ENERGY METABOLISM IN DEPENDENCE OF THE STUNNING METHOD DURING PIG SLAUGHTER PROCESSES

Manuela Peukert and Dagmar A. Brüggemann*

Max Rubner-Institute, Kulmbach, Germany

*Corresponding author email: Dagmar.Brueggemann@mri.bund.de

I. INTRODUCTION

The stunning process at slaughter aims to induce a fast unconsciousness and insensibility before exsanguination. In European countries, pigs are either electrically stunned or with a high CO_2 concentration. During electrical stunning an applied current induces a strong depolarization of nervous cell membranes, and consequently an excessive and uncoordinated activity, which resembles an epileptic seizure. In contrast to this, gas stunning with CO_2 is based on narcotic and breath-stimulating properties of the CO_2 gas. In most cases a concentration of >80% in a deep pit is used. The CO_2 rapidly accumulates in the blood and leads to an acidosis of the brain fluid, thus inducing unconsciousness in form of narcosis. Both techniques mean a huge impact on energy metabolism by either a rise in muscle activity or oxygen depletion accompanied by a high CO_2 pressure. So far, comparisons of both stunning techniques concentrated on differences of basic meat quality parameters like glycogen, lactate, pH, colour and drip loss. In addition to this, we were interested in metabolite levels related to central energy metabolism. The adenine derived energy equivalents and degradation products directly reflect impacts on energy status and are considered as candidate molecules to reflect different physiological effects of both stunning techniques.

II. MATERIALS AND METHODS

The animals were treated with 90% CO_2 for 30 seconds. The time frame after CO_2 application and exsanguination was ca 20 seconds. The electrically stunned pigs were exsanguinated ca 20 seconds after the head and head-to-chest stunning (each for 6 seconds). Purines were extracted from freeze-dried total blood, sampled at exsanguination. For extraction and re-extraction a total of 8 volumes methanol:water (2:8 v/v) was used. For clean-up, samples were applied to a solid phase extraction using Oasis PRiME HLB 1cc Vac cartridges (Waters, Germany). The flow-through containing polar metabolites were analysed by HPLC-ESI-QToF-MS (MaXis, Bruker, Germany). Compounds were separated with an elution gradient on a HILIC column (Z-HILIC, Waters, Germany) with eluent A (water:acetonitrile, 95:5, v/v and 10 mM ammonium acetate) and B (acetonitrile:water, 97:3, v/v and 10 mM ammonium acetate). Accurate masses of analyte ions were used for data integration. Statistical analysis was performed using the JMP software (17.0.0, SAS Institute Inc., USA).

III. RESULTS AND DISCUSSION

The stunning methods resulted in large differences in the adenine derived purine metabolites with the exception of ATP and ADP (Figure 1). The intermediate compounds AMP and adenosine were lower concentrated after CO_2 stunning, whereas the degradation products adenine, inosine and hypoxanthine were much higher concentrated after CO_2 stunning compared to electrical stunning.
As a consequence of the sudden drop in O_2 during CO_2 stunning the electron transport chain in the mitochondria is disturbed. Stabilization of ATP supply for energy consuming processes is existential, and different metabolic strategies exist to overcome acute ATP shortage – the phosphagen system, anaerobic glycolysis, interconversion of purine phosphates and substrate-level phosphorylation. The

same levels of ATP and ADP after both stunning techniques might result from very fast ATP supply from the phosphagen system, which is the most rapid available energy source. The increased speed of degradation to purine bases under CO2 stunning might demonstrate additional interconversion of purine phosphates by adenylate kinase (AK), which catalyses the reaction 2ADP -> ATP + AMP. AMP is quickly deaminated to IMP to further favour the AK reaction, and IMP might in a following step be degraded to inosine and hypoxanthine [1]. Another explanation is the purinergetic signalling cascade. Besides its role as energy equivalent, extracellular ATP and intermediates are important signalling molecules [2]. In response to acute hypoxia, ATP is quickly exported and inactivated to adenosine in the vasculature. Adenosine is further inactivated through deamination to inosine and hypoxanthine, which might explain the very low levels of adenosine under CO2 stunning in blood.

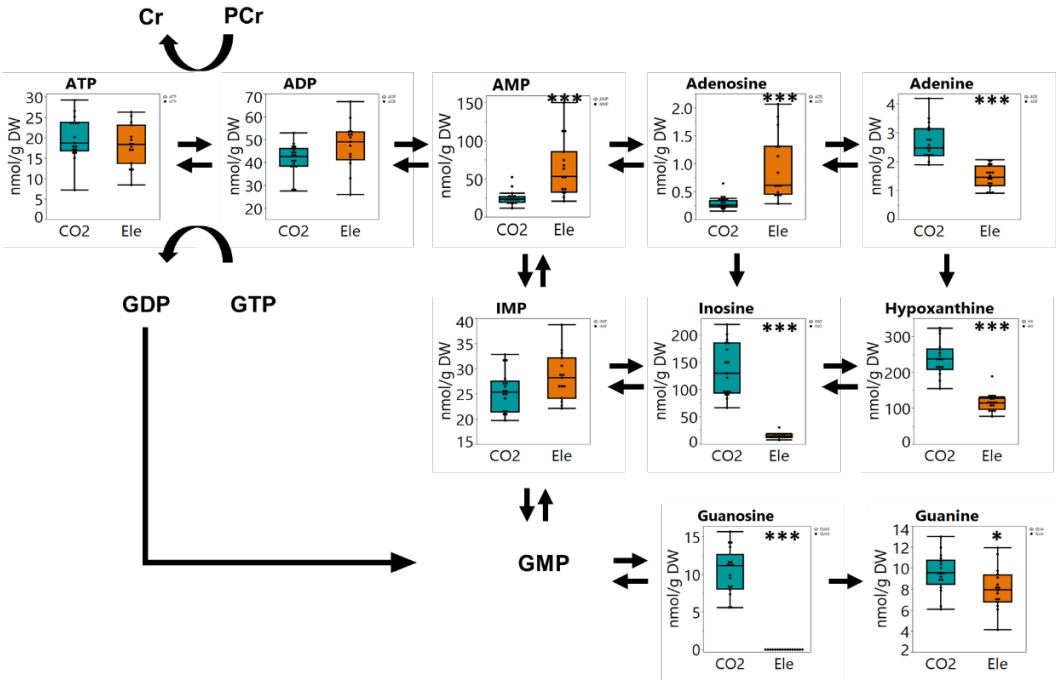

Figure 1. Concentrations of purine metabolites in blood in dependence of the stunning method. Significant differences between sample groups are labelled with * (P < 0.005) and *** (P < 0.0001)

IV. CONCLUSION

Electrical and CO_2 stunning affect physiological reactions in the animal differently. Within seconds the central energy metabolism showed huge differences in the accumulation of purine metabolites. Both techniques induce acute stresses to cellular respiration, and thus to ATP provision for energy consuming processes. From the herein observed data, we conclude that alternative pathways for further ATP production, and signalling cascades are differentially activated and mirror different grades of hypoxia – either extreme anoxia under CO_2 stunning or muscle hypoxia as a consequence of electrical stunning.

REFERENCES

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2. Burnstock, G., 1972. Purinergic nerves. Pharmacological Reviews 24: 509-581.