardium further contributing to the increase in diastolic force and slowed relaxation. It is also noteworthy that even though ORG increased Ca^{++} release (whereas EMD did not), its effects on force potentiation was only one third of the effects of EMD. This would suggest that myofilament calcium sensitization may be a more effective mode of enhancing force production than increasing peak $[Ca^{++}]_i$ (*i.e.*, EMD 57033).

The relaxation phase of the $[Ca^{++}]_i$ transient is controlled mainly by two events in the cardiac myocyte: 1) the rate of Ca^{++} release from the myofilaments and 2) the rate of Ca^{++} uptake into the SR and extrusion of calcium *via* the Na^+/Ca^{++} exchanger (Moss, 1992; Gwathmey and Hajjar, 1991). Calcium sensitizers should slow the rate of Ca^{++} release from the myofilaments (Brenner, 1988). As observed in our preparations, the Ca^{++} transient was prolonged in the presence of both ORG 30029 and EMD 57033. Because Ca^{++} release from the myofilaments is delayed, cross-bridges spend more time in the attached state, prolonging the relaxation of the force and enhancing force generation. In failing myocardium, cross-bridge cycling rate is decreased (Hajjar and Gwathmey, 1992); therefore, in the presence of Ca^{++} sensitizers, the slower time course of myocardial relaxation is further accentuated. ORG's phosphodiesterase inhibitory activity would tend to offset this effect by increasing the rate of SR Ca^{++} uptake (through the phosphorylation of phospho-

lamban). For this reason the Ca^{++} transient was less prolonged in the presence of ORG than EMD. ORG did not further increase diastolic $[Ca^{++}]_i$ in human myocardium as opposed to EMD, which increased diastolic $[Ca^{++}]_i$.

The effect of isoproterenol on preventing the prolongation of the time to relaxation by ORG has also been described in studies on isolated canine ventricular trabeculae (Kawabata and Endoh, 1993). In this study, isoproterenol was shown to inhibit the increase of diastolic force caused by ORG 30029 and EMD 57033. These effects of isoproterenol may be caused by its ability to increase the activity of the SR Ca^{++} pump *via* the cAMP-dependent phoshorylation of phospholamban. This would lead to a rapid sequestration of Ca^{++} during relaxation, even though a larger amount of calcium is entering the cell with every beat, through the phosphorylation of sarcolemmal calcium channels. The increase in cAMP, in the presence of isoproterenol, would also affect the myofilaments. cAMP-dependent phosphorylation of troponin I would reduce the sensitivity of the myofilaments to Ca^{++}, thereby countering the effects of the Ca^{++} sensitizer. This would allow Ca^{++} to be released from the myofilaments more quickly, allowing a more rapid relaxation. However, any increase in cAMP would increase energy consumption thereby worsening the energy state in the failing heart. Our results showed that ORG 30029 significantly increased peak $[Ca^{++}]_i$, most likely *via* cAMP-dependent phosphorylation of Ca^{++} channels thereby increasing Ca^{++} entry with each depolarization. An increase in intracellular Ca^{++} would augment the energy requirement to mobilize the additional Ca^{++} and would therefore worsen the energy balance in a failing heart. De Tombe and Hunter (1992) found that EMD 53998, which is a Ca^{++} sensitizer with strong PDE inhibitory effects, is not energy sparing in isolated left ventricles. This may be because of the fact that an increase in cAMP would offset any energy-sparing properties of Ca^{++} sensitization. Recently, Grandis *et al.* (1995) showed that the presence of EMD 57033 (at concentrations that did not inhibit PDE) in isolated rat hearts did not change significantly myocardial oxygen consumption and did not alter the phosphate metabolite concentrations as detected by ^{31}P nuclear magnetic resonance.

Mechanisms of action of ORG 30029 and EMD 57033. Ca^{++} sensitizers act directly on the myofilaments to exert their inotropic actions. There is little known about the mechanism of action of ORG 30029 on the myofilaments; however, the thiadiazones have been studied extensively (Solaro *et al.*, 1993; Grandis *et al.*, 1995; Pan and Johnson, 1996). It has been suggested by Solaro *et al.* (1993) that EMD 57033's action may be directed at a site on the actin-myosin interface which would promote the interaction of myosin with the thin filaments increasing myofibrillar ATPase rate. Furthermore, Solaro *et al.* (1993) found that actin sliding velocity was increased in the presence of EMD 57033 with a motility assay. Increasing cross-bridge rate would, however, decrease the apparent Ca^{++} sensitivity of the myofilaments (Brenner, 1988) and would also enhance relaxation, effects that are opposite to the ones observed with EMD 57033. It is unclear whether results from the motility assay, which is not regulated by troponin-tropomyosin, can be related to regulated isometrically contracting muscles (Solaro *et al.*, 1993). More recently, with recombinant human TnC, Pan and Johnson (1996) found that EMD 57033 binds to the Ca^{++}/Mg^{++} sites of TnC in a Ca^{++}-dependent and stereo-selective manner. Furthermore, Leijendekker and Herzig (1992) found that a compound related to EMD 57033 (EMD 53998) actually decreases the rate of actin-cross-bridge reaction in isolated fibers, and Strauss *et al.* (1992) found that the same compound (EMD 53998) antagonizes phosphate action on cross-bridges. From our present studies, it is not possible to deduce whether EMD 57033 changes cross-bridge kinetics. However, because failing hearts have decreased energy reserve, Ca^{++}-sensitizing agents that increase cross-bridge kinetics would increase energy consumption and would therefore further impair the energy balance in these hearts (Ingwall *et al.*, 1993; Gwathmey and Ingwall, 1995).

Limitations of the study. In our studies we used aequorin to measure intracellular $[Ca^{++}]_i$. As a calcium indicator, aequorin is characterized by a high sensitivity, a high signal-to-noise ratio, a fast response time and no Ca^{++}-buffering action (Blinks, 1982). However, aequorin has a nonlinear $[Ca^{++}]$ *versus* light relationship with the curve flattening at low $[Ca^{++}]$ in the range of 100 to 150 nmol/l. In our preparations, diastolic $[Ca^{++}]_i$ was measured to be 100 to 300 nmol/l, which is well within the linear range of the aequorin calibration curve and agrees well with reports using microelectrodes and other calcium indicators (Blinks *et al.*, 1982). Furthermore this range of diastolic Ca^{++} (200–300 nmol/l) is in good agreement with measurements of diastolic Ca^{++} in human myocardium with the fluorescent indicator, Fura-2 (Beuckelmann *et al.*, 1992).

In this study, we used nonfailing human myocardial tissue explanted from brain-dead subjects without antecedent heart failure as controls. There are significant limitations with the use of such tissues as true controls. Brain-dead patients often require treatment with large doses of inotropic drugs which are known to have profound effects on cardiac performance. Head trauma patients also have large surges of catecholamines which have been shown to be deleterious to myocardial function (Mann *et al.*, 1992). In our studies, nonfailing myocardial strips did not exhibit characteristic features of myopathic preparations such as prolongation of the time of contraction or $[Ca^{++}]_i$ transient, elevated diastolic $[Ca^{++}]_i$ or abnormal force-frequency relationship.

Conclusion. Taken together, the data presented herein provide evidence that Ca^{++} sensitizers have the potential to increase the force of contraction to the same extent in nonfailing and failing myocardium. However, the benefit in treatment of heart failure may be limited by a worsening of diastolic relaxation and increased diastolic force in the presence of such agents. A combination of agents that increase cAMP and sensitize the myofilaments to Ca^{++} may be helpful in the therapy of heart failure because of their ability to increase the force of contraction and to prevent an increase in diastolic $[Ca^{++}]_i$ and force.

Acknowledgments

The authors acknowledge National Disease Research Interchange for assisting in making these studies possible.

References

ARAI, M., ALPERT, N. R., MACLENNAN, D. H., BARTON, P. AND PERIASAMY, M.: Alterations in sarcoplasmic reticulum gene expression in human heart failure. A possible mechanism for alterations in systolic and diastolic properties of the failing myocardium. Circ. Res. **72:** 463–469, 1993.

BAUDET, S. AND VENTURA-CLAPIER, R.: Differential effects of caffeine on skinned fibers from control and hypertrophied ferret hearts. Am. J. Physiol. **259:** H1803–H1808, 1990.

BEUCKELMANN, D. J., NÄBAUER, M. AND ERDMANN, E.: Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation **85:** 1046–1055, 1992.

BLINKS, J. R.: Methods for monitoring calcium concentrations with photoproteins in living cardiac cells. *In* Methods for Studying Heart Membranes, ed. by N. S. Dhalla, vol. 2, pp. 237–264, CRC Press, Boca Raton, FL, 1984.

BLINKS, J. R., WIER, W. G., HESS, P. AND PRENDERGAST, F. G.: Measurement of Ca^{2+} concentrations in living cells. Prog. Biophys. Mol. Biol. **40:** 1–114, 1982.

BÖHM, M., GIERSCHIK, P., JAKOBS, K. H., PIESKE, B., SCHNABEL, P., UNGERER, M. AND ERDMANN, E.: Increase of Giα in human hearts with dilated but not ischemic cardiomyopathy. Circulation **82:** 189–197, 1990.

BRENNER, B.: Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: Implications for regulation of muscle contraction. Proc. Natl. Acad. Sci. U.S.A. **85:** 3265–3269, 1988.

BRISTOW, M. R., GINSBURG, R., MINOBE, W., CUBICOTTI, R. S., SAGEMAN, W. S., LURIE, K., BILLINGHAM, M. E., HARRISON, D. C. AND STINSON, E. B.: Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human heart. N. Engl. J. Med. **307:** 205–211, 1982.

DANIELSEN, W., VAN DER LEYDEN, H., MEYER, W., NEUMANN, J., SHMITZ, W., SCHOLZ, H., STARBATTY, J., STEIN, B., DÖRING, V. AND KALMAR, P.: Basal and isoprealine-stimulated cAMP content in failing *versus* nonfailing human cardiac preparation. J. Cardiovasc. Pharmacol. **14:** 171–173, 1989.

DE TOMBE, P. AND HUNTER, W. C.: Effect of EMD on myocardial oxygen consumption. Circulation **86:** 789–799, 1992.

FABIATO, A.: Computer programs for calculating total or free ions from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods Enzymol. **157:** 378–391, 1988.

FELDMAN, A. M., CATES, A. E., VEAZEY, W. B., HERSBERGER, R. E., BRISTOW, M. R., BAUGHMAN, K. L., BAUMGARTNER, W. A. AND VAN DROP, C.: Increase of the 40000-mol wt pertussis toxin substrate (G-protein) in the failing human heart. J. Clin. Invest. **82:** 189–197, 1988.

FELDMAN, M. D., COPELAS, L., GWATHMEY, J. K., PHILLIPS, P. J., SCHOEN, F. J., GROSSMAN, W. AND MORGAN, J. P.: Deficient production of cyclic AMP: Pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation **75:** 331–339, 1987.

FERRONI, C., HANO, O., VENTURA, C., LAKATTA, E. G., KLOCKOW, M., SPURGEON, H. AND CAPOGROSSI, M. C.: A novel positive inotropic substance enhances contractility without increasing the Ca^{2+} transient in rat myocardium. J. Mol. Cell. Cardiol. **23:** 325–331, 1991.

FUJINO, K., SPERELAKIS, N. AND SOLARO, R. J.: Sensitization of dog and guinea pig cardiac myofilaments to Ca^{2+}-activation and the inotropic effect of pimebondan: comparison with milrinone. Circ. Res. **63:** 911–922, 1988.

GAMBASSI, G., CAPAGROSSI, M. C., KLOCKOW, M., LUES, I. AND WOLF, G.: Enantiomeric dissection of the effects of the inotropic agent, EMD 53998, in single cardiac myocytes. Am. J. Physiol. **264:** H728–H738, 1993.

GRANDIS, D. J., DELNIDO, P. J. AND KORETSKY, A. P.: Functional and energetic effects of the inotropic agents EMD-57033 and BAPTA on the isolated rat heart. Am. J. Physiol. **269:** C472–C479, 1995.

GWATHMEY, J. K. AND HAJJAR, R. J.: Relation between steady state force and intracellular $[Ca^{2+}]$ in intact human myocardium. Index of myofibrillar responsiveness to Ca^{2+}. Circulation **82:** 1266–1276, 1990.

GWATHMEY, J. K. AND HAJJAR, R. J.: Intracellular calcium related to force development in twitch contraction of mammalian myocardium. Cell Calcium **11:** 531–538, 1991.

GWATHMEY, J. K. AND HAJJAR, R. J.: Calcium-activated force in a turkey model of spontaneous dilated cardiomyopathy: Adaptive changes in thin myofilament Ca^{2+} regulation with resultant implications on contractile performance. J. Mol. Cell. Cardiol. **24:** 1459–1470, 1992.

GWATHMEY, J. K. AND INGWALL, J. S.: Basic pathobiology of congestive heart failure. Cardiol. Rev. **3:** 282–291, 1995.

GWATHMEY, J. K., COPELAS, L., MAC KINNON, R., SCHOEN, F. J., FELDMAN, M. D., GROSSMAN, W. AND MORGAN, J. P.: Abnormal intracellular calcium handling in myocardium from patient with endstage heart failure. Circ. Res. **61:** 70–76, 1987.

GWATHMEY, J. K., SLAWSKY, M. T., HAJJAR, R. J., BRIGGS, G. M. AND MORGAN, J. P.: Role of intracellular calcium handling in force-interval relationship of human ventricular myocardium. J. Clin. Invest. **85:** 1599–1613, 1990.

GWATHMEY, J. K., SLAWSKY, M. T., BRIGGS, G. M. AND MORGAN, J. P.: Role of intracellular sodium in the regulation of intracellular calcium and contractility. Effect of DPI 201–106 on excitation-contraction coupling in human ventricular myocardium. J. Clin. Invest **82:** 1592–1605, 1988.

HAJJAR, R. J. AND GWATHMEY, J. K.: Calcium-sensitizing inotropic agents in the treatment of heart failure: A critical view. Cardiovasc. Drugs Ther. **5:** 961–966, 1991.

HAJJAR, R. J. AND GWATHMEY, J. K.: Cross-bridge dynamics in human ventricular myocardium. Regulation of contractility in the failing heart. Circulation **86:** 1819–1826, 1992.

HAJJAR, R. J., GWATHMEY, J. K., BRIGGS, G. M. AND MORGAN, J. P.: Differential effect of DPI 201-106 on the sensitivity of the myofilaments to Ca^{2+} in intact and skinned trabeculae from control and myopathic human hearts. J. Clin. Invest. **82:** 1578–1584, 1988.

HARRISON, S. M. AND BERS, D. M.: Influence of temperature on the calcium sensitivity of the myofilaments of skinned ventricular muscle from the rabbit. J. Gen. Physiol. **93:** 411–28, 1989.

HASENFUSS, G., REINECKE, H., STUDER, R., MEYER, M., PIESKE, B., HOLTZ, J., HOLUBARSCH, C., POSIVAL, H., JUST, H. AND DREXLER, H.: Relation between myocardial function and expression of sarcoplasmic reticulum Ca^{2+}-ATPase in failing and nonfailing myocardium. Circ. Res. **75:** 434–442, 1994.

HGASHIYAMA, A., WATKINS, M. W., CHEN, Z. AND LEWINTER, M. M.: Effects of EMD 57033 on contraction and relaxation in isolated rabbit hearts. Circulation **92:** 3094–3104, 1995.

HONERJÄGER, P., KLOCKOW, M., SCHÖNSTEINER, G. AND JONAS, R.: Imidazopyridines: roles of pyridine nitrogen position and methyhlsulfinyloxygen for *in vitro* positive inotropic mechanism and chronotropic activity. J. Cardiovasc. Pharmacol. **13:** 673–681, 1989.

INGWALL, J. S., NASCIMBEN, L. AND GWATHMEY, J. K.: Heart failure: Is the pathology due to calcium overload or to mismatch in energy supply and demand. *In* Heart Failure: Basic Research and Clinical Aspects, ed. by J. K. Gwathmey, G. M. Briggs and P. D. Allen, pp. 566–589, Marcel Dekker, New York, 1993.

KAWABATA, Y. AND ENDOH, M.: Effects of the positive inotropic agent Org 30029 on developed force and aequorin light transients in intact canine ventricular myocardium. Circ. Res. **72:** 597–606, 1993.

LEE, J. A. AND ALLEN, D. G.: EMD 53998 sensitizes the contactile proteins to calcium in intact ferret ventricular muscle. Circ. Res. **69:** 927–936, 1991.

LEIJENDEKKER, W. J. AND HERZIG, J. W.: Reduction of myocardial cross-bridge turnover rate in presence of EMD 53998, a novel Ca^{2+}-sensitizing agent. Eur. J. Physiol. **421:** 387–389, 1992.

LUES, I., BEIER, N., JONAS, R., KLOCKOW, M. AND HÄUSLER, G.: The two mechanisms of action of racemic cardiotonic EMD 53998, calcium sensitization and phosphodiesterase inhibition, reside in different enantiomers. J. Cardiovasc. Pharmacol. **21:** 883–892, 1993.

MANN, D. L., KENT, R. L., PARSONS, B. AND COOPER G.: Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation **85:** 790–804, 1992.

MOIESCU, D. G. AND THIELECZEK, R.: Calcium and strontium concentration changes with skinned muscle preparations following a change in the external bathing solution. J. Physiol. (Lond.) **275:** 241–262, 1978.

MOSS, R. L.: Ca^{2+} regulation of mechanical properties of striated muscle: mechanistic studies using extraction and replacement of regulatory proteins. Circ. Res. **70:** 865–884, 1992.

NANKERVIS, R., LUES, I. AND BROWN, L.: Calcium sensitization as a positive inotropic mechanism in diseased rat and human heart. J. Cardiovasc. Res. **24:** 612–617, 1994.

NEUMANN, J., BOKNIK, P., SCHMITZ, W., SCHOLZ, H. AND ZIMMERMANN, N.: Comparison of the stereoselective effects of a thidiazinone derivative on contractile parameters and protein phosphorylation in the mammalian ventricle. J. Cardiovasc. Pharmacol. **25:** 789–793, 1995.

O'BRIEN, P. J. AND GWATHMEY, J. K.: Myocardial Ca^{2+}- and ATP-cycling imbalances in end-stage dilated and ischemic cardiomyopathies. Cardiovasc. Res. **30:** 394–404, 1995.

PACKER, M., CARVER, J. R., RODEHEFFER, R. J., IVANHOE, R. J., DIBIANCO, R., ZELDIS, S. M., HENDRIX, G. H., BOMMER, W. J., ELKAYAM, U., KUKIN, M. L., MALLIS, G. I., SOLLANO, J. A., SHANNON, J., TANDON, P. K. AND DEMETS, D. L.: Effect of oral milrinone on mortality in severe chronic heart failure. N. Engl. J. Med. **325:** 1468–1475, 1991.

PAN, B.-S. AND JOHNSON, R. G.: Interaction of cardiotonic thiadiazinone derivatives with cardiac troponin C. J. Biol. Chem. **271:** 817–823, 1996.

PESATURO, J. AND GWATHMEY, J. K.: The role of mitochondria and sarcoplasmic reticulum calcium handling upon reoxygenation in mammalian myocardium. Circ. Res. **66:** 696–709, 1990.

SAHID, M. AND NICHOLSON, C. D.: Comparison of cyclic nucleotide phosphodiesterase isoenzymes in rat and rabbit ventricular myocardium: positive inotropic and phosphodiesterase inhibitory effects of ORG 30029, milrinone and rolipram. Naunyn-Schmiedebergs Arch. Pharmacol. **342:** 698–705, 1990.

SOLARO, R. J., GAMBASSI, G., WARSHAW, D. M., KELLER, M. R., SPURGEON, H. A., BEIER, N. AND LAKATTA, E. G.: Stereoselective actions of thiadiazones on canine cardiac myocytes and myofilaments. Circ. Res. **73:** 981–990, 1993.

STRAUSS, J. D., ZEUGNER, C. AND RUEGG, J. C.: The positive inotropic calcium sensitizer EMD 53998 antagonizes phosphate action on cross-bridges in cardiac skinned fibers. Eur. J. Pharmacol. **227:** 437–441, 1992.

V. MEEL, J. C. A., ZIMMERMANN, R., DIDEREN, W., ERDMANN, E. AND MERWA, U.: Increase in calcium sensitivity of cardiac myofibrils contributes to the cardiotonic action of sulmazole. Biochem. Pharmacol. **37:** 213–220, 1988.

VENTURA, C., MILLER, R., WOLF, H.-P., BEIER, N., JONAS, R., KLOCKOW, M., LUES, I., HANO, O., SPURGEON, H. A., LAKATTA, E. G. AND CAPAGROSSI, M. C.: Novel diazinone derivatives separate myofilament Ca^{2+} sensitization and phosphodiesterase III inhibitory effects in guinae pig myocardium. Circ. Res. **70:** 1081–1090, 1992.

Send reprint requests to: Dr. J. K. Gwathmey, Cardiovascular Diseases & Muscle Research Laboratories, Harvard Medical School, Bldg B1, Room 146, 220 Longwood Avenue, Boston, MA 02115.