HISTOCHEMICAL ANALYSES OF PSE PORCINE MUSCLE FIBRES: UNUSUAL FINDINGS IN FIBRE TYPE DISTRIBUTION AND ENZYMOLOGY

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Keywords: pig, muscle, PSE muscle, histochemistry, fibre typing, ATPase activity

INTRODUCTION

Fibre type distribution is a relevant factor affecting meat quality and numerous studies have been carried out to define this relationship. Muscles of stress susceptible pigs have been found to account for a higher number of IIB fibres showing a greater diameter and a characteristic glycolytic metabolism (Rahelic and Puac, 1980-81; Bader, 1987).

Fibre typing is usually based on histochemical detection of the activity of the alkaline myosine-ATPase after alkaline or acid preincubation and/or the main enzymes involved in the oxidative metabolism, such as SDH and NADH-TR. A high sensitivity to pH and temperature has been observed for the myosin ATPase (Khan et al., 1974; Suzuki and Cassens, 1980).

The different methods allow for a varying degree of accuracy in fibre typing, thus leading to the identification of types I, IIA, IIB and sometimes IIC. The evaluation of fibre type distribution can be performed by analysing serial sections individually treated for a specific enzyme or examining a single section treated for various enzymes with a combination of methods. Each procedure, however, still has its drawbaks.

In recent years we have had the opportunity to examine a number of Longissimus dorsi muscles from pigs of different breeds and hybrids for histochemical fibre typing. We observed that fibre typing by detecting ATPase and SDH activity was very difficult in a number of PSE cases due to the unusual staining in sections treated for ATPase activity.

OBJECTIVE

In this paper unusual histochemical findings observed in PSE L. dorsi muscles are described and discussed.

MATERIALS AND METHODS

A total of 211 Longissimus dorsi muscles from Large White (49), Landrace x Large White (41), Hylete hybrid (50) and Goland hybrid (52) pigs were taken into consideration.

The muscles were classified as normal or slightly PSE when they showed a pH 1 higher than 5.8, and PSE when the pH 1 was 5.8 or.

Muscle samples collected at 1 hour after slaughter were sprinkled with talcum powder, wrapped in aluminium foil and immediately frozen and stored in liquid nitrogen until analyses were performed. Serial 10µm thick sections were obtained in a cryostat at -20°C and histochemically processed to determine ATPase at pH 9.4 (Padykula and Herman, 1955) after alkaline (pH 10.4) preincubation and Succinate dehydrogenase (SDH) activity (Nachlas et al., 1957). The PSE cases with an unusual reaction for ATPase and a representative sample of the remaining cases were processed for ATPase activity also after acid preincubation (pH 4.75, 4.60, and 4.35)

Photomicrographs of serial sections were taken and at least 150 single fibres were evaluated for each of the histochemical reactions.

RESULTS AND DISCUSSION

A relatively high percentage of the PSE porcine L. dorsi muscles examined showed a similar unusual finding of the ATPase activity after alkaline preincubation.

The pattern was characterized by the presence of a large number of fibres, ranging from about 20% up to about 80%, which did not show any, or only very weak, staining. These fibres were mainly located at the periphery of the bundles where the majority of type IIB fibres are usually detected. Mostly, this finding included all bundles in the section but in a few cases (2 muscles) only a number of bundles located in a large area involving about a half of the section were involved. No evidence of reversal in the staining pattern for ATPase activity was detected in these fibres after various acid preincubation and no SDH activity was observed.

The myosin ATPase of type IIB fibres is alkali-stable and acid labile and the SDH activity is barely or not detectable. This was the histochemical pattern observed in the majority of normal, moderate PSE and even severe PSE muscles considered in the present study.

In the PSE muscles with an unusual histochemical finding it seemed that the alkali-stable myosin ATPase of type II fibres, especially type IIB, was inactivated. A similar pattern was described by Fazarinc et al. (1995) who attributed the loss to the very low pH (<5.5) and high temperature which probably lead to the inactivation of the alkali-stable myosin ATPase in PSE porcine muscle. In our study this unusual finding was observed in a large number of PSE muscles with pH 1 lower than 5.5 (52.7%), but never in normal, slightly PSE and severe PSE muscles with pH 1 higher than 5.5 (Table 1), thus supporting the observation of the above mentioned researchers. However, it should be clarified whether the negative reaction for alkali-stable ATPase depends on an acid inhibition of the enzyme occurring in the muscle. Furthermore, the question arises as to why only 52.7% of muscles with pH 1 lower than 5.5 were involved.

The remaining fibres showed various types of reactions. A number of them were positive for ATPase activity after alkaline preincubation and acid preincubation at pH 4.75 and 4.60, even if with varying degrees of intensity, and were negative for ATPase after preincubation at 4.35 and for SDH activity. Thus, they showed the typical histochemical characteristics of type IIB fibres. They were mainly located at the pheriphery of the bundles.

A limited number of fibres showed strong staining for SDH activity and ATPase activity after preincubation at pH 4.35, but no ATPase activity after alkaline preincubation. The ATPase activity after acid preincubation at pH 4.60 and 4.75 showed varying degree of

staining intensity. These fibres could be classified as type I, but we cannot exclude that part of them were type IIC fibres with an unusual finding due to the inactivation of the alkali-stable ATPase.
Fibres with a positive reaction for SDH activity and ATPase activity after alkaline preincubation, even if with varying degrees of staining, were also detected. Depending on the intensity of staining for these enzymes and negativity or positivity for ATPase activity after preincubation at pH 4.35, 4.60 and 4.75 these fibres might be classified as IC, IIC, IIAC, IIA, IIAB types, respectively (Staron and Hikida, 1992). However, in nearly all cases only one or two of these possible types of fibres were detected in the same muscle. Probably the majotity of them could be better and more simply classified as intermediate fibres (type IIA) and a few as type C fibres. However, abnormal reversal staining in any of the fibres cannot be excluded.
In a few cases a small number of fibres only reacting for SDH were detected. It is somewhat difficult to classify these fibres and typing them as IIA could be in agreement with the previous hypothesis concerning the inactivation of alkali-stable ATPase of type II fibres.

CONCLUSIONS

PSE muscles with a pH 1 lower than 5.5 may frequently show an unusual histochemical finding due to the absence of detectable alkali-stable ATPase activity in a high percentage of fibres, probably belonging to the type II group. The histochemical fibre typing and the evaluation of the fibre type distribution in these cases is generally difficult. Of great importance is understanding whether and how the low pH and high temperature of the muscle itself affect the alkaline preincubation effect on the ATPase activity.
Moreover, the question arises as to whether a relationship between the histochemical appearance of alkali-stable ATPase inactivation and quality traits of PSE meat exists. Further investigations are needed to clarify this point. The possibility that the inactivation of this enzyme occurs and depends on the denaturation of fast myosin should also be taken into consideration.

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Table 1

Normal and PSE porcine Longissimus dorsi muscles.
Distribution of cases with unusual staining for ATPase activity after alkaline preincubation.

	PSE muscle pH 1h <5.51		PSE muscle pH 1h 5.51-5.80		slightly PSE/normal m. pH 1h >5.8	
cases / breed/hybrid	total	unusual	total	unusual	total	unusual.
Large White	19	8	10	0	20	0
Landrace x Large White	2	2	4	0	35	0
Hylete	15	9	17	0	18	0
Goland	0	0	2	0	50	0
TOTAL	36	19	33	0	123	0