Interpretation of the Inotropic Effect of 2,3-Butanedione Monoxime on the Isometric Twitch of Guinea-pig Papillary Muscle

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Abstract. The negative inotropic effect of 2,3-butanedione monoxime (BDM) on the isometric twitch of guinea-pig papillary muscle was analysed by parameters characterizing the time course of the mechanogram. BDM at concentrations of up to 4 mmol/l produced a clear negative inotropic effect, whereas the Ca transient measured in isolated cardiomyocytes was only slightly affected. Peak force was more reduced than dF/dt_{max} and dF/dt_{min}. This led to an earlier, more narrow peak and a shortening of twitch duration. Based on a reaction scheme for the cross-bridge cycle, a mathematical model using a Ca transient and mechanograms as input data has been developed. The kinetic parameters were estimated by fitting the model to various time courses of force obtained at rising concentrations of BDM. BDM decreased the ratio of rate constants for cross-bridge attachment and detachment in a concentration-dependent manner: the formation of cross-bridges became inhibited, whereas dissociation was promoted. Above 4 mmol/l BDM the more marked alterations of the parameters of the mechanogram indicated an additional suppressing effect on intracellular Ca supply. The computer analysis suggests how the cellular mechanism(s) of the BDM-induced negative inotropic effect are reflected in the time course of the mechanogram.

Key words: 2,3-Butanedione monoxime — Ca transient — Isometric contraction — Mathematical model — Guinea-pig papillary muscle

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Introduction

Force development of the myocardium is determined by inotropic mechanisms influencing the release and the removal of intracellular Ca (Ca_i) and the sensitivity of the myofilaments for Ca. The time course of force is modified in a characteristic manner, i.e. different inotropic mechanisms show a characteristic pattern of parameters of the mechanogram (Bogdanov et al. 1979; Honoré et al. 1987; Gross et al. 1989). A computer programme was developed to estimate parameters for contraction and relaxation which can be used to characterize inotropic effects on isolated cardiac preparations (Lammerich 1992).

The intracellular Ca transient results from processes regulating the Ca homoeostasis. Inotropic effects with an only modest modification of Ca_i supply should have affected the utilization of Ca_i by the myofilaments. Fulfilling this, the negative inotropic effect of low BDM concentration on the myocardium of several species (Blanchard et al. 1990; Perreault et al. 1992; Spurgeon et al. 1992; Steele and Smith 1993; Kotsanas et al. 1993; Backx et al. 1994) and on that of the guinea-pig (Marijic et al. 1991; Gambassi et al. 1993) is not attributed to insufficient Ca_i supply (but cf. Gwathmey et al. 1991). We measured nearly unchanged Ca transients in guinea-pig cardiomyocytes at low BDM concentration. As a consequence, alterations in Ca sensitivity of the myofilaments have to be expected. This should be recognizable by a detailed analysis of the mechanogram. In order to interpret the changes in the time course of a twitch appearing at low concentrations of BDM, a reaction scheme was employed including the main steps of force development within the sarcomere (Yue 1987) instead of more complicated and complex approaches (Backx et al. 1994; Zhao and Kawai 1994). A mathematical model using a Ca transient and mechanograms as input data has been developed to calculate the rate constants for cross-bridge attachment and detachment as the essential determinants for the alteration of force development due to BDM.

Materials and Methods

The experiments were performed on 10 guinea-pig right ventricular papillary muscles. The bathing solution, oxygenated with pure O_2 at 31 °C, had the following composition (in mmol/l): NaCl 140, KCl 5.4, $CaCl_2$ 0.5, $MgCl_2$ 1.1, Tris-HCl 10.0, glucose 11.1 at pH 7.43.

Electrical stimulation and measurement of isometric force F at a preload of 2 mN were performed using the programmable stimulator module PSM 676 and the force transducer F10 connected to the bridge amplifier DBA 660 of the Plugsys system 603 (Hugo Sachs Elektronik, Germany). The analogue force signal was digitized and stored by a personal computer. The preparations were stimulated with biphasic rectangular impulses of 10 ms duration and a strength of 30% above threshold.

At a stimulation frequency of 0.25 Hz, peak force (PF) became stable after about 25 min. During the subsequent experiment at a stimulation frequency of 0.5 Hz, extra-

cellular Ca was elevated up to 18 mmol/l by adding $CaCl_2$ stock solution in order to reach maximum PF as starting-point for the expected negative inotropic effect of BDM (purchased from Sigma). [BDM] was gradually increased as seen in the results.

Osmolarity was not compensated after the addition of Ca, because only extreme changes of intracellular volume and of ionic strength are known to influence the cross-bridge cycling (Allen and Smith 1987). Therefore, high extracellular Ca concentration is frequently used without compensation of osmolarity (e.g. Gwathmey et al.; Marijic et al. 1991) to avoid other consequences of disturbed distribution of physiologically important ionic concentrations.

Furthermore, these control conditions remain constant throughout the whole following protocol. BDM is likely to be distributed uniformly in the intra- and extracellular fluid, therefore its effects should not be modified by a shift of water.

Isolated cardiomyocytes were prepared by enzymatic dissociation according to Lewartowski et al. (1994). For the measurement of Ca transients cells were loaded with indo-1-ester (Lewartowski et al. 1994). Indo-1 Ca transients were monitored as a ratio 405/495 during rhythmical field stimulation.

Software

The software in Turbo Pascal developed in our laboratory (Lammerich 1992) includes control of the experimental setup and data acquisition, the analysis of the mechanogram and statistics. A programme for control of the experiment and data acquisition permits stimulation with any chosen stimulus by the Plugsys module PSM 676. The registration of force for a desired number of twitches is synchronously started with the stimulus. The experimental protocol can be preselected. Support for calibration and adjustment of the base line is available. Values of PF and contraction curves are shown on the screen, condensed and stored. All information concerning the experimental procedure is saved in a protocol file allowing a fast survey of the experiment.

Analysis

The following parameters for contraction and relaxation were estimated from the filtered and averaged data by the analysis programme:
PF- peak isometric force; dF/dt_{max} – maximum dF/dt; dF/dt_{min} – minimum dF/dt; TL – time of latency; $T50$ – time to 50% of PF; TPF – time to peak force; $RT50$ and $RT97$ – relaxation time for the decline of force by 50% and 97% of PF, respectively; $T(dF/dt_{max})$ – time at dF/dt_{max}; $(dF/dt_{max})/PF$; $(dF/dt_{min})/PF$; $(dF/dt_{max})/(dF/dt_{min})$; $(dF/dt_{max})/F$, $(dF/dt_{min})/F$ – quotient by dF/dt_{max} and dF/dt_{min}, respectively, and the corresponding instantaneous force; CPC – coefficient for the phasic component of the contraction, being the quotient of maximum and mean rate of force development: $CPC = (dF/dt_{max}) \cdot (TPF - TL)/PF$; CPR – coefficient for the phasic component of the relaxation, being the quotient of maximum and mean rate of force decline: $CPR = (dF/dt_{min}) \cdot RT97/PF$.

The 0.1% level of PF served as the onset of force development at TL.

The dF/dt signal was calculated by a tested algorithm (Lammerich 1992) resulting in errors of about 1%. The dF/dt signal yielded dF/dt_{max}, dF/dt_{min}, $T(dF/dt_{max})$ and $T(dF/dt_{min})$.

The appearance of an early phasic and late tonic component during the isometric contraction was assumed to arise from Ca_i being delivered from different sources during

activation (Bogdanov et al. 1979; Honoré et al. 1987). The relation between dF/dt_{max}, representing the phasic component, and the mean rate of force development, representing both phasic and tonic components, was expressed as the coefficient of the phasic components of the contraction (*CPC*). The phasic behaviour of the relaxation, expressed by *CPR*, was determined by various interactions of mechanisms leading to detachment of cross-bridges (Brutsaert and Sys 1989).

The results are given as mean value ±SEM together with the results of the statistical proof by the Wilcoxon test for independent samples at $p \pm 0.05$.

Scheme for cross-bridge cycling

The following scheme (Yue 1987), describing 4 states (S1..S4) of the cross-bridge cycle, was used to relate the effects of BDM on the isometric mechanogram to alterations of cross-bridge kinetics. To quantify these suggested alterations, we employed a mathematical model based on the scheme (see the appendix).

$$\begin{array}{ccc} \text{(S1)} & k_1 & \text{(S2)} \\ \text{Ca + TA} & \rightleftharpoons & \text{Ca-TA} \\ & k_{-1} & \\ \uparrow\ g & & \uparrow\ g' \downarrow\ f \\ & k_2 & \\ \text{Ca + TA-M} & \rightleftharpoons & \text{Ca-TA-M} \\ \text{(S4)} & k_{-2} & \text{(S3)} \end{array}$$

Force F is assumed to be proportional to the instantaneously existing number of myosin- (M) actin- (A) tioponin- (T) complexes in S3 and S4, i.e. $F \sim$ [Ca-TA-M] + [TA-M].

At the resting level of Ca_i, A and M are separated in S1. Synchronously with increasing Ca_i, Ca binds to T (Housmans 1991; Peterson et al. 1991), because the high values for k_1 and k_{-1} lead rapidly to an equilibrium between S1 and S2. S2 dominates because of $k_1 \gg k_{-1}$. Ca-TA is a prerequisite for the binding of M to A and for the delayed transition into the force producing state S3, corresponding to a low value of f. The constant g' is so low that the reverse reaction is virtually impossible (Yue 1987). Thus, the transition S2→S3 also occurs at low Ca_i and low [Ca-TA] at low rate. The instantaneous value of dF/dt is the difference between the rate of cross-bridge attachment, expressed by dF/dt_+, and detachment, expressed by dF/dt_-. It follows:

$$\begin{aligned} dF/dt &= dF/dt_+ - dF/dt_- \sim \frac{d[\text{Ca-TA-M}]}{dt} + \frac{d[\text{TA-M}]}{dt} \\ dF/dt_+ &\sim \frac{k_1}{k_{-1}} f\,[\text{Ca}]\,[\text{TA}] \end{aligned} \tag{1}$$

The detachment of active cross-bridges requires dissociation of Ca from TnC (S4). S3 is favoured in the equilibrium between S3 and S4 by the high affinity of TnC for Ca ($k_2 \gg k_{-2}$). Therefore, the dissociation of Ca from TnC is only possible at very low Ca_i. The decline of the number of active cross-bridges is expressed by:

$$dF/dt_- \sim \frac{k_{-2}}{k_2}\, g\,[\text{Ca-TA-M}] \tag{2}$$

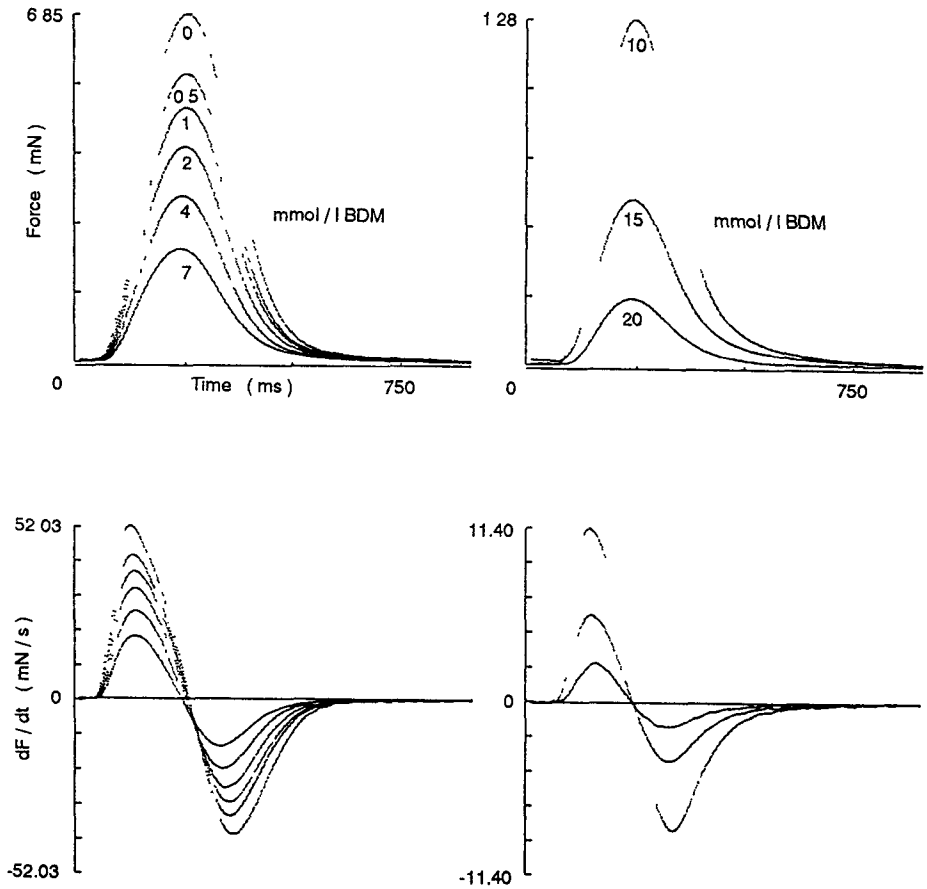


Figure 1. Typical negative inotropic effect of increasing BDM concentrations on the isometric mechanogram of guinea-pig papillary muscle at 18 mmol/l extracellular Ca (experiment MBD 10).

The value dF/dt_{max} should mainly be determined by dF/dt_+ when the detachment rate is still negligible, and analogous dF/dt_{min} by dF/dt_- when the attachment rate is expected to be already very low. PF is reached when the rates of attachment and detachment of cross-bridges become equal.

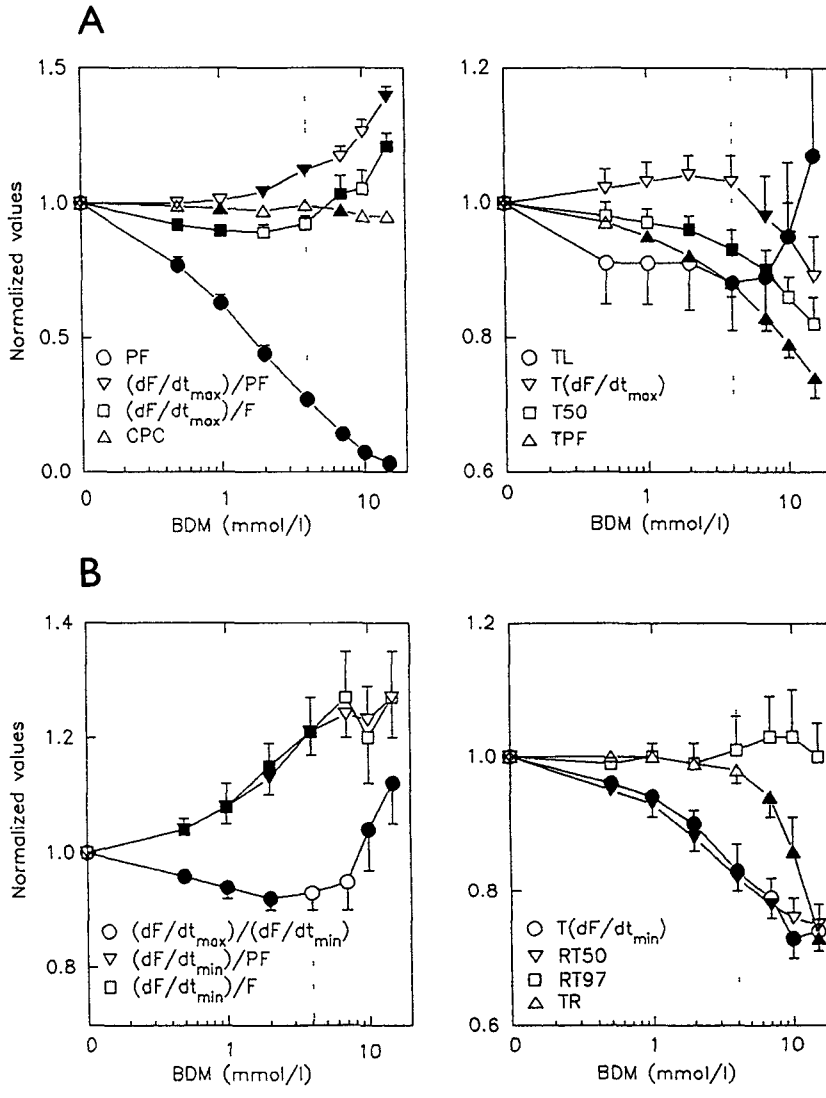
Results

In papillary muscles PF reached a mean value of 7.8 mN at 18 mmol/l Ca_e. The cumulative addition of BDM produced a clear concentration-dependent negative inotropic effect (Fig. 1). The amplitude of twitch was more influenced than its time course.

Relative changes of isometric twitch parameters produced by BDM are shown in Fig. 2. Up to 4 mmol/l BDM, $T(\mathrm{d}F/\mathrm{d}t_{\max})$, TL and CPC remained nearly constant. BDM shortened TPF mainly at the cost of $TPF - T50$. The increase in $(\mathrm{d}F/\mathrm{d}t_{\max})/PF$ demonstrated a more prominent decrease of PF compared with $\mathrm{d}F/\mathrm{d}t_{\max}$, whereas $(\mathrm{d}F/\mathrm{d}t_{\max})/F$ remained unchanged.

$T(\mathrm{d}F/\mathrm{d}t_{\min})$ and $RT50$, representing the early relaxation, were shortened. Together with the late phase of contraction, this resulted in the narrow peak of the twitch (Figs. 1 and 3).

$RT97$ was hardly influenced. The decrease of PF and of corresponding instan-



taneous F was greater than that of dF/dt_{min}, resulting in a similar enhancement of $(dF/dt_{min})/PF$ and $(dF/dt_{min})/F$.

At BDM concentrations exceeding 4 mmol/l additional effects appeared. The mechanical refractory period TR, i.e. the shortest stimulus interval eliciting a new contraction, was diminished.

Concerning the contraction, $(dF/dt_{max})/F$ and TL were increased, whereas $T(dF/dt_{max})$ and CPC were decreased.

Concerning the relaxation, $(dF/dt_{min})/PF$, $(dF/dt_{min})/F$, $T(dF/dt_{min})$, $RT50$ and CPR remained relatively constant. After a decrease at low BDM concentration, $(dF/dt_{max})/(dF/dt_{min})$ rose with increasing [BDM].

In isolated cardiomyocytes 4 mmol/l BDM depressed cellular contraction by about 80% (Fig. 4), i.e. to a similar extent as PF in papillary muscle (Fig. 1). The amplitude and the time course of the Ca transients (Fig. 4) as indices of the change in Ca_i were less affected as reported by others (Marijic et al. 1991; Gambassi et al. 1993).

The rate constants f for attachment and g for detachment of cross-bridges were estimated by a fitting procedure (see the appendix) from 9 mechanograms of

Figure 2. Mean normalized values and standard errors of parameters for the time courses of contraction (A) and relaxation (B) of guinea-pig papillary muscle at 18 mmol/l extracellular Ca as influenced by rising concentration of BDM; $n = 10$. Statistical significance versus the preceding value is marked by filled symbols. CPR is not shown in the figure because of its close correspondence to $(dF/dt_{min})/PF$ at constant $RT97$. Absolute values of parameters for the time courses of contraction (A) and relaxation (B) at 18 mmol/l extracellular Ca before the addition of BDM serving as control (corresponding to 1 as normalized values).

A

	PF	dF/dt_{max}	$\frac{dF/dt_{max}}{PF}$	$\frac{dF/dt_{max}}{F}$	TL	$T(dF/dt_{max})$	$T50$	TPF	CPC
	mN	mN/s	1/s	1/s	ms	ms	ms	ms	
mean	7.84	61.73	7.89	20.63	38.8	110.4	125.2	240.4	1.59
±SEM	0.95	7.65	0.14	0.60	4.1	5.4	4.5	4.6	0.01

B

	dF/dt_{min}	$\frac{dF/dt_{max}}{dF/dt_{min}}$	$\frac{dF/dt_{min}}{PF}$	$\frac{dF/dt_{min}}{F}$	$T(dF/dt_{min})$	$RT50$	$RT97$	CPR	TR
	mN/s		1/s	1/s	ms	ms	ms		ms
mean	43.68	1.40	5.69	8.91	98.8	125.4	289.8	1.64	243.0
±SEM	4.78	0.05	0.22	0.47	3.4	3.5	7.3	0.03	5.6

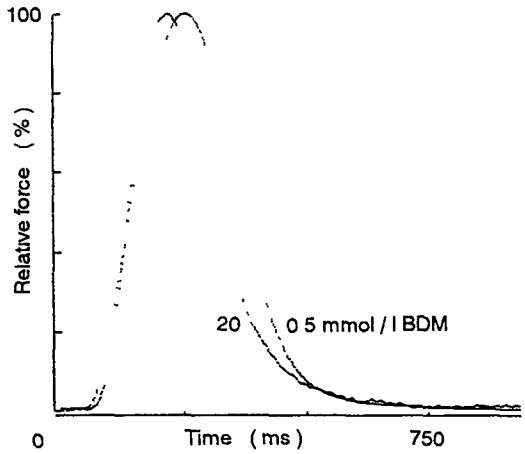


Figure 3. Comparison of the normalized mechanograms of guinea-pig papillary muscle at 0.5 and 20 mmol/l BDM (experiment MBD 10)

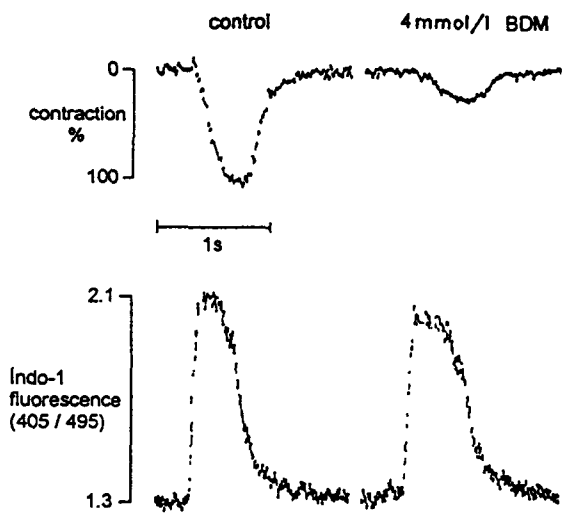


Figure 4. Example of a Ca transient and contraction of isolated guinea-pig cardiomyocyte at 0 (control) and 4 mmol/l BDM. Under these conditions the contraction amplitude was reduced to 20.2 ± 1 8%, whereas the nearly unchanged Ca transient reached an amplitude of 106.1 ± 3 6% (mean values ±SEM compared to control = 100%; $n = 6$).

a typical experiment recorded at rising concentrations of BDM from 0 up to 20 mmol/l. These mechanograms were combined with a physiological Ca transient measured in isolated guinea-pig cardiomyocytes in the absence of BDM (Fig. 5) which fulfils two important characteristics for the fitting procedure, e.g. the coincidence between peak Ca_i (Ca_imax) and $T(dF/dt_{max})$ (Yue 1987) and the slow decline of Ca_i. This approach based on the cross-bridge scheme yielded fitted curves in close conformity with the original signals (Fig. 6). Low BDM induced a decrease of f and an increase of g to a similar extent, leading to a clear decrease of their ratio (Fig. 7).

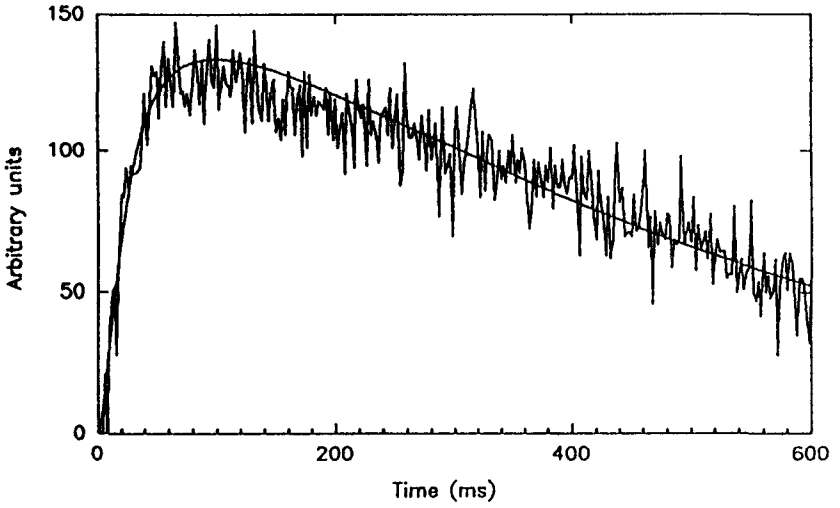


Figure 5. Intracellular calcium under normal conditions (no BDM). The experimental data were fitted to the phenomenological expression (6) (see appendix).

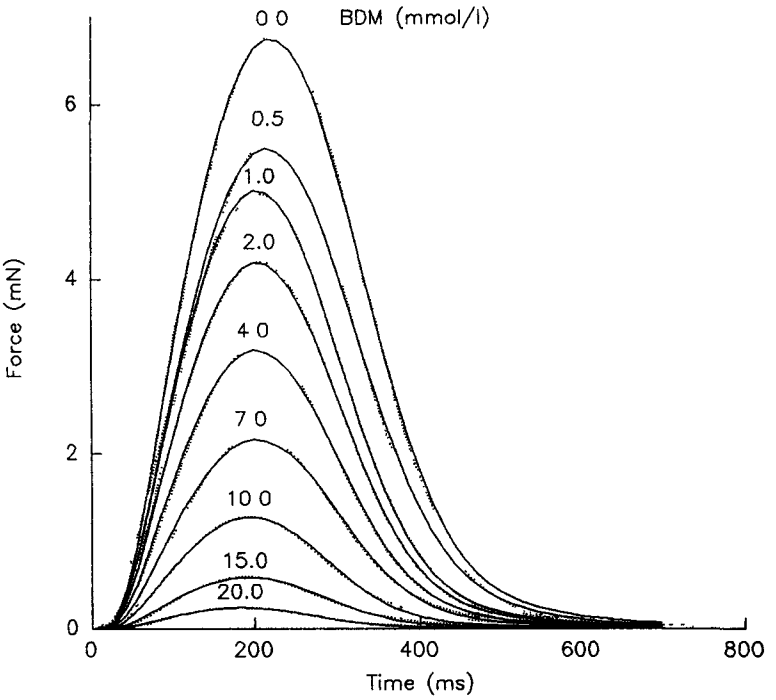


Figure 6. Mechanograms recorded at various concentrations of BDM (dotted line) and fitted solutions of differential equation (5) (solid line) (see appendix).

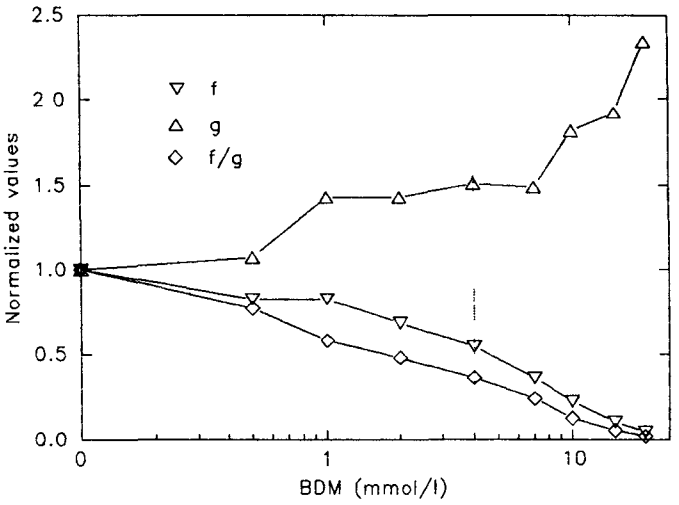


Figure 7. Change of the rate constants f and g at increasing concentrations of BDM. Plot of the relative rate constants f and g (normalized to their values without BDM). There is a monotonous decline of the rate constant f, whereas the rate constant g after abrupt increase at low concentration of BDM remains practically constant for BDM concentrations of up to 8 mmol/l. The absolute values are:

BDM (mmol/l)	0.0	0.5	1.0	2.0	4.0	7.0	10.0	15.0	20.0
f (1/s)	3.5	2.9	2.9	2.4	1.9	1.25	0.79	0.37	0.16
g (1/s)	25.2	27.0	36.0	36.0	38.0	37.50	45.70	48.40	59.00

Discussion

Suppression of PF and modifications of the mechanogram observed at low concentrations of BDM (up to 4 mmol/l) are regarded to be related to its effects on the Ca sensitivity of the myofilaments, because within this range the Ca_i transient was not reduced in papillary muscle of the guinea-pig (Marijic et al.1991) as confirmed by our experiments in isolated cardiomyocytes.

An assumed decrease in the affinity of TnC for Ca (Gwathmey and Solaro 1990; Wang and Lee 1990; Marijic et al. 1991) was not confirmed by direct measurement of Ca binding to TnC under BDM in heart (Mulieri and Alpert 1984; Gwathmey et al. 1991) and skeletal muscle (Fuchs and Wang 1991). Therefore, other components involved in the cross-bridge kinetics are most probably responsible (Li et al. 1985; Higuchi and Takemori 1989; West and Stephenson 1989; Kurihara et al. 1990; Dantzig et al. 1991; Herrmann et al. 1992; Perreault et al. 1992; Gambassi et al. 1993; Venema et al. 1993; Backx et al. 1994; Zhao and Kawai 1994).

Contraction

$T(\mathrm{d}F/\mathrm{d}t_{\max})$ reflects the moment of Ca_imax (Yue 1987; Housmans 1991; Peterson et al.1991), because maximum $[Ca_i]$ produces maximum $\mathrm{d}F/\mathrm{d}t_+$, i.e. $\mathrm{d}F/\mathrm{d}t_{\max}$ (cf. equation 1). Therefore, the constant value of $T(\mathrm{d}F/\mathrm{d}t_{\max})$ at BDM concentrations of up to 4 mmol/l is well in agreement with a less influenced Ca_i transient measured in isolated cardiomyocytes. The unchanged value of CPC means that at low BDM concentration the sources of Ca are not significantly influenced.

Assuming a reduced Ca sensitivity of myofilaments under BDM, a higher Ca_i would be required to reach the mechanical threshold, accompanied by prolonged TL, which is indeed measurable at high BDM concentrations.

At low Ca sensitivity, expressed by low ratio of f and g, the fall in Ca_i after $T(\mathrm{d}F/\mathrm{d}t_{\max})$ and Ca_imax, respectively, (Yue 1987; Housmans 1991; Peterson et al. 1991) decelerates further increase in force. When the diminished rate of cross-bridge attachment is exceeded by the rising rate of detachment, PF will be reached earlier. Thus, points of time following $T(\mathrm{d}F/\mathrm{d}t_{\max})$, like $T50$ and TPF, are shifted towards $T(\mathrm{d}F/\mathrm{d}t_{\max})$, as especially seen for the interval between $T50$ and TPF.

Both the earlier termination of the increase in force and the lower $\mathrm{d}F/\mathrm{d}t_+$ are responsible for the strong negative inotropic effect of BDM, as seen by the discrepancy between $(\mathrm{d}F/\mathrm{d}t_{\max})/\mathrm{F}$ and $(\mathrm{d}F/\mathrm{d}t_{\max})/PF$. The reduced rate constant f causes a slower transition into force generating states, followed by a decline of $\mathrm{d}F/\mathrm{d}t_+$ (equation 1). Then $\mathrm{d}F/\mathrm{d}t_{\max}$ and F are reduced in proportion after an identical $T(\mathrm{d}F/\mathrm{d}t_{\max})$. Consequently, $(\mathrm{d}F/\mathrm{d}t_{\max})/F$ remains nearly constant.

The stronger decrease in PF compared to $\mathrm{d}F/\mathrm{d}t_{\max}$ increases $(\mathrm{d}F/\mathrm{d}t_{\max})/PF$. The constant value of CPC, defined as $CPC = (\mathrm{d}F/\mathrm{d}t_{\max})/PF \cdot (TPF - TL)$, means that the increase in $(\mathrm{d}F/\mathrm{d}t_{\max})/PF$ is compensated by the decrease in $(TPF - TL)$. The same is seen in the normalized curves under high BDM (Fig. 3), where, after a prolonged TL, the peak force is reached earlier, i.e. $\mathrm{d}F/\mathrm{d}t$ remains relatively greater. Therefore, the earlier termination of the increase in F and the lower $\mathrm{d}F/\mathrm{d}t$ interact synergistically.

In the early termination of the increase in force, two processes are involved. The BDM dependent decline of f reduces the rate of the formation of active cross-bridges (equation 1). Otherwise, the increase in g indicates a higher rate of the dissociation of active cross-bridges (equation 2). Thus, the formation and dissociation of cross-bridges become already equal at higher Ca_i. The shift of contraction to relaxation appears therefore earlier, at unchanged Ca_i.

Relaxation

The decreases in $T(\mathrm{d}F/\mathrm{d}t_{\min})$ and $RT50$ demonstrate the short duration of the early relaxation. $\mathrm{d}F/\mathrm{d}t_{\min}$ is reached when F is $0.66 \cdot PF$, given by $F/PF = 0.66$ with a correlation coefficient of $r = 0.99$. Obviously, F and PF are influenced in the same manner by the lowered Ca sensitivity of myofilaments.

The shortening of $T(dF/dt_{min})$ is linearly related to the increases in $(dF/dt_{min})/PF$ and $(dF/dt_{min})/F$ ($r = 0.7$). The lower diminishing of dF/dt_{min} compared with PF and F leads to a faster decrease in force to $F = 0.66 \cdot PF$ at $T(dF/dt_{min})$. Consequently, F corresponds to a relatively higher dF/dt_{min} under BDM, and the percentage of active cross-bridges detaching per time grows.

During relaxation, the decline of Ca_i proceeds and, as seen in the reaction scheme, the formation of cross-bridges becomes more and more negligible. Relaxation then mainly depends on the detachment of the existing cross-bridges. The increase in $(dF/dt_{min})/F$ with increasing BDM concentrations means that, related to the corresponding F (e.g. normalized to the number of active cross-bridges at $0.66 \cdot PF$), the rate of cross-bridge detachment grows. This is possible, if the rate constant g is elevated (equation 2). Therefore, dF/dt_- is enhanced synergistically to a diminishing of dF/dt_+ by BDM.

Comparing the maximum rates of formation and detachment of cross-bridges, low BDM diminishes dF/dt_{max} more than dF/dt_{min}, as seen by the decrease in $(dF/dt_{max})/(dF/dt_{min})$. This also indicates a reduced ratio of f and g.

High BDM concentration

At BDM concentrations exceeding 4 mmol/l, most of the twitch parameters are markedly influenced. The shortening of $T(dF/dt_{max})$ suggests a shortening of the time to peak of Ca_i (Yue 1987). The shorter $T(dF/dt_{max})$ is explained by a probably reduced Ca_i supply (Li et al. 1985; Horiutu et al. 1988; Chapman 1993; Liu et al. 1993; Steele and Smith 1993) at shorter action potential duration (Chapman 1993; Liu et al. 1993) together with an earlier onset of removal of Ca from the cytosol brought about by the Ca outward transport via Na/Ca exchange (Brutsaert and Sys 1989). In the guinea-pig myocardium, release and reuptake of Ca by the SR is less developed (Horackova 1989) and additionally inhibited by high BDM in myocardium (Steele and Smith 1993). On the other hand, the Na/Ca exchange is the main mechanism for the removal of Ca_i (Horackova 1989). Therefore, influences on its driving force are expected to become visible in the mechanogram. A shortening of the action potential by high BDM concentrations (Li et al. 1985; Chapman 1993; Liu et al. 1993) is in agreement with the significant shortening of the mechanical refractory period TR. Thereby the reversal of Ca entry to Ca outward transport starts earlier, followed by an earlier peak of Ca_i and an earlier onset of its decline.

Under high BDM concentrations the formation and detachment of cross-bridges, due to the lowered ratio of f and g, was modified by a diminished but long lasting Ca transient (Blanchard et al. 1990; Perreault et al. 1992; Spurgeon et al. 1992; Gambassi et al. 1993; Kotsanas et al. 1993). Relaxation rate decreases now in proportion to PF without significant shortening of time. So dF/dt_{max} is less reduced than dF/dt_{min}, resulting in increased $(dF/dt_{max})/(dF/dt_{min})$. The

tonic and phasic components of contraction are less sharply separated, as seen by the decline of CPC. Especially the very early onset and the fast decline of F is responsible for the continuous increase in CPR.

In conclusion, the results showed how the cellular mechanism(s) of the BDM induced negative inotropic effect, i.e. mainly the lowered ratio of rate constants for cross-bridge attachment and detachment, leads to a characteristic modification of the isometric mechanogram. The reaction scheme (Yue 1987) for the formation and detachment of cross-bridges is a suitable tool to explain the causal connection between the cellular effect and the modified mechanogram.

Appendix

The kinetic equations related to the scheme of the cross-bridge cycle (see above) read

$$\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{TA}] &= g\,[\mathrm{TA\text{-}M}] - k_1\,[\mathrm{Ca}]^n[\mathrm{TA}] + k_{-1}\,[\mathrm{Ca\text{-}TA}] \\
\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{Ca\text{-}TA}] &= k_1\,[\mathrm{Ca}]^n[\mathrm{TA}] - (k_{-1}+f)\,[\mathrm{Ca\text{-}TA}] \\
\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{Ca\text{-}TA\text{-}M}] &= f\,[\mathrm{Ca\text{-}TA}] - k_{-2}\,[\mathrm{Ca\text{-}TA\text{-}M}] + k_2\,[\mathrm{Ca}]^n[\mathrm{TA\text{-}M}] \\
\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{TA\text{-}M}] &= k_{-2}\,[\mathrm{Ca\text{-}TA\text{-}M}] - (k_2+g)\,[\mathrm{TA\text{-}M}]
\end{aligned} \tag{1}$$

Under the assumption

$$k_1, k_{-1}, k_2, k_{-2} \gg f, g \tag{2}$$

equation system (1) can be simplified by means of the quasi-steady-state approximation (Schauer and Heinrich 1983) describing the binding of calcium to [TA] and [TA-M] by mass-action relations,

$$\frac{[\mathrm{Ca\text{-}TA}]}{[\mathrm{Ca}]^n[\mathrm{TA}]} = \frac{k_1}{k_{-1}} = \Gamma_1; \qquad \frac{[\mathrm{Ca\text{-}TA\text{-}M}]}{[\mathrm{Ca}]^n[\mathrm{TA\text{-}M}]} = \frac{k_2}{k_{-2}} = \Gamma_2 \tag{3}$$

Introducing new variables $[\mathrm{X}_1] = [\mathrm{TA}] + [\mathrm{Ca\text{-}TA}]$ and $[\mathrm{X}_2] = [\mathrm{TA\text{-}M}] + [\mathrm{Ca\text{-}TA\text{-}M}]$ which fulfil the conservation condition $[\mathrm{X}_1] + [\mathrm{X}_2] = \mathrm{const} = [\mathrm{X}]$ and taking into account the relations (3) we arrive at the following differential equation for the variable $[\mathrm{X}_2]$,

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{X}_2] = f\,\frac{\Gamma_1[\mathrm{Ca}]^n}{1+\Gamma_1[\mathrm{Ca}]^n}\,([\mathrm{X}]-[\mathrm{X}_2]) - g\,\frac{[\mathrm{X}_2]}{1+\Gamma_2[\mathrm{Ca}]^n} \tag{4}$$

The contractile force is assumed to be proportional to $[\mathrm{X}_2]$, i.e. $F = \gamma[\mathrm{X}_2]$, so that equation (4) can be easily transformed into a differential equation for F,

$$\frac{\mathrm{d}}{\mathrm{d}t}F = F_0\frac{f}{1+\left(\frac{K_1}{[\mathrm{Ca}]}\right)^n} - \left(\frac{f}{1+\left(\frac{K_1}{[\mathrm{Ca}]}\right)^n} + \frac{g}{1+\left(\frac{[\mathrm{Ca}]}{K_2}\right)^n}\right)F \tag{5}$$

where $F_0 = \gamma[X]$ and $K_t = 1/\Gamma_t^{1/n}$. In order to solve this equation one needs to know the time dependent intracellular calcium concentration [Ca]. We employed the phenomenological expression

$$[\mathrm{Ca}] = \frac{[\mathrm{Ca}]_0}{\left(1 + \left(\frac{T_0}{t}\right)^{n_c}\right)(1 + \exp(a[t - T_0]))} \tag{6}$$

for [Ca] which was fitted to experimental data. Estimated parameters are: $\mathrm{Ca}_0 = 332.4$ (Ca-units), $\mathrm{T}_0 = 25.1$ ms, $n_c = 1.6$, $a = 0.0029$ 1/ms. The introduction of expression (6) with these estimated parameter values into equation (5) results in a single differential equation for F which contains the unknown parameters f, g, K_1, K_2, n and F_0. Recent findings (Li et al. 1985; Higuchi and Takemori 1989; Fuchs and Wang 1991; Perreault et al. 1992; Gambassi et al. 1993; Khandoudi et al. 1993; Venema et al. 1993; Backx et al. 1994) suggest that BDM influences mainly the rate constants f and g, whereas the half-saturation constants K_1, K_2, the cooperativity index n for calcium binding and the maximal force F_0 are assumed to remain unaltered. Thus there were $4 + 2 \times 8 = 20$ adjustable parameters to be estimated by fitting the numerical solutions of differential equation (5) simultaneously to 9 different isometric mechanograms recorded at varying concentrations of BDM between 0 and 20 mmol/l. The fit was performed by using the software package "SIMFIT" (Holzhütter and Colosimo 1990). The estimated parameter values are: $K_1 = 199.4$ (Ca-units), $K_2 = 99.7$ (Ca-units), $n = 3.8$, $F_0 = 101$ mN, rate constants f and g cf. absolute values in legend to Fig. 7.

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