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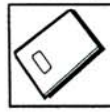
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RESEARCH FILE



Effects of slaughter at the cellular level

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Introduction

The quality of meat depends largely on the rate of pH fall in postmortem muscle. Large variations in the rate of postmortem pH fall can be observed as a function of slaughter conditions and/or genetic differences, especially in pigs. Slaughter induces ischaemia in muscle tissue, i.e. deprivation of oxygen and nutrients. The oxygen deprivation leads to a virtual, absolute anoxia within a few minutes. Such an anoxia totally inhibits mitochondrial phosphorylation. The subsequent decrease in ATP and increase in ADP and AMP stimulates glycolysis and glycolysis. This breakdown of ATP, accompanied by proton release, and the accumulation of lactate generated by glycolysis, induces acidification in postmortem muscle. The mechanisms responsible for variation in this acidification are not clear. Various hypotheses have been put forward to explain cellular injury under conditions of enhanced ATP depletion. Ca^{2+} ions seem to be involved as an accelerating factor in ATP turnover and anaerobic breakdown of glycogen, but direct proof of the involvement of calcium in early postmortem metabolism is still lacking.

Studies at the cellular level

Zuurveld *et al.* (1985) demonstrated that skeletal muscle cells in culture show oxidative capacity and enzyme activities which are comparable with those observed in mature muscle. Differentiated muscle cells (or myotubes) in culture, in the absence of nerves, display relatively high levels of glycolytic enzymes and are similar metabolically to glycolytic fibres (Lawrence and Salsgiver, 1983). Such

a model allows the extracellular environment to be controlled via both chemical (hormones, substrates, etc.) and physical (temperature, oxygen, etc.) conditions. The myotubes can therefore provide a model to study the intracellular disturbances occurring in the muscle at slaughter.

In our studies, muscle cells in culture have been used as a model to investigate the effects of hypoxia on skeletal muscle metabolism and ionic balance. In order to mimic the effects of anoxia occurring in the muscle at slaughter, cell metabolism was inhibited by the use of chemicals. The aim of our work was to investigate intracellular calcium and pH variations in response to metabolic inhibition. Variations in ATP concentration and lactate dehydrogenase (LDH) release were assessed as indicators of energy depletion and of membrane injury, respectively.

The myogenic precursors were satellite cells isolated from the masseter muscle of adult normal (HN) pigs and from halothane susceptible (HS) Pietrain pigs. Cells were grown until a differentiation stage where the

myofibrillar features were largely developed (Figure 1). Prior to the experiment, the cells were loaded with specific fluorescent probes, fura2-AM and BCECF-AM, in order to estimate the intracellular free calcium ($[\text{Ca}^{2+}]_i$) and pH (pH_i), respectively.

Ca and pH in a muscle cell under chemical hypoxia

Whatever the cell type, intracellular pH (pH_i) decreased abruptly in response to chemical inhibition within the first 10 minutes and then stabilised at 6.5.

There was a transient and early increase of intracellular calcium level ($[\text{Ca}^{2+}]_i$) in normal cells, while $[\text{Ca}^{2+}]_i$ increased slowly and progressively in HS cells. Ultimate $[\text{Ca}^{2+}]_i$ was about the same in both type of cells. ATP levels decreased dramatically in the first minutes of hypoxia whatever the cell type. The release of LDH was not significantly increased during the experiment. This suggests that, under normal conditions, the metabolism in cells under hypoxia does not depend on the genetic background of the cell, although the HS



Figure 1. Electron microscopy of a muscle cell in culture (myotube), after five weeks of differentiation. Note the presence of myofibril with regular sarcomeres, nucleus and numerous mitochondria. ($\times 5700$).

NOTES & DIGESTS

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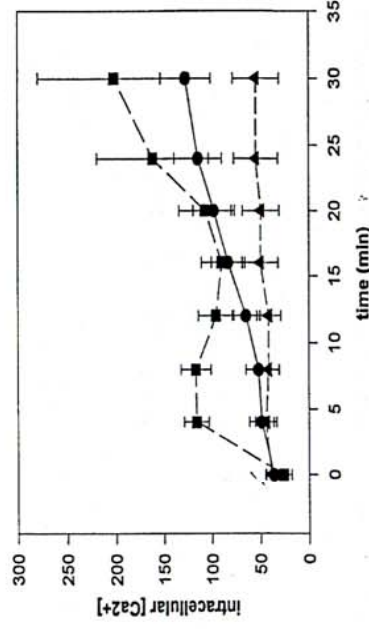
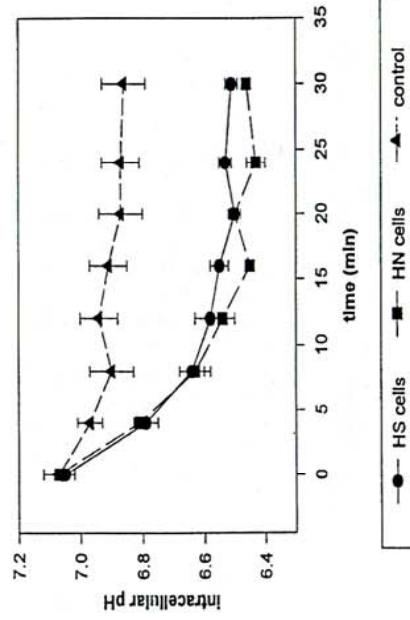


Figure 2. Intracellular pH and $[Ca^{2+}]$ variations of myotubes, in response to chemical hypoxia (buffer containing sodium cyanide; NaCN). HN = cells from normal pigs; HS = cells from halothane sensitive pigs after treatment with NaCN. Means and SEM are presented.

cells have been previously shown to have an altered Ca regulation in comparison with HN cells (Archer *et al.*, 1992). Other triggering agent(s) are needed for the expression of a disturbed metabolic behaviour in HS cells compared with normal cells. Blebs (membrane swelling) and cell death, observed after 30 minutes of chemical hypoxia, occurred more frequently in normal cells than in HS cells.

These results are in accordance with those obtained under the same conditions but using a mouse muscle cell line. They suggest that cell death is not directly related to $[Ca^{2+}]$ increase in muscle cells although intracellular calcium increase is known to be associated with ultrastructural and membrane damage in isolated muscle

(Duncan and Jackson, 1987). Acidosis reflects the metabolism in hypoxic cells. It occurred very quickly in our cells but our results suggest that it is not governed by $[Ca^{2+}]$ and/or extracellular calcium. The rapid drop of pH, associated with the depletion of high energy phosphates may determine cell injury leading to death in muscle cells.

Conclusions

Preslaughter stress leads to various physiological changes, among which increased body temperature could be of practical significance. Indeed, at slaughter this parameter varies considerably from one animal to another and it may influence the rate of postmortem glycolysis (Monin *et al.*,

1995). Another important parameter is the increase in circulating hormones, especially catecholamines, in response to preslaughter stress and electrical stunning (Van der Wal, 1978). The work presented in outline here suggests that it may well be these different physiological features that are critically important in the control of biochemical changes, such as ionic translocations in response to hypoxia, in muscle postmortem and these should be a focus of attention in the future. This fundamental approach is needed to help our understanding of the factors which control postmortem biochemical processes and thus, to optimise slaughter conditions from a meat quality point of view.

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