PHOSPHOLIPASE A_2 PRODUCTS ARE INVOLVED IN THE LOSS OF OSMOLYTES AND CELL WATER IN MEAT

Niels Ørtenblad[1], Ian Henry Lambert[2], Jacob Holm Nielsen[1] and Henrik Jørgen Andersen[1]

[1]Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark, [2]The August Krogh Institute, Biochemical Department, Universitetsparken 13, DK-2100 Copenhagen Ø

Key words: drip loss, volume regulation, phospholipase A_2, Ca^{2+}, taurine, ROS, 5-lipoxygenase

Background

Lean meat at slaughter contains about 75% water. Slaughtering and subsequent manipulation of the meat is associated with the loss of muscle water (drip loss). The water loss is estimated at about 1% from carcasses, mostly as evaporation, after two days in chill and increases to 2-10% of the lean meat weight, when muscles are cut. Water content and distribution in the meat have both a profound influence on the quality (i.e. juiciness and appearance) of the meat, and furthermore, the financial marked value of the meat. Limiting water loss during slaughter and subsequent manipulations is accordingly important. The biochemical, structural and physiological events which are initiated by slaughtering and which lead to drip loss are complex and poorly understood. In contrast, loss of osmolytes and cell water, following anoxia, osmotic perturbation and hormonal stimulation, has been described in a variety of mammalian cells. Mammalian cells exposed to a hypotonic solution swell initially as more or less perfect osmometers, whereupon they regulate their cell volume back towards the original value. Taurine, 2-amino-ethane sulfonic acid is an important organic osmolyte in many types of mammalian cells, and loss of taurine is often taken to indicate a reduction in cell volume. In cultured cells it has been demonstrated that swelling-induced activation of taurine loss involves phospholipase A_2 (PLA_2) and 5-lipoxygenase (5-LO) activity.

Objective

The aim of the present studies was to set up a model for the initial and early events in the formation of drip loss, taking our data and previous observations into account. We have (i) characterised the drip loss and (ii) investigated the effect of anoxia, acidification, osmotic perturbation and exposure to lysophospholipids on regulation of $[Ca^{2+}]_i$ and the cellular content of organic osmolytes.

Methods

The content of taurine, alanine, glycine and aspartic acid in the drip loss from Danish landrace pigs and meat samples was estimated by HPLC separation. The potassium content in the drip was estimated by atomic flame photometry, and protein content was determined using a standard Lowry procedure.

The mouse myoblastic cell line C2C12, originally derived from the thigh muscle of the mouse, was used as a representative of the muscle cell and characterised systems responsible for anoxia-induced increase in $[Ca^{2+}]_i$ as well as signal pathways involved in release of the organic osmolyte taurine. The intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by loading C2C12 cells grown on coverslips with the Ca^{2+} sensitive probe Fura-2. The$[Ca^{2+}]_i$ was followed fluorometrically in the time range 2 to 20 min following anoxia. Anoxia was induced either by gassing the experimental solutions with N_2 or by addition of Na-azide (10 mM) or Na-dithionite (20 mM). The muscle cell volume regulation was studied by measuring taurine efflux from [^{14}C]-taurine-loaded C2C12 cells.

Results and discussion

Drip loss from porcine meat (*M. Longissimus dorsi*) was found to contain high concentrations of K^+, free amino acids (Ala, Gly & Asp), taurine, as well as a high protein content, reflecting significant amounts of intracellular components in the drip (Table 1).

Table 1. Weight loss and K^+, amino acid and protein concentrations in drip loss from *M. Longissimus dorsi*

Time *post mortem* hours	Pig species	Weight loss in %	K^+ mM	Taurine mM	Alanine mM	Glycine mM	Aspartic acid mM	Protein mg/ml	n
24–48	Normal	4.8 ± 0.4	136 ± 3	13 ± 3	10 ± 3	7 ± 2	6 ± 2	129 ± 4	8
48–72	Normal	7.4 ± 0.9	131 ± 3	18 ± 4	11 ± 3	8 ± 1	5.7 ± 0.3	123 ± 2	3

To simulate the early events, which take place at the time of slaughter and lead to release of osmolytes to the extra-cellular space and subsequent formation of drip loss, C2C12 myotubes were introduced to anoxia and change in pH (7.4 → 6.0). Acidification caused a gradual increase in $[Ca^{2+}]_i$ (24 ± 1 nM·min^{-1}). Following induction of anoxia, $[Ca^{2+}]_i$ increased linearly by 32 ± 1 nM·min^{-1}. Using specific channel channel blocking agents, the increase in $[Ca^{2+}]_i$ was found mainly to take place via sarcolemma Na^+ channels.

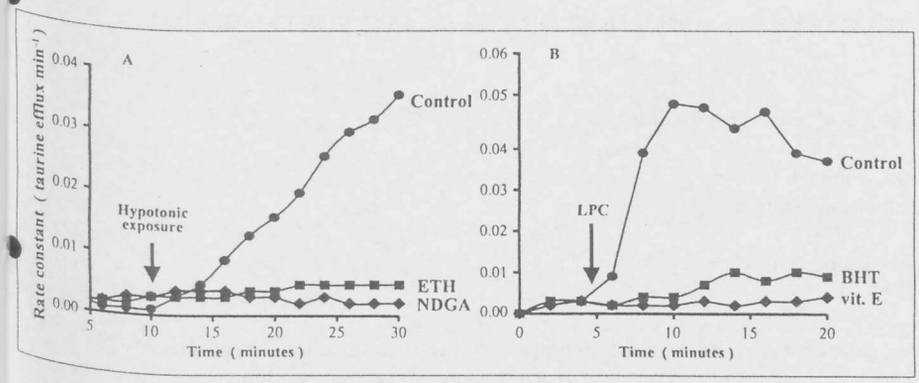

Figure 1. Regulation of swelling- and LPC-induced taurine release from C2C12 myotubes. Cells were loaded with ^{14}C-labelled taurine for 2 hours. The efflux was followed in isotonic KCl media with a shift, as indicated by the arrow, to either hypotonic KCl medium (210 mOsm, Panel A) or isotonic KCl medium containing LPC (5 μM, Panel B). The 5-LO inhibitors ETH (10 μM), NDGA (50 μM), and the antioxidants Vit E (100 μg/ml), BHT (10 mM), were added at the time of initiation of the release experiments, and the absolute rate constant (min^{-1}) for the taurine efflux was calculated.

In order to obtain a basic understanding of the mechanisms leading to cell swelling and the simultaneous release of intracellular organic osmolytes, e.g. taurine, C2C12 cells were added lysophosphatidylcholine (LPC) or exposed to hypotonic shock (Fig. 1). Addition of LPC or hypotonic exposure induced release of the organic osmolyte taurine from C2C12 cells (Fig. 1A and B). The hypotonically induced taurine efflux was blocked in the presence of anion channel blocker (DIDS) and 5-lipooxygenase inhibitors (ETH 615-139 and NDGA) (Fig. 1A). However, the hypotonically induced taurine efflux was not affected by the antioxidant butylated hydroxy toluene (BHT) and only weakly affected by vitamin E (vit. E). In contrast, LPC-induced taurine release was unaffected by the anion channel blocker, however, reduced by the antioxidants BHT and vit. E (Fig. 1B). These data indicate that slaughter stress-induced cell swelling and simultaneous taurine release may proceed by two different mechanisms, one being mainly 5-lipoxygenace dependence and the other involving reactive oxygen species (Fig. 2). These processes are potentially enhanced by the anoxia-induced increase in $[Ca^{2+}]_i$ and the subsequent activation of PLA_2.

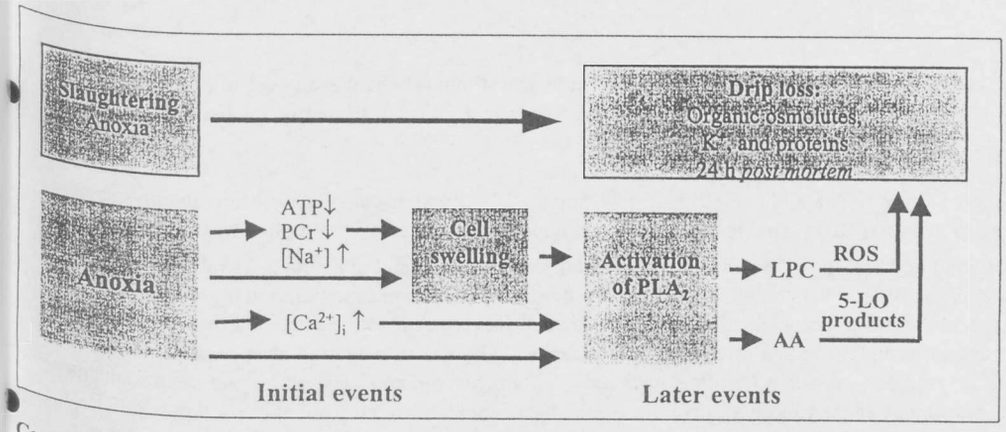

Figure 2. Hypothetical model illustrating the events involved in the formation of drip loss. According to the model, anoxia following slaughter induces a rise in $[Ca^{2+}]_i$, activation of PLA_2 and subsequent formation of Arachidonic acid (AA) and LPC. AA and LPC lead to net loss of cellular osmolytes via 5-LO products and ROS, respectively.

Conclusions

Using (i) the quantitative and qualitative characterisation of drip and (ii) a C2C12 myotube muscle cell system to simulate the physiological events taking place during slaughter, we present a model for events preceding the formation of drip (Fig. 2). Following anoxia, an initial cell swelling is most probably due to the reduction in the availability of energy rich phosphate compounds (ATP, PCr) and a subsequent net uptake of Na^+ (Cl^-) and water. Furthermore, anoxia induces an increase in $[Ca^{2+}]_i$. According to the model these events lead to an increased PLA_2 activity with the subsequent formation of arachidonic acid (AA) and lysophospho-lipids, i.e. LPC, and thereby play a key role in the swelling-induced as well as in the LPC-mediated release of osmolytes. It is assumed that in the later phase of drip loss formation, LPC and AA are involved in the formation of drip loss and that the sequences are amplified by a positive feedback mechanism involving reactive oxygen species (ROS) and 5-lipooxygenase (5-LO), respectively.

Acknowledgement

This work was supported by Danish Bacon and Meat Council and the Danish Natural Sciences Research Council.