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A role for melanocortin peptides in modulating oxidative stress induced inflammation in A549 cells

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Melanocortin peptides exert their anti-inflammatory effects *via* activation of a subgroup of G-protein coupled receptors[1]. To date five melanocortin receptors (MCR) have been identified with the MC1 and MC3R being proposed to bring about the anti-inflammatory effects of melanocortins[2]. Here we have used an *in vitro* model of oxidative stress to determine whether melanocortin peptides can switch off inflammatory cytokine release from activated epithelial cells.

RT-PCR and western blotting were used to determine MC1 and 3R expression in A549 cells. Cells were plated at 1 $x10^6$/well in 24 well plates and pretreated with 1-30 μg/ml of the pan melanocortin agonist α-MSH and the selective MC3R agonist $dTrp^8$-γ-MSH[1] for 30 mins prior to determination of cAMP accumulation by EIA. In separate experiments cells were pretreated with 1-30 μg/ml of α-MSH and $dTrp^8$-γ-MSH for 30 mins prior to stimulation with either PBS or H_2O_2 (20-200μM) for 4h. Cell free supernatants were collected and nitric oxide (NO) levels assessed by nitrite quantification by the Greiss method and the cytokines IL-8 and MCP-1 levels determined in cell-free supernatants by commercially available ELISA. Data is expressed as Mean ± SD of n=4 determination in triplicate. *P<0.05 vs. appropriate control.

RT-PCR and western blotting showed the presence of mRNA and protein for MC1 and MC3R in A549 cells. Functionality of the receptors was determined by stimulating the cells with α-MSH and $dTrp^8$-γ-MSH, resulting in a concentration dependent increase in cAMP accumulation, with a maximal accumulation of 330 ± 20 and 180 ± 10 fmol/well at 10 μg/ml for α-MSH and $dTrp^8$-γ-MSH respectively (n=4, *P<0.05). We next determined whether α-MSH and $dTrp^8$-γ-MSH could modulate H_2O_2 stimulated release of nitrite, IL-8 and MCP-1. Stimulation of cells with H_2O_2 resulted in a maximal release of IL-8 (610 ± 50 pg/ml, *P<0.05 vs. control, n=4) and MCP-1 (1435 ± 53 pg/ml, * P<0.05 vs. control, n=4) at 200 μM. Pre-treatment of cells with 10 μg/ml α-MSH and $dTrp^8$-γ-MSH prior to H_2O_2 stimulation resulted in significant reductions in all parameters measured with an 85% and 82% reduction in IL-8 and MCP-1 by α-MSH, whilst the selective MC3R agonist $dTrp^8$-γ-MSH caused a significant 88% and 71% (*P<0.05 vs. control n=4) reduction in both IL-8 and MCP-1 respectively.

Collectively these data have identified functionally active MC1 and MC3R on the human epithelial cell line A549. Hydrogen peroxide induced nitrite; IL-8 and MCP-1 were abrogated in the presence of α-MSH and $dTrp^8$-γ-MSH. Therefore, therapeutic targeting of MCR may be beneficial in the treatment of inflammatory lung disease, such as in asthma and COPD.

Getting SJ, *et al.,* FASEB J 20:2234-41, 2006.

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