Mechanical and Structural Correlates of Contracture Induced by Metabolic Blockade in Cardiac Muscle from the Rat

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SUMMARY We performed morphological studies of myocardial contracture to define its nature and relationship to mechanical changes occurring during metabolic blockade. Isolated rat papillary and trabecular muscles were stretched to the apices of their length-tension curves and stimulated to contract isometrically 12 times a minute at a temperature of 28°C. Incomplete and total metabolic blockade were induced by 1 hour of hypoxia (95% N₂, 5% CO₂) or by hypoxia plus glycolytic blockade with iodoacetic acid, 10⁻⁴M, respectively. In oxygenated control preparations, mechanical performance was stable for the 60-minute experimental period. In preparations exposed to hypoxia, developed tension fell to 7 ± 2% of prehypoxia values at 60 minutes. Contracture tension increased progressively to 2.5 ± 0.4 g/mm¹. With total metabolic blockade, developed tension declined to zero by 10 minutes, contracture tension rose to an average peak value of 5.3 ± 0.4 g/mm¹ by 15 minutes, and subsequently slowly declined. All preparations were fixed at 24°C, in the muscle bath. Light and electron microscopic studies revealed focal irregularities of A, I, and Z bands with sarcomere malalignment, hypercontraction, and fiber disruption, which increased in severity with increasing metabolic blockade. Linear densities appeared in mitochondria following total metabolic blockade, but mitochondria appeared normal otherwise. Thus, myocardial contracture after metabolic blockade is a focal process beginning within the sarcomere; morphological alterations in the contractile apparatus correlate with mechanical changes and are more severe than those in the mitochondria. Circ Res 45: 298-308, 1979

INCREASING interest recently has been focused on the process of contracture of the myocardium. This event is seen in isolated muscle during hypoxia (Bing et al., 1975; Greene and Weisfeldt, 1977) and also appears following global ischemia of the intact heart (MacGregor et al., 1975; Cooper, 1975). The “stone heart” syndrome, which appears in occasional patients after prolonged periods of ischemic arrest during open heart surgery (Cooley et al., 1972), probably is due to contracture of the myocardium (Katz and Tada, 1972; Hearse et al., 1977). In the present study of isometrically contracting isolated left ventricular muscle preparations, contracture has been induced by incomplete (with hypoxia alone) and total (hypoxia plus glycolytic blockade) metabolic blockade. We used graded metabolic blockade of myocardium held at a fixed length to gain further insight into the structural basis for mechanical events that occur during contracture.

Methods

After decapitation of male Charles River CD rats weighing 150-250 g, the heart was removed rapidly and placed in oxygenated Krebs-Henseleit solution (Krebs and Henseleit, 1932) at 30°C. Trabeculae carnea and papillary muscles were carefully dissected from the left ventricle and mounted vertically between two spring clips in a chamber containing Krebs-Henseleit solution with 5.5 mM glucose. The solution was gassed with 95% O₂ and 5% CO₂ (pH 7.4), and the temperature was kept at 28°C by a Lauda model K-2 circulating pump. The lower spring clip attached to the muscle preparation was connected to a Statham G7B-0.75-350 force transducer by a hollow stainless steel section of 30-gauge needle tubing, which passed through a mercury seal at the bottom of the muscle chamber. The upper clip was connected by a thin gold chain to a lever arm, above which a micrometer stop was mounted to adjust muscle length. The preparations were stimulated 12 times/minute by a Grass model SD-9 stimulator which delivered 7-msec rectangular pulses through parallel platinum electrodes at voltages that were approximately 10% greater than the minimum required to produce a maximum mechanical response.

Studies on Mechanical Activity

After a 30-minute equilibration period during which the preparations shortened while carrying light loads, the muscles were loaded to contract
isometrically and were stretched carefully to the apices of their length-tension curves. After a 15-minute period during which preparations performed afterloaded isotonic contractions, muscles again were placed under isometric conditions, and a final careful determination of the apex of the length tension curve (L_max) was made. This muscle length was maintained for the remainder of the experiment. A 15-minute period of stable isometric contractions was present prior to the 60-minute experimental period. Hypoxia was induced by vigorously gassing the superfusate with 95% N_2 and 5% CO_2. In experiments using glycolytic blockade, 10^{-4} M iodoacetic acid (Eastman) was added to the bath 15 minutes prior to hypoxia.

Three groups of muscles were studied: a control group, a group of muscles exposed to hypoxia alone, and a third group in which muscles were exposed to total metabolic blockade consisting of hypoxia and iodoacetic acid, 10^{-4} M. At selected times the Krebs-Henseleit bath solution was replaced by fixative (see below), and all preparations were fixed for microscopic studies while in the muscle chambers.

**Studies of Morphology**

At the completion of the studies on mechanical activity, all muscle preparations were fixed at L_max at room temperature for 1 hour in the evacuated bath chamber by the addition of fixative (2.7% glutaraldehyde and 2% paraformaldehyde in 0.066 M cacodylate buffer at pH 7.3 to 7.4. Fixation was continued for an additional 8–12 hours after removal of the preparations from the muscle chambers. Tissues then were cut into 1- to 2-mm cubes and placed in 0.2 M cacodylate buffer prior to postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The tissues then were dehydrated in increasing concentrations of ethyl alcohol and placed into propylene oxide and mixtures of propylene oxide and Epon prior to final embedding in Epon 812. Attempts were made to embed all tissue samples so that longitudinal orientation of myocardial fibers was obtained. Sections were cut on an LKB ultratome III. Toluidine blue-stained 0.5- to 1-μm thick sections were examined by light microscopy to select areas to be studied by electron microscopy. Tissue samples, which by light microscopy appeared well-fixed, free of artifacts, and oriented longitudinally were studied further by cutting thin sections which were mounted on plain copper grids, stained with uranyl acetate and lead citrate, and examined with a Phillips 201 electron microscope. Since light microscopic evaluation of toluidine blue-stained thick sections revealed consistent artificial contraction bands in cut fibers at the edges of each specimen and less than optimal fixation in the very center of these specimens, these areas were excluded from further study.

To measure sarcomere length, the following procedures were carried out: electron micrographs were prepared at calibrated magnifications of either 3032X or 7024X by using a calibration grid containing 28,800 lines per inch, and measurements of sarcomeres were made directly from negatives (3032X) by measuring the Z-to-Z band distance at the midpoint of the sarcomeres. Because shrinkage of tissues occurs during processing (Page, 1974), measured sarcomere lengths differed from in vivo values. A correction factor was calculated and used to determine a "corrected" sarcomere length measurement. Measurements of tissue samples before and after dehydration revealed 10% shrinkage. As our measurements of control sarcomeres at L_max were 16% less than reported in vivo values (Page, 1974; Grimm and Whitehorn, 1968), a 6% shrinkage due to initial fixation was assumed. Thus, corrected sarcomere lengths were calculated by assuming 16% shrinkage due to tissue processing.

**Results**

**Mechanical Activity**

During the equilibration period prior to hypoxia, mechanical activity was stable. At this time contracture did not appear in any preparation; in fact, resting tension frequently fell slightly (Fig. 1). In the 15-minute period after addition of iodoacetate acid to oxygenated preparations, no change in mechanical performance was seen. Similar observations have been made previously which suggest that no portion of mechanical activity is supported by glycology under oxygenated conditions (Evans, 1939; Frezza and Bing, 1976).

In six control preparations, a 1-hour period of isometric contractions resulted in no significant change in developed tension, and contracture did not appear (Figs. 1 and 2). In six preparations subjected to 60 minutes of hypoxia, developed tension declined in an approximately exponential manner. At 60 minutes, developed tension had fallen to 7 ± 2% of prehypoxia values. Five minutes after hypoxia was initiated, contracture began to appear and increased progressively to produce a tension of 2.5 ± 0.4 g/mm² after 60 minutes. In six additional preparations subjected to combined hypoxia and glycolytic blockade, developed tension fell promptly and declined to zero by 10 minutes. Shortly after hypoxia was initiated, contracture appeared and increased to cause an average peak tension of 5.3 ± 0.4 g/mm² by 15 minutes; subsequently, contracture declined slowly so that tension at 60 minutes was 2.7 ± 0.1 g/mm².

**Morphology**

**Controls**

By light and electron microscopy, control muscle fibers showed well-aligned parallel myofibrils (Figs. 3A and 4). Sarcomeres had well-defined and uniform A, I, and Z bands. Mitochondria showed normal matrix density, and cristae were compact. Gly-
cogen granules were present. Sarcomeres were very uniform and measured $1.93 \pm 0.01 \mu m$ (2.30 $\mu m$, corrected) in length (mean $\pm$ SEM, $n = 100$) (Table 1).

Hypoxia

Samples subjected to 1 hour of hypoxia (95% $N_2$, 5% $CO_2$) showed variability in morphology when compared with controls. By light microscopy, malalignment of sarcomeres was evident (Fig. 3B). Dark staining areas were noted to consist of contracted sarcomeres with no I bands. Light staining areas consisted of elongated sarcomeres with wide I bands. Abrupt changes in sarcomere length often were noted to occur at intercalated discs. Ultrastructural examination confirmed the above findings. In addition, there also were focal irregularities of A, I, and Z bands within individual sarcomeres (Fig. 5). Some areas demonstrated marked irregularities of Z bands with focal absence of I band material between A bands. In some sarcomeres only one I band, or only a fraction of an I band, was seen. Mitochondria were similar in appearance to those of controls, but glycogen granules were less frequent than in control tissue. Mean sarcomere length was identical to controls, 1.93 $\mu m$ (2.30 $\mu m$, corrected); however, there was greater variability ($SEM = \pm 0.12$). Two populations of sarcomeres could be separated: (1) contracted sarcomeres with absence of I bands, measuring $1.51 \pm 0.02 \mu m$ (1.80 $\mu m$, corrected), and (2) sarcomeres with I bands or portions of I bands visible. A number of these were elongated with wide I bands. In this group, sarcomere length averaged $2.12 \pm 0.03 \mu m$ (2.52 $\mu m$, corrected).

Hypoxia Plus Glycolytic Blockade

Muscles were fixed after 5 minutes of total metabolic blockade (95% $N_2$, 5% $CO_2$ plus iodoacetic acid, $10^{-4} M$) when contracture tension was increasing rapidly (Fig. 2). Light microscopy demonstrated narrowing of I bands in some areas and widening in others, whereas other areas appeared near normal (Fig. 6A). Electron microscopic studies showed marked irregularities of the Z bands (Fig. 6B), and changes were generally quite similar to the studies of 60 minutes of hypoxia alone. Sarcomeres averaged $1.93 \pm 0.06 \mu m$ (uncorrected); those with no I bands visible measured $1.57 \pm 0.02 \mu m$, and those in which I bands were visible measured $2.28 \pm 0.02 \mu m$.

After 15 minutes of total metabolic blockade when contracture tension was near its peak, light microscopic studies demonstrated focal areas of hypercontraction, and other areas appeared to have
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FIGURE 3 Light micrograph of 0.5- to 1.0-μm thick sections of rat papillary muscle. In the control preparations (A), the sarcomeres are quite uniform and well-aligned, and the I, A, and Z bands are distinct. In myocardium subjected to 1 hour of hypoxia (B), there is loss of general uniformity. Sarcomeres that appear dark lack visible I bands; those that are lighter show wide I bands. Note that a distinct change in appearance occurs at the intercalated disc (arrow). In myocardium subjected to hypoxia and glycolytic blockade (C), the fibers are disrupted at the intercalated disc (arrow), and intact sarcomeres are severely contracted and lack I bands (toluidine blue, original magnification 252X). In this and all subsequent micrographs, the scale bar equals 5 μm.

widened I bands (Fig. 7a). On electron microscopy, areas of severe hypercontraction were seen, and there was distortion of adjacent structures (Fig. 7b). Average sarcomere length in these studies was 1.84 ± 0.06 μm (uncorrected). Sarcomeres without I bands measured 1.46 ± 0.02 μm, whereas those in which I bands were visible measured 2.22 ± 0.02 μm.

At 60 minutes, samples showed the most severe changes. Some areas resembled those from muscles subjected to 5 minutes of total metabolic blockade but showed greater variability in sarcomere morphology. Other areas showed complete disruption of myocardial fibers, which sometimes could be recognized to have occurred at the intercalated disc (Fig. 3C). Most intact sarcomeres were severely distorted with marked irregularities of A and I bands and malalignment of myofilaments. Occasional mitochondria contained linear densities (Fig. 8). Glycogen was more abundant than in muscles subjected to only 1 hour of hypoxia. Sarcomeres generally were contracted severely; mean sarcomere length was 1.65 ± 0.03 μm (1.96 μm, corrected). In those in which no I bands were visible, sarcomeres measured only 1.40 ± 0.03 μm (1.67 μm, corrected). Those that had I bands visible within the sarcomere also were somewhat more contracted than controls and measured only 1.89 ± 0.03 μm (2.25 μm, corrected).

Isotonic Contractions

To exclude the possibility that hypercontraction seen in the isometric studies may have been the result of post-tear artifact, additional studies were carried out with muscles contracting isotonically. Preparations were lengthened to Lmax under baseline conditions, subjected to 60 minutes of total metabolic blockade, and then fixed in the muscle chambers. Light microscopy demonstrated a general wavy pattern of the myofibers, and narrow-to-absent I bands were present throughout (Fig. 9A).
FIGURE 4 Electron micrograph of control myocardium demonstrating well-aligned sarcomeres with distinct A, I, and Z bands and normal mitochondrial (M) morphology (uranyl acetate and lead citrate, A = 3032×, B = 7024×).

On electron microscopy all sarcomeres appear contracted uniformly (Fig. 9B), with average sarcomere lengths of 1.38 ± 0.07 μm. Mitochondria demonstrated a few linear densities but otherwise appeared relatively normal despite the presence of markedly contracted sarcomeres.

Studies with Surface Markers

To exclude the possibility that the slow decline in force might be due to artifactual lengthening at the damaged ends of the preparations, surface markers were attached to delineate the central one-

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<th>Sarcomere Z-to-Z Band Distance at 60 Minutes</th>
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* Values corrected for 16% shrinkage.
third of four additional preparations. During maximum contracture and, subsequently, after the decline in force development at 60 minutes, no change in the distance between surface markers was seen in three of four preparations. In one study, intermarker distance decreased 10%; however, we noted excess ventricular wall tissue at one end that was not present in the other three preparations or in the preparations in which microscopic studies had been carried out. These additional studies with surface markers suggest that the fall in tension with prolonged contracture is not due to lengthening at the damaged ends of the muscle preparation. The slow decline in force after contracture tension had reached a peak, and the decrease in mean sarcomere length in muscle preparations fixed at $L_{\text{max}}$ most likely can be attributed to the observed tearing apart of sarcomeres. Disruption occurred most commonly in the region of the intercalated disc.

**Discussion**

The process of contracture in skeletal muscle has been studied and appears to be the result of rigor complex formation secondary to the depletion of high energy phosphate stores (Weber and Murray, 1973). Studies of cardiac muscle likewise indicate ATP depletion to be responsible for the development of contracture (Hearse et al., 1977). The results of our studies of incomplete and total metabolic blockade are consistent with this concept. Although investigations of the mechanics and biochemistry of cardiac contracture have been carried...
out, morphological studies have been suboptimal because, in excised biopsies, myocardium contracts and ultrastructural features of the sarcomeres are altered. In the present investigation, the effects of contracture on myocardium fixed at $L_{\text{max}}$ are demonstrated.

Contracture appears to be a relatively early event in the sequence of injury to hypoxic rat myocardium because it is present at a time when mitochondrial morphology appears to be relatively normal. The process appears to begin as a focal event because some areas of the myocardium are severely affected, whereas others appear relatively normal. There even are differences in the degree of contracture...
within the individual sarcomere. Variations in the degree of involvement of myofilaments may be the reason for the marked irregularity of the Z band that is commonly seen. Although differences in sarcomere length probably reflect variability in the degree of contracture, the presence of very elongated sarcomeres probably represents the stretching of sarcomeres adjacent to those which are severely contracted. On the other hand, studies carried out under isotonic conditions in which shortening is allowed reveal uniformly contracted sarcomeres. Thus, variations in the degree of contracture

FIGURE 7  Myocardium after 15 minutes of hypoxia and glycolytic blockade. Light microscopy (A) shows more variability in sarcomere length than at 5 minutes. Severely contracted and stretched sarcomeres are evident (toluidine blue, original magnification = 252×). Electron micrograph (B) of one of the most severely distorted areas shows the beginning of tearing apart of fibers with loss of normal pattern of A, I, and Z bands. Such areas were infrequently found at 15 minutes of total metabolic blockade (uranyl acetate and lead citrate, original magnification = 3032×).
seen in isometric studies likely reflect minor inhomogeneities, which are brought out by maintaining the ends of the preparation at fixed length.

In the isometric studies, two populations of sarcomeres arbitrarily were separated: those with total absence of I bands and those in which an I band was visible within the sarcomere. After 60 minutes of hypoxia, despite the presence of both shortened and elongated sarcomeres, it was found that the average sarcomere length of these two populations was no different from the population of normal sarcomeres (Table 1). This may represent the average of shortened contracted sarcomeres and those which are actively or passively elongated.

With 60 minutes of total metabolic blockade, the two populations of sarcomeres described above both were shorter than those from normally oxygenated preparations, and contracture tension at 15 minutes was considerably greater than after 1 hour. It is unlikely that the slow decline in force after contracture tension had reached a peak is due to a lessening of the contracture process, as sarcomere length would be expected to increase rather than decrease between 15 and 60 minutes of total metabolic blockade. If slippage of myofibers was occurring, sarcomere length might decrease, but rupture or tear, as observed (Fig. 3C), might not be expected.

Since rupture occurs most commonly at the intercalated disc, this region of the myofibril appears most susceptible to the stresses imposed by sarcomeres in rigor. Rupture was seen only in isometric studies and may reflect a direct effect of mechanical stress. Local weaknesses also may be induced in part by energy depletion secondary to augmented mechanical activity associated with the contracture process.

Whereas caution must be used in extrapolating from studies on metabolic blockade in isolated muscle to the intact heart, it might be pointed out that, although ischemic myocardium is not held at fixed length as in these studies, poorly contracting ischemic areas of the heart may be subjected to considerable stress repeatedly imposed by contractions of the normal myocardium. It would not be

**Figure 8** Myocardium subjected to 1 hour of hypoxia and glycolytic blockade. There is marked irregularity of sarcomeres and A, I, and Z bands. Mitochondria (M) contain linear densities (uranyl acetate and lead citrate, $A = 3032 \times$, $B = 7024 \times$).
FIGURE 9  Myocardium allowed to shorten (isotonic studies) while subjected to 60 minutes of hypoxia and glycolytic blockade. Light microscopy (A) shows the uniformly contracted sarcomeres. Fibers also exhibit waviness associated with marked shortening (toluidine blue, original magnification = 252×). Electron microscopy (B) shows the severely contracted sarcomeres with no visible I bands (uranyl acetate and lead citrate, original magnification = 3032×).

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increased mechanical support of ischemic myocardium resulting from the contracture process may decrease the ischemic bulge (Tennant and Wiggers, 1935) and have a salutary role by improving overall cardiac performance.

Acknowledgments

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