Myocardial Ischemic Contracture
Metabolites Affect Rigor Tension Development and Stiffness

Renée Ventura-Clapier, Vladimir Veksl

Abstract
Myocardial ischemia is characterized by a decrease in phosphocreatine (PCr) and Mg2+-ATP contents as well as an accumulation of myosin ATPase reaction products (inorganic phosphate [Pi], protons, and Mg2+-ADP). The possibility that these metabolites play a role in rigor tension development was checked in rat ventricular Triton X-100-skinned fibers. Rigor tension was induced by stepwise decreasing Mg2+-ATP in the presence or in the absence of 12 mmol/L PCr. To mimic the diastolic ionic environment of the myofibrils, [free Ca2+] was set at 100 mmol/L (pCa 7); [free Mg2+], at 1 mmol/L; and ionic strength, at 160 mmol/L. In control conditions (pH 7.1, with no added Pi, or Mg2+-ADP), the pMg2+-ATP for half-maximal rigor tension (pMg2+-ATP50) was 5.07±0.03 in the presence of PCr. After withdrawal of PCr, the pMg2+-ATP50 value was shifted toward higher Mg2+-ATP values (5.57±0.03). Addition of 20 mmol/L Pi, shifted the pMg2+-ATP50 to 3.71±0.04 (P<.05) in the absence of PCr and in the opposite direction to 4.98±0.02 (P<.01) in the presence of PCr. Acidic pH (6.6) strongly increased pMg2+-ATP50 in both the absence (3.90±0.03, P<.001) and presence (5.44±0.02, P<.001) of PCr. Conversely, Mg2+-ADP (250 μmol/L) decreased pMg2+-ATP50 to 3.26±0.06 (P<.001) in the absence of PCr; at pMg2+-ATP 4, no rigor tension was observed until PCr concentration was decreased to <2 mmol/L. At acidic pH, maximal rigor tension was lower by 29% compared with control conditions, whereas in the presence of Mg2+-ADP, maximal rigor tension developed to 143% of the control value; Pi had no effect. The tension-to-stiffness (measured by the quick length-change technique) ratio was lower in rigor (no PCr and pMg2+-ATP 6) than during Ca2+ activation in the presence of both PCr and ATP. Compared with control rigor conditions, this parameter was unchanged by Mg2+-ADP and decreased by acidic pH, suggesting a proton-induced decrease in the amount of force per crossbridge. In addition to their known effects on active tension, Mg2+-ADP and protons affect rigor tension and influence ischemic contracture development. It is concluded that ischemic contracture and increased myocardial stiffness may be mediated by a decreased PCr and local Mg2+-ADP accumulation. This emphasizes the importance of myofibrillar creatine kinase activity in preventing ischemic contracture.

(Circ Res. 1994;74:920-929.)

Key Words • skinned fibers • acidosis • creatine kinase • phosphocreatine • adenine nucleotides

Contractile dysfunction is one of the earliest consequences of myocardial ischemia. Contractile depression is observed within 10 seconds after the onset of ischemia and is generally abolished after 5 to 10 minutes. After 10 to 20 minutes of ischemia, a gradual rise in diastolic tension, the ischemic contracture, occurs. Identification of the ischemic contracture in the human heart led Katz and Tada1 to propose that the “stone heart,” which is due to a failure of the ischemic myocardium to relax, reflected a state of rigor that could be attributed to loss of myocardial energy stores. Despite extensive description, the fundamental basis of the development of ischemic “contracture” is still under debate. An alteration of the contractile proteins themselves can be ruled out, since no evidence of myofibrillar damage could be shown after severe ischemia and reperfusion.2-3 This rise in tension may be attributed either to an increase in internal Ca2+, leading to actively cycling crossbridges or to the formation of rigor crossbridges after local ATP depletion. In fact, arguments have been accumulating in favor of the second hypothesis. Ischemic or hypoxic contracture may develop even in the absence of any rise in internal Ca2+ and many authors pointed out a dissociation between a rise in internal Ca2+ and the rise in resting tension.4,5 Recent data obtained using nuclear magnetic resonance to measure internal Ca2+ and ATP in the same preparations indicate that ATP depletion correlates much better with the occurrence of ischemic contracture than do changes in internal Ca2+.6 Indeed, there is an increasing amount of evidence indicating the rigor origin of this increase in tension. Using the quick length-change method, we were able to show that the rise in resting tension after hypoxia or metabolic inhibition was not related to an activation of contractile proteins by myoplasmic Ca2+ but was rather mediated by the formation of rigor bridges, suggesting low ATP availability at myofilaments.7 More recently, Leijendeker et al8 showed that the development of unstimulated force during severe hypoxia in rat trabecula was completely due to the formation of rigor links, whereas Ca2+-dependent cross-bridges contribute to the rise in force during less severe hypoxia.

However, the exact relation between ATP depletion and rise in rigor tension is not fully understood. During ischemia, phosphocreatine (PCr) rapidly drops while Mg2+-ATP declines more slowly, and the ATP threshold for rigor tension development has been estimated to be 2 mmol/L.9 Skinned-fiber studies revealed that a decrease in [PCr] shifts the dependence of rigor tension development toward higher [Mg2+-ATP] levels in skinned fibers.10-12

Received August 27, 1993; accepted January 14, 1994.

From Cardiologie Cellulaire et Moleculaire (R.V.-C.), CJE INSERM 92-11, Université Paris-Sud, Faculte de Pharmacie, Chatenay-Malabry, France; and the Laboratory of Experimental Cardiac Pathology (V.V.), Cardiology Research Center, Moscow, Russia.

Correspondence to Dr R. Ventura-Clapier, Cardiologie Cellulaire et Moleculaire, CJE INSERM 92-11, Université Paris-Sud, Faculte de Pharmacie, 92 296 Chatenay-Malabry, France.

Downloaded from http://circres.ahajournals.org/ by guest on April 19, 2017
More recently, similar results have been acquired in skinned isolated cells.\textsuperscript{13-15} The products of a variety of catabolic pathways accumulate during myocardial ischemia. Among them are the products of ATPase reactions such as Mg\textsuperscript{2+}-ADP, protons, and inorganic phosphate (P\textsubscript{i}), which are known to significantly influence active force. It is thus reasonable to suppose that these products can modulate rigor force as well.

The purpose of the present study was to evaluate the role of PCr and Mg\textsuperscript{2+}-ATP as well as the influence of Mg\textsuperscript{2+}-ADP, protons, and P\textsubscript{i}, separately and in combination, on the generation of rigor tension and stiffness. Experiments were performed in ventricular rat cardiac fibers skinned by Triton X-100. Results suggest that PCr depletion and Mg\textsuperscript{2+}-ADP accumulation can be among the major factors contributing to the development of rigor tension and stiffness in ischemic or hypoxic myocardium. These results also provide additional evidence for the role of myofibrillar creatine kinase (CK) in maintaining a high local ATP-to-ADP ratio.

**Materials and Methods**

**Mechanical Experiments**

Muscle fiber bundles were dissected in papillary muscles from the left ventricle of rats in a zero-Ca\textsuperscript{2+} Krebs' solution, pH 7.4. The mean diameter of the preparations was 194±7 \( \mu \)m (n=46), and the mean length was 1406±51 \( \mu \)m (n=46). Fibers were incubated for 1 hour in a relaxing solution (pCa 7; see solutions below) containing 1% Triton X-100 to solubilize the membranes and were then transferred to the relaxing solution without detergent. After the skinning procedure, the fiber was mounted in the experimental apparatus. It was adjusted to slack length, stretched by 20%, and subjected to an activation/relaxation cycle. For stiffness measurements, sarcomere length was adjusted to 2.1 to 2.2 \( \mu \)m using laser diffraction (10 mW He-Ne laser, Spectra Physics Inc, Mountain View, Calif) after the first activation cycle. In most cases, the dissection pattern was lost during activation and could not be followed. However, it was possible to observe that when sarcomere length was adjusted after the first activation/relaxation cycle, a better preservation of the diffraction pattern could be obtained; in these cases, sarcomere length variation was <1 \( \mu \)m. The length and diameter of the muscles were measured by use of a graticule in the dissecting microscope. Muscles were immersed in 2.5-mL chambers arranged around a disk and immersed in a temperature-controlled bath positioned on a magnetic stirrer. Each solution was well stirred at high speed (>1000 rpm).

All experiments were performed at 22°C.

**Experimental Apparatus**

A fiber was snared at both ends with hair emerging from stainless-steel tubes, between a transducer (model AE 801, SensoNor Microelectronics, Horten, Norway) and a vibrator. The bandwidth of the transducer and tube was 2 kHz. The permanent magnet and coil came from a standard loudspeaker (model TS-130A, Pioneer Electric and Research Corp, Forest Park, Ill). The fiber was glued to a glass tube axis (2 mm in diameter) driven in an axial ball bearing (total moving mass, <1.5 g). A flag with a narrow window was glued on the glass axis between a lamp and a position detector (type S1543, Hamamatsu, Japan), allowing measurements of the displacement length. A feedback with the length signal combined with a power amplifier allowed control of muscle length. The system had a rise time of ~1 millisecond without overshoot.

Length and tension changes were monitored on a digital storage oscilloscope (model OS4020, Gould, Inc, Cleveland, Ohio). Tension tracings were digitized at 20 kHz (12-bit analog/digital converter), analyzed on-line by use of a computer (Deskpro 386, COMPAQ Corp, Houston, Tex), and stored on videotape.

**Solutions**

Solutions were calculated by use of the computer program of Fabiato.\textsuperscript{10} All solutions were calculated to contain (mmol/L) EGTA 10, imidazole 30, Na\textsuperscript{+} 30.6, Mg\textsuperscript{2+} 1, and dithiothreitol 0.3; ionic strength was adjusted to 0.16 mol/L with potassium acetate. pH was adjusted with acetic acid. In relaxing and rigor solutions, pCa was 7. In activating solution, pCa was 4.5 at pH 7.1. Relaxing and activating solutions also contained 3.16 mmol/L Mg\textsuperscript{2+}-ATP and 12 mmol/L PCr. Rigor solutions were obtained by mixing two solutions of pMg\textsuperscript{2+}-ATP 6 and 2.5 in the absence of PCr and pMg\textsuperscript{2+}-ATP 4 and 6 in the presence of PCr. Rigor solutions were calculated in the absence or presence of 250 mmol/L Mg\textsuperscript{2+}-ADP and/or 20 mmol/L pCa, at pH 6.6 or 7.1. As previously shown\textsuperscript{12} and as indicated by some experiments in the present study, addition of P\textsuperscript{i}, P\textsuperscript{i}-di(adenosine-5')pentaphosphate (an inhibitor of myokinase activity) did not affect the results. EGTA was obtained from Sigma Chemical Co, St Louis, Mo. PCr (Neoton, Schiaparelli Farmaceutica, Turin, Italy) was a kind gift of Prof E. Strumia.

**Experimental Protocol**

\( pMg^{2+}\textsuperscript{-ATP/Rigor Tension Relations} \)

Maximal active tension of each fiber was obtained at pCa 4.5, pMg\textsuperscript{2+}-ATP/rigor tension relations were established by stepwise decreasing [ATP] until maximal rigor tension was obtained. The fiber was then placed in the relaxing solution for 10 to 15 minutes before a new set of rigor solutions was applied. Each experimental condition was randomly applied. No more than five different experimental conditions were tested on an individual fiber. Data were fitted using the Hill equation:

\[
T = \frac{[Mg^{2+}\textsuperscript{-ATP}]^{n_{H}}/K + [Mg^{2+}\textsuperscript{-ATP}]^{n_{H}}}{K + [Mg^{2+}\textsuperscript{-ATP}]^{n_{H}}}
\]

where T is relative rigor tension, K is a constant, and \( n_{H} \) is the Hill coefficient. The pMg\textsuperscript{2+}-ATP for half-maximal activation (pMg\textsuperscript{2+}-ATP\textsubscript{50} = \(-\log_{10}(K)\)) was calculated for each experimental condition (between 10% and 90%) by linear regression analysis. Resting tension was recorded in the relaxing solution (pCa 7, 12 mmol/L PCr, and pMg\textsuperscript{2+}-ATP 2.5) at a sarcomere length of 2.1 \( \mu \)m. Maximal rigor tension was total tension minus resting tension. Tension was expressed as millinewtons per square millimeter. Maximal rigor tension values were expressed as percentage of maximal active tension obtained at pCa 4.5 just before inducing rigor.

**Stiffness Measurements**

Fiber stiffness was estimated by the quick length-change technique. The spike of tension in phase with the length change characterizes the elastic phase.\textsuperscript{17} Stiffness was the extreme tension reached during stretch (millinewtons per square millimeter) divided by the length change (micrometers). A series of seven stretches and five releases of 0.3% to 3% of the initial muscle length were applied in relaxing, activating, or rigor solutions. Only responses to stretches were used for calculations. Each value is the mean of five to seven determinations after stretches of varying amplitudes in a given experimental condition. A first series of length changes was imposed in the relaxing solution to assess passive properties of the fiber. Resting stiffness was calculated by linear regression analysis on the responses to stretches. Then a second series of length changes was initiated in control activating solution. Tension level before the first stretch was taken as maximal tension and used for normalizations. Active or rigor stiffness values were calculated as the difference between total stiffness minus resting stiffness. The fiber was then immersed in control rigor solution (pMg\textsuperscript{2+}-ATP 6, pCa 7, no P\textsubscript{i},...
no PCr, no Mg\textsuperscript{2+}-ADP, and pH 7.1) and then in a test rigor solution. To control the reversibility in the mechanical parameters, the fiber was transferred back to the control rigor solution at pH 7.1. Control rigor stiffness represented the mean of the stiffness in control rigor solution before and after the test rigor solutions. To minimize the possible influence of damaged ends on stiffness measurements, each experimental condition was compared with the control condition in the same fiber. Stiffness was thus expressed relative to the control value at pCa 4.5.

**Statistical Analysis**

Values were expressed as mean±SEM. Student's $t$ test was used to compare the means. Statistical significance was reached at $P \leq 0.05$. Linear regression analysis was carried out by use of the least-squares method.

**Results**

**Effects of Mg\textsuperscript{2+}-ATP and PCr on Rigor Tension Development**

Rigor tension was investigated in ventricular skinned fibers in a medium whose composition was chosen to be close to the intracellular milieu. [Ca\textsuperscript{2+}] was fixed at 100 nmol/L (pCa 7), [free Mg\textsuperscript{2+}] was set at 1 mmol/L, pH was adjusted to 7.1, and ionic strength was 160 mmol/L. Chloride ions were avoided and replaced by acetate, since chloride is known to inhibit different enzymes, particularly CK.\textsuperscript{16}

After activation at pCa 4.5 (Fig 1), the fiber developed maximal tension and fully relaxed at pCa 7. When subjected to a rigor solution containing 12 mmol/L PCr but only 1 mmol/L Mg\textsuperscript{2+}-ATP (pMg\textsuperscript{2+}-ATP 6), rigor tension developed. On reintroduction of Mg\textsuperscript{2+}-ATP, resting tension quickly resumed. To establish a pMg\textsuperscript{2+}-ATP tension relation, [Mg\textsuperscript{2+}-ATP] was stepwise decreased from 3.16 mmol/L (pMg\textsuperscript{2+}-ATP 2.5) to 1 mmol/L (pMg\textsuperscript{2+}-ATP 6). As Mg\textsuperscript{2+}-ATP decreased, rigor tension increased in a rather stepwise fashion. However, in some cases (see Figs 1 and 4), transient force developments were observed, after which force reached a steady state, which was used for calculations. As can be seen (Fig 1), in the presence of 12 mmol/L PCr no rigor tension was observed for 100 μmol/L Mg\textsuperscript{2+}-ATP, and maximal rigor tension was obtained for [Mg\textsuperscript{2+}-ATP] of <5 mmol/L. In the absence of PCr, rigor tension developed for [Mg\textsuperscript{2+}-ATP] of ≈1 mmol/L and reached a maximum for 100 μmol/L Mg\textsuperscript{2+}-ATP.

The mean maximal rigor tension was 56±5% of the maximal active tension in the absence and 58±4% in the presence of PCr (Table 1). pMg\textsuperscript{2+}-ATP\textsubscript{50} was 3.57±0.03 (ie, 0.27 mmol/L) in the absence and 5.07±0.03 (ie, 8.5 μmol/L) in the presence of 12 mmol/L PCr. The difference between the pMg\textsuperscript{2+}-ATP\textsubscript{50} in the presence and in the absence of PCr was taken as the efficacy of myofibrillar-bound CK to locally rephosphorylate Mg\textsuperscript{2+}-ADP and to create a microcompartment of adenine nucleotides inside the myofibrils.\textsuperscript{19} CK efficacy represented 1.46±0.02 pMg\textsuperscript{2+}-ATP units in control conditions.

During a contraction, the substrate concentration in the core of fibers of large diameter is more likely to fall because the distance for ATP diffusion is high. Fibers having the largest diameter should therefore have a higher sensitivity to Mg\textsuperscript{2+}-ATP. To test whether diffusion distance plays a critical role in determining pMg\textsuperscript{2+}-ATP\textsubscript{50} values, we have studied the relation between pMg\textsuperscript{2+}-ATP\textsubscript{50} and fiber diameter. The data showed that there was no significant correlation for diameters ranging from 150 to 280 μm ($r = -0.426$, $n = 18$, $P > 0.05$).

**Effect of Acidification on Rigor Tension Development**

Intracellular acidification is a hallmark of the ischemic insult.\textsuperscript{20} We studied the effects of acidification on maximal rigor tension and on Mg\textsuperscript{2+}-ATP sensitivity in the presence and absence of PCr (Fig 2A and Table 1).
Acidification decreased maximal tension and shifted the two relations toward lower [Mg\(^{2+}\)-ATP] values, suggesting a direct effect of pH on myosin ATPase. Thus, intracellular acidosis may be one factor antagonizing the development of ischemic contracture and reducing its amplitude. Although Mg\(^{2+}\)-ATP sensitivity was shifted by \(0.4\ \text{pMg}^{2+}\)-ATP unit, CK efficacy was not affected by pH changes, since it was not significantly different from control conditions.

### Effects of \(P_i\) on Rigor Tension Development

Another feature of ischemia is an early increase in \(P_i\), concomitant with the decrease in PCr and Mg\(^{2+}\)-ATP. We have thus studied the influence of this ion on rigor tension development (Fig 2B and Table 1). At control pH, 20 mmol/L \(P_i\) did not affect maximal rigor tension development but slightly altered Mg\(^{2+}\)-ATP sensitivity. Rigor tension development was less sensitive to decreased [Mg\(^{2+}\)-ATP] in the absence of PCr and slightly more sensitive to Mg\(^{2+}\)-ATP decrease in the presence of PCr. This showed that CK efficacy was decreased by \(P_i\) in accordance to the inhibition of CK activity by \(P_i\).

We then studied the combined action of acidification and high \(P_i\) (Fig 2C and Table 1). At pH 6.6, \(P_i\) shifted the pMg\(^{2+}\)-ATP/tension relation toward the right only in the presence of PCr (from 5.44±0.02 to 5.06±0.03). As a result, at acidic pH, the CK efficacy was decreased from 1.55±0.04 to 1.18±0.05 (\(P<.001\)) in the presence of 20 mmol/L \(P_i\) (Fig 2C and Table 1). Thus, the combination of both acidification and high \(P_i\) exerted a depressing effect on myofibrillar CK activity. This effect can be due to either decreased CK activity or the detachment of CK from myofibrils. To clarify this question, some fibers were transferred back from solutions with PCr at pH 6.6 and 20 mmol/L \(P_i\) to solutions without phosphate at varying [Mg\(^{2+}\)-ATP] levels; in this case, the pMg\(^{2+}\)-ATP\(_{50}\) value (5.43±0.06) returned to initial values at pH 6.6, showing that the decrease in CK efficacy was due to CK inhibition rather than detachment.

### Effect of Mg\(^{2+}\)-ADP and PCr on Rigor Tension Development

The rapid depletion of PCr during ischemia leads to an inability of CK to rephosphorylate ADP and thus to an accumulation of this compound. In skinned fibers, the addition of 250 \(\mu\text{mol}/\text{L} \ Mg^{2+}\)-ADP in the absence of PCr induced a rightward shift of the pMg\(^{2+}\)-ADP/tension relation, showing an increased sensitivity of rigor tension development to Mg\(^{2+}\)-ADP depletion (Fig 3 and Table 1). Moreover, maximal rigor tension was increased from 56±5% to 80±7% of active tension by the presence of Mg\(^{2+}\)-ADP. In the presence of Mg\(^{2+}\)-ADP, no rigor tension could be elicited in the presence of 12 mmol/L PCr, showing the effective rephosphorylation capacity of myofibrillar CK. It was further interesting to determine the threshold for rigor tension development when PCr concentration was decreased. For a pMg\(^{2+}\)-ADP value giving maximal rigor tension in the absence of PCr (pMg\(^{2+}\)-ADP 4), [PCr] was varied in the absence and presence of 250 \(\mu\text{mol}/\text{L} \ Mg^{2+}\)-ADP (Fig 4). Rigor tension developed for PCr in the millimolar range, strongly depending on [Mg\(^{2+}\)-ADP]. The threshold for rigor tension development was 0.5 mmol/L PCr in the absence and 2 mmol/L in the presence of 250 \(\mu\text{mol}/\text{L} \ Mg^{2+}\)-ADP. Relatively low concentrations of Mg\(^{2+}\)-ADP could be responsible for rigor tension development when PCr is dropped below its \(K_v\) value for CK in solution (2 mmol/L). Thus, rigor tension development depends on the complex interaction between [Mg\(^{2+}\)-ADP], [Mg\(^{2+}\)-ATP], and [PCr].

### Combined Effects of Mg\(^{2+}\)-ADP, \(P_i\), and Acidic pH on Rigor Tension Development

In the presence of \(P_i\), Mg\(^{2+}\)-ADP was still able to increase rigor tension as well as to shift pMg\(^{2+}\)-ATP\(_{50}\) (Fig 5 and Table 1). In the presence of Mg\(^{2+}\)-ADP and \(P_i\), acidic pH did not induce a decrease in maximal rigor tension, whereas the development of rigor tension was hastened compared with control conditions. No rigor
tension could be elicited in the presence of high [PCr] because of the effective rephosphorylation of Mg$^{2+}$-ADP by CK reaction. These results show that Mg$^{2+}$-
ADP strongly affects rigor tension development in the absence of PCr.

Effects of Mg$^{2+}$-ADP and Acidic pH on Stiffness

Changes in maximal force can originate from an altered number of attached crossbridges or a lower force generated per crossbridge or both. To understand the reasons for the increase in maximal rigor tension by Mg$^{2+}$-ADP and for the decrease in acidic medium, fiber stiffness was measured to assess the number of attached crossbridges in the different conditions. Series of quick length changes were applied at rest, during Ca$^{2+}$ activation, and in rigor conditions. Between each series, the fiber was bathed in the relaxing solution before the induction of a new rigor tension. The plots of tension as a function of length changes are shown in Fig 6. To bring about a change in the stiffness-to-force ratio, rigor tension was scaled to maximal activated tension. In these conditions, it can be seen that the slope of the relation between tension and length in rigor conditions was steeper than in Ca$^{2+}$-activated tension. On the other hand, stiffness in control rigor was not different from active stiffness (Table 2), showing that no additional crossbridges were attached in rigor compared with Ca$^{2+}$-activated conditions. Since tension was lower (37.0±2.3 versus 9.48±1.05 mN/mm$^2$ in rigor, P<.001), the tension-to-stiffness ratio decreased from 49.9±1.2 to 12.6±1.0 nm in rigor, indicating that more rigor bridges would be needed to induce a tension equal to active tension; ie, on average, each crossbridge in rigor would develop lower force.

At acidic pHe (pH 6.6), the slope of the length/rigor tension relation scaled to activated tension was increased compared with control rigor conditions (Fig 6B). Rigor tension was decreased by 52%, whereas absolute stiffness was decreased by only 20% (Table 2). Consequently, the tension-to-stiffness ratio decreased to 62% of the control rigor value. Acidic pH markedly affected rigor crossbridges; it induced a small decrease in the number of attached crossbridges together with a marked drop in the force per crossbridge. These results
indicate that although rigor tension developing at acidic pH was lower than in control rigor conditions, the stiffness of the fiber was high and thus the compliance of the fiber would have been much decreased. This high stiffness in acidic medium was still observed in the presence of Mg 2+-ADP (Table 2). In these experiments, where maximal rigor tension was induced without decreasing stepwise [Mg 2+-ATP], 250 μmol/L Mg 2+-ADP did not induce a significant increase in rigor tension. Similarly, neither stiffness nor the stiffness-to-tension ratio was significantly changed.

Discussion

We have investigated the influence of metabolite changes associated with ischemia on the development of rigor tension using skinned ventricular fibers of rat heart in experimental solutions mimicking the intracellular milieu during ischemia. The results suggest that the decrease in PCr and the local accumulation of ADP could be the main factors involved in the increase in diastolic tension and stiffness observed during metabolic impairment of myocardium. These results emphasize the role of myofibrillar CK for the maintenance of relaxation and distensibility and optimal left ventricular filling in the normal myocardium.

Skinned Fibers and Diffusion

One potential problem is whether it is really possible to study Mg 2+-ATP depletion impact on contraction in skinned fibers. The concentration of Mg 2+-ATP producing half of the maximal rigor tension was 270 μmol/L in the absence of the endogenous Mg 2+-ATP regenerating system (bound MM-CK) and was decreased to 8.5 μmol/L in the presence of PCr (32-fold change). It can be argued that diffusional limitations due to the size of the preparations and Mg 2+-ATP consumption inside skinned fibers would produce an artifactual shift in the apparent K_m for Mg 2+-ATP toward higher [Mg 2+-ATP] levels. Although such a problem does exist, we think that it does not interfere to a high extent with the results reported below for the following reasons: (1) Mg 2+-ATP consumption in rigor is expected to be lower than in active contraction. (2) There is no significant influence of fiber diameter on pMg 2+-ATP (Reference 19 and the present study) either in the presence or absence of PCr. (3) When PCr is varied in the presence of Mg 2+-ADP, the apparent K_m for the development of rigor tension is very close to the real K_m of CK for PCr, showing no limitation of diffusion for this substrate inside our preparations in rigor conditions; since the diffusion coefficients for PCr and Mg 2+-ATP are not largely different, it can be argued that this is not the major reason for this increased K_m value for Mg 2+-ATP compared with PCr. (4) In studies involving isolated skinned cardiomyocytes, where intercellular diffusion distances have been abolished, results from Nichols and Lederer14 and preliminary results from our laboratory15 showed that the threshold for rigor tension development in isolated cells was close to the value determined.
in the present study using skinned fibers; interestingly, an effect of the endogenous CK system on the apparent $K_m$ of rigor tension development for Mg$^{2+}$-ATP was also observed in these studies. This would imply that the problem of concentration gradients of Mg$^{2+}$-ATP is an intracellular or an intramyofibrillar phenomenon. For all these reasons, we considered the use of skinned cardiac fibers appropriate for the study of rigor tension development with a limited influence of intercellular diffusion of metabolites.

**Rigor Tension Development in Muscle**

Muscle contraction or shortening occurs as a result of cyclic interactions between actin (thin) and myosin (thick) filaments with the consumption of chemical energy liberated from ATP hydrolysis in the presence of Ca$^{2+}$ binding to troponin. Many efforts have been made to connect the mechanical changes of muscle fibers with the kinetics of ATP hydrolysis by the actomyosin complex. The current theory is that crossbridges can be classified into two types, i.e., a weakly bound state in which myosin binds ATP or rather its products (ADP · P$_i$) and a strongly bound state with ADP or without bound nucleotides (rigor bonds). The transition between these two states is coupled to the release of P$_i$ from the complex and leads to the generation of force.$^{22}$ ADP release appears to limit the rate of crossbridge detachment and thus limits maximum shortening velocity.$^{23}$ Effects of the ATPase reaction products (Mg$^{2+}$-ADP, P$_i$, and H$^+$) on the development of active tension have been studied. Mg$^{2+}$-ADP potentiates isometric tension and slows kinetics$^{19,24,25}$ whereas P$_i$ and protons have a depressing effect on force generation.$^{22,24,26,27}$

In the absence of Ca$^{2+}$, the relation between myosin ATPase activity or force and Mg$^{2+}$-ATP shows substrate inhibition for millimolar [ATP].$^{28-30}$ Bremel and Weber$^{31}$ further demonstrated that myosin free of bound nucleotides has a high affinity for actin molecules, even in the presence of Ca$^{2+}$-free troponin. Thus, rigor tension development seems to be associated with an increase in myosin ATPase activity linked to the presence of rigor crossbridges. Rigor tension developed stepwise when [Mg$^{2+}$-ATP] was decreased. The present study shows that the effects of the three products of the ATPase reaction on rigor tension development are strikingly different and could be related to their release step in the ATPase reaction.

**Effects of Acidification on Rigor Tension Development**

We have shown that as for Ca$^{2+}$-activated tension,$^{32}$ acidification has a larger depressing action on rigor tension than on stiffness. Additionally, rigor tension development became less sensitive to Mg$^{2+}$-ATP decrease, independent of PCr. These results show that protons decrease the number of crossbridges (smaller stiffness) as well as the force per crossbridge (smaller tension-to-stiffness ratio). This suggests that the ATPase itself is modified by acidification. Immediate stiffness is assumed to be a measure of the number of actin-bound crossbridges. The proportionality between tension and stiffness indicates that force generation is

**TABLE 2. Mechanical Characteristics of Active and Rigor Tension in Rat Skinned Ventricular Fibers**

<table>
<thead>
<tr>
<th>Rigor Solutions (pMg$^{2+}$-ATP 6, pCa 7, and 0 mmol/L PCr)</th>
<th>Activating Solution (pCa 4.5, pMg$^{2+}$-ATP 2.5, and 12 mmol/L PCr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>pH 6.6</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Tension, mN · mm$^{-1}$</td>
<td>37.0±2.3 (17)</td>
</tr>
<tr>
<td>Stiffness, relative value</td>
<td>1 (15)</td>
</tr>
<tr>
<td>Tension-to-stiffness ratio, nm</td>
<td>48.6±1.2 (15)</td>
</tr>
</tbody>
</table>

PCr indicates phosphocreatine. Values are mean±SEM. Numbers in parentheses indicate the number of fibers.

Absolute stiffness value in activating solution was 749±54 mN · mm$^{-2}$ · µm$^{-1}$.

*P<.001 vs activating solution; †P<.001 vs control rigor solution; and ‡P<.01 vs control rigor solution.
caused by a recruitment of crossbridges interacting with actin. Bagshaw and Trentham\(^3\) showed that three steps were involved in the interaction between protons and the actomyosin reaction: (1) release of H\(^+\) at the myosin-product isomerization step or P\(_i\) dissociation step, (2) release of H\(^+\) when ATP binds to myosin, and (3) take up of H\(^+\) when ADP dissociates. One can hypothesize that the number of crossbridges would be affected by an increase in the population of weakly bound crossbridges due to the reversal of the weak-to-strong binding states and that force per cross bridge would be decreased because of the decrease in the free energy change of ATP hydrolysis in the presence of protons. In addition, the decreased sensitivity to Mg\(^{2+}\)-ATP depletion could be explained by a facilitating effect of protons on the Mg\(^{2+}\)-ADP release step.

**ADP and Rigor Tension Development**

ADP acts as a competitive inhibitor of skeletal fiber shortening velocity\(^24\) with a \(K\_0\) of 200 \(\mu\)mol/L. This value is close to the \(K\_0\) for the binding of ADP to the actomyosin–subfragment-1 (acto-S1) complex in solution and in myofibrils.\(^3^4-3^7\) In cardiac myofibrils, a lower value has been obtained for the \(K\_0\) of acto-S1 or myofibril (14 \(\mu\)mol/L), showing that ADP binds much more strongly to cardiac myosin.\(^3^4\) This is an indication of a possible physiological role of ADP in the regulation of contraction in cardiac muscle. Addition of Mg\(^{2+}\)-ADP potentiates the isometric tension and decreases tension kinetics in skeletal\(^2^4,3^6\) as well as cardiac\(^1^9\) fibers. This is thought to result from the slowing of product detachment rate and from an increase in the population of strongly bound force-generating crossbridges at the end of the power stroke. The results presented in the present study show that Mg\(^{2+}\)-ADP has a strong effect on rigor tension development: Mg\(^{2+}\)-ADP increases maximal rigor force when rigor tension is induced by a stepwise decrease in [Mg\(^{2+}\)-ATP]. In addition, Mg\(^{2+}\)-ADP increases the susceptibility of rigor tension development to decreased levels of Mg\(^{2+}\)-ATP. This can be interpreted as an inhibitory effect of Mg\(^{2+}\)-ADP on dissociation steps and/or on further Mg\(^{2+}\)-ATP binding and crossbridge detachment. This effect persists even in the presence of both \(P\_i\) and acidosis. It is completely removed by high PCr and appears increasingly important as PCr is decreased in the millimolar range.

**Effects of \(P\_i\) on Rigor Tension Development**

Although the effects of \(P\_i\) are strikingly similar to those of acidosis on maximal force and Ca\(^{2+}\) sensitivity of active tension and stiffness, \(P\_i\) ions increase the crossbridge cycling rate, an effect that is not observed during acidosis in cardiac muscle.\(^5^0,6^2,3^2,3^8\) The results presented in the present study show that \(P\_i\) ions, as well as acidosis, tend to decrease the sensitivity of rigor tension development to Mg\(^{2+}\)-ATP decrease; the effects of both factors are not additive. However, \(P\_i\) ions do not significantly affect maximal rigor tension, showing that protons and \(P\_i\) affect different steps in the crossbridge cycle. Indeed, release of \(P\_i\) precedes the release of Mg\(^{2+}\)-ADP. That \(P\_i\) ions do not affect rigor tension might be due to the fact that in rigor, the release of Mg\(^{2+}\)-ADP becomes the main rate-limiting step in the actomyosin reaction, thus minimizing the effects of \(P\_i\) accumulation.

**CK and Rigor Tension Development**

The results show that rigor tension is very sensitive to the presence of PCr. Activation of myofibrillar CK shifts the dependence of rigor tension on [Mg\(^{2+}\)-ATP] by \(\approx 1.5\) log units. Myofibrillar CK is reversibly bound to cardiac myofibrils; its activity is \(\approx 2\) IU/mg protein, which represents \(\approx 20\%\) of the total CK; this CK activity can sustain normal tension, stiffness, and kinetics of crossbridge cycling in the absence of added Mg\(^{2+}\)-ATP provided that high PCr and some ADP are present.\(^1^9\) Nichols and Lederer\(^1^4\) showed, in saponin-permeabilized myocytes, that the Mg\(^{2+}\)-ATP dependence of shortening velocity had a threshold of 1 mmol/L and a half shortening velocity (\(K\_i/2\)) of 100 \(\mu\)mol/L Mg\(^{2+}\)-ATP; these values were shifted to 100 and 10 \(\mu\)M, respectively, in the presence of PCr. It was previously shown that PCr inhibits rigor tension development in the [ADP] range of 250 \(\mu\)mol/L to 20 mmol/L, showing the efficacy of myofibrillar CK in reporphosphorylating Mg\(^{2+}\)-ADP at the vicinity of the active site of myosin ATPase.\(^1^9\) Biochemical as well as physiological studies have pointed out the compartmentation of adenine nucleotides in myofibrils and the role of myofibrillar CK in the energy supply for cardiac contraction.\(^1^1,1^2,3^0,3^9,4^0\)

The fact that acidic pH is able to shift the two curves (with and without PCr) to the same extent suggests that protons do not affect myofibrillar CK activity. Moreover, it also suggests that rigor tension development in the presence of PCr is limited by the \(K\_m\) of myosin ATPase for Mg\(^{2+}\)-ATP rather than by the CK reaction itself. Indeed, the apparent \(K\_m\) is 10 \(\mu\)mol/L, a value close to the \(K\_m\) of myosin ATPase in myofibrils in the presence of an ATP-regenerating system.\(^3^5,4^0\)

The presence of \(P\_i\) decreases the CK efficacy at both normal and acidic pH. This effect cannot be related to the detachment of CK from myofibrils as is the case in mitochondria.\(^4^1\) This effect should rather be related to the inhibiting effect of anions on CK activity,\(^1^8\) as was previously suggested.\(^2^7\)

**Ischemic Contracture**

Ischemic contracture develops when PCr falls to very low levels, followed by a slow decline in ATP and a severalfold increase in [ADP]. The occurrence of rigor force and stiffness, after PCr fall and an increased myofibrillar ADP-to-ATP ratio, provides a plausible explanation for the development of contracture. Indeed, our data show that rigor tension development depends on a complex interplay between PCr, Mg\(^{2+}\)-ATP, and Mg\(^{2+}\)-ADP and that even with [PCr] in the millimolar range, an increased ADP-to-ATP ratio can produce substantial rigor tension. Moreover, the delayed onset and reduced amplitude of ischemic contracture by acidosis\(^4^2\) is also explained by the direct effect of protons on rigor tension, with the indication that stiffness would nonetheless be elevated. In contrary to active tension, rigor tension is not inhibited by \(P\_i\) that accumulates during ischemia. Although the relation between the development of ischemic contracture and the rise in internal Ca\(^{2+}\) is not unique, it was generally observed that contracture development is potentiated by a rise in [Ca\(^{2+}\)].\(^6^2,1^3,4^2-4^6\) Indeed, a rise in internal Ca\(^{2+}\), by activating ATPases, will accelerate ATP depletion and hasten rigor tension development.
Left ventricular chamber stiffness increases when myocardial O₂ demand exceeds supply; this can be associated with a substantial prolongation of the time constant of left ventricular relaxation.47 The diastolic properties of the left ventricle are important determinants of cardiac function. An increase in diastolic tension will decrease cell perfusion, impede relaxation, and decrease ventricular filling and cardiac output. A decreased ATP-to-ADP ratio in the myofibrillar compartment, by slowing crossbridge kinetics, may decrease the rate and extent of relaxation. Impairment of the PCr shuttle may be one of the numerous factors responsible for diastolic abnormalities in the failing myocardium.

In the severely ischemic zones, an increase in diastolic tension and stiffness occurs at a time when systolic pressure is already abolished or greatly impaired. Under these circumstances, rigor tension or stiffness in the absence of PCr may appear as a “switch off” of energy expenditure for contraction. It would permit sustained tension at low energy cost. This maintained tension may be of vital importance for ischemic cardiac cells, preventing overstretching and membrane disruption. Furthermore, for the whole heart, the development of rigor tension in the ischemic zone may help to avoid local dilation and the development of aneurysm; it will also preserve mechanical coupling between adjacent contracting regions. Different structures participate in the increased resistance to stretch in cardiac tissue; among them are a higher content in connectin (titin) filaments48 and the extracellular matrix of collagen.49 It is tempting to speculate that in cardiac muscle the low Kᵦ value of myosin ATPase for Mg²⁺-ADP also represents an adaptive mechanism against cell overstretching.

Acknowledgments

This study was supported by Centre National de la Recherche Scientifique (Dr Ventura-Clapier), by an INSERM fellowship (Dr Vekslér), and in part by a grant from the “Association Française contre les Myopathies” and “Fondation de France.” The authors wish to acknowledge F. Doucet-Lefèvre for skillful technical assistance, P. Lechène for engineering assistance, S. Baudet for helpful discussion, and R. Fischmeister for continuous support.

References

25. Hoar PE, Mahoney CW, Kerrluc CG, Montague D. MgADP-increases maximum tension and Ca²⁺ sensitivity in skinned rabbit soleus fibers. Pflugers Arch. 1987;410:30-36.
32. Ventura-Clapier R, Mayoux E, Coutry N, Lechene P, Marotte F. Effects of acidosis and alkalosis on mechanical properties of...


Myocardial ischemic contracture. Metabolites affect rigor tension development and stiffness.

R Ventura-Clapier and V Vekslar

doi: 10.1161/01.RES.74.5.920

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/5/920

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org//subscriptions/