INFLUENCE OF TEMPERATURE AND HALOTHANE SENSITIVITY ON CONTRACTION TRAITS AND METABOLISM IN THE *TIBIALIS CRANIALIS* OF PIGS BEFORE AND AFTER EXCISION

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SUMMARY

The experiment involved 6 halothane-positive (HP) and 6 halothane-negative (HN) pigs. The twitch characteristics and the levels of PC, ATP, glycogen, lactate and pH were examined in the *tibialis cranialis* muscle maintained at either 35 °C or 40 °C, *in situ* and after excision. Both temperatures were applied to each animal and to each excised muscle in a balanced design. Halothane sensitivity influenced most biochemical traits in the muscle *in situ*. Temperature affected only the contraction time. In the excised muscle, both halothane sensitivity and post-excision temperature affected the biochemical changes; by contrast, the *in situ* temperature treatments had no effect. It was concluded that the effects of the various treatments on the rate of metabolism in the excised muscle were wholly explainable in terms of temperature from the time of excision.

Introduction

Several authors have shown that an increase in the body or muscle temperature of pigs at the time of slaughter has unfavourable effects on *post mortem* muscle metabolism and meat quality (Sayre *et al.* 1963; Monin, 1973; Gariepy *et al.*, 1989; Warriss, 1991; Klont and Lambooy, 1994). There is a question whether the *post mortem* acceleration of muscle metabolism is due purely to the activating effect of temperature on biochemical reactions, or if it is elicited by the continuation of the action of *ante mortem* physiological factors. Indeed, changes in the body temperature are generally induced by placing the animal in stressful situations, and so they are associated with other physiological disturbances susceptible to affect muscle metabolism, such as circulatory and respiratory changes (Forrest *et al.*, 1968; Charpentier *et al.*, 1971; Judge *et al.*, 1973) as well as hormonal changes (Aberle *et al.*, 1974; Lundström, 1976).

The aim of the present experiment was to assess specifically the effects of temperature variations on the metabolism of the muscle *in situ* and of the muscle made anoxic by excision. We used the experimental preparation described by Campion *et al.* (1972). The *tibialis cranialis* was exposed and prepared to measure contraction characteristics. It was stimulated to work isometrically at a rate inducing a moderate fatigue, and the effect of a temperature rise of 5 °C above the normal resting value (about 35 °C) on contraction and metabolic traits was investigated *in situ* and after excision.

Material and methods

Six halothane-positive Belgian Landrace pigs and 6 halothane-negative Large White x Dutch Landrace crossbred pigs of about 100 kg liveweight were used. The animals were injected intramuscularly with azaperone about 30 min before being anaesthetized by an intravenous injection of sodium pentobarbital. They were intubated and ventilated with N2O/O2. An intravenous pentobarbital infusion was applied *via* the ear vein to maintain anaesthesia. The *tibialis cranialis* was exposed and dissected free from surrounding muscles. The distal tendon was cut and attached to a force transducer. The temperature of the muscle was brought to either 35 or 40 °C (*in situ* temperature) by an infrared lamp, then kept at this temperature (± 0.5 °C). Twitch contractions were elicited by stimulation of the peroneal nerve (stimulation: 1 ms; 0.1 Hz; 50 V). The electric signal was recorded using a MAC II microcomputer equipped with a MacAdios card. The nerve was stimulated at 0.1 Hz for 10 min, then at 1 Hz for 10 further min. Then the muscle was carefully excised, weighed and split into 2 parts. Each part was put into paraffin oil at either 35 or 40 °C ('post-excision temperature'). Muscle pieces were taken just before and after the stimulation period, and at 60 and 120 min after excision for

determination of pH and metabolites. As soon as one muscle was excised, the operation was repeated on the other leg.

At sampling, one muscle sample was put into an Eppendorf plastic tube and dipped into liquid nitrogen. The other one was put into 0.5 ml of 0.005 M Na iodoacetate in a glass microextractor and immediately crushed with a glass pestle for pH measurement. The frozen samples were freeze-dried and stored at -20 °C under vacuum until they were used for determination of glycogen, G-6-P and lactate by enzymatic techniques, and of phosphocreatine (PC) and adenosine triphosphate (ATP) by HPLC.

Statistics

Data were obtained from 12 muscles for the 35 °C *in situ* temperature and from 11 muscles for the 40 °C *in situ* temperature (6 halothane-negative and 5 halothane-positive animals). They were analyzed by variance analysis. When significant interactions were found, differences between temperatures and times were assessed by t-test. All calculations were carried out using the Statview SE+Graphics programme.

Results

The changes in contraction parameters during muscle work are illustrated in Table 1. The peak tension remained approximately constant during stimulation at 0.1 Hz whatever genetic type and temperature, then it decreased during stimulation at 1 Hz. Half relaxation time increased with time. There were interactions between genetic type and time for the peak tension ($P < 0.01$) and the contraction time ($P < 0.05$) (Table 2). The peak tension decreased more in halothane-positive pigs than in halothane-negative pigs (-50 % vs. -19 %, $P < 0.01$) during 1 Hz stimulation. The contraction time tended to decrease in halothane-negative pigs while it tended to increase in halothane-positive pigs ($P < 0.10$) with time.

The biochemical characteristics measured from the muscle *in situ* are reported in Table 3. The temperature had no significant effect on any trait. There was a significant interaction between genetic type and temperature ($P < 0.05$) for the lactate content (Table 4) which was higher in halothane-positive pigs than in halothane-negative pigs at 40 °C.

In excised muscle, the levels of most compounds and the pH values were influenced by the genetic type, the post-excision temperature and the time (Table 5). Again, the *in situ* temperature had no effect on any trait. There was an interaction between genetic type and *in situ* temperature for the lactate content (Table 4).

Discussion and conclusion

The fact that the peak tension was more decreased in halothane-positive pigs than in halothane-negative pigs during 1 Hz stimulation indicated that the former were more sensitive to muscle fatigue. The increase in the contraction time in halothane-positive pigs during the 1 Hz stimulation is suggestive of some disturbance in the contraction process, maybe in the intracellular calcium regulation (Campion *et al.*, 1974). The increase of muscle temperature from 35 °C to 40 °C can be considered as very large from a physiological point of view. However, it did not affect significantly the function and the metabolism of the muscle, except that it shortened slightly the contraction time and it increased the lactate content in the halothane-positive animals. This indicates that the metabolic changes observed in muscles of pigs whose body temperature is increased by exposition to stressful conditions are probably not determined mainly by the temperature itself, but are mediated by the physiological changes (hormonal, respiratory, circulatory) associated with the efforts of the animal organism to adapt to an aversive situation.

Although it was significant, the effect of the genetic type on the biochemical changes following excision was reduced, compared to the differences reported elsewhere for muscles of slaughtered halothane-negative and halothane-positive pigs. This may be due to the general anaesthesia, as Klont *et al.* (1993) showed that the *post mortem* changes are much slowed down in halothane-positive pigs slaughtered under anaesthesia.

Klont *et al.* (1994) reported that an increase of the temperature of 4 °C (from 38 °C to 42 °C) doubled approximately the rate of *post mortem* biochemical changes in isolated muscle strips of pig semitendinosus. In the present study, the lactate content increased on average from 21 μmol/g to 29 and 39 μmol/g ($P < 0.01$) after 1 h incubation at 35 °C and 40 °C, respectively, while the ATP content decreased from 6.4 μmol/g to 5.2 and 4.2 μmol/g ($P < 0.05$), respectively. Therefore, the present results confirm the conclusion of Klont *et al.* (1994) that the effect of temperature variation, in the upper physiological range, is higher than it could be expected from the known relations between temperature and rate of biochemical changes in anoxic muscle.

Finally, the effects of the various treatments on the rate of metabolism in the excised muscle were wholly explainable in terms of temperature from the time of excision. The temperature treatments in situ may not be responsible for the differences after excision. The present results suggest that the control of muscle temperature is less important before than after the death of the animals from the point of view of meat quality. However, in practice, the muscle temperature of slaughter pigs should be kept as low as possible before killing, as it determines directly the muscle temperature in the immediate *post mortem* period.

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