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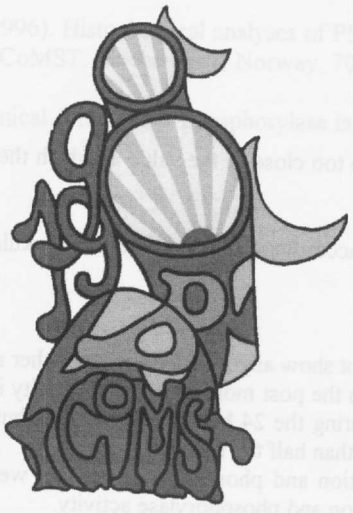
PS 8

Poster session and workshop 8

Muscle biology and biochemistry



Tuesday, September 1st
17:15h-18:45h

INVESTIGATION ON PORCINE PSE MUSCLES WITH VERY LOW pH VALUE

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INTRODUCTION

Fibres with unusual histochemical patterns have been observed in PSE pig muscles with very low pH value at one hour post mortem (Severini et al. 1996). These fibres were not reactive for ATPase at pH 9.4 both after alkaline and acid preincubation. On the basis of the location in the bundles they were supposed to be type II fibres.
The need of further investigations to clarify whether the absence of any ATPase activity was due to intra vitam pathological condition or to the post mortem effect of the very low pH when the muscle temperature is still high was stressed.
The ultrastructural characteristics and other histochemical patterns of those muscles are described in this paper and compared with the results of the histochemical reaction for ATPase. The results of tests carried out to evaluate the effect of extreme post mortem pH and temperature values on alkali-stable myosin ATPase activity, which is characteristics of type II fibres, are also reported.

MATERIALS AND METHODS

a)
Samples from porcine Longissimus dorsi muscles showing very low pH value (< pH 5.5) at 1 hr post mortem were collected at this time, sprinkled with talcum power, wrapped in aluminium foil and immediately frozen and stored in liquid nitrogen until analyses were performed. Slices of 10μ were used to evaluate ATPase activity after alkaline (pH 10.4), and acid preincubation (pH 4.75, 4.60 and 4.35), and phosphorylase activity (Takeuchi and Kuriaki, 1955; Severini and Aglietti, 1994), and those of 12μ to evaluate glycogen content by means of PAS reaction.
Samples were also taken for E.M. examination. They were fixed for 2 hr in 2.5% glutaraldehyde in 0.2M phosphate buffer, pH 7.3, at room temperature. They were post fixed in a solution of 1% osmium tetroxide, phosphate buffer 0,1M, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a Philips EM 400 transmission electron microscope.

b)
A trial was carried out to determine the concomitant effect of post mortem low pH value and high temperature on the histochemically detectable alkali-stable myosin ATPase activity. Samples were taken at 1 hr post mortem from a normal Longissimus dorsi porcine muscle and frozen in liquid nitrogen, as previously described. Slices of 10μ were used to evaluate ATPase activity at pH 9.4 after alkaline preincubation at pH 10.4 and acid preincubation, as follows:

- pH 5.43 T° 41°C for 1 hr
- pH 5.43 T° 41°C for 2 hr
- pH 5.15 T° 41°C for 1 hr
- pH 5.15 T° 41°C for 2 hr
- pH 4.95 T° 41°C for 1 hr
- pH 4.95 T° 41°C for 2 hr

Lower pH values were not used because they were too close to the value at which the activity of this enzyme is inhibited after a short-period in vitro incubation.

The myosin ATPase activity at pH 9.4 was tested according to the method of Padykula and Herman (1955).

RESULTS AND DISCUSSION

A large number of fibres in the PSE muscles did not show any ATPase activity either after alkaline or acid preincubation. Greaser et al. (1969) observed a considerably different change in the post mortem ATPase activity in myofibrils of normal and PSE porcine muscles. The former showed an increase of this activity during the 24 hours post mortem, but the latter a significant drop within 30 min after death and by 24 hr reached a level which was less than half the initial activity.
Only very few fibres showing positive PAS reaction and phosphorylase activity were detected. The fibres which did not show any ATPase activity were also negative for PAS reaction and phosphorylase activity.
The presence of very few or no fibres with detectable PAS reaction and phosphorylase activity at 1 hr hour post mortem is a typical finding in PSE muscles (Severini and Aglietti, 1994). Therefore, it is not surprising that also the fibres in question were negative for PAS and phosphorylase reaction.
The absence of the phosphorylase activity might depend on the inactivation of the enzyme caused by the low pH. On the other hand, the low pH value quickly reached after death might prove that this enzyme was effective in metabolising muscle glycogen during the post mortem period.

On the basis of our experiment there is no way to evaluate if phosphorylase and other glycolytic enzymes were effective in causing a rapid pH drop in a specific fibre. In fact, the pH value was detected in the whole muscle and not in a single fibre.
Ultrastructural changes were observed in a relatively large number of glycolytic fibres (type II), but an exact correlation between E.M. and histochemical findings in specific fibres was not established. Fragmentation of myofibrils, destruction of myofilaments, hydropic swelling of l-tubules and mitochondria were frequently detected.
These ultrastructural changes were not clearly observed at the level of histological examination, but weak morphological modifications and the presence of a greater number of giant fibres were reported in these muscles (Severini and Loschi, 1997). The presence of a latent myopathy has been supposed in agreement with other authors (Bergmann, 1979) .
The results of the trial carried out to evaluate the resistance of alkali-stable myosin ATPase activity to post mortem low pH and high temperature showed that the activity of this enzyme was still detectable in a large number of type II fibres after extreme treatments such as preincubation of 10μ slices for 2 hr at pH 5.15 and 41°C. A weak inhibition was observed at pH 4.95 already after a preincubation of 1 hr. The alkali-stable myosin ATPase is usually present in type II fibres of normal porcine muscle and is inactivated after acid preincubation for 5 min at pH 4.6.
The pH values and temperatures adopted in this experiment were higher than those detected in the PSE muscles under observation and were applied for a longer period of time than with the pig carcasses after slaughter. Therefore, the experimental conditions were supposed to be much more stressful than in field. The relative high stability post mortem of this ATPase activity to long-period acid incubation in normal muscle seems to indicate that its absence in the PSE muscle with very low pH should be not attributed to post mortem acid inactivation.

CONCLUSIONS

Our results lead to the conclusion that the most probable cause of the presence of a large number of fibres (feasibly type II fibres) showing no myosin ATPase activity in the examined PSE muscles with very low pH might be due to an intra vitam latent myopathy.

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