

## Anti-inflammatory peptide approaches for preventing vascular inflammation

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**INTRODUCTION:** Inflammation is implicated in the development of atherosclerotic lesions and restenosis [1]. It is proposed that by inhibiting the local inflammatory process e.g. using a drug-eluting stent platform, neointima formation will be reduced. Melanocyte stimulating hormone (MSH) peptides are potent inhibitors of inflammation, and act via the melanocortin-1 receptor (MC1R) [2]. Therefore, the aim of this work was to confirm whether MSH peptides can inhibit inflammatory signaling *in vitro* and upregulation of the E-selectin adhesion molecule.

**METHODS:** To confirm that  $\alpha$ -MSH inhibits TNF- $\alpha$  stimulated NF- $\kappa$ B activation porcine vascular smooth muscle (VSM) cells were transiently transfected *in vitro* with an NF- $\kappa$ B dependent luciferase reporter construct. After transfection cells were stimulated with pTNF- $\alpha$  +/-  $\alpha$ -MSH ( $10^{-6}$ / $10^{-9}$  M) for 4 hours and luciferase activity analysed. In addition, E-selectin expression was assessed by flow cytometry (Guava PCA) using porcine endothelial cells grown in culture for 3 days and pre-incubated with  $\alpha$ -MSH ( $10^{-8}$  M/ $10^{-10}$  M/ $10^{-12}$  M) for 15 minutes prior to stimulation with pTNF- $\alpha$  (2 ng/ml) for 24 hours.

### RESULTS:

**Transient Transfection:** Stimulation with pTNF- $\alpha$  increased the relative NF- $\kappa$ B dependant luciferase activation by 37 % (Figure 1).  $\alpha$ -MSH ( $10^{-9}$  M) was found to significantly inhibit pTNF- $\alpha$  stimulated relative NF- $\kappa$ B dependant luciferase activation by 29 % (n=3, p>0.01).  $\alpha$ -MSH alone (without TNF- $\alpha$ ) did not alter the activation.

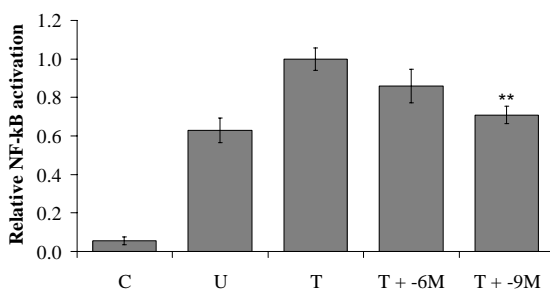


Fig. 1:  $\alpha$ -MSH inhibits TNF- $\alpha$  stimulated relative NF- $\kappa$ B dependant luciferase activation. C, Control;

U, Unstimulated; T, pTNF- $\alpha$  (2 ng/ml); M,  $\alpha$ -MSH ( $10^{-6}$ / $10^{-9}$ M). n=3. \*\*p>0.01

**E-selectin upregulation:**  $\alpha$ -MSH inhibited TNF- $\alpha$  stimulated E-selectin upregulation in a dose responsive manner from  $10^{-8}$  M to  $10^{-12}$  M (Figure 2). Complete inhibition was observed at  $10^{-8}$  M.

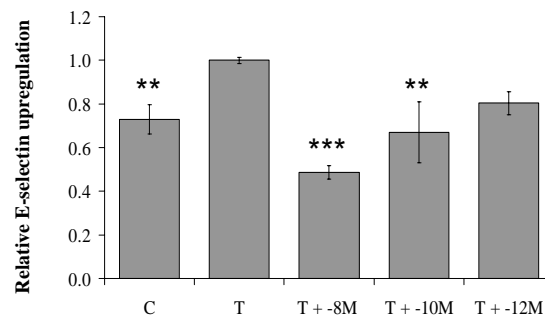


Fig. 2:  $\alpha$ -MSH inhibits TNF- $\alpha$  stimulated E-selectin upregulation. C, Control (unstimulated); T, pTNF- $\alpha$  (2 ng/ml); M,  $\alpha$ -MSH ( $10^{-8}$ / $10^{-10}$ / $10^{-12}$ M). n=3. \*\*p>0.01, \*\*\*p>0.0001

**DISCUSSION & CONCLUSIONS:**  $\alpha$ -MSH decreased inflammatory signaling and adhesion molecule upregulation in TNF- $\alpha$  stimulated VSM and endothelial cells. Work is currently underway to: (1) extend previous work on adhesion molecules with E-selectin to ICAM-1 and P-selectin on endothelial cells and ICAM-1 on VSM cells; (2) to look at the effect of  $\alpha$ -MSH on cellular apoptosis; (3) to investigate the effect of MSH on inflammatory signaling *in vivo*. This work suggests that MSH may be of potential therapeutic value in the prevention of vascular inflammation, especially in restenosis from drug eluting stents.

**REFERENCES:** <sup>1</sup> T. Inoue et al (1996) *J Am Coll Cardiol* **28**:1127-33. <sup>2</sup> J. Wikberg et al (2000) *Pharmacol Res* **42**:393-420.

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