P-09-05

Protective effects of carnosine on hydrogen peroxide–induced oxidative stress in myoblast cells derived from porcine skeletal muscle (#152)

Marie-France Palin[1], Claude Gariepy[2], Daniele Beaudry[3], Jerome Lapointe[4], Claudia Kalbe[5]

[1] Agriculture and Agri-Food Canada, Sherbrooke, Canada; [2] Agriculture and Agri-Food Canada, St-Hyacinthe, Canada; [3] Agriculture and Agri-Food Canada, Sherbrooke, Canada; [4] Agriculture and Agri-Food Canada, Sherbrooke, Canada; [5] Leibniz-Institut für Nutztierbiologie (FBN), Dummerstorf, Germany

Introduction

Carnosine (β-alanyl-L-histidine) is a naturally-occurring molecule found exclusively in meat, poultry and some fish. The biochemical properties of carnosine include among others pH-buffering, antioxidant and carbonyl scavenging effects [1]. Since these properties are important in controlling meat physico-chemical parameters, it can be hypothesized that muscle carnosine may improve pork quality. Ma et al. [2] recently reported that pigs supplemented with carnosine had reduced oxidative damage to lipids and proteins and improved antioxidant capacity in the *Longissimus* muscle. However, because muscle carnosine content was not reported in that study, a direct link between muscle carnosine and observed antioxidant properties was not established. Therefore, the current study was undertaken to determine the effect of carnosine in preventing oxidative damage in porcine myoblasts (muscle cells) and to characterize the mechanisms in play.

Methods

Satellite cells were isolated from the *Longissimus* muscle of newborn piglets (n = 34) using a percoll gradient method [3]. Cells were pooled and grown in growth medium (DMEM with 10% FBS, 10% horse serum, antibiotics/antimycotics) for 48 h and then treated with carnosine (0, 10, 25 and 50 mM) for 48 h. These cells were then either treated with H_2O_2 (0.3 mM, 1 h) to induce an oxidative stress or collected immediately to perform analyses described below. Cell proliferation was measured using the MTS tetrazolium (Promega) and BRDU (Roche Applied Science) assays. Reactive oxygen species (ROS) were measured with the OxiSelect™ Intracellular ROS assay (Cells Biolab) and protein carbonyls levels were quantified with the OxiSelect™ Protein Carbonyl ELISA Kit (Cells Biolab). Antioxidant activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) and mRNA abundance of catalase (CAT), GPX1, GPX3, GPX4, glutathione-disulfide reductase (GSR), heme oxygenase 1 (HMOX1), nuclear factor, erythroid 2 like 2 (NEF2L2), NAD(P)H quinone dehydrogenase 1 (NQO1), peroxiredoxin (PRDX1, PRDX3, PRDX4), SOD1, SOD2, SOD4 and thioredoxin reductase (TXNRD1, TXNRD2) were also determined. Characterization of activated intracellular pathways by H_2O_2 and carnosine was performed by measuring phosphorylated p38 MAPK, p44/42 MAPK, SAPK-JNK, mTOR and its downstream targets 4E-BP1 and P70 S6 (Western Blots). Data were analyzed by the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, USA).

Results

Treating myoblast cells with H_2O_2 alone reduced cell proliferation and this effect was reversed when myoblasts were pre-treated for 48 h with carnosine (MTS and BRDU assays, $P<0.001$). Addition of carnosine (10, 25 and 50 mM) to myoblasts decreased damage to proteins (carbonyls; $P<0.004$) and reduced total intracellular ROS ($P<0.001$) when compared with untreated cells. There was a marked increase of ROS ($P<0.001$) and protein carbonyls ($P<0.001$) with H_2O_2 alone and this increase was prevented by a pre-treatment of myoblasts with carnosine (ROS and carbonyls, $P<0.001$). Addition of carnosine (no H_2O_2) to myoblasts increased the mRNA abundance of *SOD2* ($P<0.001$), *PRDX3* ($P<0.05$) and *PRDX4* ($P<0.01$), but decreased *SOD3* ($P<0.001$) and *GPX3* ($P<0.001$) mRNA abundance. Lower mRNA abundances were observed for the CAT, GPX1, GPX3, NEF2L2, PRDX3 ($P<0.001$) and PRDX 4 ($P<0.05$) genes when myoblasts were exposed to H_2O_2 alone and pre-treating cells with carnosine prevented this H_2O_2–associated decline ($P<0.01$). A similar decline was observed for SOD ($P<0.001$) and GPX ($P<0.001$) activities when cells were treated with H_2O_2 alone and this was also prevented by carnosine pre-treatment($P<0.01$). The p38 MAPK phosphorylation increased with H_2O_2 alone ($P<0.01$), whereas this increase was abrogated when cells were pre-treated with carnosine ($P<0.01$).

Conclusion

These results suggest that carnosine prevents oxidative damage and modulates the intracellular antioxidant system in porcine muscle cells. Western blot analyses further suggest that the protective effect of carnosine on H_2O_2-induced oxidative stress is mediated through the p38 MAPK intracellular pathway.

Acknowledgments

Authors are grateful to Agriculture and Agri-Food Canada and the Leibniz Institute for Farm Animal Biology for financial support and to S. Methot for statistical analyses.

References

[1] Boldyrev et al., (2013) Physiology and pathophysiology of carnosine. Physiol. Rev. 93:1803-1845.

[2] Ma et al., (2010) Dietary supplementation with carnosine improves antioxidant capacity and meat quality of finishing pigs. J. Anim. Physiol. Anim.

Notes

Nutr. 94:e286-e295.
[3] Mau et al., (2008) Establishment and conditions for growth and differentiation of a myoblast cell line derived from the *semimembranosus* muscle of newborn piglets. In Vitro Cell. Dev. Biol. Animal. 44:1-5.

Notes