J Mol Cell Cardiol **24**, 783–797 (1992)

Comparison Between The Effects of 2–3 Butanedione Monoxime (BDM) and Calcium Chloride on Myocardial Oxygen Consumption

Pieter P. de Tombe[1], Daniel Burkhoff[2] and William C. Hunter[1]

[1]Department of Biomedical Engineering and [2]Cardiology Division, Johns Hopkins University, School of Medicine, Baltimore MD 21205, USA

(Received 6 September 1991, accepted in revised form 13 January 1992)

PIETER P. DE TOMBE, DANIEL BURKHOFF AND WILLIAM C. HUNTER. Comparison Between The Effects of 2–3 Butanedione Monoxime (BDM) and Calcium Chloride on Myocardial Oxygen Consumption. *Journal of Molecular and Cellular Cardiology* (1992) **24**, 783–797. The agent 2,3-butanedione monoxime (BDM) has been reported to reduce the sensitivity of myofilament force development to calcium ions, without affecting the calcium transient in myocardium. One would predict, therefore, that BDM should reduce the contractile state of the heart without reducing the amount of oxygen that is consumed to fuel the process of excitation–contraction coupling. The purpose of the present experiment was to test this hypothesis using isovolumically contracting, isolated, blood perfused canine hearts during β-blockade induced by continuous intra-coronary infusion of propranolol (1 mg/h). Contractile state was increased in seven hearts by $CaCl_2$ infusion. Subsequently, while the $CaCl_2$ infusion was continued at the highest rate, contractile state was reduced by BDM infusion. At each contractile state, we measured the left-ventricular end-systolic pressure–volume relation (ESPVR), the relation between myocardial oxygen consumption and its mechanical correlate, pressure–volume area (MVO_2 *vs* PVA), and the duration of the LV pressure waveform. Contractile state was quantified by interpolated developed pressure at a reference ventricular volume of 25 ml (P_{25}). BDM infusion (0.5–7 mM) caused a dose-dependent reduction in contractile state (50% reduction in P_{25} at 2.4 ± 0.3 mM), and a dose-independent increase in coronary blood flow. Furthermore, BDM significantly reduced the duration of the pressure waveform up to 40% at the highest rate of BDM infusion compared to the pressure waveform duration measured at maximum $CaCl_2$ infusion. We observed a direct relationship between MVO_2 of the mechanically unloaded heart and contractility; this relation was unaffected by BDM infusion ($P > 0.3$). The slope of the MVO_2–PVA relation decreased with increases in contractile state, but this decrease was unaffected by BDM ($P \geqslant 0.4$). We conclude that in the isolated canine heart, BDM does not act energetically as expected for a myofibrillar calcium desensitizing agent.

KEY WORDS: Pressure–volume area; Pressure–volume relationship; BDM; Myocardial oxygen consumption; Contractility; Canine.

Introduction

Results of previous studies have shown that oxygen consumption of the externally unloaded left ventricle varies directly with ventricular contractile state [*1–3*]. This finding has been hypothesized to reflect altered energy demands for calcium cycling, since changes in contractile state are generally thought to be brought about by changes in the amount of calcium cycled with each beat [*4–7*].

However, new classes of inotropic agents have emerged recently that reportedly affect contractile state by altering the sensitivity of the myofilaments to calcium instead of – or in addition to – altering the amount of calcium available for contraction [*8*]. That is, these agents have been reported to alter the amount of force that is developed at a given concentration of free calcium in the cytosol. For example, it has been reported that the compound 2,3-butanedione monoxime (BDM) re-

Please address all correspondence to: Pieter P. de Tombe, Department of Medicine, Division of Cardiology, Bowman-Gray School of Medicine, Wake Forest University, Medical Center Boulevard, Winston-Salem, North Carolina 27157–1045, USA.

Please address reprint requests to: William C. Hunter, Department of Biomedical Engineering, Johns Hopkins University, 720 Rutland Avenue, Traylor 726, Baltimore MD 21205, USA.

0022–2828/92/080783 + 15 $08.00/0

duces the calcium sensitivity of the myofilaments [*9–11*], without affecting the intracellular calcium transient [*12*]. Hence, it would be expected that in the presence of BDM, a higher peak calcium concentration would need to be reached during the calcium transient in order to achieve a certain contractile state. This would require cycling of an additional amount of calcium with each beat, and thus would be expected to be accompanied by increased myocardial oxygen demand. Hence, one would predict that the relation between unloaded myocardial oxygen consumption ($MVO_{2\text{-unl}}$) and contractile state would be shifted upwards by BDM. The purpose of the present study was to test this prediction in isolated, blood perfused canine hearts.

Contrary to our prediction, we found that the relationship between $MVO_{2\text{-unl}}$ and contractile state was similar when inotropic state was altered either by infusion of $CaCl_2$ alone or by infusion of BDM in combination with maintained $CaCl_2$ infusion. These results indicate that one effect of BDM in the isolated canine heart is to reduce the energy consumed for calcium handling during excitation-contraction coupling.

A preliminary report of this study has been published previously [*13*].

Methods

Surgical preparation

A total of nine isolated canine ventricles were studied. Of these, seven hearts satisfied the selection criterion (see below) and were included in the analysis. The procedures used to isolate and support a canine heart were similar to those described by Suga and Sagawa [*14*]. Briefly, two mongrel dogs (25–35 kg), anesthetized with sodium pentobarbital (30 mg/kg), were used for each experiment. The femoral arteries and veins of one dog (support dog) were cannulated and connected to a perfusion system used to supply oxygenated blood to the isolated heart. This dog was medicated with hydrocortisone (500 mg im), diphenhydramine (50 mg iv) and indomethacin (25 mg suppository). Heparin (5000 IU iv bolus) was administered after the arteries and veins were cannulated with the tubing of the perfusion system. The chest of the second dog (heart donor dog) was opened under artificial ventilation. The subclavian artery and right atrium were cannulated and connected to the perfusion system. After ligation of the azygos vein, superior and inferior venae cavae, brachiocephalic artery, descending aorta, and pulmonary hili, the heart was removed from the chest. From this time onward, coronary perfusion was maintained with arterial blood from the support dog, the temperature of which was maintained at approximately 37°C with a heat exchanger. The left ventricle was vented, the left atrium was opened, and the chordae tendineae were cut. A ring adapter was sewn into the mitral annulus, and a thin latex balloon (5 ml balloon material volume) which was connected to a volume control system was placed in the left ventricle. Left ventricular pressure was measured in the balloon with a tip micromanometer (model PC 380, Millar, Houston, TX, USA).

Coronary perfusion pressure (CPP) was measured via a catheter placed through the brachiocephalic trunk into the aortic root. A two pump (model 1215, Harvard Apparatus, South Natick, MA) system was used to servo control mean CPP at 80 mmHg. The configuration used for this system has been described recently [*15*]. Briefly, the first pump withdrew arterial blood from the support dog at a constant rate, while the second pump diverted blood flow so as to bypass the isolated heart. Using this arrangement, the hemodynamic demand on the support dog was constant, i.e. independent of coronary flow to the isolated heart. This procedure was used in an attempt to minimize fluctuations of support dog catecholamine levels, which could potentially result from alterations in cross-circulated blood flow demand and cause instability of the contractile state of the isolated heart.

It has been shown that β-adrenergic stimulation induces a decrease in the sensitivity of cardiac myofilaments to calcium [*16*], i.e. an action similar to that proposed for BDM. In order to exclude possible confounding effects of alterations in the level of β-adrenergic stimulation in the isolated heart and in order to assure maximum calcium sensitivity of the myofilaments, we performed the experiments under β-blockade. For this purpose, a 2 mg

bolus of propranolol, followed by an additional 1 mg/h, was infused into the coronary perfusion line of the isolated heart. Preliminary studies showed that this procedure resulted in an apparent complete β-blockade of the isolated heart during the course of the entire experiment (that is, absence of an inotropic response to a bolus or 5 μg dobutamine injected into the coronary perfusion line).

The support dog was ventilated mechanically. Blood pH, pO_2, and pCO_2 were maintained in the normal range by adjusting the ventilation rate or by administration of sodium bicarbonate or oxygen as dictated by the results of periodic blood gas samples (every 30 mins).

The right ventricle and atrium of the isolated heart were drained via a wide bore catheter with side holes, placed through the RV apex. The right side of the heart was made air tight by a purse string suture around the cannula in the apex and ligation of the venae cavae. The flow of coronary venous blood (CBF) draining from the right side of the heart was measured continuously by an ultrasonic flow probe (Transonics, Ithaca, NY, USA) connected in series with the wide bore catheter. This flow represents total coronary blood flow to both the right and left ventricles, except for the small amount of Thebesian flow draining directly into the left ventricle. This Thebesian flow was neglected since the amount of this flow has been shown to be relatively small [*17*].

This difference in oxygen content between arterial and coronary venous blood (AVO_2) was measured continuously by absorption spectrophotometry (A-VOX Systems [*18*]). Total heart oxygen consumption was calculated as the product of CBF and AVO_2; this amount was divided into left and right heart components (cf Data Analysis). Heart rate was fixed by atrial pacing at 10 to 15 beats/min higher than the spontaneous heart rate.

Experimental protocol

For each heart, data were collected in the baseline contractile state and at one or more levels of increased contractile state induced by $CaCl_2$ infusion (1 M solution) at increasing rates. Note that the baseline contractile state was reached after β-blockade; this contractile state was, on average, 50% of the contractile state measured before β-blockade. The maximum rate of $CaCl_2$ infusion was chosen such that left ventricular pressure development was increased by about 3 to 4 times the baseline level. Subsequently, while maintaining the infusion of $CaCl_2$ at the highest level, contractile state was reduced to one or more levels by BDM infusion (0.5 M solution). Hence, a substantially wide and overlapping range of contractile states was examined in each heart, both with $CaCl_2$ infusion alone and with $CaCl_2$ plus BDM infusion. The final concentration of BDM in the blood perfusing the isolated heart was calculated from the infusion rate and the measured CBF. Thus, in this calculation we ignored the small contributions of Thebesian flow and leakage from surgical incisions to the total flow of blood delivered to the isolated heart. Each experimental run at each contractile state consisted of making measurements at a minimum of four randomly chosen preload volumes in the range of LVV = 5–7 ml to LVV = 40–60 ml. The latter (maximum) volume was chosen on basis of the actual size of the left ventricle; care was taken to ensure that end-diastolic pressure did not exceed 18 mmHg. The hearts were contracting isovolumically during the entire protocol. After each change in preload, between 2 and 3 min were required to assure that data were obtained at steady state. Recorded signals included left ventricular pressure (LVP), left ventricular volume (LVV; corrected for the 5 ml volume of the balloon material), coronary perfusion pressure (CPP), CBF, AVO_2, and a surface ECG. Signals were digitized (12 bit resolution) at a sampling rate of 200 Hz, stored on diskettes, and analyzed off-line. Several beats of data were recorded at each preload. At the end of the experiment, the weights of the right ventricular wall and the left ventricle (left ventricular free wall plus septum) were measured.

Data analysis

The end-systolic and end-diastolic pressure–volume coordinates of the 4 or more preload volumes at each contractile state were fit by non-linear regression analysis of the Marquardt type to non-linear power functions:

$$LVP_{es} = a_{es} \cdot (LVV - V_o)^{Ces} \quad [1a]$$
$$LVP_{ed} = a_{ed} \cdot (LVV - V_o)^{Ced} \quad [1b]$$

These functions form the end-systolic (ESPVR; equation 1a) and the end-diastolic (EDPVR; equation 1b) pressure–volume relation. In these equations, 'a', 'c', and 'V_o' are constants. LVP_{es} and LVP_{ed} are end-systolic and end-diastolic left ventricular pressure, respectively. This procedure was adopted to account for the non-linearities in the ESPVR that were apparent at high and low contractile states (Fig. 2(a), Results, and reference [*19*]). Therefore, contractile state, which traditionally has been expressed by the slope of the linear ESPVR in our preparation, was expressed in the present study by interpolated end-systolic pressure at a reference ventricular volume of 25 ml (P_{25}). Changing the value of this reference volume to 20 ml or 30 ml did not alter the results of the present study.

At each preload volume and contractile state, we calculated pressure–volume area (PVA) of each beat by determining the area contained within the ESPVR and EDPVR (equations 1a and 1b) between V_o and LVV of that beat [*1, 3*]. PVA is considered to be a measure of the total mechanical energy generated by the left ventricle during a beat [*1, 3*]. Next, the MVO_2–PVA coordinates were fit by linear regression, resulting in both a slope and an intercept (*Cf.* Fig. 2(b)). The slope parameter of the MVO_2–PVA relation has been considered to reflect the product of (*i*) the efficiency of the myofibrillar proteins to convert chemical energy into mechanical energy and (*ii*) the metabolic efficiency to produce ATP by consuming oxygen; the intercept parameter of the MVO_2–PVA relation represents the amount of oxygen consumed by the heart to sustain basal metabolism and excitation–contraction coupling [*3*]. Oxygen consumption of the entire heart, however, expressed in ml O_2/beat, represents the sum of the oxygen consumed by the work-performing left ventricle plus the unloaded, but still oxygen consuming, right ventricle. To correct for this, we assumed that the right ventricle consumed an amount of the total unloaded oxygen consumption – measured when the left ventricle was also unloaded that was proportional to its weight, and we subtracted this amount of consumed oxygen from all the data at each contractile state [*1, 3*]. Note that the amount of this correction was assessed at each contractile state. These updated MVO_2 values were used to describe the MVO_2–PVA relation of the left ventricle; the intercept of this relation represents the oxygen consumption of the unloaded left ventricle ($MVO_{2\text{-unl}}$).

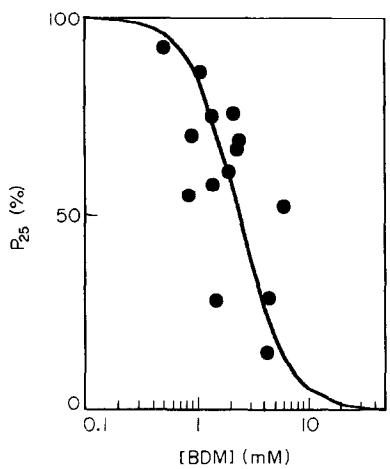

FIGURE 1. BDM dose-response curve. Contractile state was quantified by pressure development at a fixed ventricular volume of 25 ml (P_{25}). Data were normalized to the state just prior to BDM infusion and at the highest level of $CaCl_2$ infusion; On average, $P_{25} = 100\%$ was 132 ± 11.7 mmHg. Pooled data from seven hearts were fitted to a modified Hill equation: P_{25} (%) $= 100 \cdot [BDM]^{-2} / ([BDM]^{-2} + 2.38^{-2})$; $r = 0.83$; $n = 14$.

In addition to examining the parameters of the MVO_2–PVA relation, we also wished to compare the effects of $CaCl_2$ and BDM on myocardial oxygen consumption in terms other than the MVO_2–PVA framework. For this purpose we examined the effect of contractile state on left ventricular oxygen consumption when the heart contracted at the reference volume (25 ml; $MVO_{2\text{-}25}$). Since data was not always collected precisely at the 25 ml reference volume in each heart, and in order to allow comparison between hearts, $MVO_{2\text{-}25}$ was obtained by interpolation in the following manner. First, PVA at LVV = 25 ml was calculated from the non-linear ESPVR and EDPVR relations (Fig. 2(a)). Next, left ventricular $MVO_{2\text{-}25}$ was interpolated from the linear MVO_2–PVA relation [Fig. 2(b)].

Recently, it has been shown that BDM

increases the cycling rate of attached cross-bridges in isolated myocardium [*20*]. Since increased cross-bridge cycling rate may affect the rate of pressure development and relaxation, we measured the effect of $CaCl_2$ and BDM infusion on the time course of the pressure waveform. For this purpose, we determined at each contractile state and preload volume (*1*) Peak developed pressure (PP); (*2*) the time (TTP) required for pressure to develop from 10% PP to PP and (*3*) the time (RELAX) required for developed pressure to decay from PP to 10% PP. Total duration (TD) of the pressure waveform was defined as TTP+RELAX. Next, TTP, RELAX, and TD were fit as function of preload volume at each contractile state by linear regression, which allowed for interpolation of TTP, RELAX, and TD to the reference preload volume (25 ml).

BDM caused an increase in CBF, even at the lowest concentration that was encountered (0.5 mM). There was, however, no apparent relation between the BDM concentration and CBF increase. It has been reported that an increase in CBF *per se* can cause an increase in MVO_2 [*21, 22*]. We sought to reduce the impact of this confounding factor in the present study. Hence, if upon BDM infusion CBF increased by a factor of two compared the CBF obtained at the highest contractile state induced by $CaCl_2$, the data from that level of BDM infusion were rejected. Because of this criterion, all data from two hearts and data from 3 BDM states in two additional hearts were excluded from the present study.

Statistical analysis

We first tested whether contractile state affected the slope and intercept of the MVO_2–PVA relations. For this purpose multiple linear regression [*23*] was employed in each individual heart, both for $CaCl_2$ and BDM infusion (Table 1). The regression equation used had the following form:

$$MVO_2 = a \cdot \text{constant} + b \cdot PVA + c \cdot \Delta P_{25} + d \cdot \Delta P_{25} \cdot PVA$$

ΔP_{25} was defined as change of P_{25} upon infusion of either $CaCl_2$ or BDM. Note that the coefficient 'c' in this equation indicates the magnitude of the effect of ΔP_{25} on the intercept of the MVO_2–PVA relation in each heart. Similarly, the coefficient 'd' is related to the effect of ΔP_{25} on the slope of the MVO_2–PVA relation.

Next, we extended this equation, using 6 dummy variables [*23*] that coded for the individual hearts, and hence allowed for variation in slope and intercept of the MVO_2–PVA relation during the pre-drug infusion state between the seven hearts. Thus, this equation allowed for multiple linear regression analysis of the MVO_2–PVA–P_{25} interrelations measured in all hearts, either during $CaCl_2$ or BDM infusion (Table 1). This test revealed that during $CaCl_2$ infusion both intercept and slope parameters of the MVO_2–PVA relation were significantly affected by contractile state; on the other hand, during BDM infusion, only the intercept parameter was significantly affected by contractile state (Table 1).

The effect of BDM on the MVO_2–PVA–P_{25} relation can in principle be tested directly by further expanding the regression equation to also contain the actual BDM concentration. However, the regression equation then becomes quite complicated and possibly less sensitive for detecting an effect of BDM on MVO_2. Therefore, we instead opted to plot the various parameters (i.e. $MVO_{2\ unl}$, $MVO_{2\text{-}25}$, MVO_2–PVA slope, TTP, RELAX, and TD) as a function of contractile state (P_{25}) in each individual heart. Next, in order to test whether BDM affected the relation between P_{25} and these parameters, we employed multiple linear regression analysis. In the regression model, one dummy variable coded for the presence or absence of BDM and another dummy variable coded for the experiment number [*23*]. In addition, for the purpose of presenting the combined data from all hearts, we devised the following normalization scheme for pooling the data from the seven hearts. For each heart we determined mean P_{25}, $MVO_{2\text{-}unl}$, $MVO_{2\ 25}$, MVO_2–PVA slope, TTP, RELAX, and TD over the range of $CaCl_2$ infusions, that is from baseline (no infusion) to maximal $CaCl_2$ infusion. Thus, none of the data obtained during BDM infusion were included in the calculation of these mean values. Subsequently, all parameter values obtained in each heart were

TABLE 1. Coefficients of multiple linear regression between MVO_2, PVA, and P_{25}

Heart	$CaCl_2$ infusion					BDM infusion				
	# $CaCl_2$ states	Constant $\cdot 10^{-3}$	PVA $\cdot 10^{-5}$	ΔP_{25} $\cdot 10^{-4}$	ΔP_{25}.PVA $\cdot 10^{-7}$	# BDM states	Constant $\cdot 10^{-3}$	PVA $\cdot 10^{-5}$	ΔP_{25} $\cdot 10^{-4}$	ΔP_{25}.PVA $\cdot 10^{-7}$
1	4	2.9*	2.1*	3.5*	−1.4*	1	4.9*	1.2*	6.1*	−0.8
2	3	5.4*	3.1*	3.5*	−1.9*	1	6.5*	2.6*	2.2*	1.9*
3	4	2.9*	2.4*	4.3*	0.5	2	6.9*	2.6*	1.2	−0.2
4	3	3.2*	3.9*	5.0*	−1.1	2	10.0*	1.6*	5.3*	−0.5
5	3	3.6*	2.8*	6.1*	−0.1	3	6.6*	2.9*	8.1*	1.9
6	5	2.2*	3.4*	3.1*	−1.4*	2	7.6*	3.2*	2.6*	−2.6
7	2	4.4*	3.2*	3.2*	−1.6*	3	6.4*	2.2*	2.0*	−0.3
Mean		3.51	2.99	4.10	−1.00		6.99	2.33	3.93	−0.09
S.E.M.		0.41	0.23	0.42	0.33		0.59	0.27	0.97	0.60
Overall model		3.6*	3.2*	3.8*	−1.1*		6.7*	2.6*	2.9*	−0.3

Pressure–volume area (PVA) and myocardial oxygen consumption (in ml O_2/beat) were normalized to 100 g LV in each heart. Multiple linear regression[23] was then performed in each individual heart using the regression equation: $MVO_2 = a \cdot \text{constant} + b \cdot PVA + c \cdot \Delta P_{25} + d \cdot \Delta P_{25} \cdot PVA$, either for the $CaCl_2$ infusion group or the BDM infusion group. ΔP_{25} indicates the difference in P_{25} compared to pre-drug infusion values of P_{25}, i.e. P_{25} either at zero $CaCl_2$ infusion, or at maximum $CaCl_2$ infusion rate just prior to BDM infusion; note that in the case of BDM infusion, all ΔP_{25} values were negative, indicative of the negative inotropic action of BDM. # $CaCl_2$ states indicates the number of different levels of $CaCl_2$ infusion rate studied in each individual heart, including the baseline contractile state; # of BDM states indicates the number of different levels of BDM infusion rate while maintaining the $CaCl_2$ infusion at the highest level.

In the overall model, an additional six dummy variables were included in the regression equation in order to code for differences in MVO_2–PVA intercept and slope in the individual hearts under base line conditions (i.e. $\Delta P_{25} = 0$); this approach allows for overall statistical testing, that is, including the data obtained in all seven hearts [*23*].

* Indicates that the coefficient is significantly different ($P < 0.05$) from zero by multiple linear regression.

expressed as a percentage of these mean normalization values. This normalization procedure removed interanimal variation in slope and intercept of the relationship between P_{25} and the various parameters; we and others have observed such between subject variation in the MVO_2–PVA parameters both in the present study and in previous studies [2, 3]. Statistical comparisons of slopes and intercepts of the relationships between P_{25} and the various parameters were then accomplished by multiple linear regression analysis applied to the data pooled from all hearts. In this regression model, a dummy variable coded for the presence or absence of BDM [23]. Whether a statistically significant difference was present for any of the parameters that were examined in the present study, was independent of the regression model employed (i.e. with or without normalization). Commercially available software was used (SYSTAT, Evanston, IL, USA). Data are presented as mean ± S.E.M. $P<0.05$ was considered significant.

Results

A total of 24 and 14 contractile states were analyzed with $CaCl_2$ and BDM infusion, respectively, in seven hearts during β-blockade induced by continuous intra-coronary infusion of propranolol (1 mg/h). Heart rate averaged 143 ± 5.4 beats/min, right ventricular free wall mass averaged 58.3 ± 2.7 g, and left ventricular mass averaged 166 ± 7.0 g. Coronary blood flow (CBF) averaged 1.3 ± 0.17 ml/g/min at the highest contractile state induced by $CaCl_2$ infusion, and increased, on average, by 40 ± 12.9% upon infusion of BDM (0.5–7 mM). This effect of BDM on CBF is consistent with the recently reported action of BDM to decrease the contractile strength of isolated smooth muscle [24].

BDM infusion, while simultaneously infusing $CaCl_2$ at the highest rate, caused a dose-dependent decrease in the contractile state of the isolated canine hearts, as is shown by the semi-logarithmic plot in Figure 1. In this figure 100% represents P_{25} measured at the highest level of $CaCl_2$ infusion, which was the contractile state just prior to initiating BDM infusion; this P_{25} averaged 132 ± 11.7 mmHg in the seven hearts. The concentration of

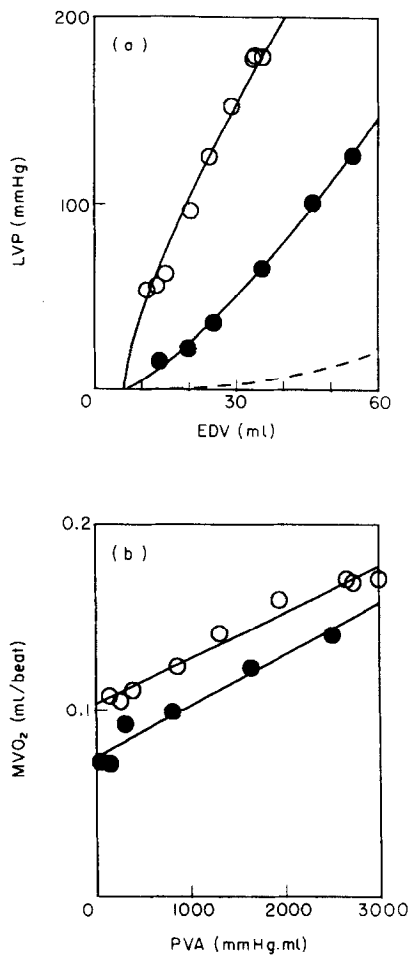

FIGURE 2. Effect of $CaCl_2$ and BDM on ESPVR and MVO_2–PVA relation (a) End-systolic pressures, measured in one representative heart, are plotted as function of ventricular volume, to form the end-systolic pressure volume relation (ESPVR). Open circles represent data collected during the highest rate of $CaCl_2$ infusion; The closed circles represent data collected during the highest rate of BDM infusion (about 4 mM final concentration), while simultaneously infusing $CaCl_2$ at the highest rate. The end-diastolic pressure–volume relation is indicated by the dashed line. ESPVR and EDPVR were fit to non-linear power functions:
ESPVR ($CaCl_2$) $LVP_{es}=11.1\cdot(V-6.0)^{0.83}$; $n=9$; $r=0.995$
ESPVR (BDM) $LVP_{es}=0.76\cdot(v-6.0)^{1.32}$; $n=6$; $r=0.999$;
EDPVR $LVP_{ed}=0.00012\cdot(V-6.0)^{3.0}$; $n=15$; $r=0.943$.
(b) Myocardial oxygen consumption is plotted as function of Pressure–Volume Area (PVA) measured during the same experimental runs as shown in panel A. The MVO_2–PVA relations (solid lines) were fit by linear regression:
($CaCl_2$) $MVO_2=0.104+2.47\cdot10^{-5}\cdot PVA$; $n=9$; $r=0.985$.
(BDM) $MVO_2=0.075+2.79\cdot10^{-5}\cdot PVA$; $n=6$; $r=0.978$.
Heart rate 148 bpm; LV weight 151 g; RV weight 47 g.

BDM required to depress contractile strength (P_{25}) by 50% was 2.4 ± 0.3 mM, which is comparable to that obtained both from isolated cardiac [*9, 12, 25, 26*] and skeletal [*10, 11, 27*] muscle preparations.

Figure 2(a) shows an example of the effect of BDM infusion (while maintaining simultaneous $CaCl_2$ infusion) on the ESPVR. The data were fit to power functions by non-linear regression (*cf* Methods). Note that for clarity of presentation, only the ESPVR corresponding to the highest level of $CaCl_2$ infusion (open circles) and the highest level of BDM infusion (filled circles) is displayed in this figure. Typically, the ESPVR was concave to the volume axis at the highest level of $CaCl_2$ infusion, and became convex to the volume axis with the highest level of BDM infusion. Thus, a significant correlation ($P<0.001$) was apparent between the constant 'c_{es}' of the non-linear ESPVR and P_{25}, consistent with our previous observations in the blood perfused canine heart [*19*]; this relation was similar whether P_{25} was varied by $CaCl_2$ or BDM infusion ($P>0.05$). Furthermore, neither $CaCl_2$ infusion nor BDM infusion significantly affected the end-diastolic pressure–volume relation.

Figure 2(b) shows the MVO_2–PVA relations corresponding to the data shown in Figure 2(a). The data were fit by linear regression (*cf.* Methods). $CaCl_2$ infusion increased the intercept of the MVO_2–PVA relation from its pre-infusion baseline (not shown), consistent with previous observations in the blood-perfused canine heart [*1–3*]. The open circles in this figure depict MVO_2–PVA coordinates measured at the highest level of $CaCl_2$ infusion. Subsequent infusion of BDM, while maintaining the $CaCl_2$ infusion at the highest level, reduced the intercept of the MVO_2–PVA relation ($P<0.05$). The closed circles in this figure depict MVO_2–PVA coordinates measured at the highest level of BDM infusion in this heart. Thus, $CaCl_2$ infusion alone, or BDM infusion combined with $CaCl_2$ infusion caused a variation in both the contractile state and the unloaded, non-work related oxygen consumption of the heart (i.e.the y-intercept of the MVO_2–PVA relation). Although the slope of the MVO_2–PVA relation was 13% higher with BDM infusion, this difference did not reach statistical significance.

We next investigated the effect of P_{25} on the MVO_2–PVA relation, both during $CaCl_2$ and BDM infusion. Table 1 shows the multiple linear regression analysis results of each individual heart (cf. Methods). Contractile state (as indexed by P_{25}) significantly affected the intercept of the MVO_2 relation (see ΔP_{25} column) during $CaCl_2$ infusion in all hearts studied, and in six out of the seven hearts during BDM infusion. The slope of the MVO_2-relation significantly decreased with increases in P_{25} (see $\Delta P_{25} \cdot$ PVA column) in four out of seven hearts during $CaCl_2$ infusion, but only in one heart out of seven hearts during BDM infusion. Overall multiple linear regression (cf. Methods), i.e. using the MVO_2–PVA-P_{25} data obtained in all seven hearts revealed that both the slope and elevation of the MVO_2–PVA relation was significantly affected by contractile state during $CaCl_2$ infusion; During BDM infusion, the elevation of the MVO_2–PVA relation, but not its slope, was significantly affectedby P_{25}. We next extended the regression equation to also contain the BDM concentration (cf. Methods). This test revealed no significant effect of BDM on either the slope or elevation of the MVO_2–PVA relation *independent* of its effect on P_{25}.

In order to better detect the possible effect of BDM on either slope or intercept of the MVO_2–PVA relation, we continued the statistical analysis using the slope and intercept of the MVO_2–PVA relation directly, i.e. as if these parameters were obtained directly by experiment, rather than being derived from linear regression at each contractile state. Figure 3(a) shows the relation between contractile state (as indexed by P_{25}) and unloaded, non-work related oxygen consumption $MVO_{2\text{-unl}}$; further analysis of data from the same heart as presented in Figure 2). Figure 3(b) shows the relation between P_{25} and $MVO_{2\text{-}25}$, a parameter that describes myocardial oxygen consumption in terms other than the MVO_2–PVA framework (cf. Methods). The relation between P_{25} and $MVO_{2\text{-}25}$ is shown in Figure 3(b). Data obtained at baseline contractile state and during $CaCl_2$ infusion are depicted by the open circles; data obtained during the BDM infusion (while maintaining the $CaCl_2$ infusion at the highest rate) are represented by the filled circles. It is clear that, in this heart, the

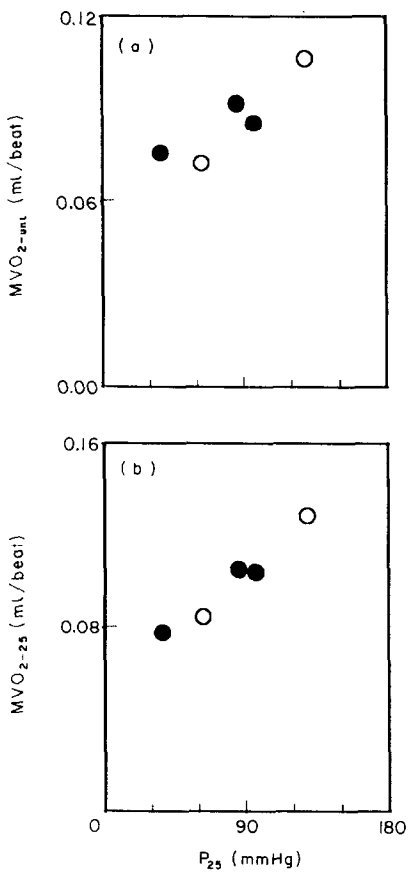

FIGURE 3. Effect of $CaCl_2$ and BDM on $MVO_{2\text{-}unl}$ and $MVO_{2\text{-}25}$ (a) Non-work related oxygen consumption ($MVO_{2\text{-}unl}$), measured in one representative heart, plotted as function of contractile state (P_{25}). The data were obained from the same heart as those presented in Figure 2. Contractile state was quantified by developed left ventricular pressure at a reference volume of 25 ml (P_{25}). Open circles represent experimental runs in which contractile state was increased from baseline contractile state by $CaCl_2$ infusion. Filled circles represent experimental runs in which contractile state was reduced by BDM infusion together with maintained $CaCl_2$ infusion at the highest rate.
(b) Oxygen consumption at 25 ml left ventricular volume ($MVO_{2\text{-}25}$) plotted as function of P_{25}. The $MVO_{2\text{-}25}$ parameter was estimated as described in the Methods. (symbols as in panel C).

relationship between contractile state and $MVO_{2\text{-unl}}$ was purely a function of contractile state and not affected independently by BDM. A similar result was observed for the $MVO_{2\text{-}25}$ parameter [Fig.3(b)], confirming the absence of an independent effect of BDM on myocardial energetics in the loaded as well as unloaded heart.

Figure 4 summarizes the results from all hearts regarding the impact of $CaCl_2$ and BDM infusion on the relationships between P_{25} and the parameters of the MVO_2–PVA relation (i.e. $MVO_{2\text{-unl}}$, $MVO_{2\text{-}25}$, and slope). The data from each individual heart were first normalized to the average results obtained with $CaCl_2$ infusion in that heart and then analyzed by multiple linear regression (cf. Methods). Similar to the results from the one heart shown in Figure 3, $CaCl_2$ infusion (open circles) caused an increase in both contractile state (P_{25}) and unloaded MVO_2 (open circles) in all hearts [Fig. 4(a)]. Subsequent infusion of BDM caused a dose-dependent decrease in P_{25}, which was associated with a decrease in unloaded MVO_2 (filled circles). Neither the slope ($P=0.9$) nor elevation ($P=0.8$) of this normalized relation was significantly different between $CaCl_2$ and BDM infusion. Also, a similar ($P=0.4$) result was obtained for the $MVO_{2\ 25}$ parameter, as is shown in Figure 4(b). Note that similar levels of statistical significance were obtained when a more extensive multiple linear regression model was applied to the data before normalization (cf. Methods). Hence, contrary to our prediction, BDM did not offset the relation between contractile state and unloaded, or even loaded, myocardial oxygen consumption.

Figure 4(c) shows the effect of contractile state on the slope of the MVO_2–PVA relation. Increases in contractile strength (p_{25}) significantly ($P=<0.001$) reduced the slope of the MVO_2–PVA relation. Furthermore, the slope of the MVO_2–PVA relation was not influenced by BDM independent of its impact on P_{25} ($P=0.7$).

Figure 5 shows the effect of $CaCl_2$ and BDM infusion on the relationship between contractile state and the parameters characterizing the duration of the pressure waveform. As in Figure 4, the data were normalized to the means of the parameters measured in each heart during $CaCl_2$ infusion only. $CaCl_2$ infusion caused both an increase in contractile state (as indexed by P_{25}) and a decrease in the duration of systole (TD). Although both the TTP and RELAX intervals significantly ($P<0.02$) decreased with increases in contractile state, the RELAX parameters was about 3 times more sensitive to contractile state than the TTP paprameter.

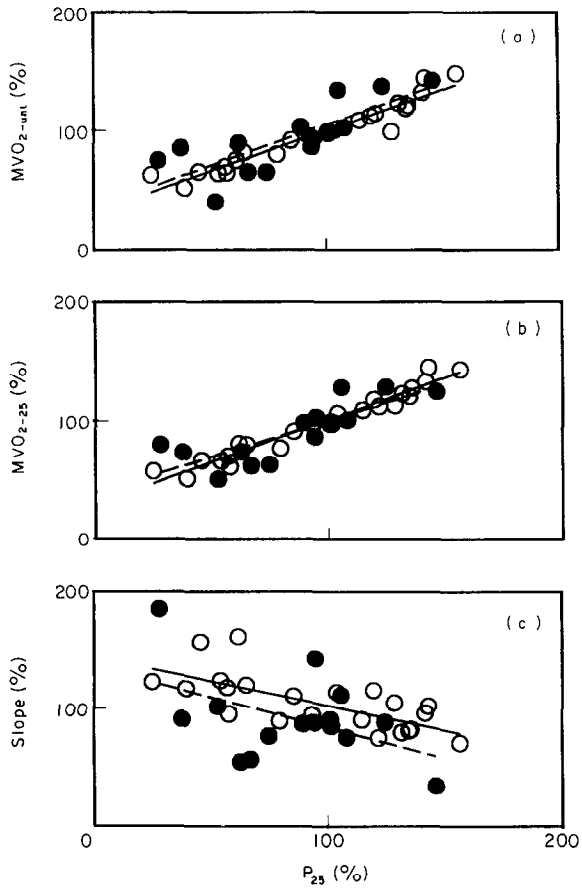

FIGURE 4. MVO_2–PVA parameters as function of contractile state in all hearts. Data from seven hearts. Contractile state is expressed as developed pressure at a reference ventricular volume of 25 ml (P_{25}). All data were normalized in each individual heart to the average result obtained with $CaCl_2$ infusion alone (see Methods). The average values used for normalization were: $P_{25} = 89.5 \pm 6.4$ mmHg, $MVO_{2-unl} = 0.50 \pm 0.02$ µl O_2/beat/g, $MVO_{2-25} = 0.65 \pm 0.04$ µl O_2/beat/g, MVO_2–PVA slope $= 2.63.10^{-5}$ ml O_2/mmHg.ml. This slope value corresponds to an average myofibrillar efficiency of 26%[3]. The data were fit by linear regression for both $CaCl_2$ infusion (open circles and solid lines; $n = 24$) and BDM infusion (closed circles and dashed lines; $n = 14$). Statistical analysis were performed by multiple linear regression analysis in which a dummy variable coded for $CaCl_2$ infusion or BDM infusion [23]. Similar analysis of the data before normalization did not affect the level of significance for any of the parameters (see also Methods).

(a) Relation between normalized unloaded oxygen consumption (MVO_{2-unl}) and normalized P_{25}. Neither the slope ($P = 0.9$) nor the elevation ($P = 0.8$) of this relation were affected by BDM. Combining the data from BDM and $CaCl_2$ infusions (overall linear regression) yielded: MVO_{2-unl} (%) $= 33.1 + 0.68 \cdot P_{25}$ ($r = 0.82$; $P < 0.001$).

(b) Relation between normalized oxygen consumption at 25 ml ventricular volume (MVO_{2-25}) and normalized P_{25}. Neither the slope ($P = 0.4$) nor the elevation ($P = 0.4$) of this relation were affected by BDM. Combining the data from BDM and $CaCl_2$ infusions (overall linear regression) yielded: MVO_{2-25} (%) $= 31.1 + 0.70 \cdot P_{25}$ ($r = 0.85$; $P < 0.001$).

(c) Relation between the slope of the normalized MVO_2–PVA relation and normalized P_{25}. Neither the slope ($P = 0.7$) nor the elevation ($P = 0.7$) of this relation were affected by BDM. Combining the data from BDM and $CaCl_2$ infusions (overall linear regression) yielded: slope (%) $= 137.2 - 0.42 \cdot P_{25}$ ($r = 0.35$; $P < 0.0001$).

Thus,the decrease in TD that was observed with increases in contractile state upon $CaCl_2$ infusion was due predominantly to an acceleration of myocardial relaxation with increases in contractile state.

BDM infusion caused a significant decrease in the duration of systole ($P < 0.01$), particularly at the higher rates of BDM infusion (and thus lower values of P_{25}). The decrease in TD upon BDM infusion was caused by both a

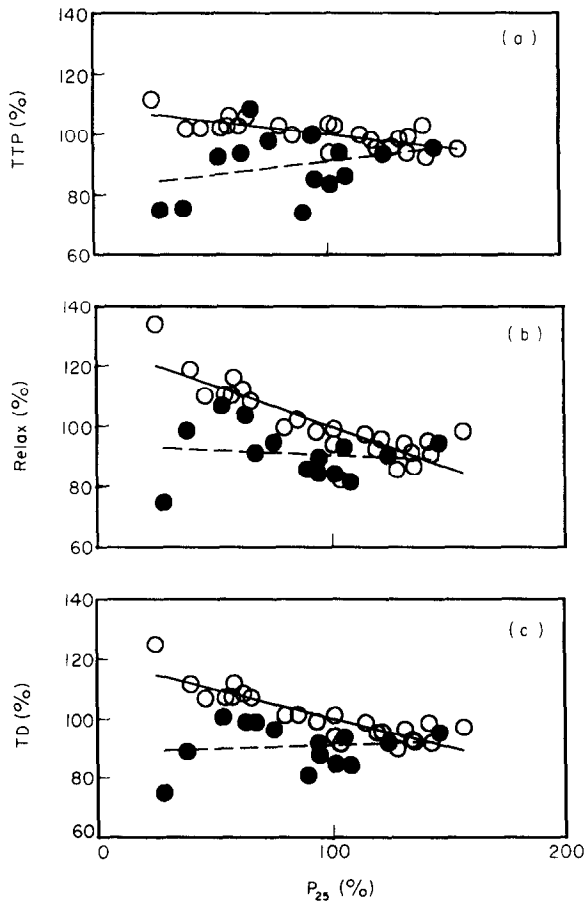

FIGURE 5. Effect of $CaCl_2$ and BDM on duration of the pressure waveform. Normalized data from seven hearts is displayed (Fig. 4 and Methods). TTP, RELAX and TD parameters are plotted as function of contractile state (P_{25}; for definition of TTP, RELAX and TD, see Methods). The data were fit by linear regression for $CaCl_2$ infusion (open circles and solid lines; $n = 24$) and BDM infusion (closed circles and dashed lines; $n = 14$). The average values used for normalization were: $P_{25} = 89.5 \pm 6.4$ mmHg, TD = 236 ± 4.7 ms, TTP = 104 ± 3.3 ms, RELAX = 132 ± 2.3 ms. Statistical analyses were performed by multiple linear regression in which a dummy variable coded for $CaCl_2$ infusion or BDM infusion [*23*].

(a) $CaCl_2$ TTP(%) = $109.0 - 0.089 \cdot P_{25}$
BDM TTP(%) = $81.7^* + 0.093^* \cdot P_{25}$
(c) $CaCl_2$ TTP(%) = $119.5 - 0.194 \cdot P_{25}$
BDM TTP(%) = $88.4^* + 0.025^* \cdot P_{25}$

(b) $CaCl_2$ TTP(%) = $127.1 - 0.271 \cdot P_{25}$
BDM TTP(%) = $93.8^* - 0.032^* \cdot P_{25}$

$^*P < 0.01$ $CaCl_2$ vs BDM by multiple linear regression.

decrease in the relaxation parameter (RELAX), and by a decrease in the TTP parameter. These results may indicate that BDM enhances the cycling rate of cross-bridges [*20*]. Alternatively, BDM may have caused abbreviation of excitation–contraction coupling at high dosages and thereby have caused a reduction in the duration of systole.

Discussion

In the present study we investigted the effect of BDM on the interrelationships between contractile state and oxygen consumption of the cross-circulated canine heart during β-blockade. BDM caused a dose-dependent decrease in contractile state. Contrary to our prediction however, we found that the relation between contractile state and unloaded myocardial oxygen consumption was not af-

fected by application of BDM to the heart. On the other hand, BDM infusion did cause a significant reduction in the duration of systole at matched contractility.

Some aspects of our results are comparable to those of Watkins *et al.* [*28*], who also found that BDM (3.5 mM) decreased the unloaded oxygen consumption of red-blood-cell perfused rabbit hearts. In contrast to the present study however, these investigators found that BDM caused a 21% reduction in the slope of the MVO_2–PVA relation (see below). However, that study was limited because no comparison was made between the effect of BDM and the effect of another inotropic agent (such as $CaCl_2$) on MVO_2. Thus, it could not be assessed whether the change in MVO_2 was simply dependent upon a change in contractile state, or whether there was an independent effect of BDM on MVO_2.

Unloaded myocardial oxygen consumption ($MVO_{2\text{-unl}}$) represents the amount of oxygen consumed by the heart to sustain basal metabolism and excitation–contraction coupling [*3*]. In addition there may also be energy expended mechanically to deform the unloaded heart, although this component is probably small [*32*]. It is generally assumed that any change in $MVO_{2\text{-unl}}$ with altered contractility is due to a change in the energy required for excitation–contraction coupling (i.e.the amount of calcium cycled during each beat) [*3*].

The failure of BDM to change the MVO_2–PVA relation independent of accompanying changes in contractile state (Figs 3 and 4) could be the result of several factors. First, BDM could alter the amount of calcium cycled during each beat. The effect of BDM on the intracellular calcium transient has been studied most extensively in isolated skeletal muscle by use of the photoprotein aequorin. Unfortunately, results of these studies have been conflicting, in that some studies have found a decrease of the calcium transient upon BDM application [*10, 27*], while others have found no effect [*11*]. In isolated rabbit myocardium, BDM up to about 4 mM has been reported to be without an affect on the calcium transient [*12*]. Similar experiments on isolated myocardium from other species however, have shown either a decrease [*20*] or an increase [*29*] in the cardiac calcium transient upon application of BDM.

A further complication, and perhaps of greater importance to the present study, is the fact that the recorded calcium transient does not measure the total amount of calcium cycled in the myocyte each beat [*30, 31*]. It should be recognized that if BDM were to decrease the myofibrillar affinity to calcium ions, one would actually expect an increase in unbound cytoplasmic calcium for a constant amount of calcium cycled each beat [*30*]. Hence, the reported actions of BDM on the calcium transient in myocardium [*12, 20, 29*] do not exclude an effect of BDM on the total amount of calcium cycles each beat. It is generally assumed that the oxygen consumed by the unloaded heart (i.e. $MVO_{2\text{-unl}}$) is proportional to the amount of calcium cycled for excitation–contraction coupling each beat [*3*]. Hence, the effect of BDM on myocardial oxygen consumption that we observed in the present study is consistent with the notion that BDM causes a reduction in the amount of calcium cycled each beat, possibly by reducing the influx of calcium into the myocytes during each beat.

A recent report by Gwathmey *et al.* [*20*] suggests that the desensitizing action of BDM in isolated cardiac muscle is due to reduced cross-bridge force development, and not due to reduced calcium binding to the myofilaments. The effect of BDM on the duration of systole, independent of the effects of contractile state *per se* (Fig. 5), would be consistent with such a mechanism because it indicates that BDM may affect the reaction rate of some step in the process of force development subsequent to bind in of calcium to the myofilaments. The energetic consequence of this effect of BDM in the present study however, appeared to be too small to be detectable by our method.

A second possible explanation of our finding could be related to the fact that even though the heart is externally unloaded at the volume at which the pressure–volume area equals zero, some energy is still required to deform the heart. Probably, the amount of deformation, and thus the energy required to accomplish this deformation, would increase in proportion to contractile state. It is difficult

to estimate the magnitude of the energy that is required for this deformational work, although indirect evidence has been put forward suggesting that this component of MVO_2 in the intact heart is small [3]. Since pressure development is minimal in the unloaded state, any energy consumed by the unloaded heart for mechanical work is utilized mostly to support shortening. Energy consumption for shortening *per se* has been shown to be minimal both in the intact heart [32] and in isolated cardiac muscle [6, 33].

Furthermore, for the purpose of the present study it is not necessary to know the exact magnitude of the deformational component of MVO_2, since we have directly compared the MVO_2–P_{25} relation between $CaCl_2$ and BDM infusion. That is, if some (unrecognized) fraction of $MVO_{2\text{-unl}}$ would be consumed to sustain deformational mechanical work of the heart, reduction of contractile state *per se* would then lead to a reduction in $MVO_{2\text{-unl}}$. However, if BDM were to reduce contractile state *without* the hypothesized reduction in calcium handling for EC-coupling, we would still detect a slope change in the $MVO_{2\text{-unl}}$–P_{25} relation compared to that observed for $CaCl_2$ infusion. Thus, only in the unlikely, limiting case in which the relation between P_{25} and $MVO_{2\text{-unl}}$ would be *entirely* due to the energy requirement of cardiac deformation, would administration of a "pure" myofibrillar calcium desensitizing agent be without an effect on the relation between P_{25} and $MVO_{2\text{-unl}}$. Our failure to find a shift in this relation implies that BDM cannot be acting as a calcium desensitizing agent in any energetically significant way, even if a measurable fraction of $MVO_{2\text{-unl}}$ were to be used for cardiac deformation. Consistent with this notion are the results shown in Figure 3(b) and 4(b). Here, we examined the effect of BDM on the relation between P_{25} and $MVO_{2\text{-}25}$, i.e. the amount of oxygen consumed by the heart at the reference volume of 25 ml, a parameter that is independent of the general framework of pressure–volume area.

It has been shown previously in the isolated canine heart that the magnitude of the increase in oxygen consumption associated with an increase in contractile state (i.e. the "energy cost" of contractility') is affected by the manner in which the positive inotropy is brought about [3]. That is, the "energy cost of contractility" is higher for epinephrine or $CaCl_2$ infusion, less for digitalis, and almost absent for cooling [3]. Similarly, it has been shown that the unloaded oxygen consumption ($MVO_{2\text{-unl}}$) in stunned myocardium is higher than that expected from the contractile state [34]. Thus, it is unlikely that the failure to detect a shift in the P_{25}–MVO_2 relation upon BDM infusion as compared to $CaCl_2$ infusion was due to an inherent lack of sensitivity of the energetic methods that we have employed in the present study. However, small changes in energy consumption by the heart induced by BDM in each beat could have been below the detection limit of our method.

During $CaCl_2$ infusion in this study, an increase in contractile state (i.e. P_{25}) correlated significantly with a reduction in the slope of the MVO_2–PVA relation in four out of seven hearts studied. This result differs from previous reports (for a recent review see Suga [3]. However, the range of contractile states explored in the present study was larger than those in the previous studies. Furthermore, in the present study we used multiple linear regression analysis [23], which greatly increased the sensitivity of the statistical tests employed. The slope of the MVO_2–PVA relation has been suggested to be inversely related to the chemo-mechanical efficiency of the myofilaments [3]. Hence, the data presented in Figure 4(c) implies that this efficiency is enhanced at higher levels of contractility. Further studies are required to investigate the mechanism by which contractility affects the chemomechanical efficiency of the heart.

BDM did not affect the relation between slope of the MVO_2–PVA relation and P_{25}. This implies that BDM does not affect the efficiency of the transduction between MVO_2 and mechanical energy produced by the heart independent of the effects of BDM on contractile state *per se* (i.e. compared to alteration in P_{25} by $CaCl_2$ infusion). This result is in contrast to the conclusion of Watkins *et al.* [28], who showed that the slope of the MVO_2–PVA relation was reduced 21% by BDM. This discrepancy could be due to dif-

ferences in animal species (rabbit *vs* canine) or experimental design between the two studies. For example, the present study was designed to compare the effects of BDM infusion to $CaCl_2$ infusion, which allowed us to discriminate between the effects of contractility *per se* and the effects of BDM.

In conclusion, the results of the present study indicate that BDM reduces both the unloaded (non-work related) and the loaded oxygen consumption of the heart. However, the relationships between contractile state and the parameters of the MVO_2–PVA relation were not influenced by BDM, which indicates that in the intact heart BDM does not act energetically as expected for a myofibrillar calcium desensitizing agent. This finding raises the possibility that a principle energetic mechanism of action of BDM in the isolated canine heart is to reduce the energy related to calcium handling for excitation–contraction coupling.

Acknowledgements

We would like to thank Mr Kenneth Rent for his excellent technical assistance and Dr Edward Lakatta for useful discussions. This study was supported, in part, by NIH research grant HL–18912, and by Postdoctoral Fellowships from the American Heart Association, Maryland Affiliate to Drs de Tombe and Burkhoff.

References

1 Suga, H., Hisano, R., Goto, Y., Yamada, O., Igarashi, Y. Effect of positive inotropic agents on the relation between oxygen consumption and systolic pressure volume area in canine left ventricle. Circ Res **53**: 306–318 (1983).
2 Burkhoff, D., Yue, D. T., Oikawa, R. W., Franz, M. R., Schaefer, J., Sagawa, K. Influence of ventricular contractility on non-work related myocardial oxygen consumption. Heart Vessels **3**: 66–72 (1987).
3 Suga, H. Ventricular energetics. Physiol Rev **709**: 247–277 (1990).
4 Allen, D. G., Kurihara, S. Calcium transients in mammalian ventricular muscle. Eur Heart J **1**(Suppl A): 5–15 (1980).
5 Kurihara, S., Konishi, M. Effects of β-adrenoceptor stimulation on intracellular Ca transients and tension in rat ventricular muscle. Pflügers Arch **409**: 427–437 (1987).
6 Gibbs, C. L., Loiselle, D. S., Wendt, I. R. Activation heat in rabbit cardiac muscle. J. Physiol (Lond) **395**: 115–130 (1988).
7 Solaro, R. J., Wise, R. M., Shiner, J. S., Briggs, F. N. Calcium requirements for cardiac myofibrillar activation. Circ Res **34**: 525–530 (1974).
8 Lee, J. A., Allen, D. G. Calcium sensitisers. A new approach to increasing the strength of the heart. Br Med J **300**: 551–552 (1990).
9 Li, T., Sperelakis, N., Teneick, R. E., Solaro, R. J. Effects of diacetyl monoxime on cardiac excitation–contraction coupling. J Pharmacol Exp Ther **232**: 688–695 (1985).
10 Fryer, M. W., Neering, I. R., Stephenson, D. G. Effects of 2,3-butanedione monoxime on the contractile activation properties of fast- and slow-twitch rat muscle fibres. J Physiol (Lond) **407**: 53–75 (1988).
11 Horiuti, K., Higuchi, H., Umazume, Y., Konishi, M., Okazaki, O., Kurihara, S. Mechanism of action of 2,3-butanedione 2-monoxime on contraction of frog skeletal muscle fibres. J Muscle Res Cell Motil **9**: 156–164 (1988).
12 Blanchard, E. M., Smith, G. L., Allen, D. G., Alpert, N. R. The effects of 2,3-butanedione monoxime on initial heat, tension, and aequorin light output of ferret papillary muscles. Pflügers Arch **416**: 219–221 (1990).
13 de Tombe, P. P., Burkhoff, D., Hunter, W. C. Effects of calcium and 2,3-butanedione monoxime on non-work-related oxygen consumption in isolated hearts. (*Abstract*) Biophysical J **59**: 421a (1991).
14 Suga, H., Sagawa, K. Instantaneous pressure–volume relationships and their ratio in the excised, supported canine left ventricle. Circ Res **35**: 117–126 (1974).
15 Burkhoff, D., de Tombe, P. P., Hunter, W. C., Kass, D. A. Contractile strength and mechanical efficiency of left ventricle are enhanced by physiological afterload. Am J Physiol **260**: H569–H578 (1991).
16 Solaro, R. J., Moir, A. J., Perry, S. V. Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. Nature **262**: 615–617 (1976).
17 Moir, T. W., Driscol, T. E., Eckstein, R. W. Thebesian drainage in the left heart of the dog. Circ Res **14**: 245–249 (1964).
18 Sheppard, A. P., Burger, C. G. A solid-state arteriovenous oxygen difference analyzer for flowing whole blood. Am J Physiol **232**: H437–H440 (1977).
19 Burkhoff, D., Sugiura, S., Yue, D. T., Sagawa, K. Contractility dependent curvilinearity of end-systolic pressure–volume relations. Am J Physiol **252**: H1218–H1227 (1987).

20 Gwathmey, J. K., Hajjar, R. J., Solaro, R. J. Contractile deactivation and uncoupling of cross-bridges: Effects of 2,3-butanedione monoxime on mammalian myocardium. Circ Res **69**: 1280–1292 (1991).
21 Gregg, D. E. Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. Circ Res **13**: 497–500 (1963).
22 Goto, Y., Slinker, B., LeWinter, M. M. Effect of coronary hyperemia on E_{max} and oxygen consumption in blood-perfused rabbit hearts. Energetic consequences of Gregg's phenomenon. Circ Res **68**: 482–492 (1991).
23 Slinker, B. K., Glantz, S. A. Multiple linear regression is a useful alternative to traditional analyses of variance. Am J Physiol **255**: R353–R367 (1988).
24 Packer, C. S., Kagan, M. L., Kagan, J. F., Robertson, S. A., Stephens, N. L. The effect of 2,3-butanedione monoxime (BDM) on smooth muscle mechanical properties. Pflügers Arch **412**: 659–664 (1988).
25 Wiggens, J. R., Reiser, J., Fitzpatrick, D. F., Bergey, J. L. Inotropic actions of diacetyl monoxime in cat ventricular muscle. J Pharmacol Exp Ther **212**: 217–224 (1980).
26 Alpert, N. R., Blanchard, E. M., Mulieri, L. A. Tension-independent heat in rabbit papillary muscle. J Physiol (Lond) **414**: 433–453 (1989).
27 Fryer, M. W., Gage, P. W., Neering, I. W., Dullhunty, A. F., Lamb, G. D. Paralysis of skeletal muscle by butanedione monoxime, a chemical phosphatase. Pflügers Arch **411**: 76–79 (1988).
28 Watkins, M. W., Slinker, B. K., LeWinter, M. M. 2,3-butanedione monoxime (BDM) increases the efficiency of chemomechanical energy transduction in the rabbit ventricle. (*Abstract*) Circulation **82**(Suppl 3): 564 (1990).
29 Marijic, J., Buljabasic, N., Stowe, D. F., Turner, L. A., Kampine, J. P., Bosnjak, Z. J. Opposing effects of diacetyl monoxime on contractility and calcium transients in isolated myocardium. Am J Physiol **260**: H1153–H1159 (1991).
30 Suga, H., Goto, Y., Futaki, S., Kawaguchi, O., Yaku, H., Hata, H., Takasago, T. Calcium kinetics and energetics in myocardium simulation study. Jpn Heart J **32**: 57–67 (1991).
31 Sipido, K. R., Wier, W. G. Flux of Ca^{2+} across the sarcoplasmic reticulum of guinea-pig cardiac cells during excitation–contraction coupling. J Physiol (Lond) **435**: 605–630 (1991).
32 Yasumura, Y., Nozawa, T., Futaki, S., Tanaka, N., Suga, H. Minor preload dependence of O_2 consumption of unloaded contraction in dog heart. Am J Physiol **256**: H1289–H1294 (1989).
33 Hisano, R., Cooper, G. Correlation of force–length area with oxygen consumption in ferret papillary muscle. Circ Res **61**: 318–328 (1987).
34 Ohgoshi, Y., Goto, Y., Futaki, S., Yaku, H., Kawaguchi, O., Suga, H. Increased oxygen cost of contractility in stunned myocardium of dog. Circ Res **69**: 975–988 (1991).