Tension Prolongation during Recovery from Myocardial Hypoxia

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ABSTRACT The mechanical behavior of isolated cat, rat, and dog ventricular muscle was examined during hypoxia and after reoxygenation. During hypoxia, an early abbreviation of tension duration was followed by a decline in the rate of tension development. After reoxygenation, a marked, early prolongation of tension development and relaxation time was invariably observed with little, if any, increase in peak tension. As recovery progressed, the duration of contraction gradually shortened as tension returned to control levels. This phenomenon was also observed in the intact dog heart after release of a coronary artery ligature. Isometric tension gauges sewn to ischemic portions of the left ventricle demonstrated that after reinstitution of coronary flow, segment tension duration "outlasts" the duration of left ventricular pressure development and is associated with ventricular irritability. Epicardial electrograms showed shortening of the QT interval within the ischemic segment with prolongation of the QT interval after release of the coronary ligature. Prolongation of tension development during recovery from hypoxia was not observed in experiments with rat skeletal muscle. These observations identify localized mechanical abnormalities during recovery from myocardial ischemia which may be important in the syndrome of acute coronary heart disease.

INTRODUCTION

The effects of hypoxia on the performance of heart muscle have been studied extensively both in vitro and in the intact organism. Little attention, however, has been given to the mechanical behavior of heart muscle during recovery from hypoxia. The present study describes an unusual alteration in the time course of tension development exhibited by mammalian heart muscle during recovery from hypoxia.' This phenomenon has now been observed both in isolated muscle preparations (1, 2) and in the intact dog heart (1).

METHODS

Isolated muscle studies. Hearts were quickly removed from cats and mongrel dogs after 25 mg/kg sodium pentobarbital anesthesia and from rats after decapitation, and placed in an oxygenated Krebs-Henseleit solution (3). Right ventricular papillary and trabecular muscles were obtained from cats and dogs respectively while left ventricular columnar carneae muscles were removed from rats. These muscles (average cross-sectional area 0.80 mm^2) were carefully suspended between two spring clips in a chamber bubbled through two scintered glass discs with 95% O_2 and 5% CO_2. Bath temperature was maintained at 28°C using a model F controlled temperature circulating pump (Chas. F. Haake, Seaford, N. Y.). The lower spring clip was connected to a Statham model G7B-0.75-350 force transducer by a 1/15,000 inch tungsten wire while the upper spring clip was attached by a gold chain to a rigid magnesium lever arm above which was placed a micrometer stop. The resting length of the muscle could thus be finely adjusted, and all experiments were performed with muscles stretched to contract at the peak of their active tension curve. Stimulation was provided by a Tektronix type 162 and 161 waveform and pulse generators which delivered 5–7 msec square wave pulses through two 0.5×2.0 cm parallel platinum electrodes at a rate of 12 per min. The minimum voltage necessary to provide supramaximal stimulation was used. Tension was recorded on a 2 channel Sanborn model 296 direct writing recorder at speeds of 100 mm/sec. Photographic recordings were obtained on a Tektronix 502A dual beam oscilloscope with speeds adjusted to convenience.

Hypoxia was induced by rapidly bubbling the muscle chamber solution with 95% N_2 and 5% CO_2 while serial Po_2's were determined using an IL meter. During recovery, the original O_2-CO_2 mixture was resubstituted.

In analyzing the data, the following measurements were made: (*a*) peak isometric tension (T); (*b*) time from onset of tension to peak isometric tension (TPT); (*c*) maximum rate of tension development (dT/dt); (*d*) the time for tension to fall from peak to 50% of that value

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($RT_{\frac{1}{2}}$) was used as an index of the relaxation time of the muscle; and (*e*) TPT + $RT_{\frac{1}{2}}$ was used as an index of the total duration of isometric contraction.

Intact dog studies. Five mongrel dogs weighing 15–20 kg were anesthetized with 25 mg/kg intravenous sodium pentobarbital. Under positive pressure respiration, a sternal-split thoracotomy was performed and a pericardial cradle was made to expose the heart. The left anterior descending coronary artery was carefully isolated; a soft cotton ligature was placed loosely about the vessel approximately 2 cm beyond its bifurcation from the circumflex artery. A Sutfin isometric strain gauge (Honeywell, Minneapolis, Minn.) was sutured directly to the myocardium perpendicular to the interior interventricular sulcus. This area was well within the distribution of the vessel to be ligated. In some animals gauges were sewn to portions of the left ventricular myocardium outside the distribution of the anterior descending coronary artery as well. The characteristics of the tension gauge were such that it responded to bending as well as compression-extension stresses. For this reason, only tension duration recorded by the gauges was analyzed. Simultaneous left ventricular pressures were recorded using a Statham SF1 catheter tip micromanometer. Tracings were obtained at a paper speed of 200 mm/sec on an Electronics for Medicine photographic recorder. In four additional dogs, epicardial electrograms were recorded from the ischemic segment of the left ventricle using saline-soaked sponge electrodes. Heart rate was maintained constant by right atrial pacing at a rate of 150 beats/min, and the QT intervals were measured before, during coronary artery ligation, and after release of the coronary ligature.

RESULTS

Isolated muscle studies

Hypoxia induction. On exposing the bathing medium to 95% nitrogen and 5% CO_2, the P_{O_2} of the solution fell exponentially with an average $t_{\frac{1}{2}}$ of approximately 5.0

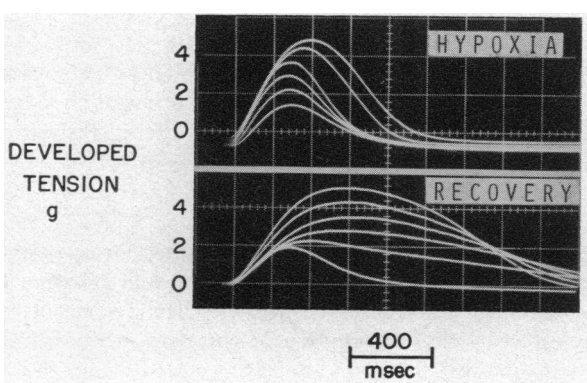

FIGURE 1 Isometric tension-time plots of cat papillary muscle. Top: top curve control (95% O_2–5% CO_2). Subsequent five curves, from above downward, represent 2, 5, 10, 15, and 20 min of hypoxia (95% N_2–5% CO_2). Bottom: bottom curve represents isometric tension after 20 min of hypoxia. Subsequent five curves from below upwards represent 2, 5, 10, 15, and 20 min of reoxygenation with 95% O_2–5% CO_2. During recovery from hypoxia, tension prolongation is striking as peak tension gradually returns to control levels.

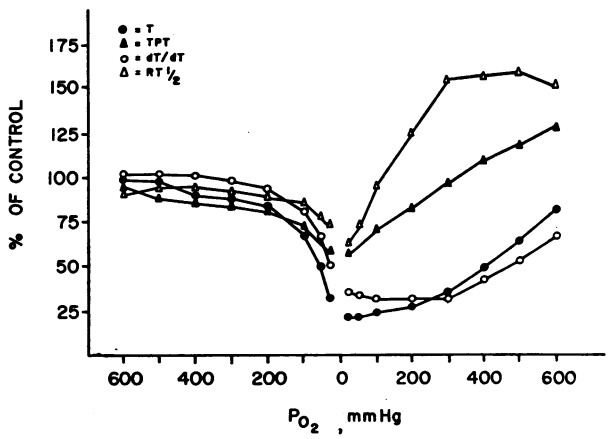

FIGURE 2 Averaged responses to hypoxia induction and recovery from eight experiments using isolated dog trabecular muscle. The mechanical parameters expressed as per cent of control (before hypoxia) are plotted against P_{O_2}. The response to decreasing P_{O_2} is illustrated on the left while the response to increasing P_{O_2}, during reoxygenation of the muscle bath, is shown on the right.

min. The effect of hypoxia upon isometric twitch tension is shown in Fig. 1 (top). While no changes in resting tension were observed, developed tension fell during hypoxia in a sigmoid fashion, at first slowly (1–3 min), then rapidly (3–6 min), and then more slowly again (greater than 7 min). Time to peak tension (TPT) and the time for tension to fall from peak to 50% of that value (relaxation time index or $RT_{\frac{1}{2}}$) fell abruptly within the first 4 min but then fell little further. The maximum rate of tension development (dT/dt) fell slowly at first, and in some experiments was seen to rise initially but then declined at a relatively constant rate after 3 min. After 7 min of hypoxia, dT/dt fell more slowly. When these parameters are plotted against P_{O_2}, the results are as illustrated in Fig. 2. In experiments using dog trabeculae muscles, the average maximal falls in TPT, $RT_{\frac{1}{2}}$, dT/dt, and T observed were 40%, 25%, 45%, and 65% respectively. Similar results with cats and rats were noted. The declines in TPT, dT/dt, and T during hypoxia are directionally similar to those reported by Buccino, Sonnenblick, Spann, Friedman, and Braunwald (4).

Recovery from hypoxia. During reoxygenation with 95% O_2 and 5% CO_2, the P_{O_2} of the solution rose exponentially to 600 mm Hg with an average $t_{\frac{1}{2}}$ of approximately 2.5 min. Within 1–2 min after the initiation of reoxygenation, when the P_{O_2} of the solution was approximately 100 mm Hg, a marked prolongation of tension duration was invariably observed (Figs. 1 and 2). This response first occurred when little or no change in the amplitude of tension development was apparent (Fig. 1, bottom). It was seen consistently in experiments from more than 15 cats, 25 dogs, and 60 rats. With the initia-

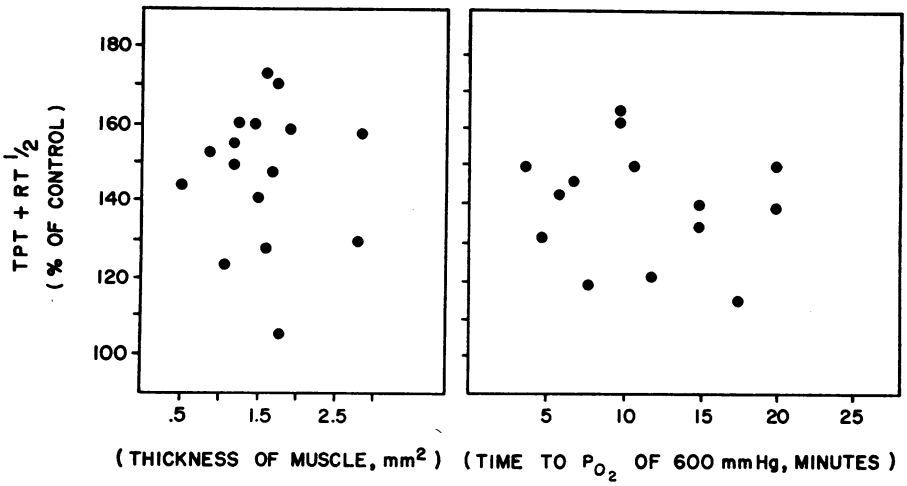

FIGURE 3 The effects of muscle thickness (left) and rate of reoxygenation (right) are plotted against the maximum duration of isometric tension (TPT and $RT_{\frac{1}{2}}$). Each point represents a single dog isolated muscle experiment. No significant relationship is apparent.

tion of tension prolongation during recovery, an additional fall in dT/dt was observed in dog and cat experiments. This additional fall in dT/dt was not seen in the rat. Because of the marked prolongation in tension it was at times difficult to measure TPT. It was clear, however, that both TPT and particularly $RT_{\frac{1}{2}}$ were markedly prolonged (Fig. 2). As recovery progressed, TPT and $RT_{\frac{1}{2}}$ gradually decreased while dT/dt and T rose. Tension generally returned to control levels within 10–15 min although TPT and $RT_{\frac{1}{2}}$ were still somewhat prolonged and dT/dt depressed. All values had returned to control levels 30–40 min after the onset of reoxygenation.

Effect of muscle thickness and rate of reoxygenation. It was considered that tension prolongation during recovery from hypoxia might be due to recovery of muscle extending from external surface to core with a gradual "reawakening" of more contractile sites. If this were the case, it might be expected that thicker muscles would exhibit more tension prolongation than thinner muscles. Similarly tension prolongation might be more pronounced with slower rates of reoxygenation. As shown in Fig. 3 no relationship between tension prolongation and muscle thickness or rate of reoxygenation was apparent in a random group of dog trabeculae muscles.

Skeletal muscle. Four experiments were performed using rat skeletal muscle (extensor digitorum longus). Muscles were subjected to the same experimental conditions as described above for cardiac muscle. While responses similar to those found in cardiac muscles were observed during the induction of hypoxia, virtually no tension prolongation was observed during recovery (Fig. 4). It will be noted that the fall in developed tension during hypoxia was of similar degree to that induced in cardiac muscle experiments.

Effect of repetitive stimulation. At selected times during induction and recovery from hypoxia, rapid stimulation at a rate of 1200/min was initiated early during isometric contraction in order to estimate the duration of the mechanical refractory period. During hypoxia induction (Fig. 5, top), repetitive electrical stimulation did not interfere with muscle relaxation, and a relatively long refractory period was apparent. During recovery from hypoxia, however, similar repetitive stimulation prevented relaxation and caused markedly prolonged tension development (Fig. 5, bottom). Control refractory periods were intermediate in duration to those observed during hypoxia and recovery. These responses were not due to an altered threshold to stimulation since similar findings were observed when the intensity of repetitive stimulation was varied.

Intact dog studies

After ligation of the anterior descending coronary artery, prompt cyanosis, a gallop rhythm, and prominent systolic bulge were observed in the ischemic segment of the left ventricle. Interruption of coronary arterial flow was maintained for periods of 15 sec to 10 min. With release of the ligature, the ischemic segment became hyperemic, and the bulge disappeared within 15–20 sec. It was at this time that tension prolongation was noted in the distribution of the previously occluded vessel. Fig. 6 depicts the time course of left ventricular pressure and segment isometric tension before, during, and 60 sec after release of coronary artery ligation. The duration of left ventricular pressure and segment tension

are concordant before and during ischemia, but with recovery from ischemia, tension prolongation is present in the previously ischemic segment. Fig. 7 summarizes the results of seven experiments in five dogs. While no change in the duration of left ventricular pressure development was noted during ligation or after release, a consistent increase in the duration of segment tension was observed after release of coronary artery ligation. Gauges sewn to nonischemic segments of the myocardium failed to exhibit tension prolongation under similar experimental conditions. It was noted that ectopic beats were common during the early period of coronary artery ligation and again particularly after release of the ligature when the phenomenon of tension prolongation was clearly evident.

In four dogs, epicardial electrograms were recorded from the ischemic area of the left ventricle, while heart rate was kept constant by atrial pacing at 150 beats/min. ½–1 min after coronary ligation, ST segment elevation was noted on the epicardial electrogram and the QT interval shortened (Fig. 8). Release of the ligature was performed 3–8 min after occlusion. The ST segments generally returned to base line within 30–60 sec and this was followed almost immediately by prolongation of the QT interval (Fig. 8) which gradually returned to control values within 10–15 min. Prolongation of the QT interval after release of the coronary ligature was not observed in epicardial electrograms from nonischemic segments of the left ventricle or in the standard limb leads of the electrocardiogram.

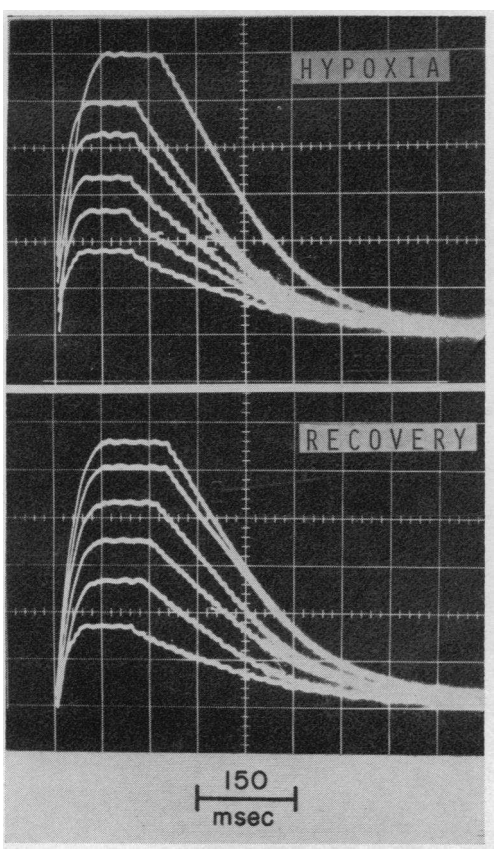

FIGURE 4 Isometric tension-time plots of isolated rat skeletal muscle recorded during hypoxia induction (top) and recovery (bottom). Temperature 13°C. Tension scale: each horizontal line represents *1.5* g. Muscle cross-sectional area 1.6 mm². The sequence of recordings is the same as that described in Fig. 1. See text.

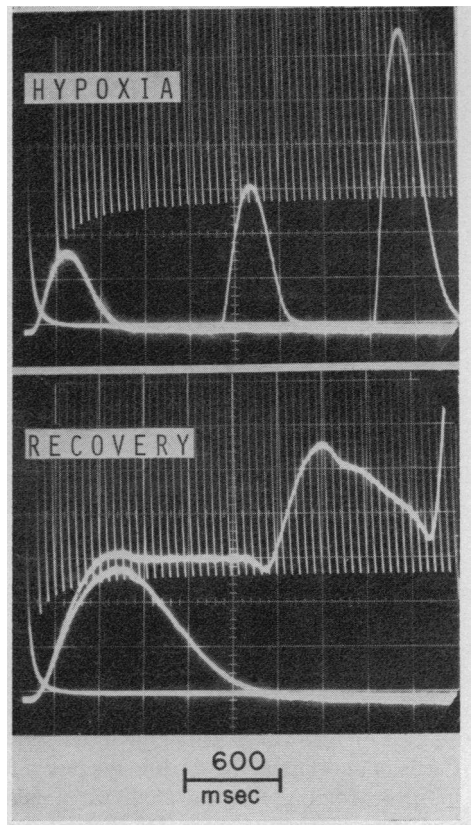

FIGURE 5 The effect of repetitive stimulation on isometric tension during hypoxia and recovery from hypoxia. Top: The first curve on the left represents two superimposed isometric contractions during hypoxia. The downslope of the stimulus artefact of the first contraction is seen at the far left of the tracing. During the second contraction (second sweep), repetitive stimulation (1200/min) was begun as indicated by the vertical lines. Muscle relaxation was complete and a relatively long refractory period is observed. As stimulation is continued two potentiated contractions are evoked. Bottom: The same procedure was repeated 10 min after reoxygenation of the muscle bath. In this instance peak isometric tension is maintained in the second contraction (second sweep) and a secondary rise in tension is observed with virtually no evidence of relaxation. Muscle cross-sectional area 0.9 mm² (dog trabeculae carneae). Temperature 28°C. Tension scale: each horizontal line represents 1 g.

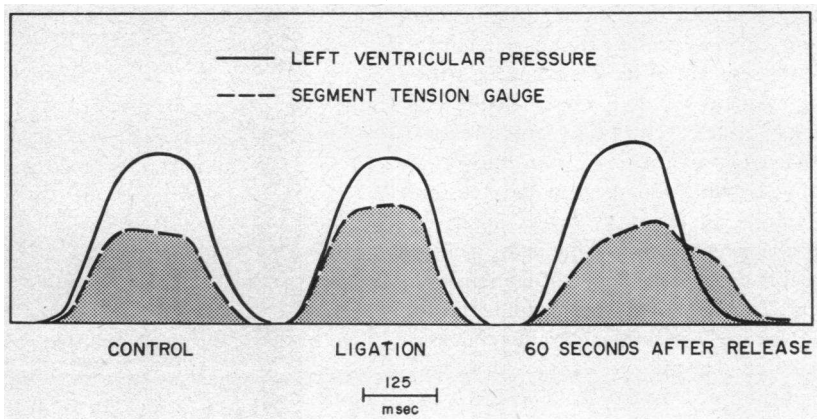

FIGURE 6 Isometric tension gauge tracings are superimposed on left ventricular pressure recordings of an open-chested dog during a control period. 3 min after coronary artery ligation and 60 sec after ligation release. See text.

DISCUSSION

Tension prolongation during recovery from myocardial hypoxia was consistently observed in more than 100 experiments. While this phenomenon was present in cardiac muscle from dogs, cats, and rats, it was not seen in rat skeletal muscle or in turtle (*Pseudemys scripta*) ventricular strips (unpublished data). The persistent presence of tension prolongation during recovery from hypoxia in myocardial specimens from three mammalian species and its virtual absence in skeletal and turtle heart muscle make it unlikely that this phenomenon is an artefact of the experimental preparation. This conclusion is further supported by the observation that tension prolongation was seen in the *intact* dog after coronary ligation release. A similar finding in the intact dog has previously been reported by Sayen, Sheldon, Peirce, and Kuo (5). Using a cinematographic technique, these authors described "delayed relaxation" of myocardial fibers after coronary artery ligation release which persisted for several minutes. It might be argued that the changes in segment tension duration observed in intact dog experiments were due to passive bulging of poorly contracting muscle rather than active tension prolongation. However, a bulge over the ischemic area during coronary artery ligation was consistently observed when tension prolongation was absent. After release, on the other

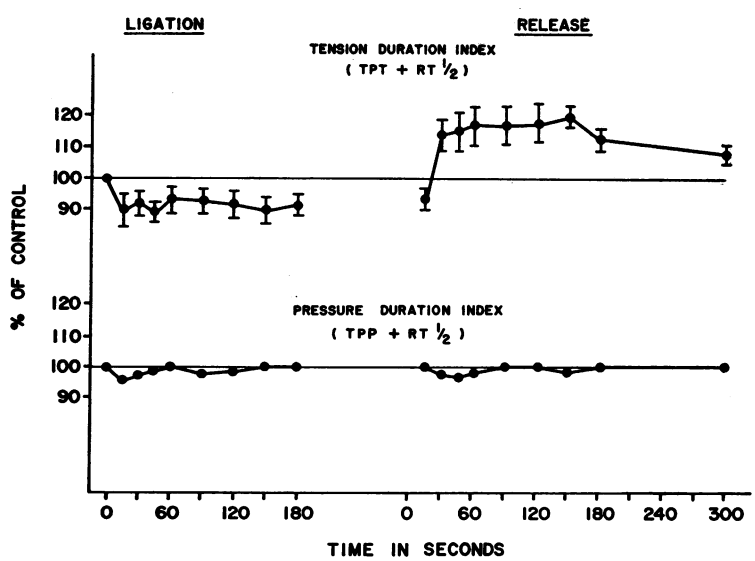

FIGURE 7 Time course of segment tension duration (above) and left ventricular pressure duration (below) after coronary artery ligation and subsequent release. TPT = time to peak tension. TPP = time to peak left ventricular pressure. Changes are expressed as per cent of control.

hand, prolongation was clearly evident at a time when the bulge was no longer visible. It might be added that this phenomenon was not seen in segment gauges sewn to nonischemic portions of the myocardium during ligation or after release.

As a consequence of studies performed by Kavaler (6) and more recently Morad and Trautwein (7), it is generally agreed that in many circumstances the duration of tension is influenced by the duration of the cardiac action potential plateau. Thus, the maintenance of a depolarized membrane favors continued entry of calcium to the myofilaments and a prolongation of contractile activity (8). Although intracellular cardiac action potentials were not measured in our studies, a review of the literature reveals that Trautwein and Dudel (9), using an isolated heart muscle preparation, demonstrated shortening of the duration of tension and the action potential plateau during hypoxia induction and transient "overshoot" prolongation of tension duration and the plateau of the cardiac action potential during recovery. In the intact dog heart, shortening of the contraction time and a fall in developed tension has been reported during myocardial ischemia (10). Kardesch, Hogancamp, and Bing (11), measuring intracellular action potentials in the intact dog during coronary artery occlusion, described abbreviation of the action potential plateau. Recovery studies, however, were not reported. While not directly analogous to myocardial transmembrane potentials, the epicardial electrograms analyzed in the present study did show changes in the duration of repolarization which were directionally the same as the changes in tension duration observed during hypoxia and subsequent reoxygenation. These experiments indicate that parallel changes in the duration of tension and the plateau of the cardiac action potential appear to be taking place during myocardial hypoxia and subsequent recovery, and suggest that changes in the duration of tension during hypoxia and recovery may be related to events taking place at the cell membrane. The fact that the action potential plateau of skeletal muscle is notably brief when compared to cardiac muscle may have some bearing on the failure of rat skeletal muscle to exhibit tension prolongation during recovery from hypoxia.

The ability of repetitive electrical stimulation to sustain tension development during recovery from hypoxia (Fig. 5) is reminiscent of the maintenance of tension development observed during voltage clamp experiments (7). Why mechanical responsiveness of the muscle to repetitive electrical stimulation is enhanced during recovery from hypoxia and diminished during the hypoxic state is presently not clear. A clarification of the interrelationships of these electromechanical events awaits further study.

Regardless of the explanation of these phenomena, the clinical implications are less unclear. While asynergic

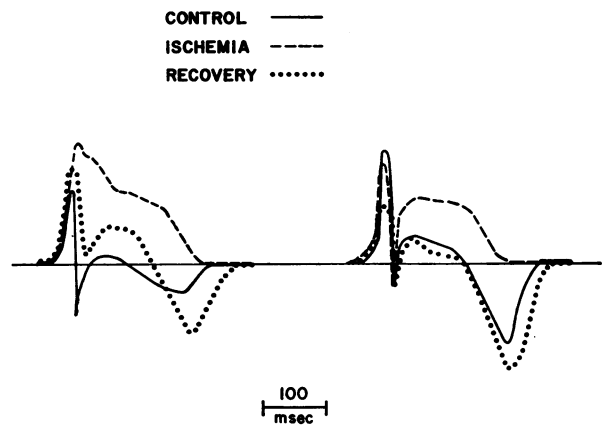

FIGURE 8 Epicardial electrograms recorded in two experiments from the ischemic segment of the left ventricle during control period, coronary ligation and 2–5 min after release of the coronary ligature. Shortening of the QT interval is seen during myocardial ischemia and prolongation is apparent during recovery from ischemia.

contraction of the ventricle is generally associated with ischemia or scar, as a consequence of tension prolongation, asynergy may also be a property of well oxygenated heart muscle after recovery from ischemia (2). After coronary occlusion, contiguous areas of the myocardium will include not only normal and hypoxic muscle but portions of the myocardium which are recovering from hypoxia via collateral vessels (12). The same principles may also apply to the patient "recovering" from an episode of angina pectoris.

It is noteworthy that ventricular irritability was associated with tension prolongation after release of a coronary artery ligature. Indeed, the danger of this moment in the course of transient myocardial ischemia has been emphasized by Beck and Leighninger (13). Han and Moe (14) have suggested that a major cause of repetitive ventricular arrhythmias during myocardial ischemia is nonuniform recovery of excitability, and in the present study repetitive electrical stimulation demonstrated a marked variation in the recovery of excitability of hypoxic and reoxygenated heart muscle. Thus, it is concluded that well oxygenated heart muscle *recovering* from hypoxia exhibits altered mechanical activity and electrical responsiveness which may have important implications in actue coronary heart disease.

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