Pflugers Arch - Eur J Physiol (2002) 445:218–223
DOI 10.1007/s00424-002-0934-6

ORIGINAL ARTICLE

Stefan Galler · Emma Puchert · Bärbel Gohlsch · Doris Schmid · Dirk Pette

Kinetic properties of cardiac myosin heavy chain isoforms in rat

Received: 26 June 2002 / Accepted: 19 August 2002 / Published online: 18 October 2002

Abstract The head portion of the myosin heavy chain (MHC) is essential in force generation. As previously shown, Ca^{2+}-activated fibres of mammalian skeletal muscle display a strong correlation between their MHC isoform complement and the kinetics of stretch activation, suggesting isoform-specific differences in kinetic properties of myosin heads. Using the same methodology on muscle strips of atria and ventricles of hyper- and hypothyroid rats, this study showed that the kinetics of cardiac αMHC are 3 times faster than those of cardiac βMHC under isometric conditions and maximal Ca^{2+} activation. Comparison of rat heart and skeletal muscle fibres revealed that 100% αMHC heart muscle strips exhibited faster stretch activation kinetics (time parameter t_3: 108±18 ms, mean±SD) than rat type-IIA fibres (t_3: 157±19 ms), but slower than type-IID fibres (t_3: 55±10 ms). The kinetics of 100% βMHC heart muscle strips (t_3: 351±44 ms) were faster than that of type-I fibres in rat skeletal muscle (t_3: 901±348 ms). This difference between the two muscle types calls in question the generally accepted identity of βMHC and MHCIβ.

Keywords Cardiac muscle · MHCIβ · αMHC · βMHC · Muscle mechanics · Myosin heavy chain isoforms · Stretch activation

Introduction

Muscle is an extremely heterogeneous tissue. Its diversity is based on the existence and differential expression of specific myofibrillar protein isoforms. In skeletal muscle, different fibre types are generally categorized according to their specific myosin heavy chain (MHC) isoforms (for review, see [27]). Thus, three fast fibre types and one slow fibre type exist in limb muscles of adult rat and rabbit: the fast fibre types IIB, IID(X), and IIA express MHCIIb, MHCIId(x) and MHCIIa isoforms, respectively. The slow type-I fibres express MHCIβ.

Cardiac MHC isoforms are also heterogeneous (for review, see [31]). Mammalian heart expresses two functionally different MHC isoforms, namely α- and βMHC. Cardiac βMHC is believed generally to correspond to the βMHCI isoform in type-I skeletal muscle fibres [25]. Cardiac αMHC is present in both atria and ventricles, whereas cardiac βMHC is present only in ventricles. βMHC predominates in the human ventricle, while αMHC is the predominant isoform in rat ventricle.

The essential component of the molecular force-generating system resides in the head portion of the MHC [15, 16, 28]. Single fibre studies have shown a clear correlation between the kinetics of force responses following a quick stretch of maximally Ca^{2+}-activated muscle fibres (stretch activation) and their MHC isoforms in rat, rabbit and human skeletal muscle [7, 9, 21]. This correlation probably points to different kinetic properties of the force-generating power strokes [22] of myosin heads of different isoforms in muscle fibres under isometric conditions and maximal Ca^{2+} activation.

Compared with skeletal muscle, only little information exists on the force-generating properties of cardiac MHC isoforms [29]. The aim of the present study was to investigate the relationship between stretch activation kinetics and MHC isoforms in rat heart muscle. For establishing a direct correlation between kinetic parameters and MHC isoforms, it appeared desirable to investigate preparations varying with regard to MHC isoform composition without differences in the isoform profiles of other myofibrillar proteins. In addition, it was desirable to compare the properties of preparations with identical MHC isoform complement in combination with different isoform patterns of other myofibrillar proteins. Both types of investigation are possible by using atria and

S. Galler (✉) · E. Puchert · D. Schmid
Zoologisches Institut, Universität Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria
e-mail: Stefan.Galler@sbg.ac.at
Tel.: +43-662-80445618
Fax: +43-662-80445698

B. Gohlsch · D. Pette
Fachbereich Biologie, Universität Konstanz, 78457 Konstanz, Germany

ventricles of rats with an experimentally altered thyroid hormone level. Atria and ventricles are known to exhibit different MHC and myosin light chain (MLC) isoforms, but identical isoforms of other myofibrillar proteins. Altered thyroid hormone levels effect MHC isoform expression in cardiac muscle without affecting the composition of other myofibrillar proteins [1, 3, 11, 30, 32] (for review, see [31]). In the rat, the spectrum of MHC expression in the ventricle shifts to 100% βMHC under conditions of hypothyroidism, whereas hyperthyroidism may induce up to 100% αMHC expression. Thus, a comparison of stretch activation between these two preparations allows the effects of different MHC isoforms to be studied against a constant background of MLC and other myofibrillar protein isoforms. Since atria and ventricles of hyperthyroid rats both contain 100% αMHC, but differ in their MLC composition, a comparison of stretch activation between these two preparations allows the influence of varied MLC composition to be studied under conditions of identical MHC isoform expression. This experimental model made it possible to investigate stretch activation kinetics of maximally Ca^{2+}-activated, skinned muscle strips prepared from hypo- and hyperthyroid atria and ventricles. Our study also included muscle strips obtained from euthyroid ventricles displaying varying ratios of α- and βMHC. Subsequent to the physiological experiments, the MHC isoform composition of the preparations was established by SDS gel electrophoresis.

Materials and methods

Muscle preparations

Adult (4 months old), male Wistar rats were utilized. Hypothyroidism was induced by feeding an iodine-poor diet containing 0.25% propylthiouracil (C1042, Altromin, Lage, Germany) for 6 weeks and by the addition of 0.9% propylthiouracil (PTU) to the drinking water. Hyperthyroidism was induced by implanting encapsulated triiodo-L-thyronine pellets with biodegradable carrier-binder (1.5 mg pellets, IRA, Toledo, Ohio, USA) 3 weeks before the animals were sacrificed. In addition to the hypo- and hyperthyroid animals, hearts from untreated euthyroid rats were examined also.

The hearts of the euthanized animals were excised quickly and perfused retrogradely by connecting a syringe with the aorta (non-circulating Langendorff perfusion). The following solutions (0–10 °C) were used for sequential perfusions: (1) Tyrode solution, containing (mM) 137 NaCl, 2.7 KCl, 0.5 $MgCl_2$, 12 $NaHCO_3$, 1.4 $CaCl_2$, 0.3 NaH_2PO_4, 5.6 glucose, 10 HEPES, approximately 2 units/ml heparin, adjusted to pH 7.4 with NaOH; (2) Tyrode solution in which $CaCl_2$ was replaced by NaCl; (3) skinning solution (mM): 132 sodium propionate, 5 EGTA, 7 Na_2H_2ATP, 2 $MgCl_2$, 10 MOPS, adjusted to pH 6.9 with KOH; (4) skinning solution in which sodium propionate was replaced by potassium propionate; (5) solution 4 with 10% v/v glycerol; (6) solution 4 with 25% v/v glycerol; (7) relaxation solution (composition see below, pH 6.9) with 50% glycerol v/v. The muscle preparations were stored in this solution for at least 1 h at approximately 5 °C. They were then transferred to a freezer at –25 °C. Before the experiments, tissue strips (below also designated as fibres) were dissected under a stereoscopic microscope on an ice-cooled plate.

Mechanical measurements

The set-up and methods for mechanical measurement have been described in detail [6]. The attachment points for the muscle strips (1.0–3.5 mm in length, 0.1–0.3 mm in diameter) on the mechanical apparatus were two vertically orientated epoxy carbon fibres of ~100 µm tip diameter. The needles were connected to the rest of the apparatus by silicon plates from force transducer elements (AE 801, SensoNor, Norway). One element, the force sensor (resonance frequency, 9 kHz) was connected mechanically to a micrometer screw and electrically to a force bridge amplifier. The other element was a dummy, glued on the lever arm of a stepping motor. Rapid changes of the fibre length (<1 ms) were achieved by a feedback-controlled stepping motor based on a Ling vibrator. The ability to make rapid solution changes (≤0.2 s) was provided by a cuvette transporting system. Laser diffractometry (He-Ne laser, 632.8 nm, 4 mW) was used for measuring the sarcomere length.

The solutions used during the mechanical measurements had an ionic strength of 0.24 M and contained (mM) 60 HEPES, 8 Na_2H_2ATP, 10 sodium creatine phosphate, 1 NaN_3, 0.1 dithioerythritol, 40 g/l dextran T-500, 30 units/ml creatine phosphokinase and 0.4 mM free Mg^{2+}. The pCa (–log $[Ca^{2+}]_{free}$) and the free $[Mg^{2+}]$ of the solutions were determined with ion-selective electrodes [5]. In addition, the relaxation solution (pCa>9) contained 50 mM EGTA. The activation solution (pCa 4.5) contained 50 mM Ca-EGTA and the pre-activation solution (low Ca^{2+}-buffering capacity, pCa 7) 50 mM hexamethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid (HDTA). pH was adjusted to 7.10 in all solutions at 22 °C.

Prior to experiments, the muscle strips were treated with 1% (v/v) Triton X-100 in relaxation solution (15 min; 22 °C). Subsequently, the length of the preparations was adjusted to exactly the slack position in relaxation solution, and both fibre dimensions and sarcomere length were recorded. After transferring the fibre from the pre-activation solution to the activation solution, quick (<1 ms) stretches (range, about 0.2–0.3% of fibre length) were applied to detect the time course of the resulting force transients.

All experiments were performed at 22 °C. Results are expressed as means±SD and analysed using two-tailed Student's *t*-tests. For checking the distribution of data, the Kolmogorov-Smirnov normality test (SIGMASTAT software, Jandel Scientific) was used.

Biochemical analysis

After completion of the mechanical measurements, the muscle preparations were cut from the apparatus for biochemical analysis. A muscle strip fragment was dissolved in SDS lysis buffer (62 mM TRIS-HCl, pH 6.8, 10% glycerol v/v, 2.3% SDS w/v, 5% 2-mercaptoethanol v/v, 19% sucrose w/v) and heated at 65 °C for 15 min. Subsequently, 2.5 µl of this extract was applied to a polyacrylamide gradient gel according to [12]. After electrophoresis, gels were silver stained [26] and the relative amounts of MHC isoforms evaluated by integrating densitometry.

Results

MHC isoforms and maximal tension

The regional distribution of MHC isoforms in rat heart muscle corresponded to previous data (e.g. [2, 14]). All preparations from hyper- and hypothyroid atria and from hyperthyroid ventricles contained αMHC exclusively. Ventricles of hypothyroid rats contained βMHC exclusively. Muscle strips from different regions of euthyroid ventricles contained 57–92% αMHC and 43–8% βMHC.

Sarcomere length (2.0–2.2 µm) could be measured successfully by laser diffraction in approximately 60% of

Table 1 Myosin heavy chain (*MHC*) isoforms, maximal tension and kinetic parameters (t_2 and t_3, see Materials and methods) of stretch activation of muscle strips from atria and ventricles of hyper- and hypothyroid rats and from ventricles of euthyroid rats. Means±SD (*n*)

	Hyperthyroid atria	Hyperthyroid ventricles	Hypothyroid atria	Hypothyroid ventricles	Euthyroid ventricles
MHC isoform	100% αMHC	100% αMHC	100% αMHC	100% βMHC	77% αMHC, 23% βMHC
Maximal tension (kN m^{-2})	15±7 (20)	21±11* (30)	5±2* (8)	11±9* (30)	23±5* (21)
t_3 (ms)	101.8±22.9 (16)	114.0±12.9 (16)	109.3±11.8 (7)	351.2±43.9* (28)	190.3±41.5* (20)
t_2 (ms)	39.6±13.2 (20)	41.0±10.6 (30)	40.5±8.7 (8)	129.4±21.5* (30)	71.0±18.4* (21)

* means significanlty different from all values given in the left column (two-tailed Student *t*-test, $P \leq 0.01$) with one single exception: Maximum tension of hyper- and euthyroid ventricles were not significantly different

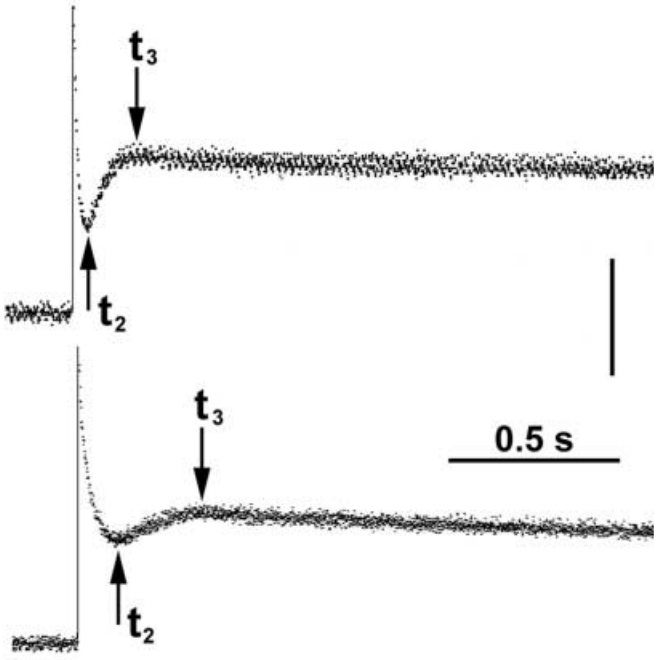

Fig. 1 Stretch experiments. Original recordings of force during a quick stretch experiment of 0.3% fibre length. *Upper trace*: fibre from hyperthyroid ventricle [100% α-myosin heavy chain (MHC)], *lower trace*: fibre from hypothyroid ventricle (100% βMHC). *Force calibration bar*: *upper trace* 3 μN; *lower trace* 10 μN

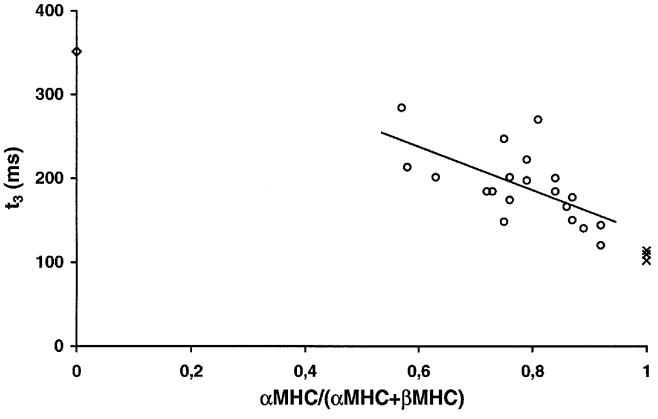

Fig. 2 The distribution of t_3 values of individual muscle strips from euthyroid ventricles (*circles*) in relation to their MHC isoform composition. For comparison the mean t_3 values of muscle strips containing 100% αMHC (atria and ventricles of hyperthyroid rats, atria of hypothyroid rats; *crosses*) and muscle strips containing 100% βMHC (ventricles of hypothyroid rats, *diamond*) are also given. The *straight line* is the linear regression line of the values of euthyroid preparations (*circles*, r^2=0.62). Including the 67 t_3 values of hyper- and hypothyroid preparations increases r^2 to 0.95

the cardiac muscle strips investigated. The remaining strips displayed no distinct diffraction pattern. The mean values of maximal isometric tension are given in Table 1. Hyperthyroid preparations exhibited higher tension values than hypothyroid preparations (P<0.001). As hyperthyroidism results in hypertrophy and hypothyroidism in atrophy of both atria and ventricles (e.g. [18, 24]), this difference most likely resulted from higher and lower myofibril densities, respectively.

Stretch activation kinetics

After maximal activation under isometric conditions, fibre length was changed stepwise (<1 ms) by stretches with amplitudes of about 0.2–0.3% of fibre length (Fig. 1). The stretches caused an immediate rise in force, followed by a decrease and, subsequently, a secondary, delayed force increase (usually designated stretch activation). To allow comparison with published data from skeletal muscle fibres [7, 10, 13], the following kinetic parameters of the force transients were evaluated: t_3, which is the time from the beginning of the stretch to the peak value of the delayed force increase, and t_2, which is the time from the beginning of the stretch to the lowest force value before the onset of delayed force increase. Independent of the tissue type, approximately 25% of all fibres exhibited no detectable force decay following the delayed force increase. t_3 could therefore not be determined accurately in these cases. t_2 was determined in all fibres.

In each of the four groups (muscle strips from both hyper- and hypothyroid atria and ventricles), the t_3 values followed a normal (Gaussian) distribution. Table 1 lists the mean t_3 and t_2 values. Preparations containing exclusively αMHC (hyperthyroid ventricles and atria, hypothyroid atria), displayed similar values, whereas preparations containing exclusively βMHC (hypothyroid ventricles), were characterized by values approximately 3 times higher (P<0.001). No overlap of the t_3 values

existed between these two groups. Muscle strips from euthyroid ventricles exhibited t_3 and t_2 values intermediate between the purely αMHC and βMHC samples (Table 1). A dependency of the t_3 values on the percentage distribution of the two isoforms is suggested in Fig. 2, in which the t_3 values of the individual preparations are shown as a function of their corresponding αMHC/(αMHC+βMHC) ratio. For comparison, the t_3 values of hypo- and hyperthyroid preparations were also included in the diagram. The correlation coefficient r^2 for the euthyroid t_3 values was 0.62. Including the 67 t_3 values for the hyper- and hypothyroid preparations increased r^2 to 0.95.

Discussion

The present findings on maximally Ca^{2+}-activated muscle strips of hyper- and hypothyroid rat hearts reveal a remarkable correlation between MHC isoform complement and kinetics of stretch activation (t_3, t_2). This correlation is validated by the results from muscle strips of euthyroid ventricles which contain both αMHC and βMHC at various ratios. Here, the kinetics of stretch activation depended on the percentage distribution of the two isoforms.

Our results suggest that the kinetic differences obtained under isometric conditions with maximal Ca^{2+} activation relate primarily to the MHC isoform and not to other components of the myofibrillar apparatus, especially the MLC complement. This suggestion is based on the following observations. (1) Preparations of atria and ventricles containing 100% αMHC, but differing in their MLC profiles (hyperthyroid ventricles and hypo- and hyperthyroid atria), exhibited similar kinetics of stretch activation. (2) Preparations with similar profiles of MLC and other myofibrillar protein isoforms, but dissimilar in their MHC isoform composition, namely hyper- and hypothyroid ventricles (for review, see [31]), exhibit great differences in their kinetic properties.

A correlation between MHC isoform composition and stretch activation kinetics has been demonstrated previously for single fibres from rat, rabbit and human skeletal muscles [7, 9, 13]. The present findings, therefore, extend our assumption of a causal relationship between MHC isoforms and stretch activation kinetics to rat cardiac muscle. In experiments in which activated muscle fibres were exposed to sinusoidal length changes at varying frequencies, maximal work output occurred at a frequency corresponding to the rate constant of the stretch-induced force increase e.g. [19, 21, 33], for skeletal muscle fibres and for heart muscle strips [20, 22]. This is in line with the suggestion that the stretch-induced delayed force increase is caused by a transient stretch synchronisation of force-generating cycles of a group of myosin heads. Consequently, it is possible that the kinetics of stretch activation is related to the kinetics of force-generating power strokes of the myosin heads in maximally activated muscle fibres under isometric conditions [20]. From this point of view our data suggest that comparable kinetic properties of myosin heads from αMHC are approximately 3 times faster than that of βMHC. These variations are most likely related to the diversity of functional requirements in the muscle tissue of atria and ventricles of (euthyroid) mammalian hearts. The varying molecular properties of the individual myosin heavy chain isoforms appear to contribute to the range of contractility in intact muscle fibres. In addition to properties of the contractile apparatus, the energy supply and regulatory factors of muscle cells play a role in fulfilling the varying functional requirements in vivo.

Using pseudo-random binary noise length changes on rat heart muscle strips Rossmanith et al. [29] have determined a kinetic parameter (f_{min}, the frequency at which dynamic stiffness is minimum) that is related to a resonance phenomenon and that may reflect kinetic aspects of force generators, similar to the stretch activation parameters t_3 and t_2 of the present study. f_{min} differed by a factor of 2 between papillary muscles from 4-week-old rats (αMHC only) and hypothyroid adult rats (βMHC only). Assuming a relationship between f_{min} and stretch activation-derived t_3 and t_2 values [19], a discrepancy exists between [29] and the present investigation. This discrepancy might result from different experimental conditions of the two studies, such as temperature (25 °C in [29], 22 °C here), pH (7.3 in [29], 7.1 here), free [Mg^{2+}] (2.3 mM in [29], 0.4 mM here), ionic strength (0.18 M in [29], 0.24 M here) and [$MgATP^{2-}$] (8.5 mM in [29], 6 mM here). The possibility exists that αMHC and βMHC differ in their sensitivities towards one ore more of these parameters which could result in different ratios of the kinetics of the two isoforms.

A comparison between heart and skeletal muscle fibres of rat revealed that heart muscle strips expressing 100% αMHC exhibited faster stretch activation kinetics (t_3: 108±18, n=39) than rat type-IIA fibres (t_3: 157±19 ms, n=10), but slower than type-IID fibres (t_3: 55±10 ms, n=27) [9]. The kinetics of heart muscle strips expressing 100% βMHC (t_3: 351±44 ms, n=28) were faster than that of type-I fibres in rat skeletal muscle (t_3: 901±348 ms, n=20). This is surprising because the βMHC isoform is thought to be identical to the MHCIβ expressed in type-I fibres [25].

A possible explanation for this discrepancy is the assumption that the kinetics of stretch activation is determined not only by the MHC but also by other myofibrillar proteins. However, this assumption is weakened by two observations. (1) Our results on 100% αMHC ventricles (hyperthyroid) and atria (hypo- and hyperthyroid) suggest that differences in MLC isoform complement do not affect the kinetics of stretch activation. (2) Studies on rat skeletal muscle fibres have shown that the kinetics of stretch activation correlates only with the MHC isoforms and not with the isoforms of troponin T [10].

An alternative explanation for the kinetic difference between cardiac βMHC and MHCIβ is that both proteins are not identical. Presently, the complete amino acid

sequence of cardiac βMHC and skeletal muscle MHCIβ have been established for the pig (*Sus scrofa*), but not yet for the rat or other mammalian species including humans. Sequence alignment analysis (using the ClustalW software, European Bioinformatics Institute, Hinxton, Cambs., UK), showed that only 10 out of the 1935 amino acids differ between pig βMHC and skeletal muscle MHCIβ and, of these ten, only one is located in the myosin head. Nevertheless, variations in the amino acid sequence of the motor domain between cardiac βMHC and MHCIβ remain a possible cause for explaining differences between the kinetics of the two isoforms in rat. In fact, two sarcomeric slow-type MHC isoforms have been identified recently in the human genome and assigned to chromosome 20 [4].

Moreover, evidence in support of the assumption that type-I fibres may not represent a functionally and/or structurally homogeneous population is found in the literature. Thus, contraction times of type-I motor units are conspicuously shorter in the tibialis anterior than in soleus muscle of the rat [23]. Also, type-I fibre subtypes have been described in several human skeletal muscles by in situ hybridization of the essential slow light chains MLC1sa and MLC1sb and their specific distribution patterns within the type-I fibre population [17]. Similarly, immunohistochemical studies with monoclonal antibodies directed against slow myosin indicate a non-homogeneous distribution of specific epitopes in myofibrillar ATPase-based type-I fibres of rat and rabbit (D. Pette, C.T. Putman, unpublished observations). Finally, two electrophoretically distinct MHC isoforms have been described in single type I fibres of rabbit plantaris muscle differing with regard to their stretch activation kinetics [8].

In summary, we have shown that the correlation between MHC isoform composition and stretch activation kinetics previously demonstrated for single skeletal muscle fibres also applies to rat cardiac muscle with regard to its αMHC and βMHC isoforms. A comparison of the stretch activation kinetics between purely βMHC-containing cardiac muscle strips and MHCIβ-containing skeletal muscle fibres revealed pronounced differences, calling in question the generally accepted identity of βMHC and MHCIβ.

Acknowledgements Supported by grants from the FWF-P14753-MOB (Austria), South Tyrolean Sparkasse, the Deutsche Forschungsgemeinschaft (Pe 62/25-1) and Fonds der Chemie.

References

1. Bottinelli R, Canepari M, Cappelli V, Reggiani C (1995) Maximum speed of shortening and ATPase activity in atrial and ventricular myocardia of hyperthyroid rats. Am J Physiol 38:C785–C790
2. Cappelli V, Moggio R, Polla B, Bottinelli R, Poggesi C, Reggiani C (1988) The dual effect of thyroid hormones on contractile properties of rat myocardium. Pflügers Arch 411:620–627
3. Dalla Libera L, Carraro U, Pauletto P (1983) Light and heavy chains of myosin from atrial and ventricular myocardium of turkey and rat. Basic Res Cardiol 78:671–678
4. Desjardins PR, Burkman JM, Shrager JB, Allmond LA, Stedman HH (2002) Evolutionary implications of three novel members of the human sarcomeric myosin heavy chain gene family. Mol Biol Evol 19:375–393
5. Galler S (1999) Ca^{2+}-, Sr^{2+}-force relationships and kinetic properties of fast-twitch rat leg muscle fibre subtypes. Acta Physiol Scand 167:131–141
6. Galler S, Hilber K (1994) Unloaded shortening of skinned mammalian skeletal muscle fibres: effects of the experimental approach and passive force. J Muscle Res Cell Motil 15:400–412
7. Galler S, Schmitt T, Pette D (1994) Stretch activation, unloaded shortening velocity, and myosin heavy chain isoforms of rat skeletal muscle fibres. J Physiol (Lond) 478:523–531
8. Galler S, Hilber K, Gohlsch B, Pette D (1997) Two functionally distinct myosin heavy chain isoforms in slow skeletal muscle fibres. FEBS Lett 410:150–152
9. Galler S, Hilber K, Pette D (1997) Stretch activation and myosin heavy chain isoforms of rat, rabbit and human skeletal muscle fibres. J Muscle Res Cell Motil 18:441–448
10. Galler S, Schmitt TL, Hilber K, Pette D (1997) Stretch activation and isoforms of myosin heavy chain and troponin-T of rat skeletal muscle fibres. J Muscle Res Cell Motil 18:555–561
11. Gorza L, Merciai N (1993) Effects of aging and hypothyroidism on troponin T and I subunit composition of the rat heart. In: Bergamini E (ed) General pathology and pathophysiology of aging. Wichting Editore, Milan, pp 175–179
12. Hämäläinen N, Pette D (1997) Coordinated fast-to-slow transitions of myosin and SERCA isoforms in chronically stimulated fast-twitch muscles of euthyroid and hyperthyroid rabbits. J Muscle Res Cell Motil 18:545–554
13. Hilber K, Galler S, Gohlsch B, Pette D (1999) Kinetic properties of myosin heavy chain isoforms in single fibres from human skeletal muscle. FEBS Lett 455:267–270
14. Hoh JFY, McGrath PA, Hale PT (1978) Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. J Mol Cell Cardiol 10:1053–1076
15. Huxley AF, Simmons RM (1971) Proposed mechanism of force generation in striated muscle. Nature 233:533–538
16. Huxley HE (1969) The mechanism of muscular contraction. Science 164:1356–1366
17. Jostarndt K, Puntschart A, Hoppeler H, Billeter R (1996) Fibre type-specific expression of essential (alkali) myosin light chains in human skeletal muscles. J Histochem Cytochem 44:1141–1152
18. Katzeff HL, Ojamaa KM, Klein I (1994) Effects of exercise on protein synthesis and myosin heavy chain gene expression in hypothyroid rats. Am J Physiol 267:E63–E67
19. Kawai M, Brandt PW (1980) Sinusoidal analysis: a high resolution method for correlating biochemical reactions with physiological processes in activated skeletal muscles of rabbit, frog and crayfish. J Muscle Res Cell Motil 1:279–303
20. Kawai M, Halvorson HR (1991) Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibres of rabbit psoas muscle. Biophys J 59:329–342
21. Kawai M, Schachat FH (1984) Differences in the transient response of fast slow skeletal muscle fibres. Correlations between complex modulus and myosin light chains. Biophys J 45:1145–1151
22. Kawai M, Zhao Y (1993) Cross-bridge scheme and force per cross-bridge state in skinned rabbit psoas muscle fibres. Biophys J 65:638–651
23. Kugelberg E, Thornell L-E (1983) Contraction time, histochemical type, and terminal cisternae volume of rat motor units. Muscle Nerve 6:149–153

24. Liu Z, Gerdes AM (1990) Influence of hypothyroidism and the reversal of hypothyroidism on hemodynamics and cell size in the adult rat heart. J Mol Cell Cardiol 22:1339–1348
25. Lompré A-M, Nadal-Ginard B, Mahdavi V (1984) Expression of the cardiac ventricular α- and β-myosin heavy chain genes is developmentally and hormonally regulated. J Biol Chem 259:6437–6446
26. Oakley BR, Kirsch DR, Morris NR (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal Biochem 105:361–363
27. Pette D, Staron RS (2000) Myosin isoforms, muscle fibre types, and transitions. Microsc Res Tech 50:500–509
28. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, Milligan RA (1993) Structure of the actin-myosin complex and its implications for muscle contraction. Science 261:58–65
29. Rossmanith GH, Hoh JF, Kirman A, Kwan LJ (1986) Influence of V1 and V3 isomyosins on the mechanical behaviour of rat papillary muscle as studied by pseudo-random binary noise modulated length perturbations. J Muscle Res Cell Motil 7:307–319
30. Saggin L, Ausoni S, Gorza L, Sartore S, Schiaffino S (1988) Troponin T switching in the developing rat heart. J Biol Chem 263:18488–18492
31. Schaub MC, Hefti MA, Zuellig RA, Morano I. Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms. Cardiovasc Res 37:381–404
32. Syrovy I, Delcayre C, Swynghedauw B (1979) Comparison of ATPase activity and light subunits in myosins from left and right ventricles and atria in seven mammalian species. J Mol Cell Cardiol 11:1129–1135
33. Zhao Y, Kawai M (1994) Kinetic and thermodynamic studies of the cross-bridge cycle in rabbit psoas muscle fibres. Biophys J 67:1655–1668