ACTIVATION OF MELANOCORTIN RECEPTORS INDUCES CYCLIC AMP-DEPENDENT HEMEOXYGENASE-1 EXPRESSION IN MURINE MACROPHAGE

Connie W. Lam, Stephen J Getting and Mauro Perretti
Department of Biochemical Pharmacology, The William Harvey Research Institute, Charterhouse Square, London.

Melanocortin peptides activate specific seven transmembrane domain G-protein coupled receptors to inhibit cell activation and down-regulate the host inflammatory response (e.g. inhibition of cytokine production and adhesion molecule expression, as well as leukocyte migration,). Agonists at melanocortin receptors (MC-R) activate adenylate cyclase causing intracellular cAMP accumulation (Getting, 2002). Here we have studied more prolonged down-stream events that follow MC-R activation in RAW264.7 cells and mouse peritoneal macrophages (MØ).

Methods. *RT-PCR*: mRNA detection for specific MC-R in primary cultured MØ and RAW264.7 cells was performed as already described (Getting *et al.,* 1999). *cAMP:* Receptor functionality was determined by stimulating RAW264.7 cells for 30 min with $ACTH_{1-39}$ (1-300 ng/ml) or the mixed MC3/4-R agonist MTII (1-100 µg/ml) before cell lysing and determination of intracellular cAMP by EIA (RPN225, Amersham, UK). *Hemeoxygenase (HO-1) determination:* Peritoneal MØ and RAW264.7 cells were cultured in 6-well plates and treated with $ACTH_{1-39}$ (1-100 ng/ml), the MC3/4-R agonist MTII (1-30 µg/ml) or LPS (10 µg/ml) for 8 h prior to cell lysing and determination of HO-1 protein (SPA-895, Stressgen, Canada) by western blotting using conventional protocols. *Cell signalling*: Peritoneal MØ or RAW264.7 cells were treated with $ACTH_{1-39}$ (100 ng/ml), MTII (10 µg/ml) or dibutyryl (Bt_2) cAMP (250 µM) alone or in the presence of the protein kinase A (PKA) inhibitor H-89 (20 µM), ERK inhibitor PD98059 (50 µM) or p38 inhibitor SB203580 (20 µM) prior to determination of HO-1 protein expression by western blotting. Data (mean ± SEM) were analysed by ANOVA and Bonferroni test.

Results. RT-PCR analysis detected MC1-R, MC3-R and MC5-R, but not MC4-R, mRNA in RAW264.7 cells and primary MØ. Receptor functionality was confirmed in either cell type by assessing the accumulation of intracellular cAMP at 30 min following cell activation with $ACTH_{1-39}$ or MTII. In three distinct experiments, a maximal effect was observed at 100 ng/ml $ACTH_{1-39}$ (567±36 fmol/well) and 10 µg/ml MTII in RAW264.7 cells (971±126 fmol/well) compared to respective control values of 228±98 and 479±74 fmol/well ($P<0.05$). Cell treatment for 8 h with either melanocortin peptide, at concentrations optimal for cAMP accumulation, led to the expression of anti-inflammatory protein HO-1. Next, we addressed the cell signalling pathway involved in melanocortin peptide induction of HO-1. The ERK inhibitor PD98059 and the p38 inhibitor SB203580 failed to inhibit HO-1 induction elicited by either $ACTH_{1-39}$ (100 ng/ml) or MTII (10 µg/ml) in RAW264.7 cells. In contrast, the PKA inhibitor H-89 blocked the inducing effect of either melanocortin (>60% inhibition, $P<0.05$). Similarly, H-89 inhibited HO-1 induction in RAW264.7 cells produced by Bt_2cAMP. Similar results were obtained with primary peritoneal MØ (data not shown).

In conclusion, this study has highlighted a previously unknown functional link between the cAMP-PKA signalling pathway and HO-1 expression following MC-R activation in M∅. This novel effect may contribute, at least in part, to the anti-inflammatory activities ascribed to this class of endogenous anti-inflammatory peptides and their synthetic derivatives, in the context of anti-inflammation and the resolution phase.

Getting, SJ (2002) *Trends Pharmacol Sci.* **23**, 447-449.
Getting, SJ *et al.*, (1999). *J. Immunol.* **162**, 7446-7453

This work was supported by a PhD studentship of the Arthritis Research Campaign UK (grant P0575).