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Fast myosin heavy chain isoforms in horse skeletal muscle: an immunohistochemical and electrophoretic study

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Summary

The aim of this study was to characterize the fast myosin heavy chain (MyHC) isoforms present in equine skeletal muscle. Muscle biopsies were removed from the superficial region of the gluteus medius muscle of 5 mature horses, and analyzed by immunohistochemistry (using a number of monoclonal antibodies specific for rat MyHC isoforms) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunohistochemistry allowed subdivision of two different muscle fibre populations containing a single fast MyHC, as well as one hybrid population, containing both fast MyHC isoforms. Electrophoresis of fast MyHC confirmed the existence of two resolvable bands, with an electrophoretic mobility parallel to type IIa and IIx rat MyHCs. The identity of one of these fast MyHCs was easily comparable with type IIa-MyHCs from rat skeletal muscle. However, a precise identification of the second fast MyHC was not made.

Keywords: muscle fibres, myosin, immunohistochemistry, electrophoresis, horse

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Isoformen schwerer, schneller Myosinketten des Skelettmuskels von Pferden: eine immunhistochemische und elektrophoretische Studie

Ziel dieser Arbeit war die Charakterisierung der Isoformen schwerer, schneller Myosinketten des equinen Skelettmuskels. Zu diesem Zweck wurden bei 5 adulten Pferden Muskelbiopsien aus den oberflächlichen Schichten des Musculus glutaeus medius entnommen und immunhistochemisch analysiert. Der Autor verwendete dazu mehrere monoklonale Antikörper mit Spezifität zu den schweren Myosinketten der Ratte. Eine Natrium-Dodecylsulphat-Polyamidgel-Elektrophorese ermöglichte die Trennung der einzelnen Isoformen.

Myosin ist das dominierende Protein im Skelettmuskel. Es macht den Hauptteil der kontraktilen Elemente der Muskelfaser aus und besteht aus vier leichten und zwei schweren Polypeptidketten. Bis jetzt wurden 9 Isoformen der schweren Myosinketten im Skelettmuskel verschiedener Tiere entdeckt. Drei Haupt-Isoformen bestimmen die Eigenschaften des Muskels : die langsame ß- oder Typ I-Kette und die zwei schnellen Myosinketten IIa und IIb. Die unterschiedliche Verteilung dieser schweren Myosinketten bestimmt die drei Hauptfasertypen. Hinzu kommen noch Hybridtypen, welche die sogenannten Typ C-Fasern und Typ IIab-Fasern bilden.

Durch Immunhistochemie konnten zwei Muskelfasertypen differenziert werden die je eine einzelne schwere Myosinkette hatten. Der Autor fand weiterhin eine Hybridform, welche beide Isoformen der schweren Myosinketten besaß.

Die elektrophoretische Auftrennung der schweren, schnellen Myosinketten ergab insgesamt zwei lösliche Banden, die eine elektrophoretische Mobilität zeigten, welche mit den Muskelfasertypen IIa und IIx der Ratte konform ging. Die Identität der Isoformen erwies sich als dieselbe des Typ IIa-Myosins des Skelettmuskels der Ratte. Die zweite Isoform der schweren Myosinkette konnte allerdings nicht präzise identifiziert werden

Schlüsselwörter: Muskelfaser, Myosin, Immunhistochemie, Elektrophorese, Pferd

Introduction

Myosin is the predominant protein in skeletal muscle and it makes up the largest portion of the contractile apparatus of muscle fibres. This protein consists of four light chains and two heavy chains. To date, a total of nine distinct MyHC isoforms have been identified in adult skeletal muscles of a number of species (for reviews see Ref. *Pette* and *Staron*, 1990). Of these, three MyHCs exist in many species: the β-, slow- or type I-MyHC and the two fast (IIa and IIb) MyHCs. The differential distribution of these MyHCs defines three main fibre types containing a single MyHC isoform (types I, IIA and IIB) and a number of hybrid fibre populations containing both I- and IIa-MyHCs (type 'C' fibres), and IIa- and IIb-MyHCs (type IIAB fibres). An additional fast MyHC isoform, termed IIx or IId, has been identified in muscles of rat, mouse, guinea pig and rabbit (*Bär* and *Pette,* 1988; *Schiaffino* et al., 1989) by using monoclonal antibodies and gel electrophoretic techniques. In humans the MyHC isoform found in IIB fibres is equivalent to rat IIx MyHC, not to rat IIb MyHC (*Smerdu* et al., 1994). Several observations have suggested that differences in MyHC content contributes significantly for the differences in both maximum shortening ve-

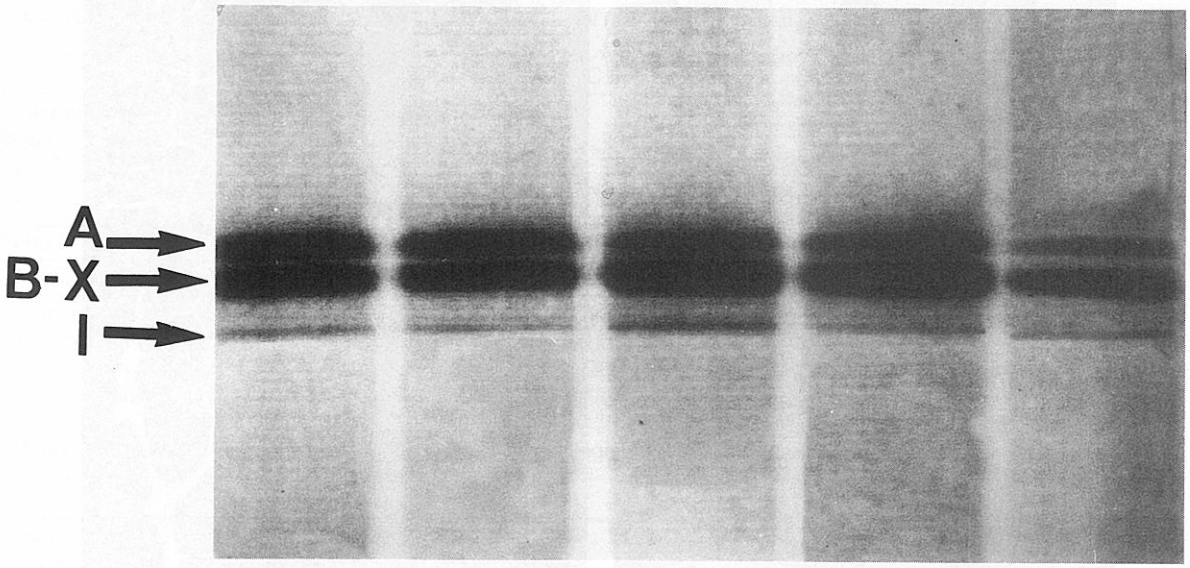

Fig. 2: 8 % Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel of normal horse gluteus medius muscle of 5 horses (lanes 1 to 5) myosin heavy chains (MyHCs). This gel shows the separation profile of the three MyHCs in adult horse muscle. The isoforms are identified as types I (I), IIa (A) and IIb or IIx (B-X).

letal muscle, in order of mobility I>IIb or IIx>IIa. Type I MyHC was the fastest-migrating band (lower band), and type IIa MyHC was the slowest-migrating band (upper band). The second fast MyHC migrated to approximately the same level as IIx-MyHC of rat muscle (*Talmadge* and *Roy,* 1993).

Discussion

Three different MyHC isoforms in horse skeletal muscle were identified using immunohistochemical and electrophoretic techniques: one slow and two fast isoforms. Using multiple MAbs against MyHC isoforms, immunohistochemistry allowed subdivision of the fast fibres in equine skeletal muscle into two different types. No previous immunohistochemical studies have reported two different fast MyHC isoforms in equine skeletal muscle (see *Snow* and *Valberg,* 1994 for a review).

In the present study, the electrophoretic method resulted in a consistent differentation of two type II horse MyHC isoforms (Fig. 2). As the slowest-migrating MyHC band comigrated with rat IIa MyHC, on the basis of this analysis, it seems clear that type IIa MyHC isoforms exist in the equine gluteus medius. By contrast, the identity of the second fast-MyHC (middle band in Fig. 2) was difficult to determine. Because its electrophoretic mobility was closer to type IIx- than type IIb-MyHC in rat muscles, a possible explanation could be that this equine MyHC is a type IIb MyHC, but with a higher molecular weight than rat type IIb-MyHC. Another plausible explanation might be that this equine MyHC could be more closely related to rat type IIx-MyHC rather than a type IIb-MyHC. There is sufficient evidence to conclude that type IIx-MyHC is coded by a distinct mRNA than type IIb-MyHC, and that its primary distribution lies within a specific fibre population termed type IIX with distinct metabolic and contractile properties.Two human skeletal MyHC genes have been identified for fast IIa and IIx MyHCs based on pattern of expression and sequence homology with corresponding rat genes (*Smerdu* et al., 1994). The distribution of these IIa and IIx MyHC transcripts defines two major fast muscle fibre types expressing a single MyHC mRNA, i.e. either IIa or IIx MyHC RNA. Fibre typing by ATPase histochemistry showed that IIa MyHC transcripts are more abundant in histochemical type IIA fibres, whereas IIx MyHC transcripts are more abundant in type IIB fibres (*Smerdu* et al., 1994). This observation strongly suggests that the so-called human IIB fibres actually express a MyHC isoform equivalent to the rat IIx MyHC isoform, and not the rat IIB isoform, and would therefore be more accurately classified as IIX fibres. Further studies are required to confirm if a similar situation occurs with those equine muscle fibres which contain a fast MyHC isoform other than IIa MyHC isoform.

The present results also indicate that, even under normal conditions, different MyHCs can coexist in single equine muscle fibres. The rarely occurring muscle fibre type containing both fast and slow myosin heavy chain substantiates the results reported by *Snow* et al. (1981) and *Sinha* et al. (1992). These fibres correspond to the type IIC muscle fibre identified histochemically, a muscle fibre type abundant in newborn foals, but extremely scarce in mature horses (*Snow* and *Valberg,* 1994). Also, a high percentage of fibres co-expressed both fast MyHCs, even in inactive animals.

In conclusion, results from the present study clearly show the existence of two fast MyHC isoforms in adult equine skeletal muscle. The differential distribution of these MyHCs defines two major fibre types containing a single MyHC (IIA and IIB or IIX) and one intermediate hybrid fibre population containing both fast MyHCs (type IIAB or IIAX fibres). Whereas the identity of one of these two fast MyHC isoforms seems to be clearly a type IIa-MyHC isoform, the present results are not conclusive regarding the second fast MyHC isoform.

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