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A common mechanism for concurrent changes of diastolic muscle length and systolic function in intact hearts

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Abstract

Mechanical properties of the myocardium at end diastole have been thought to be dominated by passive material properties rather than by active sarcomere cross-bridge interactions. This study tested the hypothesis that residual cross-bridges significantly contribute to end-diastolic mechanics in vivo and that changes in end-diastolic cross-bridge interaction parallel concurrent changes in systolic cross-bridge interaction. Open-chest anesthetized pigs were treated with intracoronary verapamil ($n = 7$) or 2,3-butanedione monoxime (BDM; $n = 8$). Regional left ventricular external work and end-diastolic pressure (EDP) versus end-diastolic segment length (EDL) relations were determined in the treated and untreated regions of each heart. Both agents reduced external work of treated regions to 31–38% of baseline and concurrently shifted EDP versus EDL relations to the right (i.e., greater EDL at a given EDP) by an average of 5% ($P < 0.05$). During washout of the drugs, EDP versus EDL returned to baseline in parallel with recovery of external work. Sarcomere length, measured by transmission electron microscopy in BDM-treated and untreated regions of the same hearts after diastolic arrest and perfusion fixation, was 8% greater in BDM-treated regions ($P < 0.01$). We concluded that residual diastolic cross-bridges significantly and reversibly influence end-diastolic mechanics in vivo. Alterations of end-diastolic and systolic cross-bridge interactions occur in parallel.

Keywords

diastole; ventricular function; calcium channel blockers; di-acetyl analogs and derivatives; sarcomeres

Cross-bridge cycling triggered by the presence of cytosolic calcium is the basis for force generation in muscle. In systole, the cross-bridge cycling rate and force generation increase in relation to the magnitude of the cytosolic calcium transient and the sensitivity of myofibrillar regulatory proteins to calcium (8). In diastole, there is an active reuptake of calcium into the sarcoplasmic reticulum; however, a low level of calcium-activated cross-bridge cycling and active tension development may persist at end diastole (17). Thus, whereas end-diastolic ventricular pressure-dimension relations are generally considered to reflect the underlying passive material properties of cardiac muscle (13), it is possible that changes in calcium availability or myofilament calcium sensitivity influence these relations. This concept is supported by a study (5) in isolated spontaneously beating cardiac myocytes demonstrating that cell length continues to increase throughout diastole. It is also supported by studies (12, 19) in isolated resting cardiac myocytes demonstrating that cell length increases when intracellular calcium concentration is reduced or when myofilament calcium

sensitivity is reduced by 2,3-butanedione monoxime (BDM). Furthermore, studies in isolated nonworking hearts demonstrate that treatment with a calcium channel antagonist (17) or BDM (20) produces a rightward shift in the left ventricular (LV) end-diastolic pressure (EDP)-volume relation. By analogy, if both systolic and end-diastolic calcium availability or myofilament calcium sensitivity are reduced in vivo, then coordinate changes in systolic function and end-diastolic muscle length would be predicted.

Recent data from our laboratory support such a prediction. In a porcine model of acute myocardial ischemia and reperfusion resulting in systolic dysfunction (stunning), we observed increased end-diastolic segment length (EDL) at low EDP in vivo and increased diastolic sarcomere length after in situ perfusion fixation at low LV cavity pressure (15). These increases in end-diastolic muscle length and diastolic sarcomere length occurred in the absence of ultrastructural signs of myocyte injury or matrix damage (16), raising the possibility that the increased lengths had a functional basis related to changes in residual end-diastolic cross-bridge interactions rather than a structural basis.

The present study tests the hypothesis that residual end-diastolic cross-bridge interactions significantly contribute to end-diastolic mechanics of the LV in vivo and that changes in end-diastolic and systolic cross-bridge interactions occur in parallel. To test this hypothesis, end-diastolic mechanics and systolic function were examined during regional intracoronary infusion of verapamil or BDM to reduce myofilament calcium availability or sensitivity, respectively.

METHODS

Experimental preparation

Fifteen Yorkshire-Landrace pigs weighing 25–34 kg were studied. *Group 1* ($n = 7$) was treated with a local (intracoronary) infusion of verapamil. *Group 2* ($n = 8$) was treated with an intracoronary infusion of BDM.

Anesthetic technique and surgical instrumentation have been described previously (15, 16). Pigs were anesthetized with α -chloralose (100 mg/kg iv, induction; 30–40 mg·kg⁻¹·h⁻¹ iv, maintenance). Atropine (0.2 mg/kg iv) and propranolol (1 mg/kg iv) were given to produce autonomic blockade. A 7-Fr introducer sheath was inserted in a carotid artery and used to measure central aortic pressure. Through the sheath, a 5-Fr solid-state micromanometer catheter was advanced retrograde across the aortic valve to measure LV pressure. Pairs of piezoelectric crystals were inserted in the subendocardium of the anterior and posterior free walls of the LV to measure regional LV segment length by sonomicrometry (Triton; San Diego, CA). Crystal pairs were inserted ~ 1 cm apart parallel to the presumed direction of local fiber orientation (4). The proximal portion of the left anterior descending coronary artery (LAD) was instrumented with a transit-time ultrasonic flow probe (Transonic Systems; Ithaca, NY). Hydraulic occluders were placed around the venae cavae to produce dynamic alteration of preload. A 0.025-in. diameter polyethylene catheter was inserted in the LAD proximal to the flow probe to infuse verapamil or BDM.

Assessment of regional LV function

Aortic and LV pressure, segment length in anterior and posterior LV regions, and LAD coronary blood flow signals were digitized at 200 Hz and analyzed using customized software as previously described (15). During brief suspension of mechanical ventilation, LV pressure versus segment length loops in both anterior and posterior LV regions were analyzed under steady-state conditions and during brief occlusion of the venae cavae. Under steady-state conditions, the area of LV pressure versus segment length loops was used as an index of regional external work. During brief occlusion of the venae cavae, LV EDP versus

EDL relations were derived. End diastole was identified from the initial upstroke of the intramyocardial electrogram recorded using the electrocardiogram (ECG) capability of the sonomicrometer. Data recorded during brief occlusion of the venae cavae were also used to derive regional Frank-Starling relations by plotting the area of consecutive pressure-segment length loops against the corresponding EDL. Under each experimental condition in each pig, data recorded during five or six occlusions of the venae cavae were pooled to derive EDP versus EDL and regional Frank-Starling relations. The slope and intercept of the Frank-Starling relations were determined by linear regression. Because the absolute values of EDL, regional external work, and slope and intercept of regional Frank-Starling relations depend in part on the distance between crystal pairs at implantation (i.e., a source of experimental rather than physiological variability), these variables are expressed as fractions of baseline.

Experimental protocol for group 1

Verapamil, an L-type calcium channel antagonist, was dissolved in normal saline solution to a concentration of 15 $\mu\text{g/ml}$. After baseline measurements of hemodynamics, regional LV function, and EDP versus EDL relations, verapamil was infused into the proximal LAD at a rate of 0.9 ml/min (13 $\mu\text{g/min}$). This dose was selected on the basis of pilot data, indicating that it produces a reduction of regional external work to approximately one-third of baseline. After ~45 min of verapamil treatment, a stable reduction of regional contractile function was achieved. Between 45 and 90 min of treatment, measurements of systemic hemodynamics, regional LV external work, and EDP versus EDL relations were repeated. After 90 min of treatment, the infusion of verapamil was discontinued. The reversibility of verapamil-induced changes in regional systolic function and EDP versus EDL relations was assessed by measuring regional LV systolic function and EDP versus EDL relations at 0, 30, 60, and 90 min of drug washout. To correlate the recovery of regional LV systolic and diastolic function during verapamil washout with the decline in tissue verapamil concentration, transmural drill biopsies of the treated region were obtained at 0, 30, 60, and 90 min of washout in five pigs. Verapamil concentration in tissue extracts was determined by gas chromatography. Controls were run by adding known quantities of verapamil to blanks or to tissue extracts from untreated hearts. The accuracy of the assay ranged from ± 6 to $\pm 17\%$ over the range of verapamil concentrations examined.

Experimental protocol for group 2

BDM was dissolved in phosphate-buffered normal saline solution at a concentration of 40 mg/ml. After baseline measurements, BDM solution was infused into the proximal LAD at a rate of 2 ml/min (80 mg/min). At any intracellular calcium concentration, BDM reduces cross-bridge cycling in a dose-dependent manner by inhibition of myosin ATPase (2, 10). The dose of BDM employed in the present experiments was chosen on the basis of pilot data indicating a reduction of regional external work to approximately one-third of baseline, similar to the effect of verapamil in *group 1*. After 45 min of BDM treatment, a stable reduction of regional contractile function was achieved. Between 45 and 90 min of treatment, measurements of systemic hemodynamics, regional LV external work, and EDP versus EDL relations were repeated. In two hearts in *group 2*, the reversibility of BDM-induced changes in EDP versus EDL relations was examined during 90 min of wash-out of the drug, similar to the measurements made during verapamil washout in *group 1*. The other six hearts in *group 2* underwent diastolic arrest and in situ perfusion fixation at the end of a 90-min BDM infusion using previously described techniques (15). Briefly, the hearts were arrested in diastole by injection of potassium chloride into the aortic root. The LV and right atrium were vented to atmospheric pressure. Through a cannula inserted in the aortic root, hearts were perfused with 200 ml of St. Thomas cardioplegic solution followed by 500 ml of phosphate-buffered 2.5% glutaraldehyde and 4% formaldehyde at a pressure of 100 mmHg. The mean LV cavity pressure measured during pressure fixation was 4 mmHg. Hearts were

rapidly excised after completion of perfusion fixation (within ~5 min of cardiac arrest). Subendocardial tissue from the anterior (BDM-treated) and posterior (untreated) LV was minced, stored in fixative, and prepared for transmission electron microscopy as previously described (15). Ultrathin sections were cut parallel to the myofiber axis and examined at $\times 2,000$ magnification with a JEOL 1200EX electron microscope. In both the anterior and posterior LV free wall of each heart, the lengths of at least 500 sarcomeres were measured and averaged. In each region of each heart, sarcomeres were measured in 6–10 nonoverlapping fields from at least 2 separate tissue blocks. To minimize error due to the angular difference between an image plane and the long axis of sarcomeres, measurements were performed only in areas where at least 10 successive sarcomeres lay in the image plane.

Statistical analysis

When a variable was examined under two conditions or in two regions in the same heart, significance of differences was assessed with a paired *t*-test. When a variable was examined under more than two conditions in the same heart, repeated measures ANOVA was employed, followed by Dunnett's test to assess differences between baseline and subsequent conditions.

RESULTS

Table 1 shows the effect of intracoronary verapamil or BDM on systemic hemodynamics, regional LV external work, and LAD coronary blood flow. Both agents produced a similar reduction of regional external work of the anterior LV: to $0.31 \pm .07$ times baseline in *group 1* during verapamil treatment and to $0.38 \pm .03$ times baseline in *group 2* during BDM treatment. Both agents also produced similar reductions in slope and increases in the dimension-axis intercept of the regional Frank-Starling relation, indicating similar depression of regional contractility. During treatment of the anterior LV with verapamil or BDM, external work of the untreated (posterior) LV declined modestly to $0.75 \pm .05$ times baseline in *group 1* and to $0.77 \pm .05$ times baseline in *group 2*. Consistent with effects as direct coronary vasodilators (7), verapamil had no significant effect on LAD coronary blood flow (despite decreases in heart rate, systolic blood pressure, and contractility), and BDM increased LAD flow.

The principal results of the study are illustrated in Fig. 1. In the anterior LV, treatment with either verapamil or BDM produced a significant rightward shift of the regional EDP versus EDL relation. This finding indicates that reduction in myofilament calcium availability (verapamil) or sensitivity (BDM) increased end-diastolic muscle length at a given EDP. Verapamil treatment produced a nearly parallel rightward shift of the EDP versus EDL relation over the range of EDP examined (2–8 mmHg), whereas BDM produced a greater rightward shift at lower EDP than at higher EDP. There was no significant shift of the regional EDP versus EDL relation in the posterior LV during infusion of either drug in the LAD.

To determine whether increases in end-diastolic muscle length during verapamil or BDM treatment reflected a fixed structural alteration or a dynamic functional alteration of the affected myocardium, the reversibility of changes in regional EDP versus EDL relations was examined during washout of the drugs. During washout of verapamil in *group 1*, there was a close correlation among the return to baseline of the regional EDP versus EDL relation (Fig. 2), the recovery of regional external work, and the decline in myocardial verapamil concentration (Table 2). The parallel recovery of EDP versus EDL relations and systolic function during verapamil washout indicates that the alteration of diastolic mechanics with this drug reflects a reversible functional alteration rather than a fixed structural alteration of the myocardium. Similarly, there was concordant recovery of regional EDP versus EDL

relation and external work in two pigs from *group 2* examined during washout of BDM (data not shown).

In six hearts from *group 2*, sarcomere length was measured by transmission electron microscopy after perfusion-fixation at low LV cavity pressure. Sarcomere length in the BDM-treated myocardium was greater than that in the untreated regions of the same hearts (2.37 ± 0.04 vs. 2.20 ± 0.04 μm , $P < 0.05$), as illustrated in Fig. 3. These findings indicate that during BDM treatment in vivo, an increase in EDL measured by sonomicrometry reflects an underlying increase in sarcomere length.

DISCUSSION

This study demonstrates that a reversible decrease in systolic function, produced by reducing myofibrillar calcium availability (verapamil) or sensitivity (BDM), is accompanied by a reversible increase in end-diastolic muscle length over a physiological range of EDP in vivo. These findings indicate that diminished systolic cross-bridge interaction, reflected by reduced slope and increased intercept of regional Frank-Starling relations, is accompanied by diminished end-diastolic cross-bridge interaction, reflected by a rightward shift in EDP versus EDL relations. Thus the end-diastolic mechanics of a normal intact heart are not solely determined by passive material properties of the myocardium but are also significantly influenced by active, residual diastolic cross-bridge interactions. This conclusion is further supported by the observation of increased sarcomere length in BDM-treated myocardium, measured after diastolic arrest and perfusion-fixation. The latter finding indicates that the increased EDL observed in vivo was due to altered myofilament interaction rather than slippage or changes in alignment between bundles of myocytes.

Despite different mechanisms of action, both verapamil and BDM produced similar effects on regional systolic function and EDP versus EDL relations in this study. Blockade of L-type sarcolemmal calcium channels by verapamil diminishes the amplitude of the myocardial calcium transient (9) and thereby decreases the contractile state. Less well recognized is the fact that verapamil may reduce diastolic intracellular calcium concentration (14, 23). A reduction in intracellular calcium concentration causes an increase in the resting length of isolated myocytes (19); by analogy, a reduction in diastolic intracellular calcium by verapamil in the present experiments likely led to diminished diastolic cross-bridge interactions and a reduced number of residual cross-bridges at end diastole, thereby allowing end-diastolic muscle length to increase. While BDM does not significantly alter the calcium transient (1), it decreases myofilament calcium sensitivity and cross-bridge interaction and contractility through inhibition of myosin ATPase (2, 10). Again, a less well-recognized, but important, prior observation is that BDM increases resting and end-diastolic lengths of isolated myocytes (19), implying a reduction in residual diastolic cross-bridge interactions in vitro. Conversely, agents that enhance myofilament calcium sensitivity have been shown to decrease the resting length of isolated myocytes (24). By analogy from these in vitro data, it is likely that diminished residual diastolic cross-bridge interaction accounts for the increase in end-diastolic muscle length and diastolic sarcomere length observed with BDM treatment in the present in vivo experiments.

In the absence of concurrent negative inotropic effects, coronary vasodilation shifts LV pressure-dimension relations to the left (22). This may reflect increased myocardial stiffness due to a garden-hose or erectile effect (6). In the present experiments, BDM increased LAD coronary blood flow, whereas verapamil did not. Consequently, BDM, but not verapamil, may have increased myocardial turgor and stiffness. This may explain the increasing slope (stiffness) of the EDP versus EDL relation during BDM treatment at higher values of EDP

(Fig. 1B), whereas the relation during verapamil treatment remained parallel with the baseline relation over the entire range of EDP examined (Fig. 1A).

Limitations of experimental design

Local (intracoronary) drug infusion allowed assessment of direct effects on regional LV mechanics with relatively little effect on systemic hemodynamics. Nonetheless, verapamil and BDM did cause a modest decline in systolic blood pressure. However, the use of an internal control region in each heart (posterior LV) allowed us to distinguish between the effects of local treatment with the drugs and those due to altered systemic hemodynamics and/or drug recirculation. We used doses of verapamil and BDM that produced severe regional systolic dysfunction; it is uncertain whether significant shifts in the EDP versus EDL relation would have occurred with doses that produce more modest reduction in systolic function. We did not assess the effects of intra-coronary verapamil or BDM on synchrony of regional LV contraction and relaxation. It is possible that asynchronous contraction contributed to the decreased external work of posterior LV during treatment of the anterior LV. However, it is unlikely that asynchronous relaxation had a significant effect at end diastole, because EDP versus EDL relations in the posterior LV were unaffected by treatment of the anterior LV.

Our findings were obtained at heart rates of ~ 110 beats/min, which prevail in this open-chest anesthetized porcine model. It is possible that the effects of residual (end diastolic) cross-bridges would have been less prominent at slower heart rates with a longer diastolic period. Verapamil caused a modest decrease in heart rate, whereas BDM caused a modest increase. Despite these directionally opposite effects on heart rate, both agents had similar effects on EDP versus EDL relations. Furthermore, the effect of BDM on sarcomere length in the perfusion-fixed hearts is independent of heart rate *in vivo*. For these reasons, heart rate changes are unlikely to affect the interpretation of the present results.

The chords subtended by crystal pairs may not have been precisely aligned with local myocardial fiber orientation. If so, there may be some inaccuracy in the assessment of changes in fiber length by sonomicrometry. However, correspondence between differences in sarcomere length measured in BDM-treated and untreated regions of the same hearts and changes in segment length recorded by sonomicrometry in treated and untreated regions provides corroboration that the sonomicrometry measurements reflected underlying changes in muscle fiber length.

Our experiments were performed with an open pericardium; it is possible that the effects of verapamil or BDM on EDP versus EDL relations would have been attenuated by pericardial restraint.

Implications

This study demonstrates a direct and immediate linkage between changes in systolic function and end-diastolic mechanics in the normal intact heart. There are several potentially important implications of these findings. While the average increase in EDL with verapamil or BDM treatment was only ~5%, the potential effect of reduced contractility on end-diastolic chamber volume may be considerably greater: because chamber volume varies as the third power of linear dimension, a 5% increase in each linear dimension at end diastole could result in a 16% increase in end-diastolic volume at a given EDP.

A reduction in the calcium sensitivity of the stunned myocardium (11), resulting in a decrease in end-diastolic cross-bridge interactions, may explain a previous finding of our laboratory (15, 16): that EDP versus EDL relations are shifted to the right after moderate LV

ischemia and reperfusion even when ultrastructural signs of injury to myocytes or interstitial matrix are absent.

The finding that systolic function and end-diastolic muscle length change concurrently may influence the interpretation of ventricular function curves that display stroke work as a function of EDP. Inherent in this formulation is the assumption that the relation between EDP and end-diastolic volume remains constant in the short term. However, the present data indicate that an acute reduction in contractility causes an immediate increase in end-diastolic dimension at a given EDP. An acute negative inotropic intervention will therefore increase preload (end-diastolic dimension) at a given EDP. Consequently, assessment of stroke work at a constant EDP will underestimate the effect of an acute negative inotropic intervention compared with assessment of stroke work at constant end-diastolic chamber dimensions.

The present findings may also reveal one of the mechanisms for clinical benefit of negative inotropic agents, such as verapamil, in some cases of “diastolic heart failure” (3, 18, 21). While reduced systolic blood pressure and increased diastolic filling time may be the most important salutary effects of these agents in this context, the present data suggest that an additional benefit may be a direct effect on end-diastolic mechanics, allowing ventricular filling to occur at lower EDP.

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Fig. 1.

End-diastolic pressure (EDP) versus end-diastolic segment length (EDL) relations in the anterior left ventricle of hearts treated with left anterior descending coronary artery infusion of verapamil (*A*) or 2,3-butanedione monoxime (BDM; *B*). Data are the average of 7 experiments (*A*) and 8 experiments (*B*). Bars indicate SE of EDL at given values of EDP. To correct for differences in intercrystal distance at implantation, EDL in each heart is normalized to the value measured under baseline conditions at EDP = 5 mmHg. At each indicated level of EDP, both drugs caused a significant rightward shift of the EDP versus EDL relation ($P < 0.05$).

Fig. 2.

Changes in EDP versus EDL relations during 90 min of washout of verapamil from myocardium. Baseline measurements were obtained before the onset of intracoronary verapamil infusion. Washout times indicate the number of minutes between cessation of verapamil infusion and measurement. Data are the average of 7 experiments. Values of EDL are normalized as described in Fig. 1. For clarity, bars indicating SE of EDL are shown only for baseline and 0-min washout conditions. At 90-min verapamil washout, the EDP versus EDL relation was not significantly different from baseline, indicating that the changes induced by verapamil were fully reversible.

Fig. 3.

Transmission electron micrographs from BDM-treated and untreated regions of the same heart. The heart was arrested in diastole and underwent in situ perfusion fixation at a left ventricular cavity pressure of ~4 mmHg. Dashed lines connecting Z bands of sarcomeres in both regions indicate greater sarcomere length in the BDM-treated region than in the untreated region.

Table 1

Hemodynamics and regional LV systolic function

	<i>Group 1</i>		<i>Group 2</i>	
	Baseline	Verapamil	Baseline	BDM
Heart rate, beats/min	103 ± 2	95 ± 2 *	109 ± 3	119 ± 2 *
LV systolic pressure, mmHg	93 ± 3	75 ± 2 *	96 ± 4	74 ± 7 *
LV EDP, mmHg	11 ± 2	15 ± 3 *	10 ± 2	13 ± 2 *
LAD coronary blood flow, ml/min	19 ± 2	20 ± 2	23 ± 3	35 ± 3 *
Anterior LV regional systolic function (fraction of baseline)				
Steady-state external work	1.00	0.31±0.07 *	1.00	0.38 ± 0.03 *
Frank-Starling relation slope	1.00	0.37±0.06 *	1.00	0.48 ± 0.05 *
Frank-Starling relation dimension axis intercept	1.00	1.13±0.03 *	1.00	1.15 ± 0.04 *

Data are means ± SE; $n = 7$ pigs in *group 1* and $n = 8$ pigs in *group 2*. LV, left ventricular; BDM, 2,3-butanedione monoxime; EDP, end-diastolic pressure; LAD, left anterior descending coronary artery.

* Significant change from baseline, $P < 0.05$.

Table 2

Reversal of verapamil-induced changes in EDP vs. EDL relation and external work during washout of drug from myocardium

Verapamil Washout Time, min	Shift of Regional EDP vs. EDL Relation (fraction of baseline EDL, averaged over EDP range 2–8 mmHg)	Regional External Work (fraction of baseline)	Tissue Concentration of Verapamil, ng/mg
0	1.05 ± 0.01 *	0.31 ± 0.07 *	2,436 ± 885
30	1.03 ± 0.02 *	0.70 ± 0.10 *	765 ± 383
60	1.02 ± 0.02	0.85 ± 0.14	258 ± 186
90	0.99 ± 0.02	0.96 ± 0.12	56 ± 12

Data are means ± SE; $n = 7$ pigs for regional EDP vs. end-diastolic segment length (EDL) relation and external work, $n = 5$ pigs for tissue concentration of verapamil. Shift of EDP vs. EDL relation expresses the average rightward shift of the curves shown in Fig. 2. The data indicate that during washout of verapamil from the myocardium, the regional EDP vs. EDL relation and regional external work return to baseline in parallel with the decline in tissue verapamil concentration.

* Significant change from baseline (before verapamil treatment), $P < 0.05$.