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The histochemical profiles of fibre types in porcine skeletal muscle

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Summary. Using a variety of histochemical methods -mATPase staining after alkaline and acid preincubations, NADH-TR and α-MGPDH- we have investigated the fibre types in porcine skeletal muscle. The results reveal that four major fibre types -I, IIA, IIB and II*- can be separated histochemically in Longissimus lumborum muscle of Landrace pigs. The histochemical properties of the muscle fibre type II* are very similar to that of type IIX described in other mammals. The existence of IIX fibres in pig muscle has been recently demonstrated by molecular biology techniques and our results validate the use of histochemistry (mATPase) as an easy methodology to differentiate the three fast myosins (type II fibres) in pig muscle.

Key words: Fibre types, Skeletal muscle, Histochemistry

Introduction

Pig skeletal muscle fibres have been routinely categorized into three major fibre types, designated I, IIA and IIB, using conventional histochemical reaction for myofibrillar actomyosin ATPase (mATPase) after acid pretreatment (Lefaucheur, 1990; Stecchini et al., 1990; Lefaucheur et al., 1991, 1998; Essén-Gustavsson et al., 1992; Karlsson, 1993; Weiler et al., 1995; Larzul et al., 1997; Ruusunen and Puolanne, 1997). These fibre types contain different myosin heavy chains (MyHC) which correlate with the specific mATPase staining profiles (La Framboise et al., 1991; Hämäläinen and Pette, 1993: Staron and Pette, 1993). In other mammals (rats, mice, guinea pigs, and rabbits), enzyme histochemistry reveals another population of type II fibres, the so-called IIX or IID (Gorza, 1990; Hämäläinen and Pette, 1993), which contain a particular MyHC denominated MyHC2X (Schiaffino et al., 1989). In a recent study, Lefaucheur et al. (1998), demonstrated the existence of three adult fast types of MyHC in pig skeletal muscle. By analogy with rat these three MyHC can be referred to as type MyHC2A, MyHC2B and MyHC2X. However, by conventional enzyme histochemistry these authors have only identified fibre types I, IIA and IIB. These results lead to the conclusion that the conventional enzyme histochemical myofiber classification in types I, IIA, and IIB, should be revised in pigs. The purpose of the present investigation was to further characterize fibre types in pig muscle by combining histochemical mATPase techniques (alkaline and acid pre-incubation) and metabolic thecniques (NADH-TR and α-MGPDH). Our results demonstrated that, with the combined use of these thecniques, four major fibre types can be identified in Longissimus lumborum muscle of Landrace pigs. Thus, the use of the mATPase reaction is a valid, fast and easy methodology to differentiate the muscle fibre types in pig skeletal muscle.

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Materials and methods

Five Landrace pigs were used in this study. The pigs were slaughtered in the week they reached 100 kg (average age at slaughter was 183±4 days). Samples of Longissimus lumborum (LL) muscle were taken 30 min after slaugter and frozen in 2-methylbutane cooled in liquid nitrogen. Transverse serial sections (10 μm) were cut in a cryostat at -20 °C, and stained for myofibrillar ATPase (mATPase) after alkaline preincubation at pH 10.2-10.6 [system A described by Snow et al. (1982), modified by Latorre et al. (1993)] and acid preincubation at pH 4.6, and 4.3 [method Dubowitz and Brooke (1973) modified by Gil (1989)]. The nicotine adenine-dicluneotide (reduced) tetrazolium reductase (NADH-TR) and menadione-linked α-glycerophosphate (α-MGPDH) was used to demonstrate the metabolic activity of muscle fibres (Dubowitz and Brooke, 1973). These methods allow the separation of three fast fibre types (IIA, IIB and IIX) in rat skeletal muscle (Latorre et al., 1993). Percentages of each muscle fibre type were obtained after counting an average of 200 fibres from randomly selected fields in each muscle sample.

Results

By histochemical reactions, four fibre types could be identified in LL muscle. According to staining intensity,

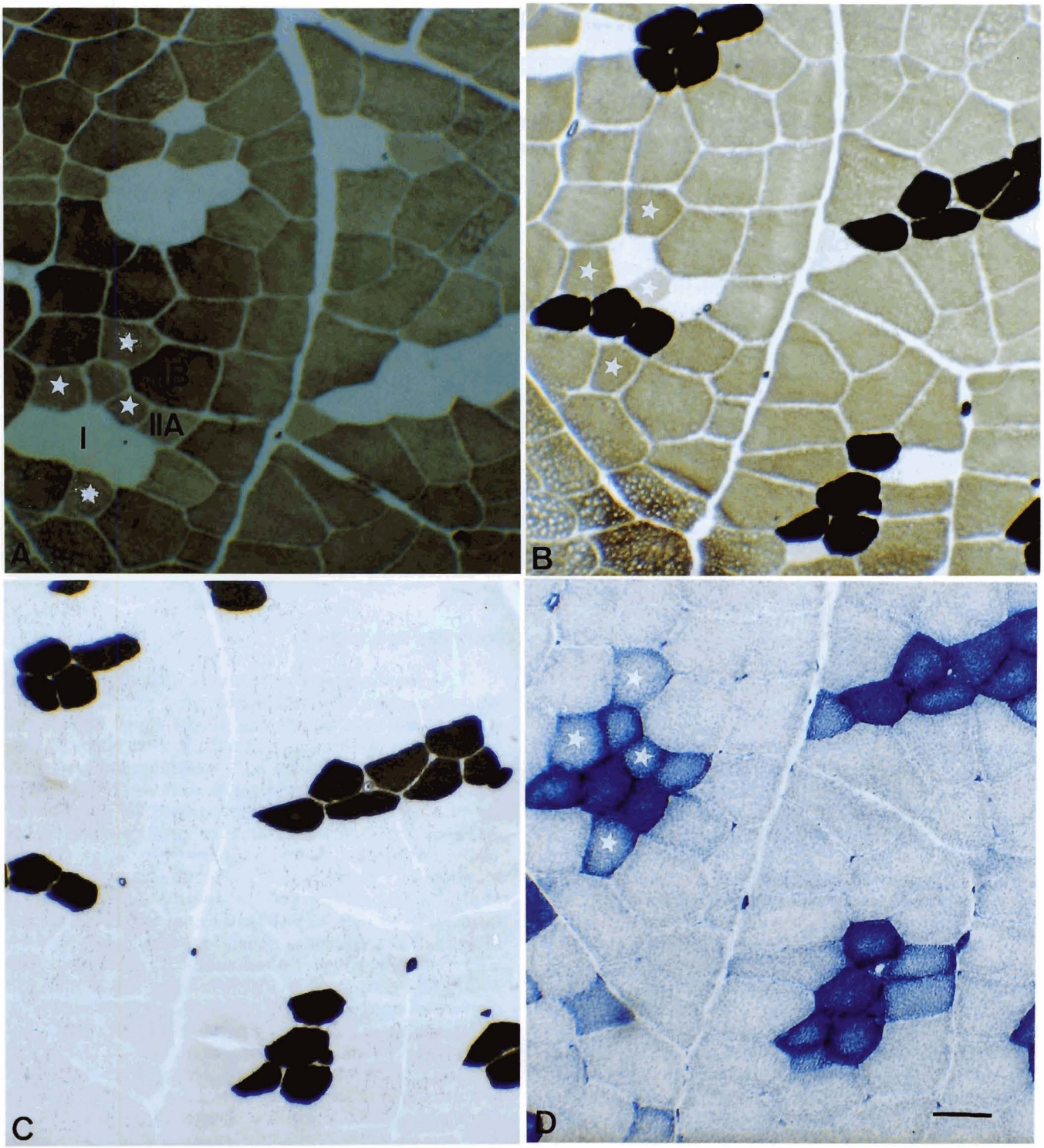

Fig. 1. Serial sections of pig Longisimus lumborum muscle. *: muscle fibre type II*. **A.** mATPase activity after alkaline preincubation at pH 10.6. Fibre types are: negative I; moderate IIA and II*; strong IIB. **B.** mATPase activity after acid preincubation at pH 4.6. Fibre types are: very strong I; moderate IIB and II*; negative IIA. **C.** mATPase activity after acid preincubation at pH 4.3. Fibre types are: very strong I, negative IIA, IIB and II*. **D.** NADH-TR activity. Fibre types are: very strong I; strong IIA; moderate II*; weak IIB. Bar: 40 μm.

they have been designated as: type I, type IIA, type IIB, and type II* fibres. By the mATPase technique alkaline preincubation at pH 10.2-10.4, types IIB and types II* appeared dark (strong staining) in contrast to type I fibres (pale or negative staining) and type IIA fibres (moderate staining). However, at pH 10.5-10.6 (Fig. 1A) muscle fibre type staining was as follows: type I fibres (pale staining); type IIB fibres (strong staining); and type IIA, II* fibres (moderate staining). The acid preincubation at pH 4.6 (Fig. 1B) allowed a clear differentiation between type I fibres (very strong staining), IIA fibres (negative staining) and IIB, II* fibres (moderate staining). At pH 4.3 (Fig. 1C), IIA, IIB and II* fibres showed negative staining in contrast with type I fibres (very strong staining). The oxidative capacity (NADH-TR technique) was high for type I and type IIA fibres, moderate for type II* fibres and low for type IIB fibres (Fig 1D). Glycolytic activity (α-MGPDH technique) was high for type IIB, IIA and II* fibres and low for type I fibres. These last muscle fibres exhibited a rosette pattern (as described by Lefaucheur et al., 1995): islets of oxidative type fibres (7.7% type I, 2.6% type IIA and 15.3% type II*, approximately), surrounded by glycolytic type non oxidative fibres (74.4 % type IIB, approximately).

Discussion

Using in situ hybridization with riboprobes, Lefaucheur et al. (1998) showed the existence of fast type 2X MyHC in pig skeletal muscle and reported that conventional IIB fibres, as defined with mATPase staining, are a heterogeneous population that should be split into pure IIX, hybrid IIX/IIB, and pure IIB fibres. In human skeletal muscle Smerdu et al. (1994) have also shown that type IIB fibres by the mATPase staining correspond to fibres containing great amounts of 2X MyHC transcripts. In this sense, it is obvious that muscle fibre types IIX have been erroneously included in the IIB fibre population because their histochemical characteristics resemble type IIB fibres of the mATPase staining after acid pre-incubation at pH 4.6, according to the Brooke and Kaiser (1970) or Dubowitz and Brooke (1973) methods. However, it is widely accepted that classical type IIB fibres have particular physiological and metabolic properties: they belong to the fast-twitch fatigable (FF) motor units; the capacity for oxidative metabolism is low; and the capacity for glycolitic metabolism is high (Buchthal and Schmalbruch, 1980). Thus, an accurate identification of fibre IIB types could be obtained using a combination of many different histochemical stainings (mATPase techniques with metabolic techniques). In addition, previous studies (Gorza, 1990; Hämäläinen and Pette, 1993) have reported that fast fibre types IIB, IIX or IID and IIB can be separated histochemically in skeletal muscle of rat, mouse, guinea pig and rabbit, modifying conditions for mATPase staining. Our results agree with these previous observations and validates the use of mATPase staining after both acid and alkaline preincubations to differentiate the three fast muscle fibres types. Although no general staining profiles can be defined among animal species, we believe that the so-called type II* in our study corresponds to type IIX (pure or hybrid) muscle fibres, because: i) the existence of adult fast type 2X MyHC in porcine skeletal muscle has been demonstrated (Lefaucheur et al., 1998); ii) fibre types called II* exhibit higher activities of enzymes related to aerobic-oxidative metabolism, than type IIB fibres; and iii) type II* fibres stained similarly to IIB fibres after pre-incubation at pH 4.6 and resembled IIA fibres at pH 10.5-10.6. In addition, the percentage of fibre type II* in LL pig muscle was 15.3%, a similar proportion to the results of Essen-Guvstason and Lindholn (1984) (15% fiber types IIB oxidative), and Lefaucheur et al. (1998) (18 % of fibers pure HC2x) in Longissimus dorsi muscles of crossbred and Yorkshire pigs, respectively.

In summary, with the combined use of mATPase techniques and the NADH-TR reaction three fast fibre types -IIA, IIB and II*- can be separated histochemically in skeletal muscle of Landrace pigs. Although a definitive demonstration of the correspondence between the II* fibres and the IIX fibres would need immunohistochemistry, electrophoresis or "in situ" hybridization, our histochemical results validate the use of the mATPase technique to easily differentiate the four major fibre types (I, IIA, IIB and II*) in porcine skeletal muscle.

Table 1. Hitochemical staining properties of muscle fibre types in the pig.

	I	IIA	IIB	II*
m ATPase pH 10.2-10.4	-	+++	++++	++++
mATPase pH 10.5-10.6	-	++	+++	++
mATPase pH 4.55-4.6	++++	-	++	++
mATPase pH 4.3	++++	-	-	-
NADH-TR	++++	+++	-	++
α-MGPDH	-	+++	+++	+++

The relative staining intensity of different fibre types is represented by: ++++ (very strong), +++ (strong), ++ (moderate), or - (very weak or negative).

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