Journal of Physiology (1991), **442**, *pp.* 527–549
With 9 figures
Printed in Great Britain

MULTIPLE ACTIONS OF 2,3-BUTANEDIONE MONOXIME ON CONTRACTILE ACTIVATION IN FROG TWITCH FIBRES

BY CHIU SHUEN HUI* AND JAMES MAYLIE†

*From the *Department of Physiology and Biophysics, Indiana University Medical Center, Indianapolis, IN 46202, USA and the †Department of Obstetrics and Gynecology, Oregon Health Science University, Portland, OR 97201, USA*

(*Received 4 January 1991*)

SUMMARY

1. The effects of 2,3-butanedione monoxime (BDM) on various steps in the excitation–contraction coupling sequence, including action potential, charge movement and twitch tension, were studied in twitch fibres of *Rana temporaria*.

2. The resting potential of intact fibres in whole muscles bathed in 20 mM-BDM was the same as control. The resting potential also remained stable after more than 100 min in 20 mM-BDM.

3. The action potential was measured in intact fibres of fibre bundles with an intracellular microelectrode. Applications of 5 and 7·5 mM-BDM had no effect on its amplitude, whereas 10 and 20 mM suppressed its amplitude by about 4 and 10%, respectively. Increasing concentrations of BDM prolonged the half-width and elevated the after-potential of the action potential progressively. The action potential was also measured in cut fibres mounted in a double Vaseline-gap chamber. Results were similar to those in intact fibres.

4. Charge movement was measured in intact fibres of halved muscles with the three-microelectrode voltage-clamp technique. The steady-state Q–V plot of the total charge measured in isotonic tetraethylammonium (TEA) Ringer solution with 20 mM-BDM appeared to be shifted about 10 mV in the depolarizing direction and to be slightly more shallow when compared with the control Q–V plot measured in hypertonic TEA Ringer solution with 350 mM-sucrose. After allowing for the voltage shift, 20 mM-BDM did not appear to affect the kinetics of both components of charge movement, but suppressed the maximum amount of total charge by about one-quarter.

5. Charge movement was also measured in cut fibres with the double Vaseline-gap voltage-clamp technique. In the presence of 20 mM-BDM, charge movement traces resembled those from intact fibres. Twenty millimolar BDM suppressed the maximum amount of total charge by about one-quarter, as in intact fibres. The steady-state Q–V plots from cut fibres were separated into Q_β (early current) and Q_γ (late hump current) components by least-squares fitting with a sum of two Boltzmann distribution functions. On average, 20 mM-BDM suppressed Q_β and Q_γ in

* To whom all correspondence and reprint requests should be sent.

MS 9055

roughly equal proportion, but did not affect the individual voltage distributions of Q_β and Q_γ.

6. Twitch tension was measured in single intact fibres stimulated extracellularly. BDM effectively reduced the peak amplitude, the time-to-peak and the half-width of twitch tension. The interaction of BDM with receptors appeared to follow more or less a simple 1 : 1 binding in fibres stretched to sarcomere lengths of about 3·6 μm. The half-blocking concentration was 0·43 mM. The dose–response relation of the binding was similar in fibres stretched to sarcomere lengths of about 3·0 μm but the interaction with receptors appeared to deviate slightly from a simple non-co-operative binding.

7. It is concluded that BDM affects the action potential, charge movement and twitch tension. However, its effect on twitch tension is much larger than that on the earlier steps in the excitation–contraction coupling sequence.

INTRODUCTION

Movement artifacts are a problem to muscle electrophysiologists. For a long time investigators have been trying to find an ideal way of blocking contraction. In early (Chandler, Rakowski & Schneider, 1976) and subsequent experiments on charge movement in intact fibres, a hypertonic bathing solution was used to block contraction. Unfortunately, hypertonicity makes microelectrode impalement more difficult. Also, if the tonicity is too high, it can suppress Ca^{2+} release from the sarcoplasmic reticulum (Taylor, Rudel & Blinks, 1975) and the Q_γ component of charge movement is obscured (see also early papers on charge movement). Tetracaine, a local anaesthetic, has also been used (Almers & Best, 1976; Gilly & Hui, 1980) but it is also not ideal because it blocks the Q_γ component (Huang, 1982; Hui, 1983*a*; Vergara & Caputo, 1983) and Ca^{2+} release (Almers & Best, 1976). For cut fibres, contraction can be blocked very effectively by a combination of moderate stretch (sarcomere length 3·5 μm) and high (20 mM) EGTA in the internal solution (Hui & Chandler, 1990), but the presence of EGTA will certainly affect the Ca^{2+}–dye signal. The best means yet is to stretch the fibres to beyond filament overlap (Melzer, Schneider, Simon & Szucs, 1986), which is found to have no observable effect on charge movement (C. S. Hui, unpublished results). In order to stretch intact fibres adequately, single fibres have to be used, but such experiments are extremely difficult with a low success rate. Thus, the search for better methods to block contraction is still on.

When Mulieri & Alpert (1984) reported that 2,3-butanedione monoxime (abbreviated by BDM), also known as diacetyl monoxime (abbreviated by DAM), is capable of blocking contraction in skeletal muscle by selectively interfering with cross-bridge formation, it opened up an exciting possibility. We decided to extend their work in characterizing the action of BDM on twitch tension and check whether BDM blocks tension by acting exclusively on cross-bridge formation without interfering with the earlier steps in the excitation–contraction coupling sequence.

At the start of our project in 1985, very little was known about the action of BDM in skeletal muscle. Chemically, BDM is a member of the oxime family. A review of the literature revealed that BDM has a variety of actions on different biological systems. For example, it suppresses the slow Na^+ current and slow Ca^{2+} current

in embryonic chick cardiac muscle (Sada, Sada & Sperelakis, 1985). The suppression of slow Ca^{2+} current is partly responsible for the negative inotropic effect in cardiac muscle (Wiggins, Reiser, Fitzpatrick & Bergey, 1980). The negative inotropic effect might also involve an inhibition of myofibrillar ATPase activity as demonstrated in skinned fibre experiments and biochemical assays (Li, Sperelakis, Teneick & Solaro, 1985). In view of the complex actions of BDM on cardiac muscle, it is difficult to imagine that its blockage of contraction in skeletal muscle could be solely at the cross-bridge level. Separately, BDM is useful for urea determination in biological fluids (Cojocaru, Serban, Nastasa, Ungureanu & Lazar, 1985). Moreover, in the clinical area, it has been known for quite a long time that BDM acts as an antidote for neuromuscular poisoning (Askew, 1956). This reactivation of acetylcholinesterase is mediated through a dephosphorylation mechanism, as is also true for the suppression of the slow Ca^{2+} current. All these pieces of information point towards a distinct possibility that BDM might have multiple actions on the excitation–contraction coupling sequence in skeletal muscle.

This paper describes the effects of BDM on twitch tension, resting potential, action potential and charge movement in frog twitch fibres. The following paper (Maylie & Hui, 1991) describes the effects of BDM on optical retardation and the transient rise in myoplasmic Ca^{2+} level as monitored with Antipyrylazo III. After the completion of our experiments and preliminary data analysis, we came across a paper published by Horiuti, Higuchi, Umazume, Konishi, Okazaki & Kurihara (1988) describing the effects of BDM on contractile activation in frog muscle. The results they reported are consistent with the findings in this paper. A preliminary report of this work has appeared (Hui & Maylie, 1988; Maylie & Hui, 1988).

METHODS

The effect of BDM on twitch tension was studied in intact fibres only because it is inconvenient to measure tension in cut fibres. The effects on action potential and charge movement were studied in both intact and cut fibres. Action potentials in cut fibres were measured simultaneously with Ca^{2+}–Antipyrylazo III signals (to be reported in the following paper, Maylie & Hui, 1991) with the optical set-up in Dr W. K. Chandler's laboratory at Yale University. All other experiments were carried out at Indiana University.

Muscle and fibre preparation

All experiments were performed on twitch fibres from English frogs, *Rana temporaria*, cold-adapted in a refrigerator at around 4 °C. Animals were killed by decapitation and pithing the spinal cord.

Resting membrane potential was measured in fibres of whole sartorius muscles. The inner surface of the pelvic end of the muscle was fine-cleaned to aid microelectrode impalement and to make solution changes more effective during the application and wash-out of BDM. The muscle was stretched to approximately 1·5 times its slack length and pinned on the bottom of the chamber. Twitch tension was measured in single intact fibres dissected from semitendinosus muscles. The dissection and mounting procedures were described in a previous paper (Hui, 1989). Action potentials were measured in bundles of three to five fibres. The method of dissection and mounting procedures were identical to those of single fibres.

For the measurement of charge movement in intact fibres, halved semitendinosus muscles were used. The dissection technique was the same as described in Hui & Milton (1987). The halved muscle was stretched to roughly 1·5 times its slack length and pinned on the bottom of an experimental chamber. The remnant of the tendon from the cut branch was pulled to one side to spread the fibres apart. As a result of this pulling, the fibres closer to the cut branch were stretched more than those closer to the other edge. The sarcomere lengths of the fibres used ranged between 2·8 and 3·4 μm.

Action potentials and charge movement were also studied in cut fibres, a preparation used originally by Hille & Campbell (1976). The procedure for dissecting and mounting cut fibres from semitendinosus muscle was similar to that used by Kovacs, Rios & Schneider (1983) and Irving, Maylie, Sizto & Chandler (1987). Briefly, a stretched muscle bundle was exposed to a Ca^{2+}-free, high-K^+ relaxing solution (solution I in Table 1) which caused a transient contraction. A 6–12 mm

TABLE 1. Composition of solutions (in mM)

Intact fibre experiments

Reference	NaCl	TEA-Cl	KCl	RbCl	PIPES	$CaCl_2$	Sucrose	BDM
A	115	—	2·5	—	2	1·8	—	—
B	115	—	2·5	—	2	1·8	—	*x*
C	—	115	—	5	2	10	350	—
D	—	115	—	5	2	10	—	*x*

Cut fibre experiments

Reference	NaCl	TEA-Cl	KCl	CsCl	Na_2HPO_4	NaH_2PO_4	$CaCl_2$	BDM
E (external)	120	—	2·5	—	2·15	0·85	1·8	—
F (external)	120	—	2·5	—	2·15	0·85	1·8	*x*
G (external)	—	120	—	2·5	2·15	0·85	1·8	—
H (external)	—	120	—	2·5	2·15	0·85	1·8	*x*

Reference	Glutamate	PIPES	EGTA	$MgSO_4$	Glucose	ATP	Creatine phosphate
I (relaxing)	120 (K)	5 (K)	0·1 (K)	1	—	—	—
J (internal)	75·5 (K)	5 (K)	0·1 (K)	6·8	5	5·5 (Na)	20 (K)
K (internal)	45·5 (Cs)	5 (Cs)	20 (Cs)	6·8	5	5·5 (Cs)	20 (Cs)

Solutions A–D are titrated to pH 7·1 with NaOH. Solutions E–H are titrated to pH 7·1 with HCl. Solutions I and J are titrated to pH 7·0 with KOH. Solution K is titrated to pH 7·0 with CsOH. *x* represents the variable concentration of BDM in test solutions. In solutions I–K, the element shown in parentheses after a number denotes the cation of the compound.

length of a single fibre was isolated and mounted in a double Vaseline-gap chamber. In order to gain electrical continuity with the myoplasm, the outer membranes of the fibre segment in the end-pools were permeabilized by a 2 min exposure to 0·01% saponin followed by thorough rinsing with solution I. The end-pool solutions were then replaced with the internal solution (either solution J or K). The centre-pool solution was changed to either normal Ringer solution (solution E) or an isotonic TEA (tetraethylammonium) solution (solution G).

Solutions

Solutions are given in Table 1. Solutions A and B are control and test solutions for measuring tension, resting potential and action potential in intact fibres. BDM was bought from Sigma (St Louis, MO, USA). The chamber for single-fibre (or bundle of fibres) experiments has a capacity of about 6 ml. Solution change from control to test, or vice versa, involves flushing the chamber twice with the preparation in place, each time with 25 ml of a solution and waiting a couple of minutes in between. Solutions C and D are control and test solutions for charge movement experiments on intact fibres. In both solutions, TEA^+ and Rb^+ were used to suppress K^+ current and the elevated Ca^{2+} concentration was for improving the seal around the microelectrodes. Tetrodotoxin (TTX; 1 μg/ml) was used to block Na^+ current (not shown in the table). Fibre contraction was blocked by 350 mM-sucrose (solution C) or 20–30 mM-BDM (solution D). Solutions E, F and J are for the measurement of action potentials and solutions G, H and K for the measurement of charge movement in cut fibres.

Tension measurements

A single intact fibre was stimulated with a pair of extracellular platinum electrodes and twitch tension was monitored with a sensitive tension transducer (Akers, Norway). The output of the tension transducer and the voltage applied to the extracellular stimulating electrode were filtered

at 1 kHz and amplified by instrumentation amplifiers (Tektronix AM 502), and digitized by an A–D converter (Data Translation 1761) at 1 kHz per channel for a period of 2 s. The data arrays were immediately compressed by keeping the average of every eight points as one point, which corresponded then to 8 ms. Compressed traces were stored in a PDP 11/73 microcomputer.

Action potential measurements

Action potentials were also measured in intact fibres stimulated with extracellular electrodes. As the time course of an action potential is very fast, the analog input signals were filtered at 10 kHz. The acquisition rate was set at 5 kHz per channel and the data arrays were stored without compression. Action potentials were also recorded in cut fibres in current-clamp mode. The signal was filtered at 10 kHz.

Charge movement measurements

For intact fibre charge movement experiments, the instrumentation, experimental protocol and method of data analysis were similar to those used by Gilly & Hui (1980) and Hui (1983*a*). Briefly, the three-microelectrode voltage-clamp technique of Adrian, Chandler & Hodgkin (1970) was applied to the end of a fibre. Two differential signals were recorded, namely $V_1 - V_0$ which gives the membrane potential and $V_2 - V_1$ (referred to as ΔV) which gives the membrane current density. The signals were filtered at 1 kHz and sampled at 120 μs per point per channel for a period of 240 ms. The data arrays were compressed by keeping the average of every eight points as one point, which corresponded then to 0·96 ms. Compressed traces were stored in a PDP 11/73 microcomputer. Command pulses fed to the voltage-clamp circuitry were generated by a programmable sequence generator (Page PSG-20) interfaced to the microcomputer. Command pulses were rounded with a time constant of typically 1 ms. Holding potential was set at −80 mV. The pulse protocol was similar to the one used previously (Hui, 1983*a*; Hui & Milton, 1987). For test pulses of magnitudes no larger than 80 mV, control pulses of an equal magnitude were used and the same number of test and control sweeps were signal averaged. For a larger test pulse, the magnitude of the control pulse was set at half of that of the test pulse and the number of control sweeps used for signal averaging was increased accordingly. The removal of the sloping baseline in the charge movement traces and the time-integration of the current transient to yield the amount of on-charge (Q_{on}) or off-charge (Q_{off}) were identical to those in previous papers. Since there is substantial residual delayed K^+ current in intact fibres, it has been customary to show current traces with the sloping baselines removed and blank out the later parts of the on-segments whenever the delayed current deviates from a straight line (Fig. 6). These traces are referred to as charge movement traces.

For cut-fibre charge movement experiments, the instrumentation, experimental protocol and method of data analysis were similar to those used by Chandler & Hui (1990) and Hui & Chandler (1990). Holding potential was set at −90 mV. Control pulses were applied from −110 to −90 mV. Each test-minus-control current trace was obtained by subtracting a scaled control current from a paired test current trace. The residual delayed K^+ current, present in intact fibres, was further suppressed in cut fibres by Cs^+ in the internal solution. Thus, uncorrected test-minus-control current traces are shown (Fig. 8).

RESULTS

Effect of BDM on twitch tension in single intact fibres

Figure 1 shows the dose-dependent and reversible blockage of twitch tension by BDM in a single isolated intact fibre. Each row shows the twitch tension before, during and after the application of BDM from left to right. The drug acted very promptly such that the reduction of peak tension almost reached steady state within a minute or two after the solution change. This suppression of tension development was fully reversible and wash-out was also very fast. The peak tension after wash-out could sometimes be even larger than before the application of the drug. This anomaly can be attributed to the loosening of the tendon so that active tension was increased slightly. In the fibre of Fig. 1, 10 mM-BDM was sufficient to block peak

tension to less than the noise level in the record. In some other fibres, small but measurable tension could be detected in the presence of the same concentration of the drug.

In addition to the suppression of peak tension, BDM also affected the time course of the tension transient. In the experiment shown in Fig. 1, the time-to-peak and

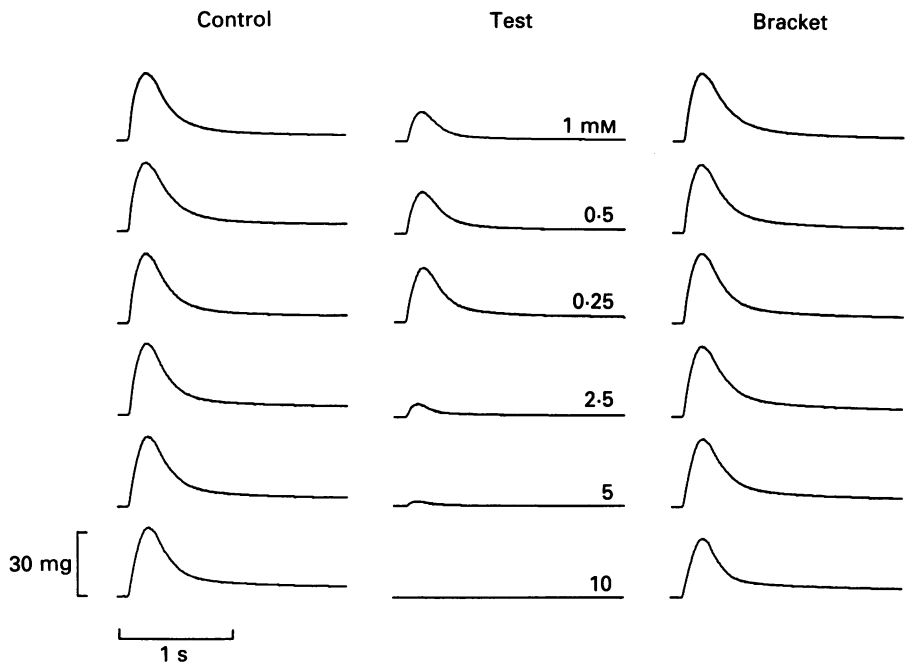

Fig. 1. Effect of BDM on twitch tension in a single intact fibre. In each row, the left tension trace was taken in normal Ringer solution. After changing the bathing solution to a Ringer solution containing BDM, 10 min were allowed for equilibration and a test trace was taken. Another one or two traces were repeated at 3 min intervals to check whether steady state had been reached. The final test trace, with the [BDM] in mM, is shown in the middle. Then the bathing solution was changed back to normal Ringer solution and, after allowing 10 min for equilibration, a bracketing control trace was taken which is shown on the right. The control trace on the left of the second row (and so forth) is different from the bracketing trace on the right of the preceding row. The rows are arranged in the temporal order in which the test solutions were applied. Fibre identification 73101; diameter 72 μm; sarcomere length 3·0 μm; temperature 4·9 °C.

half-width of twitch tension in control Ringer solution were 192 and 330 ms, respectively, on average. The time-to-peak was shortened monotonically to 172, 164, 152, 132 and 128 ms in the presence of 0·25, 0·5, 1, 2·5 and 5 mM-BDM, respectively, whereas the half-width was reduced to 296, 285, 251, 210 and 194 ms, respectively. As the time course of twitch tension is very sensitive to temperature, great care was taken to control the bath temperature to within 0·1 °C of the steady-state value after solution change before data were taken. To estimate the suppression of peak tension by each BDM concentration, the average of the values of peak tension before

the application of BDM and after wash-out was taken as the control. The residual fraction of peak tension was estimated from the ratio of peak tension in the presence of BDM to the control value.

Mulieri & Alpert (1984) reported that 7·5 mM-BDM suppressed peak tension to the same extent when the sarcomere length was either 2·3 or 3·0 μm. We investigated

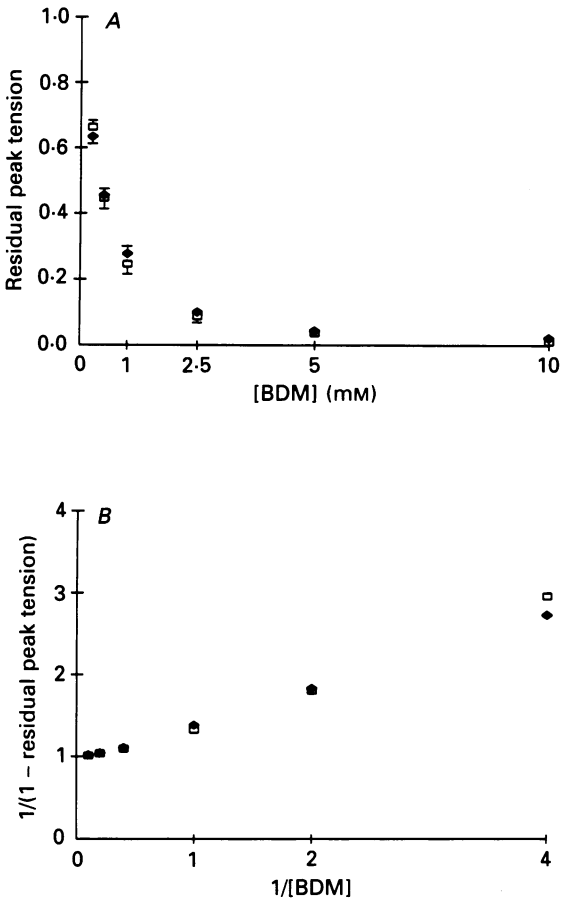

Fig. 2. Dose–response curves of the effect of BDM on peak tension. *A*, linear plot of the residual fraction of peak tension *versus* [BDM]. Each point was calculated from the ratio of peak tension in the presence of BDM to peak tension without BDM (see Methods section). □ represents the average value from seven fibres stretched to a sarcomere length of 3·0 μm whereas ◆ represents the average value from four fibres stretched to a sarcomere length of 3·6 μm. Error bars show S.E.M. and only one bar is shown on one side of each symbol to avoid overlap of the bars. Wherever omitted, the error bar is smaller than the size of the symbol. *B*, double-reciprocal plots of the two data sets in *A*. Error bars are not shown. Temperature of all experiments 5–6 °C.

further whether the dose–response curve was the same at sarcomere lengths of 3·0 and 3·6 μm, which was the range in which most of our electrical experiments were carried out. Figure 2*A* shows the residual peak tensions at six different BDM concentrations ranging from 0·25 to 10 mM at a sarcomere length of 3·0 μm (□,

average of seven fibres) and 3·6 μm (◆, average of four fibres). The two dose–response curves were almost superimposable. The difference between the two points at each BDM concentration was statistically insignificant ($P > 0{\cdot}1$ with the two-tailed t test). A closer inspection revealed that the open squares followed a curve that was more concave downward than the filled diamonds. To facilitate the comparison, the points in panel *A* are replotted in panel *B* as double-reciprocal plots. The filled diamonds for 3·6 μm sarcomere length almost followed a straight line and a linear regression line through the points yielded 0·43 mM as the half-blocking BDM concentration. It is apparent that the open squares for 3·0 μm sarcomere length deviated more from a straight line. The slope of the line segments connecting two adjacent points increased with increasing reciprocal values of BDM concentration.

One might doubt the significance of such a small difference when comparing two separate populations of fibres. In our best experiment, two dose–response curves at sarcomere lengths of 3·0 and 3·6 μm were obtained from the same fibre. Data were taken at 3·0 μm first and then at 3·6 μm. The fibre was relaxed to 3·0 μm afterwards and the residual peak tension in the presence of 0·25 mM-BDM was well bracketed. In the linear plot of residual peak tension *versus* [BDM] (similar to Fig. 2*A*), the points at 0·25 mM for both sarcomere lengths almost fell on top of each other; so did the points at 10 mM. At other concentrations, the points at 3·0 μm fell below the corresponding points at 3·6 μm, with the largest difference at 1 mM. When the two linear plots were transformed into double-reciprocal plots (similar to Fig. 2*B*), the points at 3·6 μm more or less fell on a straight line whereas the points at 3·0 μm fell on a curve that has the shape of a shallow U. The two curves intersected at 0·25 and 10 mM. Possible implications of this curvature will be described in the Discussion section.

Effect of BDM on resting potential in intact fibres

An experiment was carried out to test whether BDM has any pharmacological effect on twitch fibres by monitoring the resting potential before and after the application of BDM. In this experiment, fibres from the same sartorius muscle were used. The average resting potential in normal Ringer solution (solution A) was found to be −78·2 mV (S.E.M. = 0·7 mV, $n = 19$). After changing the bathing solution to normal Ringer solution plus 20 mM-BDM (solution B with $x = 20$), the average resting potential was −78·8 mV (S.E.M. = 1·0 mV, $n = 12$), very close to the control value. In one fibre bathed in 20 mM-BDM the resting potential was followed for a period of time. Immediately after impalement, the resting potential was −85 mV. Thirty-three minutes later, it became −84 mV and after another 74 min, it became −83 mV. It can be concluded that 20 mM-BDM had almost no effect on the resting potential and fibres stayed healthy in this BDM concentration.

Effect of BDM on action potentials in intact fibres

The action potential is the first step in excitation under normal physiological conditions. It triggers charge movement in tubular (and surface) membranes, presumably leading to Ca^{2+} release in the sarcoplasmic reticulum. Any effect of a drug on the action potential would lead to a perturbation of all the subsequent steps in contractile activation. Figure 3 shows some experiments carried out to investigate

the effect of BDM on action potentials. In each experiment, a fibre in a bundle was stimulated extracellularly to elicit action potentials, which were recorded by an intracellular microelectrode. Since the fibre twitched vigorously in normal Ringer solution and usually knocked out the microelectrode, thereby damaging the fibre, the

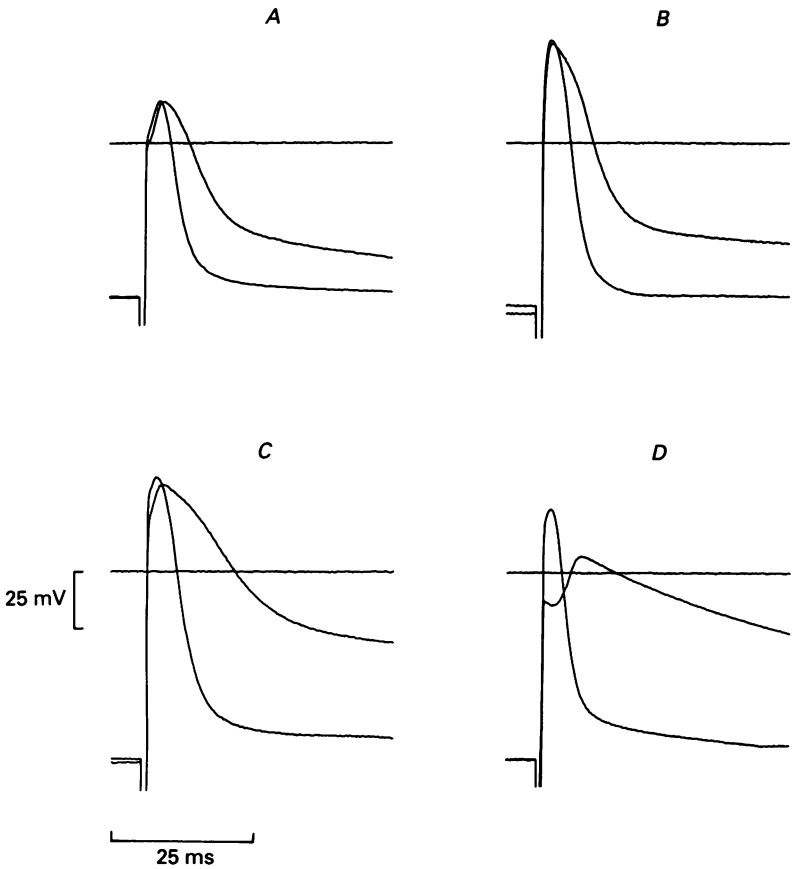

Fig. 3. Effects of BDM on action potentials in intact fibres. Each panel shows the effect of a different [BDM]. The horizontal line corresponds to the potential recorded with the microelectrode dipped into the bathing solution and serves as the zero reference. A fibre immersed in Ringer solution plus a certain [BDM] was impaled and an action potential (the broader one in each panel) was recorded. After changing the solution to normal Ringer solution without BDM and waiting for a few minutes another action potential was recorded. The two action potentials are superimposed. Fibre identifications: *A*, 62172; *B*, 62143; *C*, 62142; *D*, 62172. *A* and *D* are from the same fibre and *B* and *C* are from different fibres in the same bundle. Resting potentials, in BDM solution and in normal Ringer solution, are as follows: *A*, −67 and −66 mV; *B*, −76 and −72 mV; *C*, −83 and −81 mV; *D*, −82 and −82 mV. Temperature of all experiments 5 °C.

experiment was performed by recording an action potential in BDM solution first followed by one in normal Ringer solution. However, 7·5 and 10 mM-BDM (panels *B* and *C*) were not sufficient to block tension completely. Although the twitches did not knock out the electrode, the fibre did contract somewhat with the electrode in place,

causing a drop of a few millivolts in resting potential, as revealed by the upward shift in the baselines preceding the action potentials. After changing to normal Ringer solution, these two experiments ended in a single shot as the twitch knocked out the electrode and damaged the fibre. In the experiment shown in Fig. 3*D*, there was no

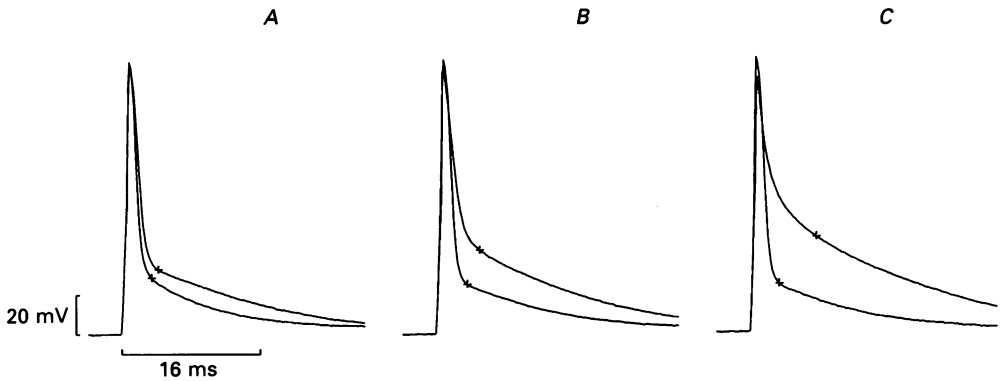

Fig. 4. Effects of BDM on action potentials in a cut fibre. Fibre identification 63271; sarcomere length 4·1 μm; diameter 94 μm; temperature 16 °C; gap factor $r_e/(r_i+r_e)$ = 0·99 (for definitions, see Irving *et al.* 1987). Fibre was held at −90 mV in current-clamp mode. In each panel, the action potential that decays faster to a lower after-potential was recorded in the absence of BDM and the other one was recorded in the presence of 5 mM (*A*), 10 mM (*B*) or 20 mM (*C*) BDM. The cursor on each action potential marks the location where the after-potential was estimated (see text for detail). The vertical and horizontal scale bars applied to all three panels.

change in resting potential after the action potential in 20 mM-BDM. Surprisingly, the subsequent action potential in normal Ringer solution did not knock out the electrode, but the fibre lost more than 10 mV in resting potential. The experiment was continued with 5 mM-BDM (Fig. 3*A*). At this stage, the action potential in 5 mM-BDM did not knock out the electrode nor did it cause a drop in resting potential.

Several observations can be made regarding Fig. 3. Low [BDM] (< 10 mM) had no apparent effect on the amplitude of the action potential but prolonged the half-width (measured at half-height) by 100% (5 mM) and 150% (7·5 mM). At 10 mM, the amplitude was suppressed by 2% whereas the half-width was prolonged by a factor of 3. At 20 mM, the amplitude was suppressed by 20% whereas the half-width was prolonged drastically and could not be measured exactly because the membrane potential did not repolarize to half-height at the end of the sample period. A rough extrapolation of the trace showed that 20 mM-BDM prolonged the half-width more than 15-fold. These experiments showed a small suppression of the amplitude and a progressive prolongation of the half-width of action potentials in intact fibres.

As the experiments on action potentials in intact fibres are very labour-intensive and with the anticipation of eliciting Ca^{2+} signals with action potentials in cut fibres, these experiments were terminated. In the cut-fibre experiments to be reported in the following paper (Maylie & Hui, 1991), action potentials were recorded simultaneously with Ca^{2+} signals. The action potential results will be presented in the next subsection.

Effect of BDM on action potentials in cut fibres

Figure 4 shows typical action potentials recorded from a cut fibre held at −90 mV under current clamp. The signal was filtered by a 10 kHz eight-pole Bessel filter before being digitized at 12·5 kHz. In panel *A*, the control action potential measured

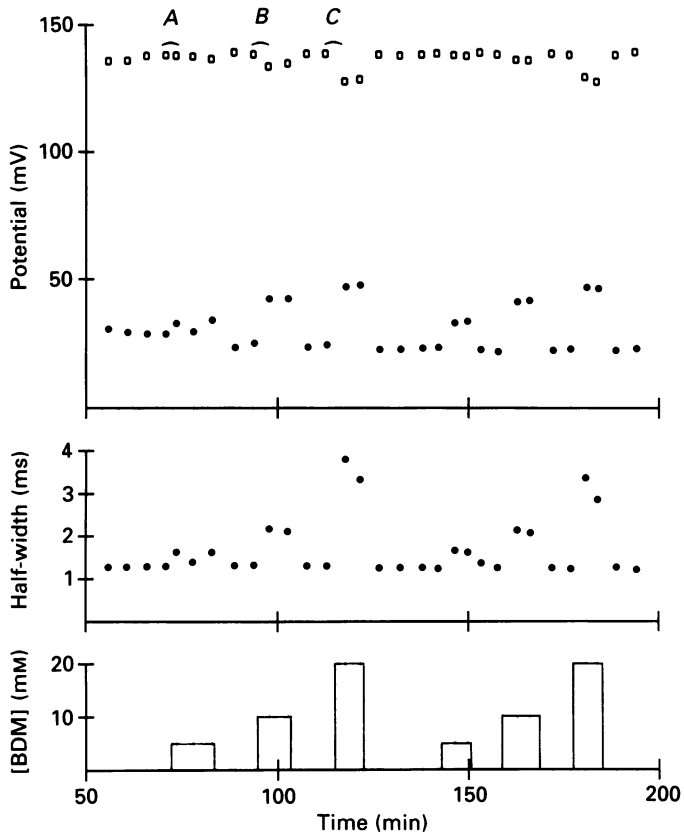

Fig. 5. Effects of BDM on the peak amplitude, after-potential and half-width of action potentials in a cut fibre. Same fibre as in Fig. 4. In each panel, the abscissa corresponds to the time after saponin treatment in the end-pools. In the upper panel, □ represents peak amplitude and ● represents after-potential estimated at the locations marked by the cursors in Fig. 4. The middle panel shows the half-width and the lower panel shows the BDM concentration. The peak amplitudes marked by *A*, *B* and *C* (and the corresponding after-potentials and half-widths) were estimated from the pairs of traces in Fig. 4*A*, *B* and *C*, respectively.

in the end-pool had a peak amplitude of 138·2 mV. The true amplitude of the action potential, if measured in the centre pool, can be obtained by dividing the above value by the gap factor, 0·99 (Irving *et al.* 1987), and should be 139·6 mV. As described in Fig. 8 of Irving *et al.* (1987), the filtering at 10 kHz delayed the action potential by 0·1 ms without perturbing its waveform noticeably. BDM (5 mM) suppressed

the peak amplitude of an action potential slightly and increased its half-width. Figure 4*B* and *C* shows that these effects of BDM were dose-dependent, as in intact fibres (Fig. 3).

Another striking feature in Fig. 4 is the elevation of the positive after-potential by BDM. For control action potentials, the after-potential could be more easily

TABLE 2. Effect of BDM on action potentials in cut fibres

Fibre reference	Peak amplitude (%)				Half-width (%)				After-potential (%)			
	2	5	10	20	2	5	10	20	2	5	10	20
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
63252	—	—	95·6	—	—	—	191·2	—	—	—	155·1	—
63271	—	99·3	96·8	92·5	—	119·5	163·5	278·9	—	120·2	170·6	202·0
66062	—	—	—	90·0	—	—	—	214·9	—	—	—	152·9
66191	—	—	90·3	79·8	—	—	196·9	216·4	—	—	193·1	202·8
66201	—	—	98·6	91·2	—	—	157·3	203·8	—	—	171·8	226·9
66202	—	—	97·0	92·3	—	—	164·9	173·7	—	—	177·5	232·0
66211	—	—	98·2	92·4	—	—	147·6	266·8	—	—	145·6	188·9
66212	—	—	—	89·9	—	—	—	142·9	—	—	—	164·1
66231	—	—	97·2	92·1	—	—	161·5	168·7	—	—	174·1	189·4
66242	99·9	99·2	97·5	92·7	101·6	134·9	147·3	174·4	127·1	158·4	197·3	174·0
Mean	99·9	99·3	96·4	90·3	101·6	127·2	166·3	204·5	127·1	139·3	173·1	192·6
S.E.M.	—	—	0·9	1·4	—	—	6·5	15·2	—	—	6·1	8·9

Column (1) gives fibre references. Columns (2)–(5) give the peak amplitudes of the action potentials in the presence of 2, 5, 10 and 20 mM-BDM, respectively. Columns (6)–(9) give the half-widths and columns (10)–(13) give the amplitudes of the after-potential (as defined in the text) with the same concentrations of BDM, as in columns (2)–(5). All values are percentages of control.

estimated when repolarization turned from a fast decay to a much slower decay. In the presence of BDM, particularly at 20 mM, the smooth transition from fast to slow decay made it difficult to define the after-potential. Thus, a criterion was introduced to estimate the after-potential at the time equal to twice the half-width after the peak of the action potential, as marked by the cursors in the traces of Fig. 4. Based on this definition, the after-potential was also increased by BDM in a dose-dependent manner.

In Fig. 5, the peak amplitude and the level of the positive after-potential (in the upper panel), the half-width (in the middle panel) and the BDM concentration (in the lower panel) are plotted as functions of time for the experiment of Fig. 4. Two sequences of solution changes to 5, 10 and 20 mM-BDM were applied. In the first sequence, these concentrations suppressed the peak amplitude to 99·3, 96·8 and 92·5%, elevated the after-potential to 120·2, 170·6 and 202·0% and prolonged the half-width to 119·5, 163·5 and 278·9% of control, respectively. These numbers are listed in the second row of Table 2 in columns (3)–(5), (7)–(9) and (11)–(13), respectively. Results in the second sequence of solution changes were slightly different from the first sequence, probably due to fibre run-down.

Nine other experiments of the same kind were performed and results are listed in Table 2. From the mean values shown at the bottom of the table, increasing BDM concentration had increasing effects in suppressing the peak amplitude, in broadening the half-width and in elevating the after-potential of action potentials in cut fibres.

Results from this and the preceding subsection show that BDM is not without effect on the first step of excitation–contraction coupling and the action of BDM is more complicated than Mulieri and Alpert hypothesized.

Effect of BDM on charge movement in intact fibres

The next step in the excitation–contraction coupling sequence is charge movement which is routinely measured under voltage-clamp conditions with Na^+ and K^+ currents blocked or minimized. Under such conditions, the effect of BDM on the action potential is bypassed. Whole muscles were used to measure charge movement in intact fibres. In early experiments, in which 20 mM-BDM was used to block fibre movement, local contraction around the electrodes could be observed under the microscope and charge movement records were contaminated by movement artifacts. On the other hand, the same BDM concentration seemed to be effective in blocking twitch tension completely in single fibres stretched to similar sarcomere lengths. A possible cause of the difference is that somehow the solution change for the superficial fibres in whole muscles may not be as effective as for single fibres, perhaps due to the presence of connective tissues in whole muscles.

In order to reduce movement artifacts further, the BDM concentration was increased from 20 to 30 mM in some experiments. In Fig. 6, charge movements in the presence and absence of 30 mM-BDM were compared in a pair of muscles from the same frog. Figure 6*A* shows charge movement traces recorded from a fibre bathed in the control TEA solution (solution C). Fibre contraction was blocked by 350 mM-sucrose. These traces resembled those obtained previously under identical conditions (Hui, 1983*a*). In the potential range −40 to −20 mV, the on-segments of the traces showed an early component and a late hump component. Following the nomenclature of Adrian & Peres (1979), the charge components carried by the early current and the late hump current will be called Q_β and Q_γ, respectively. In addition, following the nomenclature used in a previous paper (Hui, 1990), the currents associated with the movements of Q_β and Q_γ will be called I_β and I_γ, respectively. For larger depolarizations, I_γ had faster time courses and could not be visually separated from the early I_β component. The voltage distribution and the amount of total charge (listed in the figure legend) also fell within the ranges of the parameters in Table 3 of Hui (1983*b*). This implies that the muscle was probably normal. In this fibre, 350 mM-sucrose was sufficient to block fibre movement completely for depolarizations up to 0 mV but not for +19·2 mV.

Figure 6*B* shows charge movement traces recorded from a fibre in the paired muscle in the presence of 30 mM-BDM. With this BDM concentration, the fibre deteriorated rapidly. Every test pulse made the holding current increase and the membrane capacitance decrease. The trace at −30·3 mV was taken first and it appeared normal. The trace at −20·3 mV was taken second and the I_γ hump in the on-segment was small, compared with the corresponding trace in panel *A*. The traces at −25·3 and −35·2 mV were taken next and they had no I_γ hump. Several other experiments were attempted with 30 mM-BDM, but the resting potential declined even more rapidly. Hence, although 30 mM-BDM blocked contraction effectively (even up to +100 mV in one fibre), a final decision was made to return to 20 mM-BDM.

Twenty millimolar BDM is effective in suppressing movement artifact in some, but

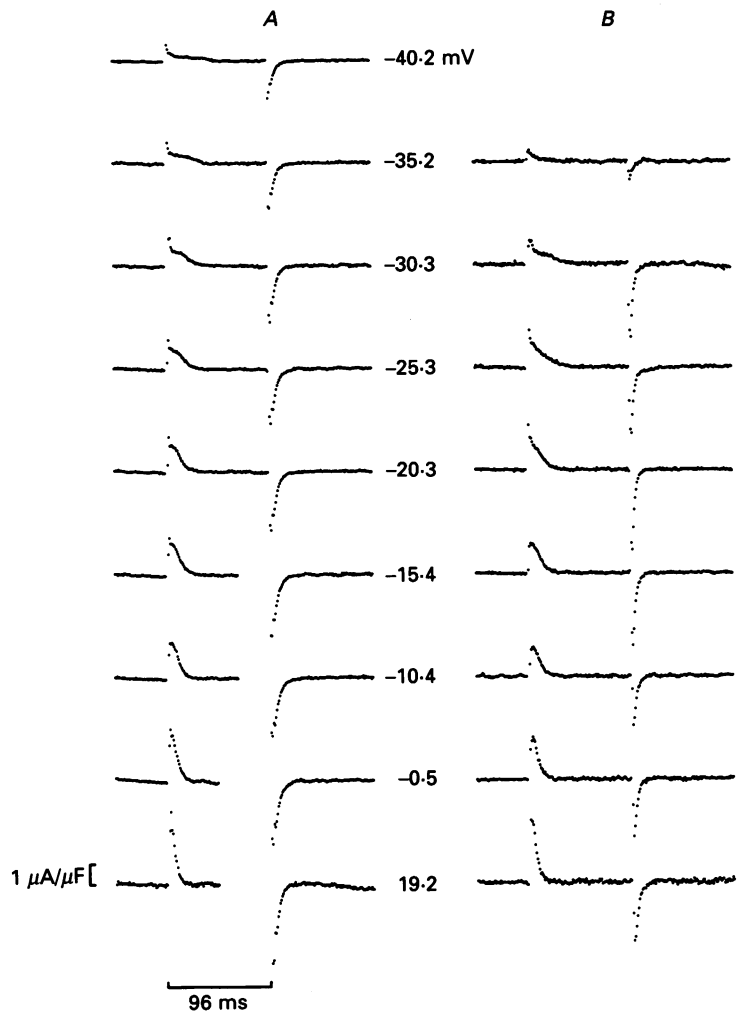

Fig. 6. Comparison of charge movement in an intact fibre in the presence and absence of BDM. *A*, charge movement traces recorded from a fibre in a semitendinosus muscle bathed in hypertonic TEA-Cl solution (solution C in Table 1). Fibre identification 59061. Temperature 5 °C. To convert ΔV signals to membrane current density, as shown by the vertical scale bar, R_i was assumed to be 363·9 Ω cm. The top trace is an average of eight sweeps and the last trace two sweeps. All other traces are averages of four sweeps. Some traces are interrupted during the 'on' of the pulse when they deviate from a straight line. The averages of on- and off-charge from the traces were plotted as a function of test-pulse potential and the Q–V plot (not shown) was least-squares fitted by eqn (1). The best-fit parameters were: $Q_{max} = 18{\cdot}0$ nC/μF, $\bar{V} = -40{\cdot}1$ mV and $k = 7{\cdot}3$ mV. *B*, charge movement traces recorded from a fibre in a semitendinosus muscle bathed in a TEA-Cl solution containing 30 mM-BDM (solution D in Table 1 with $x = 30$). Paired muscle from the same frog as in part *A*. Fibre identification 59062. Temperature 6 °C. R_i was assumed to be 263·4 Ω cm. The bottom two traces are averages of two sweeps and all other traces four sweeps. For both experiments, the numbers shown between the two columns indicate the potentials during the test pulses. Electrode spacings: $l = 200$ μm; $l' = 40$ μm; holding potential = −80 mV. In some traces one or two 'erratic' points at the 'make' or 'break' of the pulses were omitted (see Gilly & Hui, 1980).

not all, fibres. One of the successful experiments is shown in Fig. 7. Panel *A* shows charge movement traces that resembled those in the control solution (Fig. 6*A*), but the time course of the I_γ hump might be prolonged by 20 mM-BDM (for example, compare traces at −30 mV). Alternatively, since the trace at −20·4 mV in Fig. 7*A*

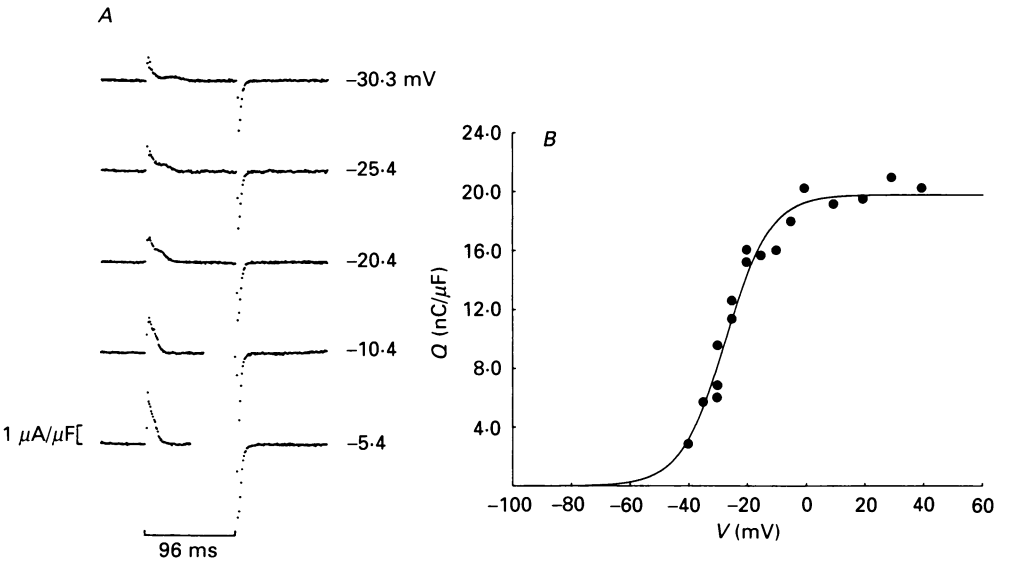

Fig. 7. Charge movement in an intact fibre in the presence of 20 mM-BDM. Semitendinosus muscle bathed in a TEA-Cl solution (solution D in Table 1 with $x = 20$). Fibre identification 59112; holding potential −80 mV; sarcomere length 3·35 μm; $l = 200$ μm; $l' = 40$ μm; temperature 5 °C. *A*, charge movement traces for depolarizations to voltages as indicated in millivolts on the right. To convert ΔV signals to membrane current density as shown by the vertical scale bar, R_i was assumed to be 271·8 Ω cm. Each trace is an average of four sweeps. The last two traces are interrupted during the 'on' of the pulse when they deviate from a straight line. *B*, steady-state Q–V plot. The average values of on- and off-charge are plotted against test pulse potential. The smooth sigmoidal curve was obtained by least-squares fitting eqn (1) to the points. The best-fit parameters are: $Q_{max} = 19{\cdot}8$ nC/μF, $\bar{V} = -27{\cdot}6$ mV and $k = 7{\cdot}6$ mV.

resembled the trace at −30·3 mV in Fig. 6*A*, 20 mM-BDM or the lower tonicity of the bathing solution might have shifted the voltage dependence of charge movement by about 10 mV in the depolarizing direction. To clarify this point, the amount of charge moved in each of the traces in Fig. 7*A* (and others not shown) is plotted in Fig. 7*B* as a function of the potential during the test pulse. The amount of charge definitely reached saturation at positive potentials. The smooth sigmoidal curve was fitted according to an equation based on a two-state Boltzmann model used by Chandler *et al.* (1976):

$$Q(V) = Q_{max}[1+\exp-(V-\bar{V})/k]^{-1}, \quad (1)$$

in which Q_{max} represents the maximum amount of charge moved to the active state, k represents the inverse steepness factor, and $\bar{V}$ represents the potential at which charges are equally distributed between the resting and active states. The best-fit

values for the parameters are listed in the sixth row of Table 3, together with those from other experiments. For comparison, the mean values of the parameters in control fibres from a previous paper (Hui, 1983*b*) are also listed in Table 3. The mean value of $\bar{V}$ in 20 mM-BDM is about 10 mV more positive than that in the control solution, confirming the observation mentioned above. The difference is highly

TABLE 3. Effect of BDM on Q–V distributions in intact fibres

Fibre reference (1)	Q_{max} (nC/μF) (2)	k (mV) (3)	$\bar{V}$ (mV) (4)
	In 20 mM-BDM		
57251	15·1	4·6	−29·5
57254	19·3	7·1	−30·5
58163	18·0	11·8	−23·2
58164	14·6	9·5	−23·1
59111	26·2	8·7	−28·6
59112	19·8	7·6	−27·6
5N012	14·6	6·6	−32·1
5N041	19·0	10·9	−23·2
Mean	18·3	8·4	−27·2
S.E.M.	1·3	0·8	1·2
	In control solution (see Hui, 1983*b*)		
Mean	23·7	7·0	−36·5
S.E.M.	1·1	0·3	0·7

significant ($P < 0.001$ with the two-tailed t test). The Q–V curve in test solution might be somewhat more shallow, as reflected by the slightly larger value of k, but the difference is not statistically significant ($P > 0.1$ with the two-tailed t test). The mean value of Q_{max} in the test solution is about 23% less than that in the control solution. The difference is statistically significant ($P < 0.01$ with the two-tailed t test). However, considering the drastic suppression of twitch tension by 20 mM-BDM, its effect on charge movement is relatively minor.

In the fibres listed in Table 3, charge movement traces showed an I_γ hump in the range of moderate depolarization and traces at large depolarizations were not accompanied by serious movement artifact. These are classified as successful experiments. An interesting observation was made in an experiment not included in Table 3. At the beginning of the experiment, an I_γ hump could be observed in the proper potential range and charge movement traces for large depolarizations showed movement artifacts. As the experiment progressed, the I_γ hump disappeared and the movement artifact subsided concomitantly, probably due to fibre deterioration. This is consistent with the suggestion that I_γ is closely associated with Ca^{2+} release and tension generation.

Effect of BDM on charge movement in cut fibres

To investigate whether the effects of BDM on Q_β and Q_γ are different, cut fibres are more convenient than intact fibres, because cut fibres have a larger proportion of Q_γ than intact fibres and the Q_β and Q_γ in cut fibres can be separated by a method developed by Hui & Chandler (1990). Figure 8*A* shows test-minus-control current

traces recorded from a cut fibre before the addition of BDM. These traces resembled those from other control cut fibres (Hui & Chandler, 1990) and also those from the intact fibre in Fig. 7*A*. The on-segments showed an early I_β component followed by an I_γ hump, whereas the off-segments showed a monotonically decaying transient.

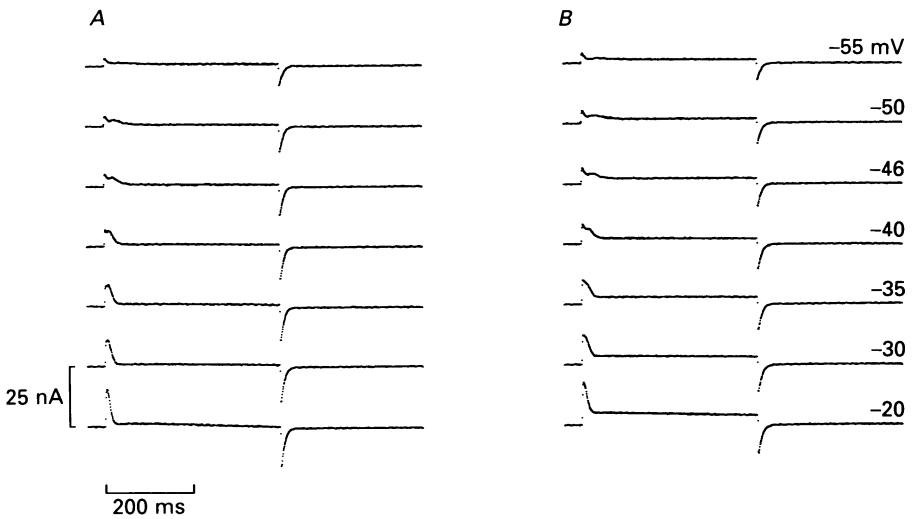

Fig. 8. Test-minus-control currents in a cut fibre with and without 20 mM-BDM in the external solution. Saponin treatment was applied to membrane segments in both end-pools at time zero. After rinsing, the solutions in the end-pools were replaced by solution K. Then, the solution in the centre pool was changed to a TEA-Cl solution (solution G). At the 16th minute, the voltage clamp was turned on and the holding potential was set at −90 mV. From the beginning to the end of the experiment, the holding current changed from −31 to −43 nA and $r_e/(r_i+r_e)$ increased from 0·982 to 0·983. *A*, traces taken in control solution (solution G) from the 58th to the 74th minutes. At the 78th minute, the external solution was changed to solution H with 20 mM-BDM. *B*, traces taken from the 88th to the 104th minute. Only representative traces are shown in each panel. The numbers at the right show the potentials during the test pulses (same for both traces in each row). Fibre identification 7D112; diameter 81 μm; temperature 13·5 °C.

The time course of the I_γ hump was shortened with increasing depolarizations. After the addition of 20 mM-BDM, the traces in panel *B* were taken. These traces only differed slightly from the corresponding ones in panel *A*: the peak of the I_γ hump was suppressed somewhat and its time-to-peak was delayed slightly.

The amount of total charge ($Q_\beta+Q_\gamma$) in each trace was estimated from the time integral of the current transient and is plotted in Fig. 9 as a function of the potential during depolarization. Symbols represent data taken in the absence of BDM (◆) and in the presence of 20 mM-BDM (■). The maximum amount of total charge was suppressed slightly by 20 mM-BDM. The two data sets were fitted by a sum of two Boltzmann distribution functions, each having the same form as eqn (1), with correction for the charge moved by the control pulse and correction for the charge moved in the membranes underneath the Vaseline seals (Hui & Chandler, 1990). The curves fitted the points very well, always better than a single Boltzmann distribution function. The best-fit parameters for the two components are listed in

the first entry in Table 4, with those for curve A in the first row and those for curve B in the second row. The two components had different voltage dependencies. The one with a smaller k value (i.e. more steeply voltage dependent) and more negative $\bar{V}$ value is identified with Q_γ and the other with Q_β. It appears that, in this fibre, the

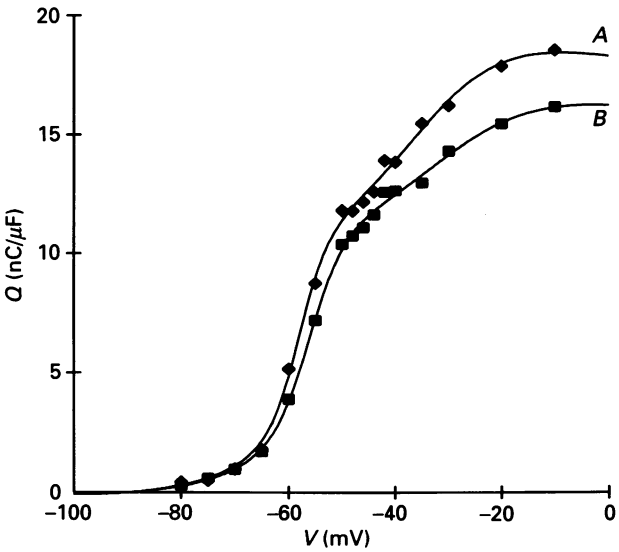

Fig. 9. Effect of 20 mM-BDM on steady-state voltage distribution of total charge ($Q_\beta + Q_\gamma$) in a cut fibre. Same fibre as in Fig. 8. ◆ and ■ show data taken without and with 20 mM-BDM, respectively, in the external solution. The points were obtained from the time integrals of off-transients in test-minus-control current traces, some of which are shown in Fig. 8. The smooth curves A and B were obtained by least-squares fits of a sum of two Boltzmann distribution functions, with corrections for charge movements in the control current trace and in membranes underneath the Vaseline seals. The values of the best-fit parameters are listed in the first two rows of Table 4.

charge suppressed by BDM can be accounted for by the reduction in Q_{max} of Q_β. A bracketing Q–V plot was also taken after wash-out (not shown in Fig. 9). and the best-fit parameters are listed in the third row of the entry.

Four other experiments of the same kind were performed and the results are also listed in Table 4. In one of the experiments, wash-out was omitted. Averaging over the five fibres, the values of $\bar{V}$ and k for both Q_β and Q_γ are not statistically different in the absence and presence of BDM ($P > 0{\cdot}05$ with the two-tailed t test). The amount of Q_β is reduced by 20 mM-BDM to 74% of control and the difference is statistically significant ($P < 0{\cdot}02$ with the two-tailed t test). The amount of Q_γ is reduced to 80% of control, but because of the scatter in values, the difference is not significant ($P > 0{\cdot}1$ with the two-tailed t test). The amount of total charge is reduced to 76% of control and the difference is marginally significant ($P < 0{\cdot}05$ with the two-tailed t test). From these numbers, it can be concluded tentatively that BDM does not differentially suppress Q_β or Q_γ.

It is worth noting that 20 mM-BDM blocks about one-quarter of the total charge in both intact and cut fibres. Since the effect of 20 mM of the drug is not substantial,

experiments with lower concentrations were not carried out. Moreover, the results from cut-fibre experiments showed that the presence of 20 mM-BDM does not affect the values of $\bar{V}$ and k for Q_β and Q_γ significantly. This implies that the shift in $\bar{V}$ and increase in k for the total charge in intact fibres by the same concentration of the drug might not be caused by the drug, but rather due to the difference in tonicity of the two solutions with and without the drug.

TABLE 4. Effect of BDM on Q–V distributions in cut fibres

		Q_β			Q_γ			Fraction of control		
Fibre reference	[BDM] (mM)	$\bar{V}$ (mV)	k (mV)	Q_{max} (nC/μF)	$\bar{V}$ (mV)	k (mV)	Q_{max} (nC/μF)	Q_β (%)	Q_γ (%)	Q_{total} (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
7D112	0	−37·1	8·9	11·8	−59·8	2·8	11·5			
	20	−33·2	10·3	9·0	−57·8	3·3	11·7	76	102	89
	0	−40·1	10·2	7·6	−50·8	3·0	10·4			
81051	0	−35·1	11·2	12·3	−57·4	3·4	7·6			
	20	−35·8	14·8	7·1	−52·1	4·2	6·7	58	88	69
	0	−33·3	15·8	10·1	−62·8	4·6	6·9			
81071	0	−38·5	10·6	15·7	−57·8	0·9	13·2			
	20	−22·1	14·4	10·6	−52·8	2·2	11·3	68	86	76
	0	−29·8	12·2	16·9	−59·4	2·6	12·2			
81072	0	−30·1	11·0	10·6	−55·8	3·9	13·4			
	20	−32·7	16·7	10·2	−42·0	3·7	10·1	96	75	85
81082	0	−28·3	11·6	11·8	−50·4	2·3	11·2			
	20	−49·9	7·9	8·6	−42·9	1·1	5·5	73	49	61
	0	−37·4	10·2	11·6	−53·5	1·7	8·5			
Mean								74	80	76
S.E.M.								6	9	5

Columns (1) and (2) give the fibre references and the concentrations of BDM in the external solution. Columns (3)–(8) give the best-fit values of the parameters in the Boltzmann distribution functions of Q_β and Q_γ, after correcting for the contributions from charge movement in control current trace and in the membranes underneath the Vaseline seals. Columns (9)–(11) are obtained by dividing, respectively, the values in column (5), column (8) and column (5) plus column (8) in the second row of each entry by the corresponding values in the first row.

DISCUSSION

Initially it was hoped that BDM blocks twitch tension in skeletal muscle by selectively interfering with cross-bridge formation while leaving all early steps in excitation–contraction coupling unaffected, as suggested by Mulieri & Alpert (1984). If that were the case, BDM would be an ideal contraction blocker for studying excitation–contraction coupling or properties of ionic channels in skeletal muscle. Unfortunately, results of our experiments showed that, except for resting potential, BDM affects other steps in the sequence, including action potential and charge movement (also Ca^{2+} signals to be reported in the following paper; Maylie & Hui, 1991). Each signal is affected by BDM quite differently and to a much lesser extent than twitch tension. The emphasis of this project was then shifted to compare and contrast the action of BDM on each step in the sequence.

Comparison of conditions of different groups of experiments

Ideally, the effects of BDM on all the signals should be compared under similar conditions, but, in reality, that is very difficult. For example, tension measurement is best performed on single fibres so that the tension transient is not dampened by the presence of non-contracting neighbouring fibres, and intact fibres (with tendons at both ends) are most suitable for mounting the fibres onto a tension transducer. Also, single intact fibres are almost free of connective tissues and so solution change is most effective. On the other hand, the Ca^{2+} signals (to be described in the following paper; Maylie & Hui, 1991) can be studied more conveniently in cut fibres, making it difficult to decide whether action potentials and charge movement should be studied in intact or cut fibres. Ultimately, these signals were studied in both preparations.

For action potential experiments on intact fibres, a muscle was dissected down to a bundle of fibres, which is not different from single fibres for electrical measurement and is probably more free of connective tissues than a whole muscle. For charge movement experiments on intact fibres, three microelectrodes have to be inserted into the end of a fibre. Very often, several fibres had to be impaled before a successful experiment could be performed. Thus, whole muscles were used in this group of experiments and the preparation is in line with all the previous work on intact fibre charge movement. The problem of the diffusion barrier caused by connective tissues was reduced by switching from sartorius muscles to halved semitendinosus muscles.

Although cut fibres and intact fibres might be in different physiological states, the effects of BDM on action potentials and charge movement were qualitatively similar in both preparations. In addition, the better signal-to-noise ratio in cut fibre records allowed more detailed data analysis of charge movement experiments. The results suggested that Q_β and Q_γ might be suppressed in equal proportion by BDM. However, cut fibres are not without complication. The kinetics of the I_γ hump in the on-segments of charge movement traces seemed to be retarded more by BDM than that in intact fibres. This latter difference could be an intrinsic property of cut fibres.

It should be noted that cut-fibre experiments were performed at around 16 °C for action potential measurement, as in Maylie, Irving, Sizto & Chandler (1987), or at around 14 °C for charge movement measurement, as in Hui & Chandler (1990). In contrast, all experiments on intact fibres were performed at a few degrees Celsius, as in Hui (1983*a*). Hence, in comparing results from different sections in this paper, readers should be aware of the differences in temperature and fibre preparation, although neither of them should affect the conclusions drastically. Horiuti *et al.* (1988) faced similar complications when they tried to correlate results from intact and skinned fibres. They observed that BDM was somewhat more potent in suppressing tension at a lower temperature.

Comparison with published results

Our experiments on twitch tension confirmed the finding of Mulieri & Alpert (1984) that BDM blocks twitch tension very effectively. There is a quantitative difference in the percentage of peak tension blocked by 7·5 mM-BDM, i.e. 85% in their experiments *versus* 98% in ours, but their experiments were at 15 °C whereas ours

were at 5 °C. We have extended their results to obtain complete dose–response curves. Our dose–response curves are similar to the one published by Horiuti *et al.* (1988), but their experiments were at 18 °C.

We also studied the effect of stretch on the effectiveness of BDM in suppressing tension. Along this line, Mulieri and Alpert found no difference in the blocking of peak tension whether the sarcomere length was 2·3 or 3·0 μm. Similarly, we found very little difference between the dose–response curves at sarcomere lengths of 3·0 and 3·6 μm. However, a transformation of the linear plots to double-reciprocal plots showed that the data at 3·0 μm deviated more from a straight line than those at 3·6 μm. Whether relaxing the fibres to sarcomere lengths $< 3{\cdot}0$ μm will make the double-reciprocal plot deviate even more from a straight line remains to be explored. A deviation of the double-reciprocal plot from a straight line is interpreted as the presence of non-identical binding sites for the ligands or of interacting binding sites (see, for example, Gutfreund, 1972). A detailed comparison of dose–response curves at a wider range of sarcomere lengths would probably provide more useful information.

Experiments on resting potential showed that fibres in whole muscles stayed healthy at rest, probably for a long time, in 20 mM-BDM. In contrast, an action potential was affected by BDM in two different ways. First, the peak amplitude of an action potential was suppressed in a dose-dependent manner, but this effect was very minor as compared to the suppressing effect on twitch tension. Second, BDM broadened the half-width of an action potential. The two effects are actually opposing each other. If it was not for the slight suppression of amplitude, BDM would almost act like a type B potentiator which enhances twitch tension rather than suppresses it. This implies that the suppression of tension would be even larger if tension was studied under voltage clamp.

As far as charge movement is concerned, the maximum amount of movable charge was suppressed by about one-quarter by 20 mM-BDM in both intact and cut fibres. In intact fibres, the k factor was increased somewhat by BDM and $\bar{V}$ was shifted about 10 mV in the depolarizing direction. As these changes were not observed in cut fibres, the most likely explanation for the changes in intact fibres is a reduction in the tonicity of the bathing solution. Fryer, Gage, Neering, Dulhunty & Lamb (1988) reported a negative effect of BDM on charge movement in rat soleus muscle which is different from its positive effect in frog muscle described in this paper.

The overall conclusion will be given in the Discussion section of the following paper (Maylie & Hui, 1991).

The authors are indebted to Dr W. Knox Chandler for the use of his optical set-up in carrying out the cut-fibre experiments. This work was supported by grants from the National Institutes of Health (NS21955 to C.S.H. and AM35737 to J.M.) and from the Muscular Dystrophy Association and American Heart Association to C. S. H. who was a recipient of a Research Career Development Award (NS00976) from the NIH.

REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Voltage clamp experiments in striated muscle fibres. *Journal of Physiology* **208**, 607–644.

ADRIAN, R. H. & PERES, A. R. (1979). Charge movement and membrane capacity in frog muscle. *Journal of Physiology* **289**, 83–97.

ALMERS, W. & BEST, P. M. (1976). Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *Journal of Physiology* **262**, 583–611.

ASKEW, B. M. (1956). Oximes and hydroxamic acids on antidotes in anticholinesterase poisoning. *British Journal of Pharmacology* **11**, 417–423.

CHANDLER, W. K. & HUI, C. S. (1990). Membrane capacitance in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *Journal of General Physiology* **96**, 225–256.

CHANDLER, W. K., RAKOWSKI, R. F. & SCHNEIDER, M. F. (1976). A non-linear voltage dependent charge movement in frog skeletal muscle. *Journal of Physiology* **254**, 245–283.

COJOCARU, Z., SERBAN, F., NASTASA, V., UNGUREANU, M. & LAZAR, M. I. (1985). Synthesis of diacetylmonoxime, a new reagent for urea determination in biological fluids. *Revistă Medico-chirurgicală* **89**, 661–662.

FRYER, M. W., GAGE, P. W., NEERING, I. R., DULHUNTY, A. F. & LAMB, G. D. (1988). Paralysis of skeletal muscle by butanedione monoxime, a chemical phosphatase. *Pflügers Archiv* **411**, 76–79.

GILLY, W. F. & HUI, C. S. (1980). Voltage-dependent charge movement in frog slow muscle fibres. *Journal of Physiology* **301**, 175–190.

GUTFREUND, H. (1972). *Enzymes: Physical Principles*. Wiley, New York.

HILLE, B. & CAMPBELL, D. T. (1976). An improved vaseline gap voltage clamp for skeletal muscle fibers. *Journal of General Physiology* **67**, 265–293.

HORIUTI, K., HIGUCHI, H., UMAZUME, Y., KONISHI, M., OKAZAKI, O. & KURIHARA, S. (1988). Mechanism of action of 2,3-butanedione 2-monoxime on contraction of frog skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **9**, 156–164.

HUANG, C. L.-H. (1982). Pharmacological separation of charge movement components in frog skeletal muscle. *Journal of Physiology* **324**, 375–387.

HUI, C. S. (1983*a*). Pharmacological studies of charge movement in frog skeletal muscle. *Journal of Physiology* **337**, 509–529.

HUI, C. S. (1983*b*). Differential properties of two charge components in frog skeletal muscle. *Journal of Physiology* **337**, 531–552.

HUI, C. S. (1989). Contractile properties of frog twitch fibres after D600 paralysis. *Journal of Muscle Research and Cell Motility* **10**, 473–488.

HUI, C. S. (1990). D600 binding sites on voltage-sensors for excitation–contraction coupling in frog skeletal muscle are intracellular. *Journal of Muscle Research and Cell Motility* **11**, 471–488.

HUI, C. S. & CHANDLER, W. K. (1990). Intramembranous charge movement in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *Journal of General Physiology* **96**, 257–297.

HUI, C. S. & MAYLIE, J. (1988). Effects of BDM on contractile and membrane electrical properties in frog twitch fibers. *Biophysical Journal* **53**, 646a.

HUI, C. S. & MILTON, R. L. (1987). Suppression of charge movement in frog skeletal muscle by D600. *Journal of Muscle Research and Cell Motility* **8**, 195–208.

IRVING, M., MAYLIE, J., SIZTO, N. L. & CHANDLER, W. K. (1987). Intrinsic optical and passive electrical properties of cut frog twitch fibers. *Journal of General Physiology* **89**, 1–40.

KOVACS, L., RIOS, E. & SCHNEIDER, M. F. (1983). Measurement and modification of free calcium transients in frog skeletal muscle fibres by a metallochromic indicator dye. *Journal of Physiology* **343**, 161–196.

LI, T., SPERELAKIS, N., TENEICK, R. E. & SOLARO, J. (1985). Effects of diacetyl monoxime on cardiac excitation–contraction coupling. *Journal of Pharmacology and Experimental Therapeutics* **232**, 688–695.

MAYLIE, J. & HUI, C. S. (1988). Effects of BDM on antipyrylazo III calcium signals in frog cut twitch fibers. *Biophysical Journal* **53**, 646a.

MAYLIE, J. & HUI, C. S. (1991). Action of 2,3-butanedione monoxime on calcium signals in frog cut twitch fibres containing Antipyrylazo III. *Journal of Physiology* **442**, 551–567.

MAYLIE, J., IRVING, M., SIZTO, N. L. & CHANDLER, W. K. (1987). Comparison of arsenazo III optical signals in intact and cut frog twitch fibers. *Journal of General Physiology* **89**, 41–81.

MELZER, W., SCHNEIDER, M. F., SIMON, B. J. & SZUCS, G. (1986). Intramembrane charge movement and calcium release in frog skeletal muscle. *Journal of Physiology* **373**, 481–511.

MULIERI, L. A. & ALPERT, N. R. (1984). Differential effects of 2,3-butanedione monoxime (BDM) on activation and contraction. *Biophysical Journal* **45**, 47a.

SADA, H., SADA, S. & SPERELAKIS, N. (1985). Effects of diacetyl monoxime (DAM) on slow and fast action potentials of young and old embryonic chick hearts and rabbit hearts. *European Journal of Physiology* **112**, 145–152.

TAYLOR, S. R., RUDEL, R. & BLINKS, J. R. (1975). Calcium transients in amphibian muscle. *Federation Proceedings* **34**, 1379–1381.

VERGARA, J. & CAPUTO, C. (1983). Effects of tetracaine on charge movements and calcium signals in frog skeletal muscle fibers. *Proceedings of the National Academy of Sciences of the USA* **80**, 1477–1481.

WIGGINS, J. R., REISER, J., FITZPATRICK, D. F. & BERGEY, J. L. (1980). Inotropic actions of diacetyl monoxime in cat ventricular muscle. *Journal of Pharmacology and Experimental Therapeutics* **212**, 217–224.