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Tohoku Journal of Agricultural Research
Vol. 39 No. 2-4, March 1989
Printed in Japan

Inconsistency of Reactivity for Acid-stable Myosin ATPase in Subtypes of Type II Myofibers in Equine, Porcine, and Caprine Muscles

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(Received, January 24, 1989)

Summary

Differences in reactivity for acid-stable myosin ATPase among subtypes of type II myofibers classified by differences in activity of NADH tetrazolium reductase (NADH-TR) were examined in equine, porcine, and caprine muscles by histochemical methods. Myofibers that reacted strongly for acid-stable myosin ATPase and were unreactive for alkali-stable myosin ATPase were classified as type I myofibers. Myofibers that were weakly reactive or unreactive for acid-stable myosin ATPase and strongly reactive for alkali-stable myosin ATPase were classified as type II myofibers. Type II myofibers were subdivided into type IIa1 myofibers with a strong NADH-TR activity, type IIa2 with a moderate NADH-TR activity, and type IIb myofibers with a weak NADH-TR activity. Many type IIa1 myofibers were unreactive for myosin ATPase after preincubation at pH 4.4-4.5, and the remainder, in equine and porcine muscles, were weakly reactive. In the horse and pig, some muscle had all or many type IIa2 myofibers that reacted weakly for acid-stable myosin ATPase, and other muscle had all or many type IIa2 myofibers unreactive for acid-stable myosin ATPase. Equine type IIb myofibers were weakly reactive or unreactive for acid-stable myosin ATPase. All porcine type IIb myofibers reacted weakly for acid-stable myosin ATPase. In contrast, in the goat, numerous type IIb myofibers were unreactive for acid-stable myosin ATPase whereas all type IIa1 and many type IIa2 myofibers were weakly reactive. The results show that subtypes of type II myofibers classified on the basis of NADH-TR activity vary in reactivity for acid-stable myosin ATPase among muscles or species.

Introduction

Myofibers of skeletal muscles are classified into type I, IIA, and IIB myofibers by differences in histochemical reactivity for myosin ATPase after acid preincubation in the human, rabbit, and rat (1). Type I myofibers react strongly for acid-stable myosin ATPase. Type IIA myofibers are unreactive for myosin ATPase after preincubation at pH 4.5. Type IIB myofibers react weakly or

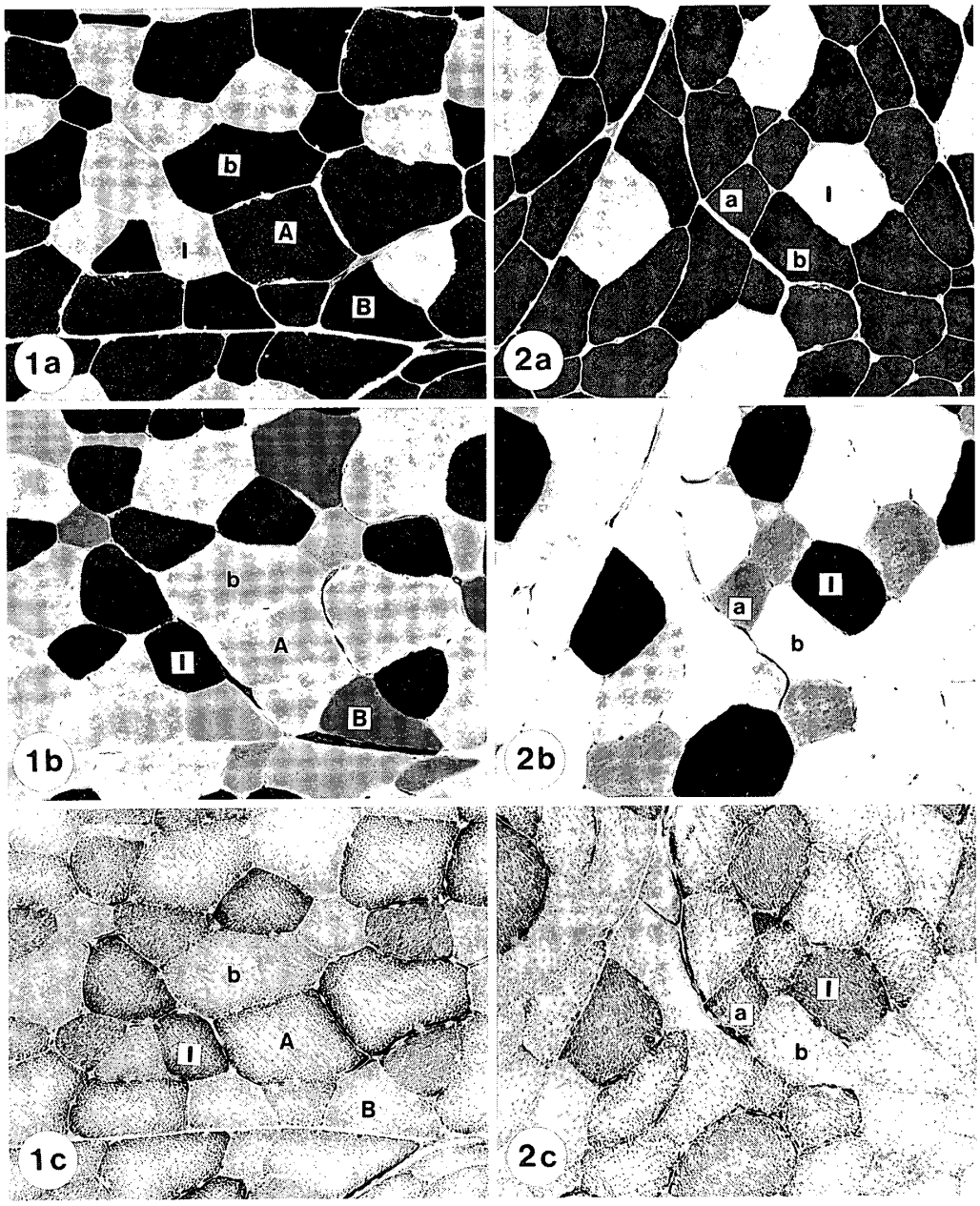
moderately for myosin ATPase after preincubation at this pH, but the myosin ATPase of type IIB myofibers is inhibited completely by preincubation at pH 4.3. Type IIA myofibers show stronger activity for NADH tetrazolium reductase (NADH-TR) than do type IIB myofibers in humans, rabbits, and rats (1). From these histochemical properties of type IIA and IIB myofibers, it is inferred that type IIA myofibers classified on the basis of differences in pH sensitivity of myosin ATPase possess a strong NADH-TR activity, whereas type IIB myofibers show a weak NADH-TR activity in these animals. However, the type IIA and IIB myofibers based on differences in pH sensitivity of myosin ATPase have been shown to vary in activity of succinate dehydrogenase in humans, rodents, and cats (2, 3).

In the horse (4, 5) and pig (6), some type IIA and IIB myofibers based on pH sensitivity of myosin ATPase show a moderate activity for NADH-TR, indicating that type IIB myofibers do not always show a weak NADH-TR activity. The reactivity for myosin ATPase after acid preincubation in type IIA and IIB myofibers classified by differences in the intensity of NADH-TR activity in one goat with foot rot (7) was opposite to that in the type IIA and IIB myofibers showing a similar reactivity for NADH-TR in humans, rabbits, and rats (1). The purpose of the present study was to reexamine pH sensitivity of myosin ATPase in subtypes of type II myofibers classified by differences in NADH-TR activity, and to confirm whether the subtypes of type II myofibers classified on the basis of NADH-TR activity are interchangeable with those based on differences in pH sensitivity of myosin ATPase in the horse, pig, and goat.

Materials and Methods

The obliquus internus abdominis and pectoralis transversus muscles were taken from one adult horse (crossbreed). The semitendinosus, semimembranosus, longissimus thoracis, and serratus ventralis thoracis muscles were taken from one adult pig (crossbreed). These muscle samples were obtained in an abattoir. The semitendinosus and semimembranosus muscles were taken from three adult goats (Saanen). The animals used were castrated. The muscle samples were frozen in a mixture of dry ice and acetone within 1 hr after slaughter. Cross sections (10 μm thick) were serially cut on a cryostat. Fresh sections were used for the demonstration of reactivity for myosin ATPase after preincubation at pH 4.3-4.5 and pH 10.4-10.6 (1, 8). Other sections were stained with NADH tetrazolium reductase (NADH-TR) (9).

Myofibers that were strongly reactive for myosin ATPase after acid preincubation and very weakly reactive or unreactive for myosin ATPase after alkaline preincubation were classified as type I (1). Myofibers that were weakly reactive or unreactive for myosin ATPase after acid preincubation and strongly reactive for myosin ATPase after alkaline preincuabtion were classified as type II. The



Figs. 1 and 2. Differences in myosin ATPase reactivity after acid preincuabtion in subtypes of type II myofibers in the M. obliquus internus abdominis of the horse (Fig. 1) and the M. semitendinosus of the goat (Fig. 2). Figs. 1a and 2a. Myosin ATPase reaction after alkaline preincubation. Light myofibers are type I myofibers (I) and dark myofibers type II myofibers. Figs. 1b and 2b. Myosin ATPase reaction after acid preincubation. All equine type IIa (A), some equine type IIb (b), and almost all caprine type IIb (b) myofibers are unreactive, whereas some equine type IIb (B) and all caprine type IIa (a) myofibers are weakly reactive. Figs. 1c and 2c. NADH tetrazolium reductase activity. Type I myofibers are strong, type IIa myofibers moderate to strong, and type IIb myofibers weak. All figures are the same magnification, $\times 150$.

type II myofibers were classified into subtypes by differences in activity for NADH-TR (10-12). Type II myofibers showing a moderate to strong NADH-TR activity were designated as type IIa. Furthermore, type IIa myofibers were subdivided into type IIa1 with a strong NADH-TR activity and type IIa2 with a moderate NADH-TR activity (Figs. 1 and 2). Type II myofibers showing a weak NADH-TR activity were designated as type IIb.

Myofibers that reacted moderately to strongly for both acid and alkaline preincubation were classified as intermediate type. 720 to 1085 myofibers were classified and counted. The myofibers unreactive for myosin ATPase after preincubation at pH 4.4-4.5 correspond to the type IIA myofiber of Brooke and Kasier (1), and the myofibers weakly reactive for myosin ATPase correspond to the type IIB myofiber of Brooke and Kaiser. The percentages of the weakly reactive and the unreactive myofibers in the type IIa1, IIa2, and IIb myofibers were calculated in addition to those of type I and intermediate type myofibers.

Results

1. *Proportion of myofiber types*

The muscles of the horse, pig, and goat had fewer type I myofibers than type II myofibers (Table 1). They had more type IIb myofibers than type IIa1 and IIa2 myofibers except in the pectoralis transversus muscle. Subtype ID of type I myofibers shown in sheep muscles (12) were found in the semitendinosus (2.3%)

Table 1. *Composition of Myofiber Types in Muscles of One Horse, Pig, and Three Goats*

Muscle	Myofiber types (%)				
	I	IIa1	IIa2	IIb	Int.[@]
Horse					
Obliquus internus abdominis	40.1	10.4	11.3	37.6	0.6
Pectoralis transversus	17.4	41.4	13.6	23.6	4.0
Pig					
Semitendinosus	1.6	9.2	7.8	81.4	0
Semimembranosus	14.4	5.1	5.9	74.2	0.4
Longissimus thoracis	12.1	11.0	5.8	70.9	0.2
Serratus ventralis thoracis	37.6	6.7	22.3	33.0	0.4
Goat					
Semitendinosus	15.4±3.0[#]	13.2±0.9	14.5±1.4	56.7±4.6	0.2±0.3
Semimembranosus	28.4±7.9	12.7±1.7	9.5±2.0	49.2±5.2	0.2±0.2

[@] Intermediate type
[#] Mean±S.D. n=3

and semimembranosus muscle (13.2%) of goats but not in the equine and porcine muscles. Intermediate type was rarely found in the muscles examined.

2. Reactivity of myosin ATPase after preincubation at pH 4.4-4.5 in type IIa1, IIa2, and IIb myofibers

Horse

In the obliquus internus abdominis muscle, all type IIa1 and IIa2 myofibers were unreactive for myosin ATPase ; they corresponded to the type IIA myofiber of Brooke and Kaiser (1). Type IIb myofibers were weakly reactive for myosin ATPase or unreactive for myosin ATPase ; they fell under the category of either the type IIA myofiber or the type IIB myofiber (Fig. 1). The pectoralis transversus muscle had type IIa1, IIa2, and IIb myofibers weakly reactive or unreactive for myosin ATPase. In this muscle almost all type IIb myofibers corresponded to the type IIB myofiber and many type IIa1 myofibers to the type IIA myofiber (Table 2). 27.8% of type IIa1 myofibers and 83.3% of type IIa2 myofibers would be designated as type IIB myofiber if they were classified on the basis of differences in pH sensitivity of myosin ATPase.

Pig

Many type IIa1 myofibers were similar to the type IIA myofiber in pH sensitivity of myosin ATPase and the remainder to the type IIB myofiber in the

Table 2. *Percentages of the Weakly Reactive and the Unreactive Myofibers for Acid-stable Myosin ATPase in Type IIa1, IIa2, and IIb Myofibers of One Horse, Pig, and Three Goats*

Muscle	Myofiber types (%)					
	IIa1		IIa2		IIb	
	weakly[@]	unreactive[=]	weakly	unreactive	weakly	unreactive
Horse						
Obliquus internus abdominis	0	100	0	100	42.3	57.7
Pectoralis transversus	27.8	72.2	83.8	16.2	94.1	5.9
Pig						
Semitendinosus	40.2	59.8	100	0	100	0
Semimembranosus	27.5	72.5	100	0	100	0
Longissmus thoracis	24.5	75.5	100	0	100	0
Serratus ventralis	1.5	98.5	5.8	94.2	100	0
Goat						
Semitendinosus	100	0	66.9	33.1	3.2	96.8
Semimembranosus	100	0	90.5	9.5	7.1	92.9

[@] Myofiber weakly reactive for myosin ATPase after preincubation at pH 4.4-4.5.
[=] Myofiber unreactive for myosin ATPase after preincubation at the same pH.

semitendinosus, semimembranosus, and longissimus thoracis muscle (Table 2). In these muscles, all type IIa2 myofibers corresponded to the type IIB myofiber. Almost all type IIa1 and IIa2 myofibers corresponded to the type IIA myofiber in the serratus ventralis thoracis muscle. In all the muscles, type IIb myofibers corresponded to the type IIB myofiber.

Goat

All type IIa1 and many type IIa2 myofibers were weakly reactive for myosin ATPase, whereas many type IIb myofibers were unreactive in the muscles examined (Fig. 2). The type IIa1 and IIa2 myofibers would be designated as the type IIB myofiber and type IIb myofibers as the type IIA myofiber if they were classified on the basis of pH sensitivity of myosin ATPase (Table 2). Few type IIa2 myofibers corresponded to the type IIA myofiber, and only a few type IIb myofibers to the type IIB myofiber.

Discussion

The reactivity for acid-stable myosin ATPase in all ovine type IIa myofibers (7) has been shown to resemble that in the type IIB myofiber based on pH sensitivity of myosin ATPase (1) and the reactivity in numerous ovine type IIb myofibers to resemble that of the type IIA myofiber. The myofibers weakly reactive for acid-stable myosin ATPase contained type IIa and IIb myofibers in equine and porcine muscles. This finding is compatible with the results of the previous studies (4-6), and indicates that the type IIB myofiber does not always show a weak NADH-TR activity. Although type IIb myofibers were similar to the type IIB myofiber in porcine muscles, type IIb myofiber and the type IIB myofiber are not always interchangable, nor are type IIa myofiber and the type IIA myofiber always interchangable because the type IIB myofiber includes some type IIa1 and IIa2 myofibers. Similar findings have been shown in muscles of humans, rodents, and cats (2, 3). The type IIB myofiber classified on the basis of pH sensitivity of myosin ATPase cannot always be regarded as myofibers with a weak NADH-TR activity : glycolytic or white myofibers. Also, the type IIA myofiber does not always correspond to myofibers with a strong NADH-TR activity : oxidative or red myofibers in domestic animals.

The intensity of NADH-TR activity in myofibers indicates their capacity for oxidative metabolism (13) and for a resistance to fatigue in movement (14). Myofibers with a strong NADH-TR activity have been classified as oxidative (13) or red myofiber (15) and those with a weak NADH-TR activity as glycolytic or white myofibers. The findings of the present study show that the type IIA and IIB myofiber classified only by difference in pH sensitivity of myosin ATPase are not always interchangable with oxidative (red) and glycolytic (white) myofiber, respectively.

Subtypes of type II myofibers are distinguished by differences in pH sensitiv-

ity of myosin ATPase in the semitendinosus muscle but not in the serratus ventralis muscle of cattle (8). Because type II myofibers of sheep show no difference in reactivity for myosin ATPase after acid preincubation, they must be classified into type IIA and IIB myofibers by differences in the intensity of NADH-TR activity (10-12). This classification of myofibers does not lead to confusion for indicating their capacity for oxidative or glycolytic metabolism among species. The results of this study together with the histochemical characteristics of bovine and ovine myofibers (8) indicate that subtypes of type II myofibers should be classified on the basis of the intensity of NADH-TR activity in domestic animals if the capacity of subtypes for oxidative and glycolytic metabolism needs to be elucidated.

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