Protection of Human Left Ventricular Myocardium From Cutting Injury With 2,3-Butanedione Monoxime

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To prevent dissection injury when cutting strip preparations from human left ventricular papillary muscle tissue, dissections were carried out with 2,3-butanedione monoxime (30 mM) added to Krebs-Ringer solution and followed by washout with normal solution. Eleven muscle strip preparations were dissected from left ventricular papillary muscle tissue of five patients undergoing mitral valve replacement surgery. The average muscle strip length was 6.8±1.4 mm, and cross-section area was 0.49±0.16 mm². Peak twitch tension was 2.02±1.33 g/mm² and ranged from 0.67 to 5.5 g/mm² at an extracellular calcium concentration of 2.5 mM (21° C, 0.16 Hz). In one muscle strip, which was stored in Krebs-Ringer plus 2,3-butanedione monoxime solution for 20 hours, peak twitch tension in normal Krebs-Ringer solution was 1.85 g/mm². When temperature was increased from 21° C, there was a continuous increase in peak twitch tension (by 38%) up to about 28° C; then peak twitch tension decreased so that at 37° C (n=3) average peak twitch tension was lower than at 21° C by 47%. The force-frequency relation exhibited a broad force plateau between 40 and 120 beats/min at 37° C. The plateau was markedly narrowed at 30° C and 24° C. Thermopile heat measurements revealed appropriate waveform characteristics in high-resolution single-beat heat records indicating minimal surface cell damage. Thus, cardioplegia with 2,3-butanedione monoxime protects human left ventricular myocardium from dissection injury facilitating dissection and preservation of strip preparations with extraordinarily low cross-sectional areas and high peak twitch tensions. These preparations are suitable for myothermal and mechanical measurements. (Circulation Research 1989;65:1441–1444)

Investigation of human myocardium under in vitro conditions is a promising approach to understanding the processes that occur when myocardial failure develops. Since left ventricular dysfunction is the most common cause of human congestive heart failure, left ventricular myocardium is of primary interest for in vitro investigation. Viable human left ventricular myocardium can be obtained from cardiac transplantation or from open heart surgery. A suitable preparation for mechanical and myothermal measurements should have normal electrical excitability and a small enough cross section for adequate oxygenation. For physiological temperature and stimulation frequency, cross sections should be less than 0.59 mm². Since naturally occurring left ventricular papillary muscles or trabeculae of this size are seldom available, left ventricular myocardial strips have been dissected from larger papillary muscles, or right ventricular myocardium has been substituted. The substitution is appropriate only if the right and left ventricular myocardium is identical. Dissecting left ventricular muscle strips is problematic because of cutting injury, which is more significant the smaller the cross section. This may explain why muscle strips with large cross-sectional areas can give high tension provided low frequencies and/or temperatures are used and why small muscle strips that can be used at physiological conditions give low tensions.

We investigated methods of preventing the dissection injury that usually occurs when muscle strips are cut from left ventricular myocardium. The rationale for the method developed is based on the...
assumptions that 1) a major part of the irreversible nature of cutting through muscle cell membranes is attributable to the self-destructive effects of the resulting contracture and supercontracture and 2) these can be prevented by 2,3-butanedione monoxime (BDM). BDM was chosen because it is an effective, quick-acting, and reversible inhibitor of cardiac contractility and it can protect myocardium from hypoxia and the calcium paradox.

Materials and Methods

The method was developed by use of rabbit and dog myocardium. In preliminary experiments with normally dissected rabbit right ventricular papillary muscles, increasing quantities of BDM were added to normal Krebs' solution until visual signs (×10 magnification) of the contracture response to cutting or pinching the tissue were minimized. Although the protective effect of BDM seemed to saturate at a concentration of 20–25 mM, we chose 30 mM because, in muscle strips cut from dog left ventricular papillary muscles, spontaneous peristaltic contractions of sarcomeres (×400 bright field microscopy) were observed unless the higher dose was used. The "protective" BDM-Krebs solution contains (mM) BDM 30, Na⁺ 152, K⁺ 3.6, Cl⁻ 135, HCO₃⁻ 25, Mg²⁺ 0.6, H₂PO₄⁻ 1.3, SO₄²⁻ 0.6, Ca²⁺ 2.5, and glucose 5.6. In later experiments, we found that doubling the glucose concentration to 11.2 mM and adding 10 IU insulin per liter of solution increased the percentage of strip preparations that gave stable performance to 80–100%.

We measured the reversibility of pretreatment with BDM on conventionally dissected right ventricular papillary muscles from rabbit hearts that were not pretreated with BDM. Isometric twitch tension was measured before soaking the muscles in 30 mM BDM-Krebs-Ringer solution for 30–60 minutes. Twitch tension was measured again after washout of the protective solution and found to decline by 2.5±2.32%. A paired t test indicated that the reversal values were not significantly different from the control values (p>0.05, n=8).

We also measured the degree of protection by the BDM solution against the injury of cutting the base of the muscle away from the ventricular wall in conventionally prepared right ventricular papillary muscles. Isometric twitch tension (0.3 Hz, 30° C) in muscles prepared from hearts that were pretreated with BDM was 10.1±1.2% (p<0.05) lower than the value obtained (1.95±0.27 g/mm², n=5) from muscles prepared in the conventional way without pretreatment of the heart.

The possibility that the protective action of BDM is achieved through an osmotic rather than a specific effect was assessed by determining experimentally whether the cells were impermeable to BDM. The effect of adding 163 mM BDM to the Krebs-Ringer solution on muscle cross-sectional area was compared with the effect of adding the same amount of mannitol to give 1.5 times normal osmotic strength. Average cross-sectional area was determined by optical measurement (×50 magnification) of four diameters at each of four places (a total of 16) along the length of the muscle. In five preparations (three human and two rabbit), addition of 163 mM mannitol caused a 10.1±1.2% (p<0.05) decrease in cross-sectional area whereas addition of the same quantity of BDM caused a slight (5.2±1.1%, p=NS) increase in cross-sectional area. This shows that the BDM used in the protective solution (30 mM) was permeable and would exert negligible osmotic effects on the myocardial cells.

Human papillary muscle tissue was obtained from mitral valve replacement surgery in three cases of severe mitral regurgitation, in one case of mitral stenosis with combined moderate mitral regurgitation, and in one case of combined aortic and mitral regurgitation. Immediately after excision of a portion of the tendinous end of the anterior papillary muscle, the tissue was submerged in preoxygenated (95% O₂-5% CO₂) BDM-Krebs-Ringer solution (21° C). After 30 minutes, the tissue was transferred to the dissection chamber containing the same solution and mounted between clamps. Muscle strips (0.49±0.16 mm² cross section, 6.8±1.4 mm length, n=11) were cut along the fiber direction by means of scissors (6-mm blade) under a ×7–10 binocular microscope. Silk ligatures containing platinum wires were attached to the muscles to facilitate end-to-end stimulation and connection to the isometric force gauge. After this, the muscle strips were either used immediately (60 minutes after excision) or stored in continuously oxygenated BDM-Krebs-Ringer solution for later experimentation. To perform force and heat measurements, the muscle strip was mounted in contact with the active region of a thermopile and connected to the force gauge. The muscle and thermopile were then submerged in normal Krebs-Ringer solution at 21° C, which washed out the BDM solution. Stimulation was begun at intervals of 6 seconds, 20% above threshold. All-or-none responses were obtained in each preparation, and twitch force increased during 60 minutes until a stable force value was reached. In later experiments, we found that raising the temperature to 30° C during BDM washout and equilibration improved muscle recovery considerably. After another 30–50 minutes, the muscle was stretched to optimum length at which maximum twitch force was reached. Thereafter, heat and mechanical measurements were performed and analyzed as previously described. Mechanical measurements were performed at 21° C (n=11), 30° C (n=5), and 37° C (n=3). Force-frequency relations were measured at 24° C, 30° C, and 37° C in two muscle strip preparations. Complete time-course heat records were corrected for heat loss by the Hill method. In a few preparations, myocyte orientation and sarcomere length were assessed by using ×400 bright field
microscopy to confirm that cell orientation was parallel to the longitudinal axis of the muscle strips. Resting sarcomere lengths of 2.3–2.4 μm were observed at optimum length. Cross sections were estimated from the blotted weight and length of the portion of muscle strip lying between the two silk ligatures assuming unity density. Values are given as mean±SD.

Results
Figure 1 shows representative records at optimum length from a human myocardium preparation. The heat record exhibits expected behavior in that it has a rapidly rising phase (initial heat), which ends near the time of complete relaxation of force. This phase is followed by a slow phase of heat evolution attributable to recovery heat. Of particular interest is the lack of time delay in onset of heat evolution since presence of as little as 50 μm (6% of muscle diameter) of dead tissue would cause the rise time to increase from 20 msec to 470 msec.12

In the human myocardium, average peak twitch tension at 21°C was 2.02±1.33 g/mm², which ranged from 0.67 to 5.5 g/mm², and average resting tension was 2.01±1.08 g/mm² (n=11). One preparation was stored in BDM-Krebs-Ringer for 20 hours before use and had a peak twitch tension of 1.85 g/mm² and a resting tension of 1.78 g/mm². When the temperature was increased above 21°C, peak twitch tension increased continuously (by 38%) up to about 28°C; above this temperature, peak twitch tension decreased so that at 37°C (n=5) it was lower than the 21°C value by 47%.

The force-frequency relations at 24°C, 30°C, and 37°C are shown in Figure 2. At 37°C, total twitch duration decreased as frequency increased so that it remained shorter than stimulus interval up to 2-Hz stimulation rate.

Discussion
The work of Sonnenblick et al3 in excising viable muscle preparations from human hearts during corrective cardiac surgery has demonstrated the feasibility of making standard mechanical measurements on isolated human myocardium. These experiments had to be performed far from physiological conditions because the thickness of the available human papillary muscle tissue (5.5±3.9 mm²) did not allow for adequate oxygenation. Although dissection of thinner muscle strip preparations allowed higher temperatures and stimulation rates to be used, the low tension values obtained still limit the usefulness of this method.4–13

Using BDM–Krebs-Ringer solution in the dissection procedure, we obtain viable human left ventricular muscle strips that are electrically excitable, produce high tension values, and have small enough cross-sectional areas (<0.59 mm², see Figure 2) for adequate oxygenation at 37°C and 120 beats/min. The peak twitch tensions obtained from BDM-dissected left ventricular muscle strips lie between those obtained from uncut whole guinea pig muscles14 and rabbit11 papillary muscles. The average peak twitch tension values obtained in BDM-dissected preparations are considerably higher than those obtained previously in small diameter (<1 mm) human myocardial strips or trabeculae. Tension values obtained from the BDM-dissected muscle strips are 2.8–6 times larger than from muscle strips cut from left ventricular papillary muscles by others4,13 without BDM protection. Muscle strips
prepared under BDM protection also give 1.7–2.9 times more tension than whole right or left ventricular papillary muscles or trabeculae prepared by others without BDM protection. The results of the control experiments on rabbit myocardium described in ‘Materials and Methods’ argue favorably that pretreatment with 30 mM BDM followed by washout is reversible and that the higher values of twitch tension reported here for human myocardial strips result from the protective action of BDM.

For the first time the force-frequency relation of human left ventricular myocardium has been demonstrated at 37° C. The peak twitch tension is constant between 40 and 120 beats/min. This shows that the previously reported plateau over the limited range of 10–50 contractions per minute and 30° C extends over the whole resting physiological range and beyond. Since the same behavior has been demonstrated in the intact right human ventricle, these findings suggest that the Bowditch effect or force-frequency staircase may not always be present in human myocardium.

The most probable mechanism by which BDM prevents dissection injury is by reducing the contracture that results from cutting cell membranes. Such a reduction diminishes the tendency of injured cells to tear themselves apart during the membrane’s “self-healing” period. BDM reduces the contractures because it decreases the sensitivity of the contractile proteins to calcium ions and exerts a direct inhibitory effect on cross-bridge interaction. 5–7 Furthermore, 30 mM BDM has been shown to preserve ATP and phosphocreatine stores in hypoxic cells to tear themselves apart during the membrane’s “self-healing” period. BDM reduces the contractures because it decreases the sensitivity of the contractile proteins to calcium ions and exerts a direct inhibitory effect on cross-bridge interaction. 5–7

The ability to cut left ventricular myocardium to dimensions suitable for thermoplebe measurements allows, for the first time, the details of beat-to-beat energetics to be measured. Such time resolution (as short as 10 msec) is not possible with currently available oxygen measurements. Myothermal measurements can now be used in investigations of disease-related changes in crossbridges, excitation-contraction coupling, recovery, and resting metabolism in living isolated human myocardium as they have in other species. 11

The demonstrated effectiveness and reversibility of BDM as a cardioplegic agent for isolating preparations of animal and human myocardium opens the possibility of developing a cardioplegic solution for open heart surgery based on BDM.

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References


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