# ENERGETICS, MECHANICS AND MOLECULAR ENGINEERING OF CALCIUM CYCLING IN SKELETAL MUSCLE

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## 1. INTRODUCTION

During skeletal muscle contraction, $Ca^{2+}$ moves through an intracellular cycle. $Ca^{2+}$ is released from the sarcoplasmic reticulum (SR) in response to stimulation and then diffuses to bind to troponin C (TnC) on the thin filaments to activate the contractile machinery. Relaxation ensues when stimulation ceases and $Ca^{2+}$ dissociates from TnC in response to $Ca^{2+}$ re-accumulation into the SR driven by the SR $Ca^{2+}$ ATPase. This review will trace the development of thought with regard to three aspects of this $Ca^{2+}$ cycle: a) the energetic sequelae of $Ca^{2+}$ cycling, b) the role of the intracellular $Ca^{2+}$ binding protein parvalbumin (PA) in the $Ca^{2+}$ cycle and c) the consequences of altering the $Ca^{2+}$ exchange kinetics with TnC on muscle relaxation rate. This review is not comprehensive but rather selective with emphasis on topics of interest to our laboratory over the years.

## 2. ENERGETICS OF CALCIUM CYCLING

In 1949 A.V. Hill[1] defined the "activation heat" as the "...a 'triggered' reaction setting the muscle in a state in which it can shorten and do work." After the discovery of the role of $Ca^{2+}$ in contraction and relaxation[2], the activation heat came to be regarded as the "sum of the thermal accompaniments of the liberation of calcium into the sarcoplasm, its movement to and from the myofibrillar binding sites, and its return to its storage site by an ATP-dependent transport process in the sarcoplasmic reticulum.[3] In a completely reversed cycle, all other processes should thermally cancel, so that the net activation heat would result from the hydrolysis of ATP associated with the cycling of calcium.[3] The problem then became how to separate the energy utilization by the cross-bridges from that associated with the cycling of $Ca^{2+}$. Two developments, one of great conceptual importance and the other entirely practical, were pivotal in isolating and investigating the energetics of the $Ca^{2+}$ cycling pathway. In 1954, A.F. Huxley and R. Niedergerke[4] and

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H.E. Huxley and J. Hanson[5] proposed the sliding filament model of muscle contraction. This theory suggested that a muscle stretched beyond myofilament overlap would generate no isometric force. Thus the energetics of $Ca^{2+}$ cycling could be separated from the energetics of force and work production assuming that the $Ca^{2+}$ cycling process was not inhibited by muscle stretch. Unfortunately the classic skeletal muscle preparation, the frog sartorius, could not be stretched to this extent without damage. But in 1972 E. Homsher[3] working in W.F.H.M. Mommaert's laboratory and I.C.H. Smith[6] working in R.C. Woledge's laboratory discovered that the frog semitendinosus muscle could be reversibly stretched to long strengths. They performed comprehensive studies that form the foundation of our present day understanding of the energetics of $Ca^{2+}$ cycling. The basic experiment is illustrated in Fig. 1. Isometric twitch force and accompanying energy liberation at optimum myofilament overlap is superimposed upon results at an extreme muscle length where twitch force is nearly zero (Fig. 1A). When energy liberation is plotted versus isometric twitch force at stretched muscle lengths, a straight line results with an intercept that is about 30% of the energy liberated in a maximum twitch (Fig. 1B). Similar results were observed in isometric tetani[3]. Furthermore, the net activation heat over repeated contraction-relaxation cycles was produced in proportion to the hydrolysis of ATP. This result suggested that the activation heat was ultimately derived from ATP hydrolysis by the SR $Ca^{2+}$ pump. Thus Homsher *et al* and Smith concluded that about 30% of the energy liberated, or ATP utilized, during a maximum isometric twitch in amphibian muscle at low temperature was associated with the cyclic movement of $Ca^{2+}$.

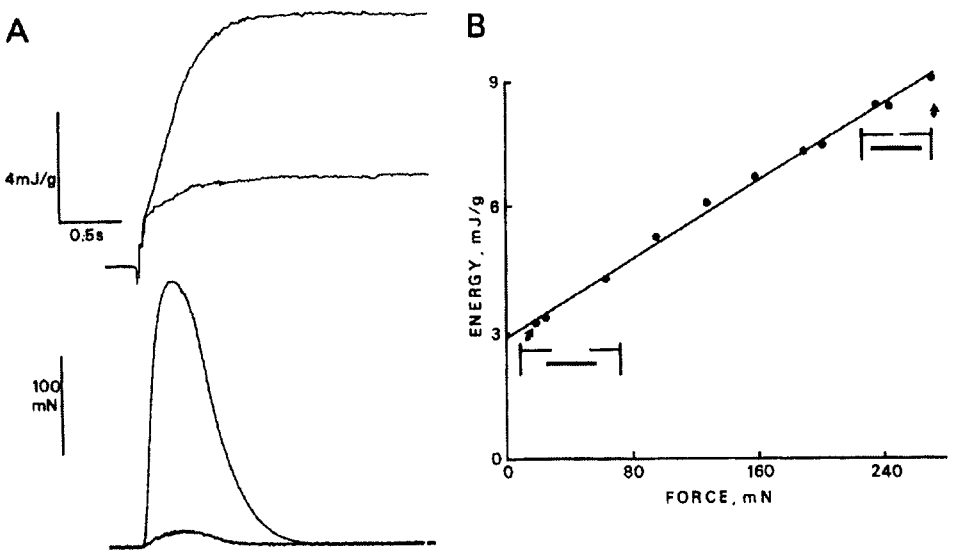


**Figure 1.** Measurement of activation heat in an isometric twitch. From reference 12 with permission.

It was of interest to test the generality of these results by measuring activation heat or $Ca^{2+}$ cycling energetics in a variety of muscles under different conditions. In 1973, B. Schottelius and I[7] characterized the activation heat in isometric twitches of phasic and

tonic skeletal muscles from the chicken at room temperature. The phasic posterior latissimus dorsi muscle exhibited kinetics of isometric contraction and relaxation and isotonic shortening that were 4 to 6-fold faster than that observed for the tonic anterior latissimus dorsi muscle. Yet in both muscles the activation heat represented about 20 – 25% of the energy liberation in a maximum isometric twitch. In the early 1970s, C. Gibbs and his laboratory were the first to extensively study the energetics of contraction and relaxation in mammalian fast- and slow-twitch muscle. They found that the energy associated with $Ca^{2+}$ cycling was about 35 – 40% of the steady rate of energy liberation in a tetanus in rat fast- and slow-twitch muscle.[8,9] In 1983, M.T. Crow and M.J. Kushmerick[10] found that the activation energetics represented about 30 to 40% of the total energy utilization in tetanic contractions of mammalian fast- and slow-twitch muscle of the mouse at 20 °C. L. Rome has shown the even in the fastest contracting and relaxing muscle known, the toadfish swimbladder muscle, the fraction of the energy utilization attributed to $Ca^{2+}$ cycling is about 25 to 40% of the total energy utilized.[11] Thus across the animal kingdom there is a significant fraction of the total energy utilized in a twitch or tetanus that is devoted to $Ca^{2+}$ cycling.

These experiments led to four fundamental conclusions. First, during a complete contraction-relaxation cycle, the amount of activation heat reflects the amount of ATP hydrolyzed to move $Ca^{2+}$ through the cycle and thus the total amount of $Ca^{2+}$ moved. Second, during an isometric twitch or tetanus of skeletal muscle, a surprisingly large fraction of the ATP (20 to 40%) utilized was devoted to pumping $Ca^{2+}$ back to the SR and not to producing force and/or work. Third, since the fraction of ATP utilized to pump $Ca^{2+}$ was approximately constant in a twitch and a tetanus where the total amounts of ATP utilized were vastly different, $Ca^{2+}$ must continually move through its cycle during the maintenance of tetanus force. Fourth, since the fraction of ATP utilized to cycle $Ca^{2+}$ was approximately the same in fast and slow contracting muscles in twitches and tetani, the rate of $Ca^{2+}$ accumulation by the SR must be "tuned" to match the rate of cross-bridge cycling. In other words, it would be pointless to have a fast contracting muscle that relaxed slowly, since it could not contract rapidly again until it relaxed.

Using the amplitude of the activation heat produced in a twitch as an indirect measure of the amount of $Ca^{2+}$ cycled has led to insights into the mechanism of action of various perturbations of muscle contraction (see review by Rall[12] and the references therein). For example, the activation heat amplitude or amount of $Ca^{2+}$ cycled in a twitch is: a) increased by agents that potentiate excitation-contraction (E-C) coupling such as $Zn^{2+}$, $NO_3^-$, caffeine and $UO_2^{2+}$, b) decreased by agents that inhibit E-C coupling such as elevated $CO_2$, strongly hypertonic solutions, $D_2O$ and dantrolene, c) unaltered by temperature in the range of 0 to 20 °C, and d) independent of muscle length. One interesting feature of the activation heat is its characteristic repriming time.[13] When a stimulus is given at varying time intervals after a twitch or tetanus, it is possible to map the repriming of the activation heat amplitude, and presumably the amount of $Ca^{2+}$ cycled, as a function of stimulus interval. This measure provides insight into the time it takes $Ca^{2+}$ to return to a releasable state in the SR. The activation heat in frog muscle at low temperature reprimes as a single exponential after a twitch but as a double exponential after a tetanus. In general the activation heat produced at any time after a tetanus is depressed to a greater extent than after a twitch. Activation heat also has been measured in cardiac muscle but other approaches have been taken since it is not possible to stretch cardiac muscle to long sarcomere lengths.[14] More recently techniques have been developed to directly measure the steady rate of ATP hydrolysis by the SR $Ca^{2+}$

pump in single skinned muscle fibers.[15] In general the activation heat has proven to be a fundamental aspect of the muscle contraction-relaxation cycle and an indirect measure of the amount of $Ca^{2+}$ cycled during contraction and relaxation.

Even though this history has focused on a narrow aspect of muscle energetics, no review of the history of muscle energetics would be complete without mention of two classic monographs in the field. In 1965 A.V. Hill published a book entitled "Trails and Trials in Physiology".[16] This book contains an annotated bibliography of his contributions to the field of muscle mechanics and energetics, a field which he dominated for over fifty years. It was considered to be a "bible" to those of us learning muscle energetics. The second monograph, published in 1985, entitled "Enegetic Aspects of Muscle Contraction" is a remarkable *tour de force* by R.C. Woledge, N.A. Curtin and E. Homsher.[17] This monograph is: a) a depository of virtually all studies on muscle energetics up to that time, b) a primer on enzyme and actomyosin kinetics, and c) an advanced treatise on muscle mechanics and energetics with integration, interpretation and theoretical speculation. These books are still valuable for novel ideas, history and inspiration.

## 3. ROLE OF PARVALBUMIN IN RELAXATION

The description of the activation heat above concentrated on its net amount which is proportional to the ATP hydrolyzed to pump $Ca^{2+}$ back to the SR and thus also proportional to the amount of $Ca^{2+}$ cycled during contraction and relaxation. But it must be remembered that the myothermal technique is nonspecific. Thus any reaction that liberates or absorbs heat will affect the time course of the energy liberation. It is only over a complete cycle that these events would be reversed and thus would be thermally neutral. Indeed during the time course of an isometric tetanus more energy is liberated than can be explained by the accompanying ATP hydrolysis.[18,19] N.A. Curtin and R.C. Woledge showed that some of this "unexplained" energy or enthalpy is associated with $Ca^{2+}$ cycling (the rest is associated with cross-bridge cycling).[20]

C. Gerday and J.M. Gillis in 1976[21] and J.-F. Pechere, J. Derancourt and J. Haiech in 1977[22] proposed that the intracellular $Ca^{2+}$ binding protein parvalbumin (PA) might promote relaxation in skeletal muscle by binding $Ca^{2+}$ in parallel with the SR. At about this time, Curtin and Woledge[23] speculated that $Ca^{2+}$ binding to PA might be the heat producing reaction that could account for the component of the unexplained energy associated with the activation heat time course. Indeed $Ca^{2+}$ binding to PA was shown to be a heat producing reaction.[24,25] PA is a soluble, intracellular $Ca^{2+}$ binding protein found in high concentration in fast contracting and relaxing skeletal muscles across the animal kingdom.[26] The affinity of $Ca^{2+}$ for PA is higher than the affinity of $Ca^{2+}$ for the regulatory sites of TnC.[21] Since PA concentration in some fish muscles can be ten-fold greater than the TnC concentration, speculation arose as to how these muscles could contract at all. The answer, as reviewed in detail by Rall,[27] is that PA also competitively binds $Mg^{2+}$, albeit with a $10^4$ fold lower affinity than exists for $Ca^{2+}$. But since the free $Mg^{2+}$ concentration in a muscle fiber at rest is about $10^4$ higher than the free $Ca^{2+}$ concentration, PA would be largely in the $Mg^{2+}$ bound form, i.e., Mg•PA. $Ca^{2+}$ cannot bind to PA until $Mg^{2+}$ dissociates and this is a relatively slow process. On the other hand, the regulatory sites of TnC do not contain $Mg^{2+}$ and $Ca^{2+}$ binds to these sites in a manner than is limited only by the rate of diffusion of $Ca^{2+}$ in the sarcoplasm. Thus muscle activation is not impaired by the presence of PA. Gerday and Gillis[21] further showed that

the SR has a higher affinity for $Ca^{2+}$ than does PA. Computer simulations showed that PA could, in principle, act to shuttle $Ca^{2+}$ from TnC to the SR and thus promote relaxation in the process.[28,29] Figure 2 shows a scheme indicating how PA might promote skeletal muscle relaxation.

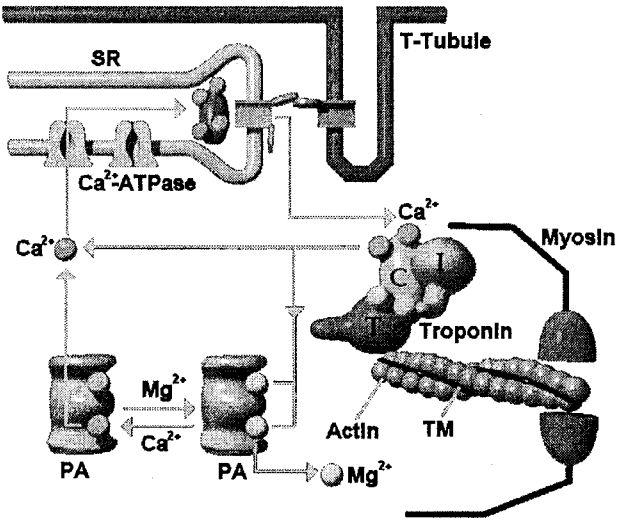


**Figure 2.** Proposed role of parvalbumin in promoting skeletal muscle relaxation. From reference 27 with permission.

Our laboratory tested and verified this scheme for the role of PA in promoting muscle relaxation in frog skeletal muscle at 0 and 10 °C.[30-32] Frog skeletal muscle was chosen to study because it has a high concentration of PA. Furthermore experiments were conducted at low temperatures because under these conditions PA would be expected to be more effective in promoting relaxation relative to the SR $Ca^{2+}$ pump. This speculation follows from the fact that the $Ca^{2+}$ pump ATPase exhibits a higher temperature sensitivity than does the exchange of $Ca^{2+}$ for $Mg^{2+}$ on PA. According to the scheme shown in Fig. 2, the effect of PA in promoting relaxation rate should diminish with increasing tetanus duration as PA becomes saturated with $Ca^{2+}$ during the tetanus and is thus unable to further bind $Ca^{2+}$ during relaxation. Furthermore the time course of diminution of the PA effect on relaxation should be determined by the rate of $Mg^{2+}$ dissociation from PA. This prediction was tested by comparing the time course of the slowing of relaxation rate in muscle with the time course of $Mg^{2+}$ dissociation from PA at the same temperature. Relaxation rate slowed progressively in frog skeletal muscle at 0 °C with increasing tetanus duration with a rate constant of 1.2 $s^{-1}$. After a four second tetanus, the relaxation rate reached a final value of about 40% of the maximum rate of relaxation. During this time there is little fatigue of maximum force development. In parallel experiments in solution, the $Mg^{2+}$ dissociation rate from PA was 0.9 $s^{-1}$, a value similar to the rate of slowing of relaxation. Also, consistent with these results, the rate of fall of the $Ca^{2+}$ transient declines with increasing tetanus duration in parallel with the decline of relaxation rate.[33]

A second prediction of the hypothesis is that the time course of recovery of the ability of PA to promote relaxation after a tetanus should reflect the time course of $Mg^{2+}$ re-association to PA which is determined by the time course of $Ca^{2+}$ dissociation from PA. In frog skeletal muscle at 0 °C, the depressed rate of relaxation after an isometric tetanus was accelerated with a rest period with a rate constant of 0.12 $s^{-1}$. This value is similar to the rate constant of $Ca^{2+}$ dissociation from isolated PA of 0.19 $s^{-1}$. These same predictions also were verified at 10 °C.[31] A final prediction is that PA should be able to cause relaxation even when the SR $Ca^{2+}$ pump is inhibited but only from a brief tetanus. This prediction was tested in frog skeletal muscle at 10 °C when the SR $Ca^{2+}$ pump was poisoned selectively with 2,5-di(tert-butyl)-1,4-benzohydroquinine (TBQ).[32] If the muscle was stimulated to produce a tetanus that would be predicted to saturate PA with $Ca^{2+}$ at 10 °C (1.1 second) in the presence of TBQ, there was little change in the rate of contraction or the amplitude of contraction but the fall of the $Ca^{2+}$ transient and relaxation required nearly five minutes instead of the usual 1 second. If instead the tetanus was brief (0.3 second), full relaxation occurred, albeit at a rate that was eight-fold slower than in the un-poisoned control muscle. Taken together, these results are consistent with the hypothesis that PA can promote relaxation in a brief contraction by binding $Ca^{2+}$ in parallel with the SR.

Recent studies have employed genetic techniques to directly test the role of PA in promoting relaxation in mammalian muscle. PA gene inactivation[34] or gene knockout[35] led to a decrease in relaxation rate in fast-twitch muscles of the mouse. Direct gene transfer of PA cDNA into rat slow-twitch muslce[36] or rat cardiac myocytes[37], which normally do not express PA, resulted in an acceleration of relaxation rate. Over-expression of PA in slow-twitch muscle of the mouse not only resulted in an acceleration of relaxation but also in other alterations in the muscle phenotype, including changes in the profile of metabolic enzymes.[38] It now is generally accepted that PA can promote relaxation in fast contracting and relaxing skeletal muscle.

## 4. CALCIUM EXCHANGE WITH TROPONIN C AND RELAXATION

The observation that PA modulates relaxation by transiently binding $Ca^{2+}$ implies that the rate of $Ca^{2+}$ sequestration must limit the rate of relaxation, especially at low temperatures in frog skeletal muscle. But what is the rate limiting process at higher temperatures? The effect of PA in frog skeletal muscle diminishes at higher temperatures, e.g., it is half as large at 10 °C as at 0 °C.[31] In fact, J.D. Johnson and Y. Jiang made the provocative observation that even though the rate of the fall of the $Ca^{2+}$ transient and rate of relaxation in frog skeletal muscle increased in the presence of a cell permeable form of the $Ca^{2+}$ chelator EGTA at 10 °C, there was no change at 20 °C.[39] It appeared that at 20 °C the rate of relaxation was not limited by $Ca^{2+}$ sequestration but rather by some myofibrillar process(es). They further noted that the rate of relaxation was similar to the rate of $Ca^{2+}$ dissociation from isolated whole troponin (Tn). Thus they proposed that at higher temperatures, relaxation rate might be limited by the rate of $Ca^{2+}$ dissociation from TnC.

This conclusion was a surprise since it had been assumed for many years that $Ca^{2+}$ dissociation from TnC in the muscle was much more rapid than the rate of mechanical relaxation and thus was not involved in controlling the rate of relaxation. This assumption was based on the fact that the $Ca^{2+}$ dissociation rate from purified TnC is more than twenty-fold faster than the rate of muscle relaxation at the same temperature.[40]

But $Ca^{2+}$ dissociation from isolated whole Tn (TnC-TnI-TnI) is much slower and similar to the rate of muscle relaxation.[41] Thus it appears that under some conditions the rate of $Ca^{2+}$ dissociation from whole Tn may be slow enough to limit the rate of muscle relaxation.

In order to test if the rate of $Ca^{2+}$ dissociation from TnC is a factor in determining the rate of muscle relaxation, we have: a) generated mutants of TnC with varying $Ca^{2+}$ affinities and $Ca^{2+}$ dissociation rates, b) developed a simplified *in vitro* system that mimics isolated whole Tn to screen mutant properties and c) begun to measure the effects of some of these TnC mutants on the rate of muscle relaxation. J.P. Davis and S.B. Tikunova in our laboratory have generated mutants of TnC by substituting hydrophobic residues in the regulatory domain of TnC with glutamine.[42] These TnC mutants exhibit a wide range of $Ca^{2+}$ affinities and $Ca^{2+}$ dissociation rates. Also they have shown that the rate of $Ca^{2+}$ dissociation from a complex of TnC and a peptide of TnI ($TnI_{96\text{-}148}$) is similar to the rate of $Ca^{2+}$ dissociation from isolated whole Tn.[43] Furthermore Figure 3 shows that the rate of $Ca^{2+}$ dissociation from the TnC-$TnI_{96\text{-}148}$ complex is much slower than the rate of $Ca^{2+}$ dissociation from isolated TnC but similar to the rate of muscle relaxation.[43] Thus the TnC-$TnI_{96\text{-}148}$ complex was utilized to screen TnC mutants for variations in $Ca^{2+}$ dissociation rates (>30-fold) and $Ca^{2+}$ affinities (>240-fold). Interestingly, mutations that affected $Ca^{2+}$ sensitivity of isolated TnC but not the TnC-$TnI_{96\text{-}148}$ complex had little affect on the $Ca^{2+}$ sensitivity of muscle force development. Thus, the effect of mutations on the $Ca^{2+}$ sensitivity of force development could be better predicted from the change in $Ca^{2+}$ affinity of the TnC-$TnI_{96\text{-}148}$ complex than from the change in affinity of the isolated TnC alone.

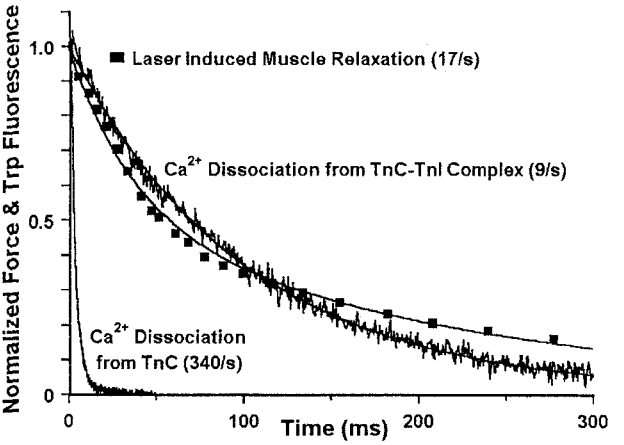


**Figure 3.** $Ca^{2+}$ dissociation from the TnC-TnI complex, but not from isolated TnC alone, is similar to the rate of relaxation in skinned psoas muscle fibers at 15 °C. $Ca^{2+}$ dissociation was measured from changes in fluorescence of trp engineered into $TnC^{F29W}$. Relaxation was induced by flash photolysis of the $Ca^{2+}$ chelator diazo-2. From reference 43 with permission.

In collaboration with L. Smillie, our laboratory has determined the effects of TnC mutants which exhibited a higher or lower $Ca^{2+}$ dissociation rate from the TnC-$TnI_{96\text{-}148}$ complex on the rate of muscle relaxation.[44] TnC mutants were incorporated into fast-twitch mammalian skinned psoas fibers after extraction of endogenous TnC. The skinning process destroyed the SR in these fibers. Contraction was induced by elevating

the free $Ca^{2+}$ concentration and relaxation was induced by flash photolysis of the photolabile $Ca^{2+}$ chelator, diazo-2. A TnC mutant with a higher $Ca^{2+}$ affinity and a two-fold slower $Ca^{2+}$ dissociation rate as measured in solution, $TnC^{M82Q}$, increased the $Ca^{2+}$ sensitivity of isometric force production and decreased the rate of relaxation in muscle fibers by two-fold. A TnC mutant with a lower $Ca^{2+}$ affinity and 1.5-fold faster $Ca^{2+}$ dissociation rate, $TnC^{NHDel}$ (deletion of residues 1-11), decreased the $Ca^{2+}$ sensitivity of force production but did not significantly accelerate muscle relaxation. The rate of cross-bridge detachment was slowed by reducing the inorganic phosphate concentration. This perturbation also slowed relaxation rate. Thus slowing of either the $Ca^{2+}$ dissociation rate from TnC or cross-bridge detachment rate slowed muscle relaxation but acceleration of one rate, $Ca^{2+}$ dissociation from TnC, had little effect on relaxation rate. These results are consistent with the interpretation that the rate of $Ca^{2+}$ dissociation from TnC and the rate of cross-bridge detachment are comparable and contribute equally to determining the rate of relaxation.

In order to test this idea more fully, mutants of TnC have been constructed with a wider range of $Ca^{2+}$ dissociation rates and these mutants will be tested in muscle fibers. Since at present, it is not possible to measure the rate of $Ca^{2+}$ dissociation from TnC in a muscle fiber, these mutants are being incorporated into isolated myofibrils to test for their influence on $Ca^{2+}$ dissociation rate from TnC in a system intermediate in structure between TnC-$TnI_{96-148}$ and the skinned fiber.[45] To date, we have found that the $Ca^{2+}$ dissociation rate from the isolated myofibrils is similar to the rate of $Ca^{2+}$ dissociation from the TnC-$TnI_{96-148}$ complex and the rate of muscle relaxation. Also, we recently have constructed mutants of cardiac TnC with varying $Ca^{2+}$ dissociation rates in order to determine their effects in cardiac muscle relaxation and to compare the results to those observed in skeletal muscle.[46] In cardiac muscle, the phosphorylation of TnI is thought to accelerate relaxation by increasing the rate of $Ca^{2+}$ dissociation from TnC.[47,48] This idea will be tested directly with the cardiac TnC mutants. In conclusion, the notion that $Ca^{2+}$ dissociation from TnC plays a role in determining the rate of skeletal muscle relaxation under certain conditions seems probable but is still speculative and more work will need to be done in this area.

## 5. SUMMARY

During muscle contraction and relaxation, $Ca^{2+}$ moves through a cycle. About 20 to 40% of the ATP utilized in a twitch or a tetanus is utilized by the SR $Ca^{2+}$ pump to sequester $Ca^{2+}$. Parvalbumin is a soluble $Ca^{2+}$ binding protein that functions in parallel with the SR $Ca^{2+}$ pump to promote relaxation in rapidly contracting and relaxing skeletal muscles, especially at low temperatures. The rate of $Ca^{2+}$ dissociation from troponin C, once thought to be much more rapid than the rate of relaxation, is likely to be similar to the rate of cross-bridge detachment and to the rate of muscle relaxation under some conditions. During the past fifty years, great progress has been made in understanding the $Ca^{2+}$ cycle during skeletal muscle contraction and relaxation. Nonetheless, there are still mysteries waiting to be unraveled.

## 6. REFERENCES


1. A.V. Hill, The heat of activation and the heat of shortening in a muscle twitch, *Proc. Roy. Soc. B* **136**, 195-211 (1949).
2. S. Ebashi, M. Endo, and I. Ohtsuki, Control of muscle contraction, *Quat. Rev. Biophys.* **2**, 351-384 (1969).
3. E. Homsher, W.F.H.M. Mommaerts, N.V. Ricchiuti, and A. Wallner, Activation heat, activation metabolism and tension-related heat in frog semintendinosus muscles, *J. Physiol.* **220**, 601-625 (1972).
4. A.F. Huxley and R. Niedergerke, Structural changes in muscle during contraction, *Nature* **173**, 971-973 (1954).
5. H.E. Huxley and J. Hanson, Changes in the cross-striations of muscle during contraction and stretch and their structural interpretations, *Nature* **173**, 973-976 (1954).
6. I.C.H. Smith, Energetics of activation in frog and toad muscle, *J. Physiol.* **220**, 583-599 (1972).
7. J.A. Rall and B.A. Schottelius, Energetics of contraction in phasic and tonic skeletal muscles of the chicken, *J. Gen. Physiol.* **62**, 303-323 (1973).
8. I.R. Wendt and C.L. Gibbs, Energy production of rat extensor digitorum longus muscle, *Am. J. Physiol.* **224**, 1081-1086 (1973).
9. C.L. Gibbs and W.R. Gibson, Energy production of rat soleus muscle, *Am. J. Physiol.* **223** 864-871 (1972).
10. M.T. Crow and M.J. Kushmerick, Correlated reduction of velocity of shortening and the rate of energy utilization in mouse fast-twitch muscle during a continuous tetanus, *J. Gen. Physiol.* **82**, 703-720 (1983).
11. L.C. Rome and A.A. Klimov, Superfast contractions without superfast energetics: ATP usage by SR-$Ca^{2+}$ pumps and crossbridges in toadfish swimbladder muscle, *J. Physiol.* **526**, 279-286 (2000).
12. J.A. Rall, Energetics of $Ca^{2+}$ cycling during skeletal muscle contraction, *Fed. Proc.* **41**, 155-160 (1982).
13. J.A. Rall, Effects of previous activity on the energetics of activation in frog skeletal muscle, *J. Gen. Physiol.* **75**, 617-631 (1980).
14. N.R. Alpert, E.M. Blanchard, and L.A. Mulieri, Tension-independent heat in rabbit papillary muscle, *J. Physiol.* **414**, 433-453 (1989).
15. G.J.M. Stienen, R. Zaremba, and G. Elzinga, ATP utilization for calcium uptake and force production in skinned muscle fibres of *Xenopus laevis*, *J. Physiol.* **482**, 109-122 (1995).
16. A.V. Hill, *Trails and Trials in Physiology* (The Williams & Wilkins Company, Baltimore, 1965).
17. R.C. Woledge, N.A. Curtin, and E. Homsher, *Energetics Aspects of Muscle Contraction* (Academic Press, London, 1985).
18. N.A. Curtin and R.C. Woledge, Chemical change and energy production during contraction of frog muscle: how are their time courses related? *J. Physiol.* **288**, 353-366 (1979).
19. E. Homsher, C.J. Kean, A. Wallner, and V. Garibian-Sarian, The time-course of energy balance in an isometric tetanus, *J. Gen. Physiol.* **73**, 553-567 (1979).
20. N.A. Curtin and R.C. Woldege, The effect of muscle length on energy balance in frog skeletal muscle, *J. Physiol.* **316**, 453-468 (1981).
21. C. Gerday and J.M. Gillis, Possible role of parvalbumin in the control of contraction, *J. Physiol.* **258**, 96-97P (1976).
22. J.-F. Pechere, J. Derancourt, and J. Haiech, The participation of parvalbumins in the activation-relaxation cycle of vertebrate fast skeletal-muscle, *FEBS Letters* **75**, 111-114 (1977).
23. N.A. Curtin and R.C. Woledge, Energy changes and muscular contraction, *Physiol. Rev.* **58**, 690-761 (1978).
24. S.J. Smith and R.C. Woledge, Thermodyamic analysis of calcium binding to frog parvalbumin, *J. Muscle Res. Cell Mot.* **6**, 757-768 (1985).
25. M. Tanokura and K. Yamada, A calorimetric study of $Ca^{2+}$ binding to two major isotypes of bullfrog parvalbumin, *FEBS* **185**, 165-169 (1985).
26. C.W. Heinzman, Parvalbumin, an intracellular calcium-binding protein; distribution, properties and possible roles in mammalian cells, *Experientia Basel* **40**, 910-921 (1984).
27. J.A. Rall, Role of parvalbumin in skeletal muscle relaxation, *News Physiol. Sci.* **11**, 249-255 (1996).
28. J.M. Gillis, D. Thomason, J. Lefevre, and R.H. Kretsinger, Parvalbumins and muscle relaxation: a computer simulation study, *J. Muscle Res. Cell Mot.* **3**, 377-398 (1982).
29. M. Cannell and D.G. Allen, Model of calcium movements during activation in the sarcomere of frog skeletal muscle, *Biophys. J.* **45**, 913-925 (1984).
30. T. Hou, J.D. Johnson, and J.A. Rall, Parvalbumin content and $Ca^{2+}$ and $Mg^{2+}$ dissociation rates correlated with changes in relaxation rate of frog muscle fibres, *J. Physiol.* **441**, 285-304 (1991).
31. T. Hou, J.D. Johnson, and J.A. Rall, Effect of temperature on relaxation rate and $Ca^{2+}$, $Mg^{2+}$ dissociation rates from parvalbumin of frog muscle fibres, *J. Physiol.* **449**, 399-410 (1992).

32. Y. Jiang, J.D. Johnson, and J.A. Rall, Parvalbumin relaxes frog skeletal muscle when the sarcoplasmic reticulum Ca-ATPase is inhibited, *Am. J. Physiol.* **270**, C411-C417 (1996).
33. M.B. Cannell, Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres, *J. Physiol.* **376**, 203-218 (1986).
34. J.M. Raymackers, P. Gailly, M.C. Schoor, D. Pette, B. Schwaller, W. Hunziker, M.R. Celio, and J.M. Gillis, Tetanus relaxation of fast skeletal muscles of the mouse made parvalbumin deficient by gene inactivation, *J. Physiol.* **527**, 355-364 (2000).
35. B. Schwaller, J. Dick, G. Dhoot, S. Carroll, G. Vrbova, P. Nicotera, D. Pette, A. Wyss, H. Bluethmann, W. Hunziker, and M.R. Celio, Prolonged contraction-relaxation cycle of fast-twitch muscles in parvalbumin knockout mice, *Am. J. Physiol.* **276**, C395-C403 (1999).
36. M. Muntener, L. Kaser, J. Weber, and M.W. Berchtold, Increase of skeletal muscle relaxation speed by direct injection of parvalbumin cDNA, *Proc. Nat. Acad. Sci.* **92**, 6504-6508 (1995).
37. P.A. Wahr, D.E. Michele, and J.M. Metzger, Parvalbumin gene transfer corrects diastolic dysfunction in diseased cardiac myocytes, *Proc. Nat. Acad. Sci.* **96**, 11982-11985 (1999).
38. E.R. Chin, R.W. Grange, F. Viau, A.R. Simard, C. Humphries, J. Shelton, R. Bassel-Duby, R.S. Williams, and R.N. Michel, Alterations in slow-twitch muscle phenotype in transgenic mice overexpressing the $Ca^{2+}$ buffering protein parvalbumin, *J. Physiol.* **547**, 649-663 (2003).
39. J.D. Johnson, Y. Jiang, and M. Flynn, Modulation of $Ca^{2+}$ transients and tension by intracellular EGTA in intact frog muscle fibers, *Am. J. Physiol.* **272**, C1437-C1444 (1997).
40. J.D. Johnson, S.C. Charlton, and J.D. Potter, A fluorescence stopped flow analysis of $Ca^{2+}$ exchange with troponin C, *J. Biol. Chem.* **254**, 3497-3502 (1979).
41. J.D. Potter and J.D. Johnson, Troponin, in: Calcium and Cell Function, Vol II, edited by W.Y. Cheung (Academic Press, New York, 1982), pp. 145-172.
42. S.B. Tikunova, J.A. Rall, and J.P. Davis, Effect of hydrophobic residue substitutions with glutamine on $Ca^{2+}$ binding and exchange with the N-domain of troponin C, *Biochem.* **41**: 6697-6705 (2002).
43. J.P.Davis, J.A. Rall, C.Alionte, and S.B. Tikunova, Mutations of hydrophobic residues in the N-domain of troponin C affect calcium binding and exchange with the troponin C-troponin $I_{(96\text{-}148)}$ complex and muscle force production, *J. Biol. Chem*, in press (2004).
44. Y. Luo, J.P. Davis, L.B. Smillie, and J.A. Rall, Determinants of relaxation rate in rabbit skinned skeletal muscle fibres, *J. Physiol.* **545**, 887-901 (2002).
45. J.P. Davis, S.B. Tikunova, D.R. Swartz, and J.A. Rall, Measurement of $Ca^{2+}$ dissociation rates from troponin C (TnC) in skeletal myofibrils, *Biophys. J.* **86**, 218a (2004).
46. S.B. Tikunova, J.P. Davis, and J.A. Rall, Engineering cardiac troponin C (cTnC) mutants with dramatically altered $Ca^{2+}$ dissociation rates as molecular tools to study cardiac muscle relaxation, *Biophys. J.* **86**, 394a (2004).
47. S.P. Robertson, J.D. Johnson, M.J. Holroyde, E.G. Kranias, J.D. Potter, and R.J. Solaro, The effect of troponin I phosphorylation on the $Ca^{2+}$-binding properties of the $Ca^{2+}$-regulatory site of bovine cardiac troponin, *J. Biol. Chem.* **257**, 260-263 (1982).
48. R. Zhang and J.D. Potter, Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation, *Circ. Res.* 76, 1028-1035 (1995).