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A re-interpretation of the rate of tension redevelopment (k_{TR}) in active muscle

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Abstract

A slackening to zero tension by large length release (~20 %) and a restretch of active muscle fibres cause a fall and a redevelopment in tension. According to the model of Brenner (Proc Natl Acad Sci USA 85(9):3265–3269, 1988), the rate constant of tension redevelopment (k_{TR}) is the sum of attachment and detachment rate constants, hence is limited by the fast reaction. Here we propose a model in which, after restretch, cross-bridges cycle many times by stretching series elastic elements, hence k_{TR} is limited by a slow reaction. To set up this model, we made an assumption that the stepping rate (v) decreases linearly with tension (F), which is consistent with the Fenn effect. The distance traveled by a cross-bridge stretches series elastic elements with stiffness σ. With these assumptions, we set up a first order differential equation, which results in an exponential time course with the rate constant $k_{TR} = \sigma\eta_0 v_0(1 - \lambda)/F_1$, where $\lambda = v_1/v_0$, η = step size, the subscript 0 indicates unloaded condition, and the subscript 1 indicate isometric condition. We demonstrate that the ATP hydrolysis rate (=[myosin head]/v_0) is proportionate to k_{TR} as the ambient temperature is changed, and that the published data fit to this relationship well if $\lambda = 0.28$. We conclude that k_{TR} is limited by the cross-bridge turnover rate; hence it represents the rate constant of the slowest reaction of the cross-bridge cycle, i.e. the ADP isomerization step before ADP is released.

Keywords

Cross-bridge cycle; ATP hydrolysis rate; Tension transient; Rate constant; Posas fibres

Introduction

Muscle contraction occurs through cyclic attachment/detachment of myosin cross-bridges to/from actin in the thin filament, thereby pulling the thin filament toward the center of sarcomeres. In order to characterize the cross-bridge kinetics, Brenner and his colleague (Brenner and Eisenberg 1986; Brenner 1988) developed a simple mechanical method, in which the length of an active muscle fibre is released by ~20 % to induce slackness in fibres. Tension in the fibre falls to zero simultaneously with the length release owing to the shortening of series elastic elements. After being held at the short length for 20–50 ms, the fibre is then restretched to its original length. With the restretch, attached cross-bridges detach instantly, tension in fibers (if any) drops to zero again, hence the series elastic elements are still at the shortened stage. Thereafter, cross-bridges start attaching to the thin filament. This is the point of synchronization of all cross-bridges, which otherwise perform

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stochastic chemical transitions. As the number of force-generating cross-bridges increases, an exponential time course of tension redevelopment follows with the apparent rate constant k_{TR} (Brenner 1988). With the tension increase, a restretch of the shortened series elasticity occurs.

In the two-state model proposed by Brenner, the rate constant of cross-bridge attachment to actin is defined as f_{app}, and that of detachment from actin is defined as g_{app} after applying the two-state model originally proposed by Huxley (1957). From these, it follows that the apparent rate constant of the system is: $f_{app} + g_{app}$, hence it was thought that the k_{TR} reflects the kinetics of cross-bridge attachment and detachment (Brenner and Eisenberg 1986; Brenner 1988). The subscript "app" was used to signify that the rate constants are not limited to a single reaction step, but rather a composite of the rate constants of several steps in the cross-bridge cycle (Brenner 1990). In this report, "app" is shortened to "a" for simplicity. In the original formalism, it must be recognized that k_{TR} is limited by the *fast* reaction. This is because, if $f_a \ll g_a$, then it follows that $k_{TR} = f_a + g_a \approx g_a$. Therefore, k_{TR} is sensitive to the fast reaction (g_a), but not sensitive to the slow reaction (f_a).

In spite of the common use of k_{TR}, there have been problems in the interpretation of k_{TR}. One problem has been that the observed k_{TR} is never faster than 40 s^{-1}, whereas many rate constants of tension transients observed with various techniques are in the range 100–1,000 s^{-1}, which suggested thatk_{TR}may be limited byone or more slow reactions (Gordon et al. 2000). Another problem has been that the length release needed to measure k_{TR} is ~20 %, which corresponds to ~250 nm at the cross-bridge level when the half sarcomere length is 1,250 nm. If the step size is 9.7 nm (Sherwood et al. 2004), change of length by ~250 nm corresponds to ~26 cross-bridge steps. Therefore, k_{TR} may represent the kinetics of the multiple cross-bridge cycles, and is very likely limited by the slow reaction(s) in cross-bridge cycle.

Here we propose a model, in which cross-bridges cycle many times during the tension recovery period by stretching series elastic elements, hence k_{TR} is limited by the slowest reaction instead of the fastest reaction of the cross-bridge cycle. Because the contractile apparatus is made of proteins, it has been known that the series elasticity is present in thick and thin filaments (Huxley et al. 1994; Wakabayashi et al. 1994), myosin heads, Z-disks, and in tendon when present. Figure 1 represents a visual representation of the model we propose. A preliminary account of the present results was presented at a recent Biophysical Society meeting Kawai and Wang (2013).

Materials and methods

Skinned skeletal muscle fibres from New Zealand white rabbit were used. The use of rabbits conformed with the current Guide for the Care and Use of Laboratory Animals (NIH publication DHSS/USPHS), and was approved by the University of Iowa's Animal Care and Use Committee. Rabbit of 3.5–5 kg weight was euthanized by injecting 150 mg/kg sodium pentobarbital into an ear vein. Strips of psoas, extensor digitorum longus (EDL) and tibialis anterior (TA) muscles were excised. Fibre bundles of ~15 mm in length and 2 mm in diameter were tied to wooden sticks at the in situ length using cotton thread and placed in the ice-cold *Na* skinning solution for 15–30 min. This was followed by *K* skinning solution for 30 min, and finally placed in the storage solution on ice for overnight. Next day the storage solution was replaced once again. The compositions of these solutions are found in Table 1 of our previous publication (Wang et al. 2013). The muscle bundles were then stored at –20 °C until used for experiments. On the day of experiments, skinned fibres (~3 mm in length and ~75 μm in diameter) were dissected from the bundles. Psoas fibres were used in the experiments to study the correlation between k_{TR} and the ATP hydrolysis rate

(ATPase). All three kinds of fibres (psoas, EDL, and TA) were used to study the correlation between k_{TR} and the slow rate constant $2\pi a$. The data of ATPase was taken from our previous publication (Zhao and Kawai 1994), which was measured on psoas fibres by the NADH fluorescence method with coupled enzyme systems, pyruvate kinase and lactate dehydrogenase.

After mounting fibres to the experimental apparatus, the sarcomere length was adjusted to 2.5 μm using He–Ne laser (wavelength: 0.6328 μm) diffraction, and the fibre length (L_0) and cross-sectional area were determined. The fibres were activated by the standard activating solution (6 mM CaEGTA, 5 mM MgATP, 1 mM Mg^{2+}, 8 mM Pi, ionic strength 200 mM with KAc (Ac = acetate), pCa 4.6, pH 7.00). When the tension plateau developed, the length was suddenly released by 20 % L_0, and restretched to the original length after 50 ms (Fig. 2a). Sinusoidal analysis was performed before the release-and-restretch experiment, because the latter disturbs fibres much more than the sinusoidal analysis. The method of sinusoidal analysis was described previously (Kawai and Brandt 1980; Wang et al. 2013). All the standard activations were performed at 20 °C, except for the temperature study. All data are expressed as mean ± standard error (SE).

The model

The model is depicted in Fig. 1. Here, x is the location of a myosin head, t is time, η is the step size, and v is the number of steps/sec (stepping rate, turnover rate, or cycling rate). From these definitions, it follows that

$$\text{Velocity}=\frac{\mathrm{d}x}{\mathrm{d}t}=\eta v \quad (1)$$

$$\therefore \mathrm{d}x=\eta v \mathrm{d}t \quad (2)$$

where dx is the distance traveled by the cross-bridge in time dt. This stretches the series elasticity with the spring constant σ by dx. The associated force increase (dF) for cross-bridges is:

$$\mathrm{d}F=\sigma \mathrm{d}x=\sigma \eta v \mathrm{d}t \quad (3)$$

Note that σ includes series elasticity of the thin filament, the thick filament, the myosin head, Z-line, and tendon if present. Our basic premise is that during force redevelopment (dF), the series elasticity elongates (dx), and filament sliding occurs according to Eq. 3.

For the model we are proposing, one assumption needed is that the stepping rate (v) decreases linearly with an increase in force in the following way (Eq. 4).

$$v=v_0\left(1-\left(1-\frac{v_1}{v_0}\right)\frac{F}{F_1}\right) \quad (4)$$

where v_1 is isometric cycling rate at $F = F_1$, v_0 is unloaded cycling rate at $F = 0$, and $0 \le F \le F_1$. We define $\lambda : v_1/v_0$ ($0 < \lambda < 1$). The step size (η) is a constant (η_0) for $F < F_1$, but it becomes 0 for $F = F_1$ (Eq. 5):

$$\eta=\begin{cases}\eta_0 & (0 \le F<F_1)\\ \eta_1=0 & (F=F_1)\end{cases} \quad (5)$$

where subscript 0 indicates the unloaded condition, and 1 indicates the isometric condition. From Eqs. 4 and 5,

$$\eta v = \text{velocity} = \begin{cases} \eta_0 v_0 \left(1 - (1-\lambda)\frac{F}{F_1}\right) & (0 \le F \le F_1) \\ 0 & (F = F_1) \end{cases} \quad (6)$$

Eq. 6 corresponds to the linearized force–velocity relationship.

Equation 4 (Fig. 3) represents one interpretation of the Fenn (1923) effect , i.e. the stepping rate becomes less as force develops. There is experimental evidence for Eq. 6 by using skeletal muscle fibres (Piazzesi et al. 2007) and single molecules (Greenberg and Moore 2010).

From Eqs. 3 and 6, we get

$$\frac{\mathrm{d}F}{\mathrm{d}t} + kF = \sigma\eta_0 v_0 = \frac{kF_1}{1-\lambda} \quad (7)$$

$$\text{where} \quad k \equiv \frac{\sigma\eta_0 (v_0 - v_1)}{F_1} = \frac{\sigma\eta_0 v_0 (1-\lambda)}{F_1} \quad (8)$$

Equation 7 can be solved with the initial condition $F(0) = 0$ to result:

$$F(t) = \frac{F_1}{1-\lambda}\left(1 - e^{-kt}\right). \quad (9)$$

In a practical data fitting of the exponential time course, a constant (C) must be added to Eq. 9:

$$F(t) = \frac{F_1}{1-\lambda}\left(1 - e^{-kt}\right) + C$$

Because $F(\infty) = F_1$, we set $C = -\frac{\lambda}{1-\lambda}F_1$

$$\text{Consequently,} \quad F(t) = F_1\left(1 - \frac{1}{1-\lambda}e^{-kt}\right) \quad (10)$$

Equation 10 is the force time course, which approximates (dotted line) the data (solid line) in Fig. 2; see also (Brenner and Eisenberg 1986; Regnier et al. 1995; Stehle et al. 2002; Piroddi et al. 2003), where $k \equiv: k_{TR}$. The ATP hydrolysis rate is:

$$\text{ATPase} = M_c v \quad (11)$$

where M_c is the concentration of the myosin head.

Isometric values

Here we set $F = F_1$. Then, $v_1 = \lambda v_0$,

$$\therefore \text{ATPase}_1 = M_c v_1 = M_c \lambda v_0 = \frac{\lambda M_c F_1}{\sigma \eta_0 (1-\lambda)} k_{TR} \quad (12)$$

Two-state model

The two-state model (Huxley 1957; Podolsky 1968; Julian 1969; Brenner 1988) has been extensively used because of its simplicity. In this model (Brenner 1988),

$$\text{Det} \underset{g_a}{\overset{f_a}{\rightleftarrows}} \text{Att} \quad (13)$$

where Det is the detached state and Att is the attached state. f_a is the apparent rate constant of attachment, and g_a is the apparent rate constant of detachment. The total concentration of myosin (M_c) equals the sum of concentrations of the detached and attached states.

$$[\text{Det}] + [\text{Att}] = M_c \quad (14)$$

Because of the steady-state,

$$f_a [\text{Det}] = g_a [\text{Att}] \quad (15)$$

From Eqs. 14 and 15,

$$[\text{Att}] = M_c \frac{f_a}{f_a + g_a}$$

$$\therefore \text{ATPase} = g_a [\text{Att}] = f_a [\text{Det}] = M_c \frac{f_a g_a}{f_a + g_a} \quad (16)$$

Except for M_c, all the variables in Eq. 16 depend on F. The average time cross-bridges spend in the Det state ($\bar{t}_{det}$) is:

$$\bar{t}_{\text{det}} = \frac{\int_0^\infty t e^{-f_a t} dt}{\int_0^\infty e^{-f_a t} dt} = \frac{\frac{1}{f_a^2}}{\frac{1}{f_a}} = \frac{1}{f_a}. \quad (17)$$

as expected ($\bar{t}_{det}$ is the time constant of the attachment step). Similarly the average time in the Att state ($\bar{t}_{att}$) is:

$$\bar{t}_{att} = \frac{1}{g_a} \quad (18)$$

One cycle time is the sum of the time spent in the two states:

$$\frac{1}{v} = \bar{t}_{\text{total}} = \bar{t}_{\text{det}} + \bar{t}_{\text{att}} = \frac{1}{f_a} + \frac{1}{g_a} = \frac{f_a + g_a}{f_a g_a}.$$

If this equation and Eq. 16 are multiplied, then Eq. 11 results, confirming the derivation of t_{total}. After combining Eqs. 12 and 16, we arrive at Eq. 19.

$$k_{TR}=\frac{1-\lambda}{\lambda}\times\frac{\eta_0}{F_1/\sigma}\times\frac{f_{a1}g_{a1}}{f_{a1}+g_{a1}} \quad (19)$$

where f_{a1} and g_{a1} represent isometric values.

The model thus proposed is one of the simplest, because it does not consider non-linear filament compliance (Edman 2009; Nocella et al. 2013). The strain sensitivity of the rate constant is considered in Eq. 4, that the stepping rate (v) decreases linearly with an increase in force. The model proposed is also a simplified version, because it does not consider multiple attached states, and transitions between them are generally considered to be strain sensitive (Huxley and Simmons, 1971; Kawai and Halvorson, 2007). The case we have considered applies to shortening and isometrically held muscles as does the Fenn (1923) effect, but its applicability to lengthening muscle remains to be seen. The model assumes that the filament sliding occurs during force redevelopment, and this sliding stretches the series compliance.

Results

One or two exponential functions were fitted to the tension time course

The tension time course at standard activation from rabbit psoas fibres recorded by the experimental computer is plotted in Fig. 2b, c with solid lines. When this time course was fitted to one exponential function (Fig. 2b, broken line), we obtained the following results: k = 8.0 s^{-1}, A = 0.93 mN, where $k = k_{TR}$ is the rate constant and A is the amplitude. When the same time course was fitted to two (fast and slow) exponential functions (Fig. 2c), the results are as follows: k_{slow} = 5.7 s^{-1}, A_{slow} = 0.58 mN, k_{fast} = 16.3 s^{-1}, and A_{fast} = 0.42 mN. In Fig. 2b, there is a small departure between the data and fitted curve, whereas Fig. 2c the departure is almost absent.

The effect of temperature on fast and slow rate constants of k_{TR}

With skinned psoas muscle fibres, we studied k_{TR} at temperatures in the range of 10–25 °C in every 5 °C. The effect of temperature on fast and slow rate constants of k_{TR} is shown in Fig. 4. As expected, the temperature-rate constant plots show increasing functions with concave upward. Here we obtained Q_{10} = 1.8 for the slow component, and Q_{10} = 1.4 for the fast component.

Relationship between k_{TR} and ATPase at different temperatures

Through combining our published data on the isometric ATP hydrolysis rate ($ATPase_1$) (Zhao and Kawai 1994) and the rate constant of the slow components in Fig. 4, ATPase is plotted against k_{TR} in Fig. 5. This plot demonstrates nearly a perfect linear relationship with the intercept going through the origin, demonstrating that k_{TR} and $ATPase_1$ are proportionately related, and as predicted by Eq. 12.

Relationship between k_{TR} and the rate constant $2\pi a$

Sinusoidal analysis was performed immediately before the k_{TR} measurement on the same preparations. The complex modulus $Y(f)$ of fast twitch skeletal muscle fibres were fitted to the equation with three exponential processes, that included exponential processes A, B, and C (Eq. 2 in (Kawai and Brandt 1980)). The results are plotted in Fig. 6a for rabbit psoas fibres in which temperature was changed between 10 °C and 25 °C. Our results demonstrate

that k_{TR} deduced from release-restretch experiments and $2\pi a$ deduced from sinusoidal analysis are linearly related. Their empirical equation is:

$$2\pi a = 0.71\, k_{TR} + 0.89\ s^{-1} \quad (20)$$

This regression line in entered in Fig. 6a as a broken line. The correlation of k_{TR} and $2\pi a$ was repeated for two other muscle types (EDL and TA) at 20 °C, and the results are plotted in Fig. 6b. The same regression line as in Fig. 6 a is entered in Fig. 6b. It is apparent that the data from two additional fast twitch fibres fall on the same regression line, except that the values for EDL and TA are about half of that of psoas fibres (Galler et al. 2005). As is well known, psoas contains 100 % type IID fibres, whereas EDL and TA contain significant amount of type IIA fibres (Hamalainen and Pette 1993; Galler et al. 2005) and may also contain a small amount of type I fibres (Lexell 1994). The experiments on EDL and TA, such as shown in Fig. 6b, were performed only on type IIA fibres by judging from the plot of complex modulus $Y(f)$ (Galler et al. 2005).

Discussion

The cross-bridge model that account for k_{TR}

Owing to its simplicity, k_{TR} has been frequently used to characterize cross-bridge kinetics for ~25 years since its inception (Brenner 1988), but very little effort was spent on how to relate this parameter to the elementary steps of the cross-bridge cycle. In this report, we reexamined the tension redevelopment time course, and found that there are no differences in the results compared to those reported earlier. However, our interpretation of the time course is different, and we presented additional evidence. Based on these results, we propose a cross-bridge model that can provide both the theoretical and experimental bases for k_{TR}. In the original model, $k_{TR} = f_a + g_a$ (Brenner 1988), hence it is limited by the fast reaction. That is, in the two-state model, if one rate constant is very much faster than the other, k_{TR} measures primarily the faster rate constant of the two. However, it has been realized that k_{TR} is never larger than ~40 s^{-1}, while the rate constants of tension transients can be as large as 100–1,000 s^{-1} (Huxley and Simmons 1971; Heinl et al. 1974; Kawai 1978; Goldman and Simmons 1984; Fortune et al. 1991), hence it has been suggested that k_{TR} may measure a slow reaction in the cross-bridge cycle (Gordon et al. 2000). The model we proposed in this report is consistent with this idea, and our result demonstrates that k_{TR} is limited by the slowest forward rate constant of the cross-bridge cycle. Eq. 12 can be rewritten as:

$$k_{TR} = \frac{v_1 \eta_0 (1-\lambda)/\lambda}{F_1/\sigma} = \frac{ATPase_1}{M_c} \times \frac{\eta_0 (1-\lambda)/\lambda}{F_1/\sigma} \quad (21)$$

Equation 21 demonstrates that k_{TR} is proportionate to the turnover rate (v_1), which is $f_a g_a/(f_a + g_a)$ in the two-state model (Eq. 19). This quantity is limited by the slower reaction, if the two rate constants are very different. Therefore, it can be concluded that $k_{TR} = f_a + g_a$ as originally proposed (Brenner 1988) does not hold.

The best evidence comes from the proportionate relationship between k_{TR} and the ATP hydrolysis rate when temperature was altered (Fig. 5). Because ATPase requires the cross-bridges to go through the entire cycle, it is easy to understand that ATPase is limited by the slowest reaction of the cycle. Eq. 21 forms the theoretical bases for this explanation.

Other evidence comes from the numerical evaluation of Eq. 21. The step size (η_0) was reported to be 5.3 nm with the papain digested chicken skeletal muscle myosin S1 and rabbit skeletal actin (Kitamura et al. 1999), 9.7 nm in chicken pectoralis muscle (Sherwood et al.,

2004), 12.6 nm in frog skeletal muscle (Wu and Nakamura, 2005), hence if we use the average value, η_0 = 9.2 nm. The isometric ATPase rate was reported to be $ATPase_1$ = 0.61 ± 0.04 mM s^{-1} at 20 °C (Kawai et al. 1987), and the myosin S – 1 concentration M_c = 0.21 ± 0.02 mM (Murakami and Uchida 1985) in rabbit psoas. Therefore, the isometric turnover rate is $v_1 = ATPase_1/M_c = 2.90\ s^{-1}$. The value for force:stiffness is reported to be F_1/σ = 9.8 nm in rabbit psoas fibres at 19 °C (Linari et al. 2007). By using single myofibrils, k_{TR} was reported to be 8 s^{-1} in rabbit psoas myofibrils at 15 °C (Piroddi et al. 2003). Our value for k_{TR} (slow) is 6 s^{-1} for 20 °C (Fig. 4). Thus, the median value is k_{TR} = 7 s^{-1}. Therefore, Eq. 17 is satisfied if λ = 0.28, which is reasonable. Consequently, an agreement is obtained to validate Eq. 21, which further validates Eq. 12. In the case of the two-state model, these constants make Eq. 19 as follows:

$$k_{TR}=2.4\times\frac{f_a g_a}{f_a+g_a} \quad (22)$$

The above analyses demonstrate that a measurement of k_{TR} provides an estimate of the cross-bridge cycling rate (Eq. 21 and Fig. 5). Brenner and Eisenberg (Brenner and Eisenberg 1986) found that the time course of force redevelopment was fitted well by a single exponential function at the low level of Ca^{2+} activation (Brenner 1988), implying that force redevelopment is a first-order process. Metzger et al. also found, at all levels of Ca^{2+} activation the rate of tension redevelopment was well fitted by a single exponential function, which was recorded under sarcomere length control (Metzger et al. 1989). However, several other studies with rabbit psoas fibres found better fitting of the tension time course at high [Ca^{2+}] by the sum of two exponential functions than by a single exponential function (Swartz and Moss 1992; Chase et al. 1994; Burton et al. 2005), although fitting with more than two exponentials did not provide a further improvement (Burton et al. 2005). The reason for the two exponentials is presumably because (1) nonlinear series elasticity (Edman 2009; Nocella et al. 2013), (2) possible nonlinear stepping rate (cf. Eq. 4), and/or (3) Eq. 5 may be more continuous than indicated. The nonlinearity introduces imperfectness in solving Eq. 7, resulting in a time course that is not a perfect exponential function, which makes the two exponential fit a better approximation.

The temperature effects

With our experiments, both fast and slow rate constant of k_{TR} exhibited an increasing function of temperature (Fig. 4): the fast rate constants increased ~1.7 times (Q_{10} = 1.4), and the slow rate constant increased ~2.4 times (Q_{10} = 1.8) at the maximal Ca^{2+} activation in the temperature range 10–25 °C. When the temperature increases, both v_0 (stepping rate) and k_{TR} increase, but η_0 (step size) and σ (stiffness of series elasticity) remain approximately unchanged. At the level of elementary steps of the cross-bridge cycle, the force generation step 4 (K_4) increases with temperature, because it involves hydrophobic interaction (Zhao and Kawai, 1994), hence this step is an endothermic process. The increase in K_4 results in an increase in the number of force-generating cross-bridges AM*ADP·Pi and AM*ADP (AM = actomyosin). This, in turn, results in an increase in the ATP hydrolysis rate, because ATPase = k_6 [AM*ADP]. k_6 is the rate constant of the ADP isomerization to AM ADP before ADP release, which is the slowest rate of the cross-bridge cycle where much of the work is performed (Kawai and Halvorson 2007), hence this step limits the cross-bridge cycling rate (v of Eq. 4).

The relationship between k_{TR} and ATPase

The linear correlation between ATPase activity and k_{TR} has been shown previously in single isolated human cardiomyocytes from atrial and ventricular tissues (Narolska et al. 2005). There is a roughly linear relationship between k_{TR} and the unloaded shortening velocity over

a tenfold range of shortening velocities in a variety of muscles at different conditions [reviewed by (Gordon et al. 2000)]. At the same time the shortening velocity was shown to have a linear relationship with actomyosin ATPase (Barany 1967), which implies a linear relationship between k_{TR} and ATPase. Our results (Fig. 5) demonstrate that they are not only linear, but proportionately related, and as predicted by Eq. 21. Therefore, we conclude that k_{TR} directly measures the steady-state cross-bridge turnover rate.

The relationship between k_{TR} and k_{act}

In single myofibril experiments, the rise of tension on Ca^{2+} activation (k_{act}) and the tension redevelopment time course after release/restretch experiment (k_{TR}) are remarkably similar, and $k_{act} = 7.9\ s^{-1}$, and $k_{TR} = 8.0\ s^{-1}$ were reported (Piroddi et al. 2003). This experiment was interpreted to mean that the same mechanochemical events take place in both time courses. If we combine the myofibril observation and our new interpretation of k_{TR}, then it follows that the tension time course on Ca^{2+} activation likewise represents a stretching of the series elastic elements by multiple cross-bridge cycles, just as in the release-re-stretch experiment. In another words, when tension increases, it accompanies a stretch of series elastic elements, which would account for a difference in the Pi transients when [Pi] was decreased (tension increases) and [Pi] was increased (tension decreases) to result in the same [Pi] (Tesi et al. 2000) in myofibril experiments. Tension decrease is faster, because it can occur simultaneously with the cross-bridge detachment, whereas tension increase is slower, because it requires multiple cross-bridge cycles which is limited by a slow reaction. The rate constants of myofibril experiments are in the range 5–18 s^{-1} on rabbit psoas. In contrast, the asymmetric tension transient discussed by Huxley and Simmons (Huxley and Simmons 1971) or by Ranatunga et al. (2002) has a very fast time course (~500 s^{-1}), and it likely represents an elementary step in the cross-bridge cycle. When one performs experiments at the slow time scale, such as carried out in this report, the fast signal is lost in the data collection process, hence not the subject of this report.

Relationship between k_{TR} and slow exponential process A (phase 4) of tension transients

In addition to the two exponential processes present in the three-state model and detected by the perturbation analysis method, we have observed that the presence of series compliance results in an extra exponential process with the rate constant $2\pi a$ of process A, which is present in fast-twitch fibres (Galler et al. 2005; Kawai and Halvorson 2007). There have been suggestions that this process may represent the rate limiting step of the cross-bridge cycle [step 6 in Scheme 8 in Kawai and Halvorson (2007)]. This possibility has not been considered previously, because the transient analysis method in principle cannot resolve the slowest step of the cycle. However, this condition is lifted by introducing the series elasticity in the model. The linear relationship between k_{TR} and $2\pi a$ has been demonstrated experimentally as the temperature was changed (Fig. 6a) or different muscle types were employed (Fig. 6b). Because the intercept value in Fig. 6 is small, and the slope is close to the unity, we can further approximate this relationship by $2\pi a \approx k_{TR}$ for simplicity and for practical purposes. Both k_{TR} and $2\pi a$ values for EDL and TA fibres are about half compared to that for psoas fibres, because EDL and TA carry MHC IIA isoform, whereas psoas belongs to type IID fibres and carry only MHC IID isoform; type IID fibres respond more quickly than do type IIA fibres (Galler et al. 2005). The linear relationship between k_{TR} and $2\pi a$ demonstrates that $2\pi a$ is also limited by the cross-bridge turnover rate.

Conclusions

1. In this paper, we have developed a cross-bridge model, in which during the force development, a cross-bridge cycles many times to stretch the series compliance to increase the force. It is a more comprehensive model than the earlier one described

in Sect. 19 of Kawai and Halvorson (2007), and presented here together with new experimental evidence.

2. k_{TR} is limited by the slowest reaction of the cross-bridge cycle, which is the ADP isomerization step 6 before its release, and not by the fastest reaction of the cycle as originally proposed (Brenner 1988). Previous model was not applicable for contracting muscle, because it assumed equilibrium between the two states, which did not consider multiple cross-bridge cycles and a consequent elongation of series elasticity. The slow component of k_{TR} and the ATP hydrolysis rate are proportionately correlated when temperature is changed; hence k_{TR} is proportionate to the cross-bridge cycling rate.

3. k_{TR} and $2\pi a$ are linearly correlated based on the studies of different temperatures and different muscle types. k_{TR} and $2\pi a$ are approximately the same.

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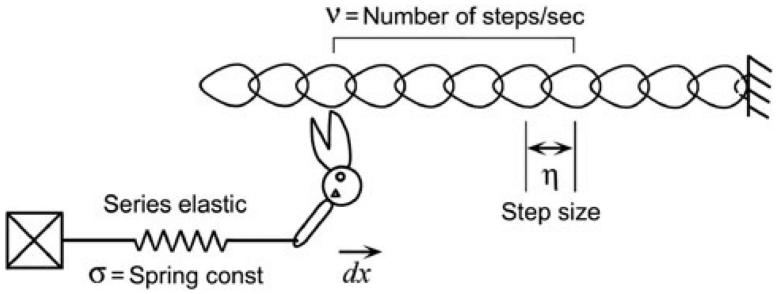

Fig. 1.

A graphic representation of the proposed cross-bridge model, in which cross-bridges cycle many times in the tension recovery period by stretching series elastic elements. Z-line is to the *right*, and M-line to the *left*

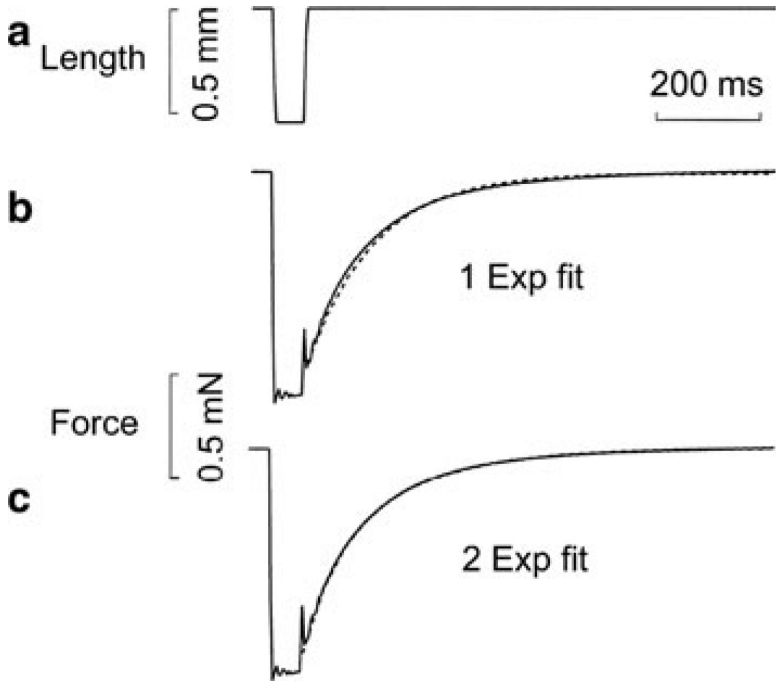

Fig. 2.

Length (in **a**) and tension (in **b** and **c**) time courses. A rabbit psoas single muscle fibre was activated in the standard activating solution at 20 °C. When the tension plateau was reached, the length was suddenly released by 20 % L_0 and restretched to L_0 after 50 ms. The *solid lines* are the actual record, and the *dotted lines* are exponential fits (one exponential fit in **b**, and two exponential fit in **c**) with the following parameters: **b** $k_{TR} = 8\ s^{-1}$, $A = 0.93$ mN; **c** $k_{TR}(slow) = 5.7\ s^{-1}$, $A_{slow} = 0.58$ mN, $k_{TR}(fast) = 16.3\ s^{-1}$, $A_{fast} = 0.42$ mN; where k_{TR} is the rate constant and A is its amplitude

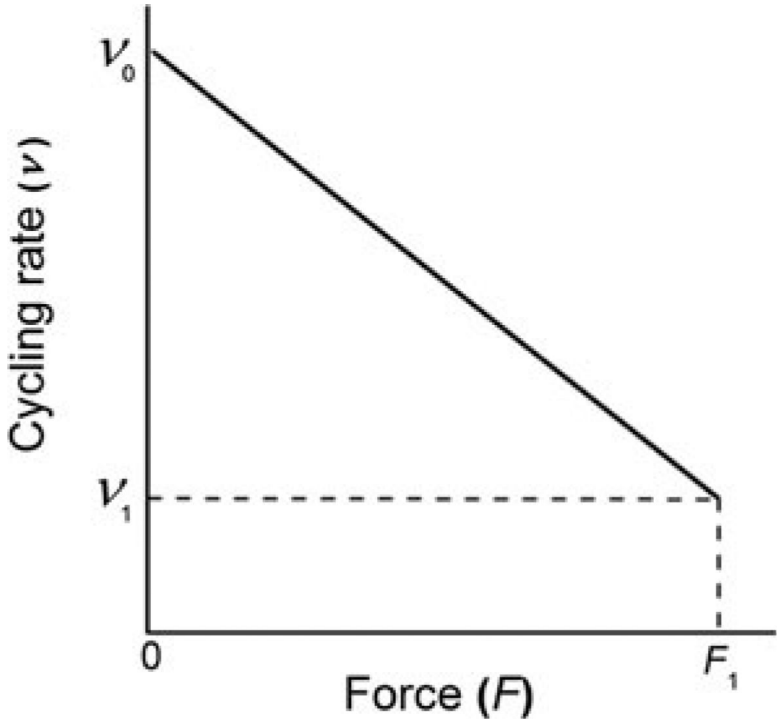

Fig. 3.
A graphic representation of the Fenn effect (Eq. 4 in the text), *i.e*. the rate of stepping (v) becomes increasingly less as force develops

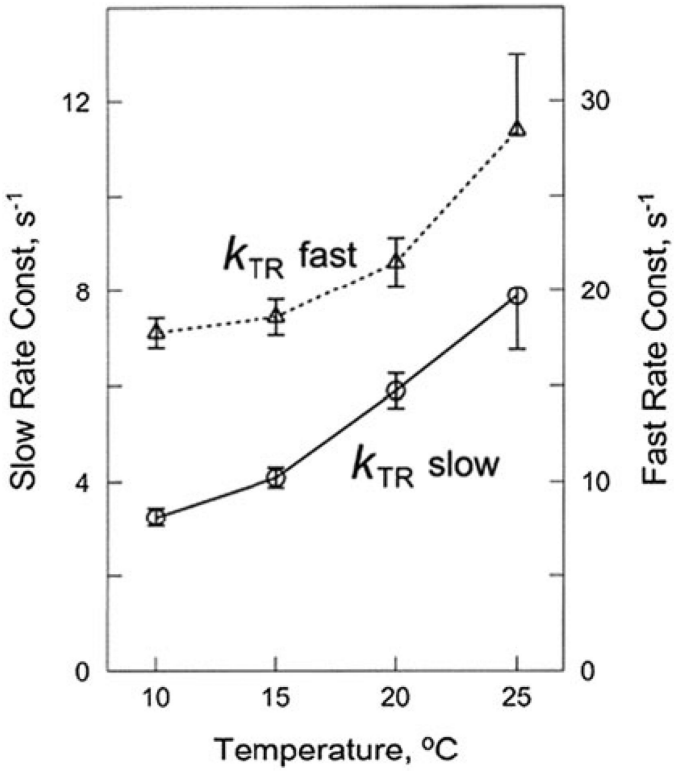

Fig. 4.
The effect of temperature on fast and slow rate constants of k_{TR} in rabbit psoas fibres

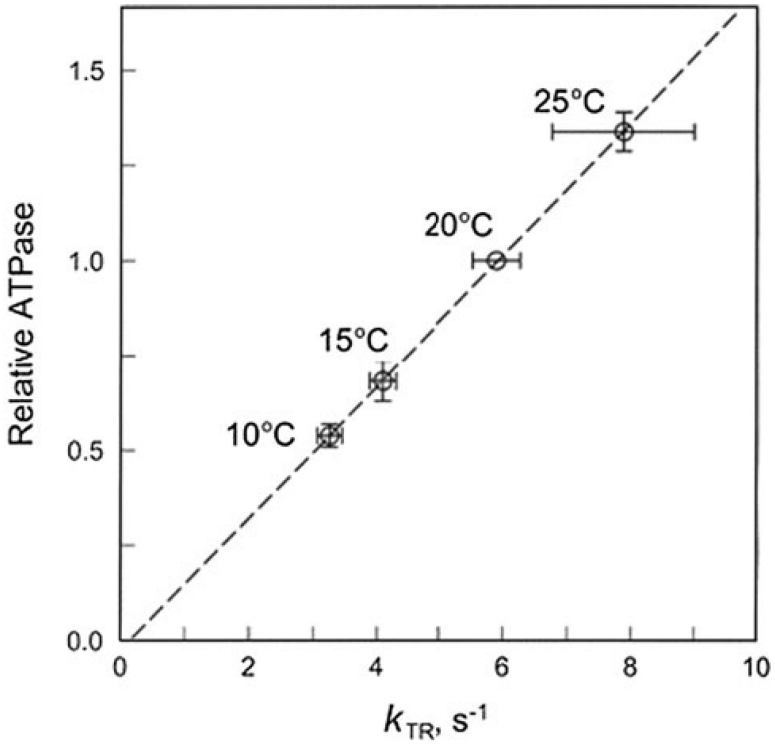

Fig. 5.

Correlation between the ATP hydrolysis rate (ordinate) and the slow component of k_{TR} (abscissa) in rabbit psoas fibres in the standard activating solution as the temperature is changed between 10 °C and 25 °C. The ATPase data were taken from our earlier publication (Zhao and Kawai, 1994), and normalized to that at 20 °C

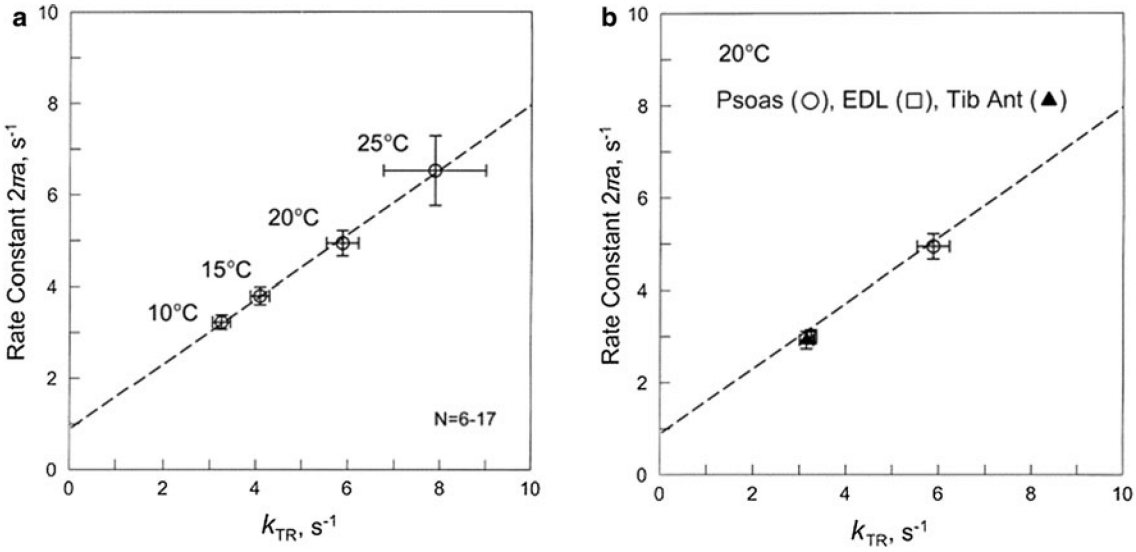

Fig. 6.

Correlation between the apparent rate constant $2\pi a$ (sinusoidal analysis) and k_{TR} as temperature is changed between 10 °C and 25 °C in rabbit psoas fibres in **a**, or different muscle fibres (psoas *open circle*, EDL *open square*, TA *filled triangle*) are compared at 20 °C in **b**. The points for EDL and TA overlap. From the regression line in **a**, Eq. 20 was deduced as the empirical relationship. The same line is drawn in **b**