METABOLIC RESPONSE AND GIANT FIBRES IN MUSCLE FROM PIGS AFTER SLAUGHTER

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SUMMARY

The cause for the appearance of giant fibres observed in post-mortem muscle samples is still not well understood. The purpose of this study was to analyse metabolic response and pH in muscle samples (M longissimus dorsi) from two earlier studies in which giant fibres were found. In study A, samples were obtained immediately at exsanguination from ten halothane-gene free and ten halothane homozygote Yorkshire pigs which had been lairaged for 2 hours before they were electrically stunned without a restrainer. In study B, samples were obtained 30 min after exsanguination from twentyone F2 animals (crosses between the European wild boar and the Swedish Yorkshire pig breed) which had no lairage and were electrically stunned using a restrainer. Eleven of these pigs were carriers of the halothane-gene. Samples were taken both for histochemical (myofibrillar ATP-ase, NADH-tetrazolium reductase, PAS and Sudan black B) and biochemical (pH, CP, ATP, ADP, AMP, IMP, lactate, glycogen) analyses. Two days post-mortem, colour of the meat was measured as surface reflectance.

In study A giant fibres were found in three of the halothane homozygote pigs. These pigs had the highest lactate and IMP concentrations and lowest glycogen, ATP and CP concentrations, with muscle pH less than 6.3. In study B, most of the pigs revealed giant fibres although a large variation was seen in the number of these fibres. The appearance of giant fibres was positively correlated to lactate and IMP concentrations and negatively correlated to pH and ADP concentrations. The results show that the halothane gene and metabolic stress-situations in muscle will give rise to giant fibres. Furthermore, the results indicate that the appearance of giant fibres is related to glycogenolysis with lactate accumulation and a marked pH fall in connection with slaughter and a pronounced ATP degradation with IMP accumulation. The appearance of giant fibres could be one factor of importance for meat quality, and a positive correlation was seen between the number of giant fibres and reflectance values of the meat.

Introduction

Giant fibres have been observed on histochemically stained sections from muscle samples obtained after slaughter from both turkeys, chicken, bovine and pigs (Klosowska et al. 1979, Bader, 1982, Sink et al. 1986, Sosnicki et al. 1991). These fibres are usually found in the periphery of a fascicle among type IIB fibres and have an oval or round shape which differ from the normal fibre pattern seen in a cross-section (Cassens et al. 1969). Giant fibres have been said to represent a different fibre type and to be an anomaly caused by muscle contraction during rigor development. They seem to appear only in muscle samples obtained after slaughter and mainly in muscle with PSE-charactheristics (Klosowska and Klosowski, 1985). Giant fibres have been observed in muscles from wild pigs but were then located in the centre of the fascicle and had properties that resembled type I and IIA fibres (Solomon and Eastridge, 1987). Giant fibres have also been found in pigs which succumbed due to a diet low in E-vitamin (Jensen et al. 1988). This was said to be related to peroxidation and free radical formation giving rise to membrane damage and tissue destruction.

The cause for the appearence of giant fibres is still not clear. One hypothesis could be that giant fibres develop due to metabolic stress-situations in connection with slaughter. The purpose of this study was therefore to analyse pH and metabolic response in muscle samples obtained after slaughter which contained different amounts of giant fibres.

Material and methods

The muscle samples (M.longissimus dorsi) investigated in the present study were obtained in two earlier studies (Essén-Gustavsson et al. 1992, Karlssson, 1993). In study A, muscle samples were obtained immediately at exsanguination from ten halothane-gene free and ten halothane homozygote Yorkshire pigs which were slaughtered the week they reached 100kg. They were transported 5 km from the research station to the abbattoir and had been lairaged for 2 hours before they were electrically stunned without a restrainer. In study B muscle samples were obtained 30 min after exsanguination from twentyone F2 animals (crosses between the European wild boar and the Swedish Yorkshire pig breed). Eleven of the F2 pigs were carriers of the halothane gene ($Hal^{N}Hal^{n}$) whereas ten pigs were free of this gene. The pigs were slaughtered the week their live weight reached 80 kg or at a minimum age of 190 days. They were transported 5 km from the research station to the abbattoir. Because of a violent behaviour of these pigs they had no lairage and were immediately electrically stunned using a restrainer. In both study A and B the samples from M.longissimus dorsi were taken both for histochemical and biochemical analyses. Except for observations of giant fibres the results from study A have been presented earlier (Essén-Gustavsson et al. 1992).
Histochemical analyses: Serial transverse sections were cut in a cryostat and stained for myofibrillar ATPase after acid (pH 4.3 for 3 min and pH 4.6 for 5 min at room temperature) and alkaline (pH 10.3 for 9 min at 37°C) preincubation (Brooke and Kaiser, 1970), NADH-tetrazolium reductase (Novikoff et al. 1961), PAS (Pearse, 1961) and Sudan black B (Dubowitz, 1985). The number of giant fibres revealed in the NADH stain was counted on a circular screen (19 cm diameter) of a Visopan-microscope with a 55 x magnification.
Biochemical analyses: Muscle samples were freeze-dried and dissected free of blood, connective tissue and fat, then weighed and extracted in perchloric acid before being neutralised with potassium hydroxide. ATP, ADP, AMP and IMP concentrations were determined using a HPLC-technique (Sellevold et al. 1986). Lactate and glycogen concentrations were determined using fluorimetric techniques (Lowry and Passoneau, 1973). Muscle pH was analysed on a pH-meter after homogenisation of muscle in iodoacetate neutralized to pH 7.0.
Meat colour was measured at 2 days post-mortem as surface reflectance (EEL, Diffusion Systems Ltd, London, England) using a Y-filter (400-700nm).

Results and Discussion.

In study A, giant fibres were seen in 3 of the halothane homozygotes which had 9, 4 and 3 giant fibres and a muscle pH of 5.98, 6.21 and 6.25 at slaughter. In samples with no giant fibres muscle pH was above 6.28. The metabolic response at exsanguination is shown to differ between halothane homozygotes and non-carriers (Table 1). The three pigs that revealed giant fibres seem to have been exposed to the highest demand for ATP in connection with slaughter as they had the highest lactate (146-219 mmol/kg) and IMP (10.9-15.7 mmol/kg) concentrations and lowest ATP (1-16 mmol/kg) and CP (0-4 mmol/kg) concentrations. The fact that they were homozygotes for the halothane gene is likely a contributing factor to this metabolic response. These pigs are shown to have large fibre areas and a low capillary density which may cause not only a limitation in oxygen supply but also in release of waste products like lactate (Essén-Gustavsson et al. 1992). In study B, most of the pigs revealed giant fibres although a large variation was seen in the occurence of these fibres (Figure 1). The appearance of giant fibres showed a significant negative correlation to pH (r= -0.52) and ADP (r= -0.61) concentration and a significant positive correlation to IMP (r= 0.60) and lactate (r= 0.57) concentrations. IMP concentration was highest in the carriers of the halothane-gene and most giant fibres were seen in two of these pigs. However, one pig that was a carrier of the halothane gene was an exception as it had no giant fibres. Notable was that this pig also had the lowest IMP (3.1 mmol/kg) and lactate (141 mmol/ kg) concentration and the highest ATP (16 mmol/kg) concentration of all the pigs. In contrast, most of the pigs revealed high lactate, IMP and ADP concentrations. The pigs were exposed to a stressful situation pre-slaughter and therefore energy demand might have been high in the muscles of these pigs. The metabolic response is related both to the post-mortem breakdown of glycogen and ATP but also to the exposure to different stressful treatments pre-slaughter. When the rate of energy utilisation exceeds the rate of production ADP concentration increase in muscle. An increased ADP content leads to AMP formation via the myokinase reaction and IMP formation via the AMP-deaminase reaction which has its pH-optimum around 6.3 (Wheeler and Lowenstein, 1979). When pH is lowered to values around 6.3 due to increased rate of glycogenolysis and lactate accumulation this seems to stimulate IMP production. A close correlation was found between muscle lactate and IMP concentrations (Figure 1). In agreement with previous results in study A, lactate concentrations around 150 mmol/kg correspond to pH values around 6.3. Results from study A has previously shown a pH linked treshold for the onset of IMP-formation in muscle (Essén-Gustavsson et al. 1991). The appearance of giant fibres in this study

and the correlation to lactate and pH support an earlier study on pigs in which giant fibres, 5 min post mortem, were rather low in M.longissimus dorsi of normal muscle but higher in PSE muscle (Klosowska et al. 1984). That study also showed that an increase had occured in giant fibres 45 min post mortem. Similar findings have been observed in chicken where samples obtained 15 min after slaughter had a higher content of giant fibres compared with samples obtained immediately after slaughter (Klosowska et al. 1979). In the present study, the samples taken 30 min after exsanguination had higher IMP and lactate concentrations and a greater number of giant fibres than the samples in study A, taken immediately at slaughter. Pigs that are carriers of the halothane gene seem to be susceptible for development of giant fibres. In both syudy A and B the highest IMP and lactate concentrations and most giant fibres were found among these pigs. The appearance of giant fibres could be one factor of importance for meat quality, and a positive correlation was seen between the number of giant fibres and reflectance values of the meat (r=0.55).

The giant fibres were shown to have an unnormal staining pattern and were usually located in the periphery of a fascicle among type IIB fibres (Figure 2). In agreement with previous observations the fibres appear as type IIB fibres as they are heavily stained with both 4.6 and 10.3 preincubations. Fibres with an oval shape on one stained section did not always appear as giant on another serial stained section. They could be quite small, still having the oval shape, or they seemed to have disappeared. This indicates that not only hypercontraction but ruptures and other structural changes may occur within a giant fibre It is likely that the unnormal staining pattern of giant fibres could be related to these changes. It can be questioned if the NADH-tetrazolium reductase and Sudan black B staining intensity in giant fibres indicate a high oxidative capacity and a high lipid content in these fibres or is an artifact due to structural changes. Unstained fibres in the PAS-stain, however, likely reflect glycogen depleted fibres.

The results in the present study indicate that the appearance of giant fibres is related to damages of cell membranes and hypercontraction due to a pronounced glycogenolysis with lactate accumulation and ATP degradation with IMP accumulation in muscle. Further studies with investigation of metabolic response in single fibres are, however, needed to better understand the cause for appearance of giant fibres.

Conclusion

The results of this study show that both the halothane gene and metabolic stress-situations, especially stressful treatment of pigs before slaughter, will give rise to giant fibres. Furthermore, the results indicate that the appearance of giant fibres is related to glycogenolysis with lactate accumulation and a marked pH fall in connection with slaughter and a marked ATP degradation and.IMP production.

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