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Cross-bridge kinetics in respiratory muscles

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ABSTRACT: In respiratory muscles, force generation and shortening depend on the cyclical interaction of actin and myosin (cross-bridge cycling). During cross-bridge cycling, adenosine triphosphate (ATP) is hydrolysed. The globular head region of the myosin heavy chain (MyHC) possesses both the binding site to actin and the site for ATP hydrolysis. Therefore, the MyHC is both a structural and enzymatic protein.

Different isoforms of MyHC are expressed in skeletal muscle fibres, and these MyHC isoforms provide mechanical and metabolic diversity. In the present study, the relationships between MyHC isoform expression in single rat diaphragm muscle fibres and their mechanical and energetic properties were evaluated. The expression of MyHC isoforms in single diaphragm muscle fibres was identified using electrophoretic and immunohistochemical techniques. Cross-bridge cycling kinetics in diaphragm muscle fibres clearly depend on MyHC isoform expression, and these differences are interpreted in the context of Huxley's two-state cross-bridge model.

It is concluded that the unique mechanical and energetic properties of myosin heavy chain isoforms are designed to accomplish different motor behaviours of the diaphragm muscle, and that, as a result of these unique properties, a selective recruitment of diaphragm muscle fibres is essential to avoid fatigue.

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In respiratory muscles, as in other skeletal muscles, mechanical action results from the cyclical interaction between two contractile proteins, actin and myosin, which is regulated by Ca^{2+} release from the sarcoplasmic reticulum (SR) and binding to troponin C. The myosin molecule, a hexameric protein, comprises two myosin heavy chains (MyHC) and four myosin light chains (MyLC). Different isoforms of MyHC exist in skeletal muscle, and the pattern of expression of MyHC isoforms provides the underlying basis for classification of different muscle fibre types. The expression of different MyHC isoforms also provides the underlying basis for the varying mechanical properties of muscle fibres.

The dependence of muscle fibre mechanical and energetic properties on MyHC isoform expression stems from the fact that each MyHC contains the binding site of the myosin molecule to actin for cross-bridge formation, as well as the site for the hydrolysis of adenosine triphosphate (ATP) (actomyosin adenosine triphosphatase (ATPase)) during cross-bridge cycling [1–3]. Indeed, a smaller proteolytic fragment of the MyHC, comprising the globular head region (subfragment 1), possesses both the actin-binding site and the site for ATP hydrolysis [4]. This review will focus on the mechanical and energetic significance of MyHC isoform expression in diaphragm muscle fibres.

Myosin heavy chain expression in skeletal muscle fibres

The genetic regulation of MyHC isoform expression in skeletal muscle fibres is responsive to a number of intrinsic factors, including preprogrammed embryonic and postnatal development, hormonal influences and the pattern of innervation, as well as a number of extrinsic factors, including mechanical loading and changes in activity [5–10]. Typically, within an adult skeletal muscle fibre, a single MyHC isoform is expressed, and this expression corresponds with the histochemical classification of fibre type based on the pH lability of myofibrillar ATPase (mATPase) staining [11–17] (fig. 1). Accordingly, in the rat diaphragm muscle, fibres histochemically classified as type I, IIa, IIx and IIb express the MyHC-slow, MyHC-IIa, MyHC-IIx and MyHC-IIb isoforms, respectively [16].

In the adult rat diaphragm muscle, ~33% of all fibres express the MyHC-slow isoform, ~26% the MyHC-IIa isoform and ~24% the MyHC-IIx isoform [16]. Very few fibres in the rat diaphragm muscle singularly express the MyHC-IIb isoform (~3%), while ~14% of all fibres co-express the MyHC-IIb isoform with MyHC-IIx [16]. These fibres co-expressing MyHC-IIb and MyHC-IIx isoforms are usually classified histochemically as type IIb.

Fig. 1. — Classification of muscle fibre types based on: a) histochemical staining for myofibrillar adenosine triphosphatase (mATPase); b) and immunohistochemical staining for antibodies of different myosin heavy chain (MyHC) isoforms. Note the correspondence between the two classification systems. Internal scale bar = 10 μ m.

In the rat diaphragm muscle, the expression of MyHC-slow and MyHC-IIa isoforms appears during late embryonic to early postnatal development [18–23]. In contrast, expression of MyHC-IIx and MyHC-IIb isoforms does not appear in the rat diaphragm muscle until the third to fourth postnatal weeks. During late embryonic and early postnatal development, embryonic (MyHC-emb) and neonatal (MyHC-neo) isoforms are also expressed in rat diaphragm muscle [18–23]. The MyHC-emb and MyHC-neo isoforms are usually not singularly expressed within muscle fibres, but co-expressed with MyHC-slow and/or MyHC-IIa isoforms. During the first 3 weeks of life, this pattern of co-expression of MyHC isoforms undergoes dramatic transformations in the rat diaphragm muscle, until the adult pattern of MyHC isoform expression emerges by postnatal day 28. The developing muscle fibres that co-express MyHC isoforms are histochemically classified as type IIc [21].

As mentioned above, co-expression of MyHC isoforms is not entirely restricted to the early postnatal period. In the adult rat diaphragm muscle, ~14% of all fibres co-express MyHC isoforms [16]. The majority of these fibres co-express the MyHC-IIb and MyHC-IIx isoforms and are histochemically classified as type IIb. However, in the adult rat diaphragm muscle, there are also a few muscle fibres (<1%) that co-express the MyHC-slow and MyHC-IIa isoforms. These adult fibres are also histochemically classified as type IIc [16]. Al-

though these type IIc fibres are rarely found in the adult rat diaphragm muscle, they do appear under pathological conditions, *e.g.* as following denervation (fig. 2) [24].

Recently, we examined the distribution of MyHC isoform expression in rat diaphragm muscle fibres that had been denervated for 2 weeks. Transverse sections of diaphragm muscle fibres were cut at 6 μm , for a total distance of 150 μm . The muscle sections were then reacted with antibodies specific for MyHC-slow and MyHC-IIa isoforms. Secondary antibodies specific to mouse immunoglobulin G and M (IgG and IgM) were conjugated with the fluorophores, Cy3 and fluorescein, which permitted double labelling of the MyHC-slow and MyHC-IIa isoforms within the same section. The co-expression of MyHC-slow and MyHC-IIa isoforms was apparent in each of the transverse sections, indicating that the MyHC-slow and MyHC-IIa isoforms are uniformly distributed in these denervated diaphragm muscle fibres. There was no evidence of localized expression of different MyHC isoforms. This observation would suggest that the co-expression of MyHC isoforms within muscle fibres reflects a signal affecting the messenger ribonucleic acid (mRNA) expression of all myonuclei rather than select myonuclei within the fibre. With denervation of the diaphragm muscle, it is likely that the signal underlying the change in MyHC isoform expression is related to muscle fibre injury, which is quite prevalent in these fibres [25].

Fig. 2. — Co-expression of myosin heavy chain (MyHC) isoforms in denervated rat diaphragm muscle. Serial muscle cross-sections were fluorescently double-labelled using antibodies to: a) MyHC-slow; and b) MyHC-IIa isoforms. The co-expression of these MyHC isoforms was found to extend for at least 150 μm along the length of individual muscle fibres. Internal scale bar = 10 μm .

Relationship between MyHC isoform expression and mechanical properties of the muscle

In the original model of muscle contraction proposed by HUXLEY [26], cross-bridges cycle between two functional states: a force-generating state, in which cross-bridges are strongly attached to actin, and a non-force-generating state, in which cross-bridges are detached from actin. The relative proportion of attached and non-attached cross-bridges within a fibre under varying conditions has been estimated by measuring the change in force induced by small amplitude (<1% optimal length, (L_0)) length perturbations, *i.e.* muscle stiffness [27–30]. The measurement of muscle stiffness assumes that the small amplitude length perturbations do not disrupt cross-bridge binding, and that the rate of imposing length perturbations far exceeds that of cross-bridge cycling. In this case, each cross-bridge acts as a spring with an incremental force opposing the imposed strain, such that each strongly-bound cross-bridge contributes an element of stiffness, and thereby the total stiffness reflects the number of strongly-bound cross-bridges. During steady state conditions, changes in force generated by a muscle fibre are closely approximated by changes in fibre stiffness, and, thus, the measurement of active stiffness provides an important estimate of the number of strongly-bound cross-bridges [27, 28, 30–33]. However, a dissociation of force and stiffness does occur during non-steady state conditions. For example, during force development and relaxation, stiffness is greater than force, suggesting that cross-bridges can exist in a strongly-bound non-force-generating state [34–37]. These observations have led to the formulation of multistate cross-bridge models instead of the simpler two-state model of HUXLEY [26]. While these multistate models may more accurately reflect the underlying biochemistry of actin-myosin interactions, especially during non-steady state conditions, the two-state model of HUXLEY [26] still provides the simplest representation of the mechanical processes underlying muscle contraction.

In the Huxley model, the transitions between the two primary functional states of cross-bridges are described by two apparent rate constants, one for cross-bridge attachment (f_{app}) and the second for cross-bridge detachment (g_{app}). The increase in isometric force with increasing myoplasmic Ca^{2+} is explained by the recruitment of cross-bridges in the force-generating state (described by f_{app}) [26, 33]. The transition of the cross-bridge from force-generating to non-force-generating states (described by g_{app}) requires the hydrolysis of ATP (actomyosin ATPase). Thus, in the Huxley model, there is an implicit transduction of chemical to mechanical energy.

BRENNER and co-workers [38–41] proposed an analytical framework based on Huxley's two-state model of cross-bridge cycling, in which this transduction of chemical to mechanical energy is more explicitly described. In the Brenner analytical framework, the steady state fraction of strongly-bound cross-bridges in the force-generating state (α_{fs}) is given by Equation (1):

$$\alpha_{fs} = f_{app} / (f_{app} + g_{app}) \quad (1)$$

By rapidly releasing the muscle fibre to a shorter length (a length step of ~20% of optimal fibre length) and then

Fig. 3. — The rate of force redevelopment (k_{tr}) differs between rat diaphragm muscle fibres expressing different myosin heavy chain (MyHC) isoforms (MyHC-slow and MyHC-IIx in this example). These differences in k_{tr} reflect phenotypic differences in the forward rate constant (f_{app}) for cross-bridge cycling.

rapidly restretching the fibre to L_0 , all cross-bridges are broken and force decreases abruptly to zero. Subsequently, force redevelops as cross-bridges reattach to actin (fig. 3)

The relationship between f_{app} , g_{app} and the rate constant for force redevelopment (k_{tr}) is given by Equation (2):

$$k_{tr} = f_{app} + g_{app} \quad (2)$$

Since k_{tr} is initially dominated by cross-bridge reattachment, it provides an approximation of f_{app} . In contrast, maximum shortening velocity (V_{max}) is dependent on g_{app} , and, thus, V_{max} provides an approximation of g_{app} .

In Brenner's analytical framework, isometric force (F) and stiffness (S) are given by Equations (3) and (4), respectively:

$$F = n f \alpha_{fs} \quad (3)$$

$$S = n s \alpha_{fs} \quad (4)$$

where n is the number of cross-bridges in parallel per half sarcomere, f is the mean force per cross-bridge in the force-generating state, and s is the mean stiffness per cross-bridge.

Assuming that during each cross-bridge cycle, there is the hydrolysis of one ATP molecule, the isometric ATP consumption rate (actomyosin ATPase activity) is given by Equation (5):

$$ATPase = n b g_{app} \alpha_{fs} \quad (5)$$

where b is the number of half sarcomeres within the fibre.

Recently, we examined the relationships between the mechanical properties of single diaphragm muscle fibres and their MyHC isoform expression. In these fibres, MyHC isoform expression was determined either by single fibre gel electrophoretic separation (fig. 4a) or by immunoreactivity of the fibres to different MyHC antibodies (fig. 4b). Since only ~3% of all diaphragm muscle fibres singularly express the MyHC-IIb isoform, no attempt was made to distinguish between the expression of the MyHC-IIb and MyHC-IIx isoforms in the present study.

Fig. 4. — The expression of myosin heavy chain (MyHC) isoforms within single diaphragm muscle fibres can be identified: a) by differences in gel electrophoretic migration and Western analysis; or b) by immunoreactivity for specific MyHC antibodies. Internal scale bar = 10 μ M.

In the rat diaphragm muscle, an association was found between MyHC isoform expression and k_{tr} (fig. 3). The k_{tr} of fibres expressing the MyHC-slow isoform is approximately half that of fibres expressing MyHC-fast isoforms. This would indicate that the f_{app} of fibres expressing the MyHC-slow isoform is also approximately half that of fibres expressing MyHC-fast isoforms. There are no apparent differences in k_{tr} (and by infer-

ence f_{app}) across fibres expressing different MyHC-fast isoforms.

An association between MyHC isoform expression and the specific force (force per fibre cross-sectional area) of rat diaphragm muscle fibres was also observed (fig. 5a). Muscle fibres expressing the MyHC-IIx isoform alone, or in combination with the MyHC-IIb isoform, generate greater specific force than fibres expressing either the MyHC-slow or MyHC-IIa isoforms (fig. 5a). Similar fibre type differences in specific force have been reported previously [42–44], but such differences remain controversial [45]. It was also found that the active stiffness of rat diaphragm muscle fibres depended on MyHC isoform expression (fig. 5b). As for specific force, muscle fibres expressing the MyHC-IIx isoform alone, or in combination with the MyHC-IIb isoform, had greater active stiffness than fibres expressing either the MyHC-slow or MyHC-IIa isoforms (fig. 5b). The fibre type differences in specific force and active stiffness that were found in the rat diaphragm muscle may simply reflect the higher mitochondrial volume densities of type I and IIa fibres, which would presumably be at the expense of a corresponding lower myofibrillar volume density and, thus, fewer cross-bridges in parallel for a given fibre cross-sectional area [26, 46]. This

Fig. 5. — a) Maximum isometric force; and b) elastic modulus (stiffness per fibre cross-sectional area) of single permeabilized rat diaphragm muscle fibres varies with myosin heavy chain (MyHC) isoform expression. When normalized for estimated myofibrillar density (■), differences in specific force between fibres expressing MyHC-IIa and MyHC-IIx/IIb are absent, while the corrected specific force of fibres expressing MyHC-slow remains lower. Fibre type differences in stiffness remain even after normalizing for estimated myofibrillar density. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

would correspond to a smaller n in Equations (3) and (4).

However, when the specific forces of different fibre types in the rat diaphragm muscle are corrected for the estimated myofibrillar volume density, the specific force of type I fibres remains significantly lower than that of type IIx and IIb fibres (fig. 5a). However, when corrected for the estimated myofibrillar volume density, the specific force of type IIa fibres in the rat diaphragm muscle is comparable to that of type IIx and IIb fibres (fig. 5a). The lower corrected specific force of type I fibres may reflect inherent differences in the force per cross-bridge of the MyHC-slow isoform. It appears unlikely that differences in cross-bridge cycling kinetics could explain this difference, since, if anything, the duty cycle for cross-bridge cycling (*i.e.* the time of cross-bridge attachment relative to detachment) would be longer in type I fibres.

The $V_{\max}$ of fibres in the rat diaphragm muscle also corresponds with the expression of different MyHC isoforms (fig. 6). A similar observation has been reported in a number of previous studies [42–44, 47–49]. Diaphragm muscle fibres expressing the MyHC-IIx isoform, either alone or in combination with the MyHC-IIb isoform, display the fastest $V_{\max}$. Diaphragm muscle fibres expressing the MyHC-IIa isoform have a $V_{\max}$ that is nearly twice as fast as that of fibres expressing the MyHC-slow isoform, but only half that of fibres expressing the MyHC-IIx and/or MyHC-IIb isoform (fig. 6). These results suggest that the g_{app} of fibres expressing the MyHC-IIx and/or MyHC-IIb isoforms is more than fourfold faster than that of fibres expressing the MyHC-slow isoform, and more than twofold faster than that of fibres expressing the MyHC-IIa isoform.

With the approximations of f_{app} and g_{app} provided by k_{tr} and $V_{\max}$, respectively, the steady-state fraction of strongly-bound cross-bridges in the force-generating state (α_{fs}) in single diaphragm muscle fibres expressing different MyHC isoforms can be determined from Equation (1). During steady state isometric force generation, the α_{fs} of fibres expressing the MyHC-IIx isoform, either alone or in combination with the MyHC-IIb isoform, would be approximately half that of type I fibres, since the higher g_{app} of fibres expressing the MyHC-

IIx and/or MyHC-IIb isoform far exceeds the relative difference in f_{app} . In contrast, the α_{fs} of type I and IIa fibres is comparable, since the higher f_{app} of type IIa fibres is matched by a higher g_{app} .

If one assumes that the force per cross-bridge is not different across MyHC isoforms, relative differences in myofibrillar density can be estimated from Equation (3) (myofibrillar density is proportionate to n in Equation (3)). Accordingly, the myofibrillar density of fibres expressing the MyHC-IIx and/or MyHC-IIb isoforms would have to be approximately threefold greater than that of fibres expressing the MyHC-slow isoform in order to account for the differences in specific force that are observed. Similarly, the myofibrillar density of fibres expressing the MyHC-IIx and/or MyHC-IIb isoform would have to be approximately twofold greater than that of fibres expressing the MyHC-IIa isoform.

In evaluating the relationship between $V_{\max}$ and MyHC isoform expression in single diaphragm muscle fibres, $V_{\max}$ was determined using both the "slack" test [50], where unloaded shortening velocity (V_0) is measured directly, and isotonic loading, where $V_{\max}$ is estimated by extrapolation of the force/velocity relationship to zero load. Typically, V_0 approximates $V_{\max}$ in single fibres, such that the terms $V_{\max}$ and V_0 can generally be used interchangeably in most single fibres. In contrast, in mixed fibre bundles, V_0 is faster than $V_{\max}$, since V_0 reflects the unloaded shortening velocity of the fastest fibres in the bundle, whereas $V_{\max}$ reflects a weighted average of the shortening velocities of all fibres in the bundle [51]. Similarly, in fibres co-expressing MyHC isoforms, V_0 and $V_{\max}$ differ, reflecting the internal loading imposed by cross-bridges cycling at different rates.

In this regard, the distribution of MyHC isoform co-expression may be particularly significant. As mentioned above, in denervated diaphragm muscle fibres, there is an abundant co-expression of MyHC-slow and MyHC-IIa isoforms, and these isoforms appear to be uniformly distributed, with no evidence of localized distribution of different MyHC isoforms (fig. 2). This observation is important, in that the internal loading imposed by a parallel distribution of different MyHC isoforms would be quite different from that imposed by a series distribution. If cross-bridges cycling at different rates are distributed in series, sarcomere inhomogeneity would rapidly develop, markedly affecting force transmission through the length of the fibre. Moreover, such a series arrangement could result in eccentric fibre injury. A parallel arrangement of cross-bridges cycling at different rates would not necessarily result in sarcomere inhomogeneity and/or eccentric injury. However, the efficacy of force generation and shortening would be impaired. Accordingly, it was observed that following denervation of the rat diaphragm muscle specific force was reduced and $V_{\max}$ was slowed [52].

Given the differences in shortening velocity and specific force across different fibre types, it is not surprising that the power output of rat diaphragm muscle fibres also depends on MyHC isoform expression (fig. 7). The maximum power output of fibres expressing the MyHC-IIx isoform alone or in combination with the MyHC-IIb isoform is nearly sevenfold greater than that of fibres expressing the MyHC-slow isoform, and over threefold

Fig. 6. — Maximum shortening velocity (muscle length (ML)) of single permeabilized rat diaphragm muscle fibres varies with myosin heavy chain (MyHC) isoform expression. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

Fig. 7. — Maximum power output of single permeabilized rat diaphragm muscle fibres varies with myosin heavy chain (MyHC) isoform expression. *: $p < 0.05$, compared to fibres expressing MyHC-slow. †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

greater than that of fibres expressing the MyHC-IIa isoform (fig. 7).

As mentioned above, different fibre types in the rat diaphragm muscle vary considerably in mitochondrial volume density and oxidative capacity [16]. For example, the succinate dehydrogenase (SDH) activity of type I and IIa fibres is significantly higher than that of type IIx and IIb fibres. The higher oxidative capacities of type I and IIa fibres certainly contribute to the greater fatigue resistance of these fibres as compared to type IIx and IIb fibres [23, 53]. However, fatigue resistance may also be related to fibre type differences in the ATP consumption rate of different MyHC isoforms.

Relationship between MyHC isoform expression and ATP consumption (actomyosin ATPase activity)

Previous analyses comparing the energetics of skeletal muscles predominantly composed of either type I or type II fibres have suggested that the energy cost for contraction of type I fibres is approximately half that of type II fibres [54]. This difference in energy utilization between fibre types undoubtedly reflects differences in cross-bridge cycling kinetics and actomyosin ATPase activities of the different MyHC isoforms [16, 55–58].

In recent studies, we utilized a nicotinamide adenine dinucleotide, reduced form (NADH)-linked fluorescence technique [59] to measure actomyosin ATPase activity in permeabilized fibres of the rat diaphragm muscle (fig. 8).

In this method, the ATP hydrolysed by actomyosin ATPase is regenerated by the biochemical reaction of adenosine diphosphate (ADP) and phosphoenol pyruvate (PEP), which is catalysed by the enzyme pyruvate kinase (PK). This reaction is coupled to the reduction of pyruvate to lactate, which is catalysed by lactate dehydrogenase (LDH), and the associated oxidation of NADH to nicotinamide adenine dinucleotide (NAD⁺). For each mole of ATP regenerated by these coupled reactions, 1 mole of NADH is oxidized to NAD⁺. Important in the quantification of ATP consumption is the fact

Fig. 8. — The adenosine triphosphate (ATP) consumption rate of skeletal muscle fibres and smooth muscle fibre bundles was determined using a nicotinamide adenine dinucleotide, reduced form (NADH)-linked fluorescence technique. In this technique permeabilized fibres are mounted in a quartz cuvette between force and displacement transducers and activated by perfusion with solutions containing varying amounts of free ionized Ca²⁺. Perfusion of the cuvette is stopped for 15 s, during which time the ATP consumed by cross-bridge cycling is regenerated by a reaction that results in the oxidation of NADH, a fluorescent compound, to nicotinamide adenine dinucleotide (NAD), which is nonfluorescent. The rate of extinction of the NADH fluorescent signal is directly proportional to ATP consumption due to actomyosin adenosine triphosphatase (ATPase) activity.

that NADH is fluorescent, whilst NAD⁺ is nonfluorescent. Thus, the rate of decrease in NADH fluorescence is proportional to the rate of ATP consumption (actomyosin ATPase activity).

In the example shown in figure 8, a single permeabilized rat diaphragm muscle fibre was mounted between force and displacement transducers in a quartz cuvette that was perfused with solutions containing a free Ca²⁺ concentration of either 1 nM (unstimulated) or 100 μM (maximal activation). Flow through the cuvette was stopped during fluorescence measurements, and the cuvette was flushed every 15 s, providing fresh constituents necessary to couple ATP hydrolysis to NADH consumption. The rate of decline in NADH fluorescence during the 15 s period was measured and used to calculate actomyosin ATPase activity based on a *a priori* calibration of the system at known NADH concentrations.

The actomyosin ATPase activity of diaphragm muscle fibres during maximal isometric activation varies with MyHC isoform expression (fig. 9). Diaphragm muscle fibres expressing the MyHC-IIx isoform, either alone or in combination with the MyHC-IIb isoform, display the highest actomyosin ATPase activity, followed in rank order by fibres expressing the MyHC-IIa and MyHC-slow isoforms (fig. 9). From Equation (5), the higher actomyosin ATPase activity of fibres expressing the MyHC-IIx and/or MyHC-IIb isoform may be related either to the greater myofibrillar density (proportionate to n in Equation (5)) or the faster g_{app} . The

Fig. 9. — Adenosine triphosphate (ATP) consumption rate (actomyosin adenosine triphosphatase (ATPase) activity) of rat diaphragm muscle fibres varies with myosin heavy chain (MyHC) isoform expression under various conditions. a) The maximum capacity of actomyosin ATPase activity of different fibre types was determined using a quantitative histochemical method. The actomyosin ATPase activities of single permeabilized fibres were determined during: b) isometric activation; and c) isovelocity shortening conditions, using the nicotinamide adenine dinucleotide, reduced form (NADH)-linked fluorescence technique. During isovelocity shortening at 33% maximum velocity (V_{max}) corresponding to peak power output, actomyosin ATPase activity of diaphragm muscle fibres increased significantly from that observed during isometric activation, and approximated their maximum capacity. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

fraction of strongly-bound cross-bridges in the force-generating state (α_{fs}) is estimated to be lower in these fibres (see above), and, thus, differences in α_{fs} cannot account for the higher actomyosin ATPase activity in fibres expressing the MyHC-IIx and/or MyHC-IIb isoforms. The higher actomyosin ATPase activity of fibres expressing the MyHC-IIa isoform relative to those expressing the MyHC-slow isoform, undoubtedly reflects the faster gapp of these fibres.

Using a quantitative histochemical procedure that we developed [16, 60], the Michaelis-Menton kinetics of the actomyosin ATPase reaction can be determined within single muscle fibres. In the rat diaphragm muscle, it was found that the maximum rate of the actomyosin ATPase reaction varies according to fibre type and MyHC isoform expression [16]. As for the ATP consumption rates measured during maximal isometric activation, we found that the maximum rate of the actomyosin ATPase reaction is lowest in type I fibres, followed in rank order by type IIa, IIx, and IIb fibres (fig. 9). However, the ATP consumption rate of each of these fibre types during maximal isometric activation is only about one third of the maximum rate of the actomyosin ATPase reaction (*i.e.* maximum capacity for ATP consumption) (fig. 9). Thus, there appears to be considerable reserve capacity for ATP consumption in skeletal muscle fibres during maximal isometric contraction.

In 1923, FENN [61] observed that energy utilization of skeletal muscle increases in proportion to work ("Fenn effect") [61, 62]. Thus, as muscle fibres are allowed to shorten and maximum power is reached, ATP consumption rate should increase. In rat diaphragm muscle fibres, maximum power is achieved at a load corresponding to ~33% of maximum tetanic force (fig. 10). Shortening velocity at maximum power output also corresponds to ~33% of V_{max} . In a recent study, we examined the ATP consumption rate of single rat diaphragm muscle fibres during isovelocity shortening using the NADH-linked fluorescence technique. During these measurements, the fibres were only allowed to shorten by 20% of L_0 , before being restretched to L_0 and then allowed to shorten again. As expected, ATP consumption rate of the fibres increased dramatically during isovelocity shortening. In the example shown in figure 10, ATP consumption rate of a permeabilized type IIx fibre in the rat diaphragm muscle was measured during isometric activation and during two isovelocity shortening conditions, one at 33% V_{max} , corresponding to maximum power output, and the second at ~90% of V_{max} . In this fibre, as well as in all other muscle fibres that were

Fig. 10. — In a single rat diaphragm muscle fibre, actomyosin adenosine triphosphatase (ATPase) activity increases with power output (P_0) (Fenn effect). ATPase activity ($\text{nmol} \cdot \text{mm}^{-3} \cdot \text{s}^{-1}$), indicated by arrows, is maximum at peak power output and minimum under isometric conditions. —■— : power; —▲— : velocity.

examined, the maximum rate of ATP consumption was achieved at a shortening velocity corresponding to peak power output of the fibre. These observations confirmed, for the first time, the Fenn effect in single muscle fibres. It is also important to note that the ATP consumption rates of diaphragm muscle fibres at peak power output approximated the maximum capacity for ATP hydrolysis, as determined by quantitative histochemistry (fig. 9).

Relationship between ATP consumption rate and skeletal muscle fatigue

In the rat diaphragm muscle, the energy costs for contraction are not always matched by the aerobic capacity of the fibre for energy production [16]. For example, using quantitative histochemical techniques to measure SDH and actomyosin ATPase activities, it was found that fibres expressing the MyHC-slow and MyHC-IIa isoforms in the rat diaphragm muscle have the highest SDH activities, whilst their actomyosin ATPase activities are lowest (fig. 11). In contrast, fibres expressing the MyHC-IIx and MyHC-IIb isoforms have the highest actomyosin ATPase activities but the lowest SDH activities [16]. Therefore, in rat diaphragm muscle, it is

Fig. 11. — a) Oxidative capacity (succinate dehydrogenase (SDH) activity); and b) actomyosin adenosine triphosphatase (ATPase) activity of rat diaphragm muscle fibres. The SDH activity of fibres expressing the MyHC-slow and MyHC-IIa isoforms is considerably higher than that of fibres expressing the MyHC-IIx and MyHC-IIb isoforms. In contrast, the actomyosin ATPase activity of fibres expressing the MyHC-slow and MyHC-IIa isoforms is lower than those expressing the MyHC-IIx and MyHC-IIb isoforms. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

possible that fatigue of muscle fibres during repetitive activation may be related to an imbalance between ATP consumption and aerobic capacity (as reflected by SDH activity). In support of this hypothesis it has been shown that more fatigable motor units comprise type IIx and IIb muscle fibres [63]. In contrast, fatigue-resistant motor units comprise type I and IIa fibres.

If fatigue of muscle fibres during repetitive activation is related to an imbalance between ATP consumption and aerobic capacity, then increasing the rate of ATP consumption should hasten fatigue. As mentioned above, the maximum rate of ATP consumption coincides with peak power output of a muscle fibre, and for all fibre types, ATP consumption rate at peak power output is

Fig. 12. — Fatigue of the rat diaphragm muscle was assessed during: a) isometric activation; and b) isotonic shortening at a load corresponding to peak power output (30% P_0). In both conditions, the muscle fibre bundle was activated repetitively at 40 Hz in trains lasting 330 ms repeated each second. c) Fatigue during isometric conditions was defined as a decrement in force, while under isotonic conditions, fatigue was defined as a decrement in total displacement of the fibre bundle during the activation period (corresponding to shortening velocity). As can be seen, fatigue was much more pronounced during isotonic conditions. —●—: isotonic conditions; —□—: isometric conditions.

more than twofold greater than that observed during isometric contractions (figs. 9 and 10). Accordingly, we compared fatigue induced in the rat diaphragm muscle by repetitive isometric contractions with that induced by repetitive isotonic contractions at peak power output (fig. 12). The rate of fatigue was more rapid and pronounced during repetitive isotonic contractions than during repetitive isometric contractions.

In skeletal muscle fibres, the increase in ATP consumption during activation is met not only by aerobic capacity, but also by the buffering capacity of the high energy phosphate pool, *e.g.* phosphocreatinine. In type I and IIa fibres, it is likely that aerobic capacity and phosphocreatinine buffering are sufficient to maintain energy supply during activation. However, the rapid fatigue of type IIx and IIb fibres suggests that ATP consumption rate far exceeds not only aerobic capacity but also phosphocreatinine buffering.

Tension cost and work efficiency of fibres expressing different MyHC isoforms

The ATP cost for generating force (*i.e.* tension cost) varies across fibres expressing different MyHC isoforms (fig. 13). For example, the tension cost for rat diaphragm muscle fibres expressing the MyHC-IIx isoform alone or in combination with MyHC-IIb is approximately twofold greater than that of fibres expressing the MyHC-slow isoform. Thus, type I fibres are better able to sustain force at lower energy cost.

The work efficiency of fibres is defined as the amount of work performed per unit ATP consumed. The work efficiency undoubtedly reflects the energetic properties of different MyHC isoforms. Accordingly, work efficiency varies across different fibre types in the rat diaphragm muscle (fig. 14). The work efficiency of fibres expressing the MyHC-IIx isoform, either alone or in combination with the MyHC-IIb isoform, is approximately fourfold greater than that of fibres expressing the MyHC-IIa isoform and nearly sixfold greater than that of fibres expressing the MyHC-slow isoform (fig. 14).

Fig. 13. – In single permeabilized rat diaphragm muscle fibres, the tension cost (adenosine triphosphate (ATP) consumed for a given level of force) varies with myosin heavy chain (MyHC) isoform expression. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

Fig. 14. – In single permeabilized rat diaphragm muscle fibres, the work efficiency (work performed for a given amount of adenosine triphosphate (ATP) consumed) varies with myosin heavy chain (MyHC) isoform expression. *: $p < 0.05$, compared to fibres expressing MyHC-slow; †: $p < 0.05$, compared to fibres expressing MyHC-IIa.

Summary

Our studies have clearly demonstrated that there are significant differences in the mechanical and energetic properties of diaphragm muscle fibres expressing different myosin heavy chain isoforms. It is also clear that different myosin heavy chain isoforms are uniquely designed to accomplish the different tasks required of the diaphragm muscle, and that selective recruitment of these fibres is absolutely necessary to avoid fatigue.

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