ANTE- AND POST MORTEM 31P NMR STUDY ON SKELETAL MUSCLE METABOLISM RESPONSE TO HALOTHANE AND SLAUGHTER IN HETEROZYGOUS AND MH NORMAL PIGS.

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An occurence of mulfunctioning ryanodine-binding Ca2+ channel in the sarcoplasmic reticulum has been connected with malignant hyperthermia (MH), (Mickelson et al., 1988, Fuji et al., 1991). The heterozygotes express both the normal and the MH allele and the MH condition in pigs causes pale, soft, and exudative (PSE) meat at significant level in heterozygotes (Jensen, Barton-Gade, 1985, Cheah et al., 1993). The advantages of the DNA-based test are that it is highly accurate and able to identify heterozygotes but is not sufficient to elicit all the MH susceptible individuals and to explain all PSE meat quality occurence (Cheah et al., 1994). It looks to be important modulating factors of the MH syndrome that must be present for full expression of the syndrome or if it is absent it is not observed (Fletcher et al., 1993). It was shown that of the capacity the muscle energetic metabolism (Chance et al., 1986) could be very important factor influencing and modulating variation in the expression of the MH syndrome . Recently also 31P NMR studies on skeletal muscle metabolism in vivo on genetically identified heterozygotes MH pigs were introduced (Moesgaard et al., 1995, Scholz et al., 1995).
The aim of the present study was to determine energetic metabolism and pH in skeletal muscle on entire heterozygous and MH normal pigs by applying of halothane and time course post mortem changes using 31P NMR spectroscopy measurements.

MATERIAL AND METHODS

DL pigs (18.5 - 55 kg live weight) were used in this experiment. Animals at approx. 20 kg were tested by halothane and the MH genotypes (normal, monomutant) were identified by DNA gene test (a ryanodine receptor - RYR 1 gene test using polymerase chain reaction technique - PCR), (Fujii et al., 1991) in laboratory of Genetic Institute of George-August University in Gottingen using tissue probe of slaughtered animals.
To prevent movement during NMR measuremnt pigs were anesthetized by intra muscular administration of Tilest (30 mg/kg body weight) and after positioning the pigs oxygen was administered. In vivo after obtaining five reference spectra pigs were exposed via face mask to halothane at concentration of 2% in the semiopen system with oxygen (100%) flow of 3L/min. The maximum time of halothane exposure was limited to 20 minutes. But the halothane administration was stopped earlier before reaching the time limit after observing significant changes in the PCr peak in consecutive spectra.
The 31P NMR experiments were performed in a 1.5 Tesla with a wide bore (60 cm) of the NMR device (Biospec 1.5/100, Bruker, Karlsruhe). Phosphorus signal were obtained at 26 MHz using 10 cm diameter two - turn surface coil placed above the lateral side of M. biceps femoris. The average of five reference spectra before halothane exposure was set to 100% (PCr signal = 100%). During the experiment, spectra were obtained every 60 s by repetition time of 600 ms.
For carcasses (slaughter weight for normal pigs average 66.3 kg, heterozygotes 58.4 kg), post mortem changes (30 min to 120 min) of inorganic phosphate (Pi), phosphocreatine (PCr), adenosine triphosphate (ATP) were observed during the experiment. pH values were calculated from the chemical - shift difference between Pi and PCr using a Henderson - Hasselbach equation (Moon, Richards, 1983).
Statistical significance between different genotypes was evaluated using the twotailed Student's test.

RESULTS AND DISCUSSION

Plots of PCr/Pi versus time obtained from the spectra of different MH genotypes have shown significant differences before the halothane exposure (Tab 1). The energy states characterised by PCr/Pi was at heterozygotes significant higher to normal pigs. During and after the halothane exposure there were only little and not significant changes at monomutant and nonmutant pigs observed. Also PCr/ATP ratio before halothane exposure showed significant differences between different MH genotypes.
From the results introduced by Meyer et al. (1985) using 31P NMR measurements followed higher PCr/Pi ratio of fast-twitch type muscle fibers to compare to slow-twitch type muscle fibers. Higher PCr content was later found in alfa W muscle fibers (Uhrin and Liptaj, 1991) and in type II fibers (Soderland et al., 1991). In agreement to others (Decanniere et al., 1993) the higher basal intracellular ratio PCr/Pi or PCr concentrations observed in heterozygotes (monomutant) might reflect a different fibre composition and/or an „endogenous" effect as a consequence of a higher than normal sensitivity (Decanniere et al., 1993) connected with an occurence of one allele (monomutant) of mutated of RYR 1 gene. After halothane administration the differences between heterozygotes and normal pigs dissapeared. At our experimental condition (using 2.0 % vol. Hal. and 20 min of administration) we were not able to find differences between heterozygotes and normal pigs during and after administration of halothane.
The post mortem changes of metabolite level and pH of carcass were followed continuously by 31P NMR spectroscopy with beginning of first spectrum at 25.3 ± 5.1 min of nonmutant (n=9) and 26.8 ± 4.8 min of monomutant (n=5) after killing untill 180 min post mortem. Kinetic parameters (non linear regression) for time course changes of PCr and Pi (Tab 2) and rate of pH fall during two hour post mortem were calculated too.
The differences between monomutant and nonmutant as follows from the results (Tab 2) on rate parameters for PCr and Pi were significant with 6.2 min longer of half time ($t_{1/2}$) breakdown of PCr at monomutant pigs. Monomutants shown also at the same time course higher decline of pH (8.51×10^{-3} unit per min) if compare to normal pigs (3.61×10^{-3} unit per min). The differences in rate of pH decline at monomutants were more as twice of normal pigs.
As followsfrom our post mortem results (Tab 2,) using kinetic 31P NMR measurements we found significant differences between heterozygotes and normal pigs in rate of PCr breakdown and pH changes. Our results confirmed that slaughtering could exagerate breakdown of PCr and glycolytic process in different level in heterozygotes and normal pigs but inspite this normal quality of meat is expected also from our heterozygotes at light slaughter weight (below 70 kg l.w.). This could be in agreement with Sather et al. (1992)

who introduced the dominance of halothane gene in heterozygous pigs reflected in meat quality values appeard to be weight dependent. Our research using 31P NMR spectroscopy measurements of entire heterozygous pigs carrying one copy of mutated RYR 1 gene is progressing in age and breed dependence.

CONCLUSION

Slaughtering could exagerate breakdown of PCr and glycolytic process in different level in heterozygotes and normal pigs also at light (below 70 kg l.w.) slaughter weight.

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Tab 1. Ratio PCr/Pi and PCr/ATP in rest biceps femoris metabolism in

Genotype	PCr/Pi	PCr/ATP
NN (n = 12)	7.17 ± 1.64	3.67 ± 0.61
Nn (n = 16)	8.56 ± 2.30	3.90 ± 0.68

Differences between genotypes are significant at $P < 0.05$

Tab. 2 Kinetic parameters for post mortem Pcr and Pi biceps femoris

Genotype	k (min $^{-1}$) (x 10^2) PCr	Pi	$t_{1/2}$(PCr) (min)
NN (n = 9)	2.46 ± 0.37	1.47 ± 0.60	28.1 ± 4.9
Nn (n = 5)	3.17 ± 0.34	0.72 ± 0.43	21.9 ± 2.6

Differences between genotype are significant at $P<0.05$