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Short communication

The Ca^{2+} sensitizer EMD 53998 antagonizes the effect of 2,3-butanedione monoxime on skinned cardiac muscle fibres

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Abstract

The effects of 2,3-butanedione monoxime (BDM) and 5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-one (EMD 53998) on cardiac muscle were studied in skinned muscle fibres from the right ventricle of the porcine heart. BDM decreases the Ca^{2+} sensitivity (pCa_{50} for 50% activation) and it exerts a dose-dependent inhibitory effect on force in troponin I (TnI)-depleted (unregulated) cardiac skinned muscle fibres ($IC_{50} \sim 20$ mM) thereby mimicking the effect of the TnI inhibitory peptide (cTnI 137–148, corresponding to the cardiac TnI inhibitory region) and that of inorganic phosphate (Pi). This inhibitory action can be antagonized by the calcium-sensitizing cardiotonic thiadiazinone derivative EMD 53998 that increases the IC_{50} to about 30 mM. In skinned fibres, BDM (10 mM) also increased the ratio of ATPase activity to isometric force (tension cost), whereas EMD 53998 (20 μM) decreased it. We propose that BDM antagonizes EMD 53998 because both compounds affect the Pi release step of the crossbridge cycle in an antagonistic manner.

Keywords: Butanedione monoxime (BDM); Ca^{2+} sensitivity; Cross-bridge; EMD 53998; Cardiac skinned fiber

1. Introduction

Butanedione monoxime (BDM), a nucleophilic oxime with a phosphatase-like activity (Coulombe et al., 1990), shortens the action potential duration and inhibits the slow inward current (Bergey et al., 1981), but it also exerts a Ca^{2+}-desensitizing effect on both cardiac and skeletal skinned muscle fibres (Fryer et al., 1988; Gwathmey et al., 1991). Thus it decreases the pCa (negative logarithm of Ca^{2+} concentration) required for 50% force generation of permeabilized cardiac muscle fibres. It also has been shown to inhibit the actomyosin ATPase (McKillop et al., 1994; Higuchi and Takemori, 1989) and it decreases contractile force of skinned fibres at maximal calcium activation, but less so than immediate stiffness (Zhao et al., 1995). The latter finding has been taken to mean that BDM increases the population of strongly attached 'preforce-generating' crossbridges, possibly by slowing and inhibiting the phosphate (Pi) release step and stabilizing the actomyosin-ADP-Pi intermediate crossbridge state (AM-ADP-Pi). Using the vanadate method developed by Strauss et al. (1992a), we extracted troponin I (TnI) from skinned fibres to find out whether BDM inhibits, like Pi (cf. Strauss et al., 1992b), contractile force of these troponin I-depleted, i.e. unregulated cardiac skinned fibres. We also investigated whether the inhibitory effect of BDM is antagonized by a crossbridge agonist, the cardiotonic thiadiazinone derivative 5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-one (EMD 53998).

EMD 53998 and its (+) enantiomer EMD 57033 are so-called calcium sensitizers as they increase the pCa value required for 50% activation in skinned fibres (Beier et al., 1991; Solaro et al., 1993), but they also increase force at maximal Ca^{2+} activation. The sensitizing effect depends on the particular troponin isoform (Barth et al., 1995), yet EMD 53998 and 57033 do not increase the Ca^{2+} affinity of troponin C (Solaro et al., 1993). Rather, these Ca^{2+}-sensitizing drugs affect the actomyosin contractile system directly, as they increase the force even in troponin I-depleted unregu-

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lated skinned fibres (Strauss et al., 1992b), as well as the sliding movement of actin in the in vitro motility assay (Solaro et al., 1993). EMD 53998 increases $f_{app.}$, the rate constant governing the rate of crossbridge attachment in the strongly bound force-generating state (Arner et al., 1995; Simnett et al., 1994), while $g_{app.}$ (the crossbridge detachment rate) is not affected (Strauss et al., 1994; Simnett et al., 1993). Thus force is increased, as it depends on $f_{app.}/(f_{app.} + g_{app.})$ (Brenner, 1990).

The transition into the force-generating state that is accelerated by EMD 53998 is associated with the release of Pi from the actomyosin-ADP-Pi ternary complex. As EMD also antagonizes the inhibitory effect of inorganic phosphate on skinned fibres (Strauss et al., 1992b), it seems likely that EMD 53998 accelerates the Pi release step, perhaps by lowering the Pi affinity for myosin (cf. Strauss et al., 1994). Therefore its action ought to be antagonistic to that of BDM that, according to McKillop et al. (1994), retards Pi release thereby stabilizing the actomyosin-ADP-Pi ternary complex. It was also of interest whether EMD 53998 and BDM have an antagonistic effect on tension cost, i.e. on the ratio of ATPase activity and force in skinned fibres.

2. Materials and methods

We used porcine cardiac muscle 'fibres' from the right ventricle. The fibre bundles (diameter < 0.2 mm) were dissected and skinned with Triton X-100 and stored in glycerol at -20°C as described by Barth et al. (1995). Triton-skinned fibre bundles were attached to an AME 801 strain gauge and the length was adjusted in 'relaxing solution' so that resting tension was just noticeable (sarcomere length = 1.95 μm). Relaxing solution contained 20 mM imidazole, 10 mM ATP, 10 mM phosphocreatine, 12.5 mM $MgCl_2$, 5 mM NaN_3, 5 mM EGTA and 380 U/ml creatine kinase. The pH was adjusted to 6.70 and ionic strength (120 mM) was adjusted with KCl; $T = 20$–22°C. In contraction solution EGTA was replaced by 5 mM Ca^{2+}-EGTA. The pCa ($-\log[Ca^{2+}]$) was varied by mixing contraction solution and relaxing solution as appropriate and pCa values were calculated according to Fabiato and Fabiato (1979), using the stability constants given by Fabiato (1981).

After testing the Ca^{2+} dependence with an initial maximal contraction at pCa 4.3, followed by a subsequent relaxation at pCa 8, skinned cardiac fibres were then maximally contracted at pCa 4.3 and then relaxed by 10 mM vanadate to extract TnI. After washing out the vanadate, they were no longer Ca^{2+}-regulated, i.e. they contracted maximally even at pCa 8. Under these conditions up to 90% of TnI was extracted according to Strauss et al. (1992a). Contracted fibres were then immersed into calcium-free solution containing butanedione monoxime (BDM) at different concentrations and (subsequently) in solutions containing BDM and EMD (100 μM) and 1% dimethyl sulfoxide (DMSO) as solvent. To avoid fibre deterioration, only one concentration of BDM and EMD 53998 was tested with each fibre bundle. Rabbit psoas fibres were skinned as described (Rüegg et al., 1989) and maximum calcium-activated force at pCa 4.3 was inhibited by increasing BDM concentration in a cumulative manner.

To determine the tension cost, we measured simultaneously force development and ATPase activity at different Ca^{2+} concentrations. The relaxing solutions contained 20 mM imidazole, 10 mM ATP, 12.5 mM $MgCl_2$, 5 mM NaN_3, 0.2 mM diadenosine pentaphosphate (an inhibitor of adenylate kinase), and 5 mM EGTA. The contraction solution also contained 5 mM $CaCl_2$. The ATP concentration was stabilized with an ATP-regenerating system, phosphoenol pyruvate (12.5 mM) and pyruvate kinase (100 U/ml). ATPase activity was measured simultaneously with force by using a linked NADH fluorescence assay (0.46 mM NADH, 140 U lactate dehydrogenase) according to the method of Güth and Wojciechowski (1986). By subtracting the basal ATPase activity (in relaxing solution) we obtained the suprabasal ATP-splitting rate. The ratio of suprabasal ATPase activity and force was taken as a measure for 'tension cost'.

3. Results

As found previously (Strauss et al., 1992a), after treatment with vanadate (10 mM) cardiac skinned fibres contract maximally even in the absence of Ca^{2+}.

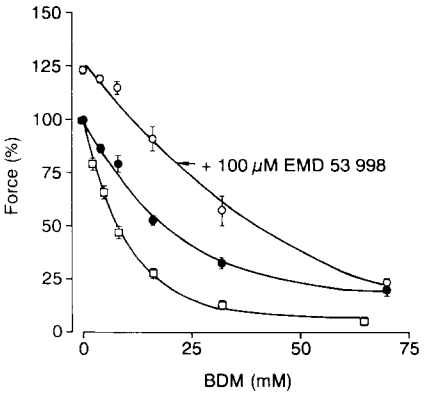

Fig. 1. The inhibitory effect of 2,3-butanedione monoxime on troponin I-depleted skinned cardiac muscle fibres (filled circles) and (Ca^{2+}-regulated) skinned psoas fibres (open squares). Each data point represents the mean relative force (in % of force at pCa 4.5 ± S.E.M.) of 5 fibre bundles incubated with the BDM concentration shown on the abscissa. Note that 100 μM EMD 53998 (open circles) increases force (by about 25%) in the absence of BDM and reduces the inhibitory effect of BDM on cardiac fibres.

As shown in Fig. 1, contractile force of these vanadate treated TnI-depleted unregulated cardiac fibres could be inhibited dose-dependently by increasing the concentrations of BDM, the IC_{50} being approx. 20 mM. This inhibition could be partly antagonized by EMD 53998 (100 μM). Thus in the presence of EMD much larger concentrations of BDM (30 mM) are required to produce half-maximal inhibition. Note that EMD 53998 increases force (by about 25%) also in the absence of BDM, as previously reported (Strauss et al., 1992b). It is noteworthy that Ca^{2+}-activated skinned fibres from rabbit psoas can be much more readily inhibited by BDM, the IC_{50} being less than 10 mM, as found by others (Zhao et al., 1995).

A cumulative increase in Ca^{2+} concentration increases both force and ATPase activity of native skinned cardiac fibres (cf. also Kuhn et al., 1990). The Ca^{2+}-activated force of these skinned fibres is also decreased by BDM that, in addition, reduces the pCa_{50} value for force generation, i.e. the apparent Ca^{2+} sensitivity (data not shown). The maximally calcium-activated force (at pCa 4.3) of skinned cardiac fibres is inhibited by BDM (10 mM) to a much larger extent (by $41.0 \pm 4.6\%$) than the contractile ATPase at pCa 4.3 which is only reduced by $15.9 \pm 7.4\%$ ($n = 5$). Thus at pCa 4.3, 10 mM BDM increased tension cost, i.e. the ratio of ATP splitting rate to force about by a factor of 1.4. In Fig. 2 the suprabasal ATPase activity at different pCa values in the presence and absence of BDM was plotted against isometric force. The slope of the ATPase/force relation appeared to be more or less linear (cf. also Kuhn et al., 1990; Strauss et al., 1994). Note that the slopes of the regression lines in the presence and absence of BDM are quite different, indicating a 1.4-fold difference in tension cost. In contrast, EMD 53998 (20 μM) reduced tension cost by 25% (cf. Strauss et al., 1994).

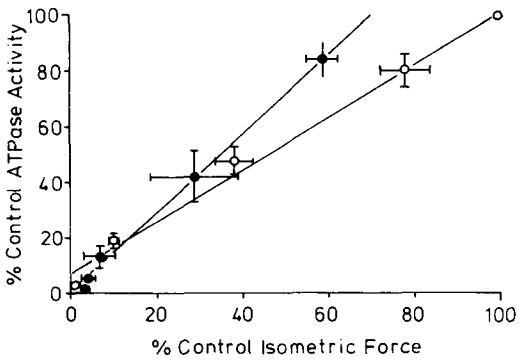

Fig. 2. Effect of BDM (10 mM) on tension cost. Isometric force and ATPase activity were measured in the presence (filled circles) and in the absence (open circles) of BDM at pCa values between 8 and 4.3. ATPase activity and force are given in % of the values obtained in the absence of BDM at pCa 4.3. Note the increase in the ratio of ATPase and force at pCa 4.3 (by a factor of 1.4) caused by BDM. Symbols represent means (±S.E.M.) of 5 fibres.

However, in interpreting the data, a word of caution is appropriate. The small intercept on the ordinate of the relationship between ATPase activity and force may indicate the presence of a calcium-activated ATPase that is unrelated to contraction, e.g. the sarcoplasmic reticulum ATPase that, despite the detergent treatment, may still be present in the fibre (cf. Strauss et al., 1994). Alternatively, it may be an indication of a curvilinear relationship between contractile ATPase and force at low levels of Ca^{2+} activation, as also found by Kerrick et al. (1991) who suggested therefore that tension cost was decreasing with increasing Ca^{2+} concentration.

4. Discussion

BDM has been shown to be an inhibitor of actin myosin interaction (McKillop et al., 1994; Zhao et al., 1995) and it also desensitizes the contractile myofilaments to calcium (Gwathmey et al., 1991). BDM is assumed to inhibit the release of inorganic phosphate (Pi) from the AM-ADP-Pi ternary complex thereby inhibiting the production of force (McKillop et al., 1994; Zhao et al., 1995). Here we showed that BDM inhibits the contractile force of troponin I-depleted unregulated skinned cardiac muscle fibres dose-dependently. However, larger concentrations of BDM are required for 50% inhibition (IC_{50}) than in the case of Ca^{2+}-activated skinned fibres from rabbit psoas. This inhibitory effect of BDM on skinned cardiac fibres is antagonized by the calcium-sensitizing drug EMD 53998 which is said to increase the apparent rate of crossbridge attachment and hence presumably the rate of Pi release (cf. Arner et al., 1995; Simnett et al., 1994). Such an antagonistic action is just what one would expect from a drug promoting Pi release from the crossbridge and hence the transition from weakly binding bridges into strongly binding crossbridges. It is also noteworthy that EMD not only antagonizes the inhibitory effect of BDM on force generation but also that of inorganic phosphate (Strauss et al., 1992b, 1994) and of the peptide TnI 137–148 (corresponding to the inhibitory region of cardiac TnI), and even that of TnI itself (Barth et al., 1995; Solaro et al., 1993). Is it possible, therefore, that these inhibitors all act in a similar way, namely by inhibiting Pi release and thus the transition of non force-generating or weakly attaching crossbridges into force-generating crossbridges?

Like phosphate (cf. Herzig et al., 1981), BDM increases tension cost, i.e. the ratio of ATPase activity to force. According to Brenner (1990), tension cost depends both on $g_{app.}$, the crossbridge detachment rate constant, as well as on $1/K$ (the reciprocal crossbridge force). The reason is that ATPase is proportional to $f_{app.} \times g_{app.}/(f_{app.} + g_{app.})$ and force is proportional to

$K \times f_{app.}/(f_{app.} + g_{app.})$. An increase in tension cost could thus be due to an increase in $g_{app.}$ or a decrease in the force per crossbridge. Since BDM slows the rate of Pi release and transition of preforce-generating strongly attached crossbridge states into force-generating ones (Zhao et al., 1995), crossbridges would spend a larger fraction of the cycle time in the preforce-generating than in the force-generating crossbridge state. Thus the average force in an ATP hydrolyzing crossbridge cycle would be reduced thereby causing a decrease in the force-per-ATPase ratio and an increase in tension cost. Contrary to BDM, EMD 53998 decreases tension cost (Strauss et al., 1994; Leijendekker and Herzig, 1992) and this could be taken to mean that it increases the fraction of crossbridges in the force-generating state while reducing that of strongly attached crossbridges in the preforce-generating state, or in the weakly attached state. In any case, EMD 53998 does not seem to affect tension cost by altering the crossbridge detachment rate constant $g_{app.}$, since the latter is not reduced by EMD 53998 (Simnett et al., 1993). In conjunction, the results reported here suggest that the antagonistic effects of EMD 53998 and BDM on force generation and tension cost may be understood in terms of an antagonistic action of the two compounds on the rates of Pi release and of the transition of non force-generating to force-generating crossbridges.

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