

Investigating the role of melanocortin receptor subtypes in mediating anti-inflammatory protection following cerebral ischemia reperfusion injury.

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Introduction

Potent anti-inflammatory circuits have been demonstrated to be centred on the melanocortin receptors (MC) and their activation has shown beneficial effects in a number of models of inflammatory diseases. With inflammation being increasingly acknowledged as playing a major role in the pathology of stroke, targeting the melanocortin receptor system may provide a sound therapeutic strategy to improve clinical outcome. However the relative contribution of each receptor subtype is still not fully understood and may vary with the cellular environment. The melanocortin receptors MC₁, MC₃ and MC₄ have previously been demonstrated to play a part in conducting anti-inflammatory processes in different tissues types and disease states. This project aims to determine the main receptor subtype/s involved in providing anti-inflammatory protection following cerebral ischemia reperfusion.

Methods

The Bilateral common carotid artery occlusion (BCCAO) mouse model of global stroke has been used to assess the inflammatory response in the cerebral microcirculation following stroke. Briefly, male C57BL/6 mice (18-25g) were anaesthetised using pentobarbital (100mg/kg i.p) and then subjected to 5 minutes global brain ischemia followed by either 40 minutes or 2 hours of reperfusion. Melanocortin treatments were given i.p either 30 minutes prior to ischemia or at the start of reperfusion. Intravital microscopy was utilised to quantify the inflammatory reaction through a real-time *in vivo* visualisation of the leukocyte adhesion cascade in the pial venules of the cerebral microcirculation. Leukocyte-endothelium interactions were quantified in terms of rolling cell flux and adherent leukocytes, and expressed as cells/mm². Leukocyte rolling velocity was expressed as $\mu\text{m}/\text{sec}$. 40 minute reperfusion Sham and saline treated BCCAO groups n = 8mice/group, All other 40 minute reperfusion groups n = 6 mice/group. Two hour reperfusion groups n = 3 mice/group. Statistical evaluation was performed using ANOVA with Bonferroni test for post hoc analyses. P<0.05 was considered to be significant. All treatments were 10 $\mu\text{g}/\text{mouse}$ i.p.

Results

In comparison to sham operated animals (rolling = 21.5 ± 6.9 cells/mm², adherence = 42.1 ± 14.4 cells/mm²) BCCAO was found to induce a significant increase in leukocyte rolling (191.0 ± 31.49 cells/mm²) and adherence (282.3 ± 49.4 cells/mm²) following 40 minutes of reperfusion. An extended reperfusion period of 2 hours resulted in a further increase in leukocyte endothelium interactions (rolling = 243.9 ± 74.0 cells/mm², adherence = 659.7 ± 115.6 cells/mm²). Prophylactic treatment 30 minutes prior to ischemia with the pan receptor agonist α -MSH resulted in a significant reduction in ischemia reperfusion induced leukocyte rolling (58.1 ± 10.4 cells/mm²) and adhesion (51.7 ± 17.2 cells/mm²) at 40 minutes into reperfusion. This effect was maintained when treatment was delayed until the start of reperfusion and these reductions remained significant in the 2 hour reperfusion model. The MC₃ selective agonist, Dtrp8- γ -MSH, similarly showed a significant reduction in the adherence of leukocytes at both time points however did not significantly reduce rolling either at 40 minutes or two hours into reperfusion. Antagonism of MC₃ and MC₄ using SHU9119 failed to increase adherence and rolling following 40 minutes of reperfusion. Furthermore combination treatments revealed that SHU9119 did not significantly abrogate the anti-inflammatory effects of α -MSH or Dtrp8- γ -MSH. However at 2 hours of reperfusion SHU9119 treatment blunted the anti-inflammatory actions of α -MSH and prevented the Dtrp8- γ -MSH mediated reduction in rolling and adhesion.

Conclusions

These results suggest that in the early stages of reperfusion MC₁ activation may be of particular importance in mediating these protective effects. However as the inflammatory reaction progresses the role of MC₃ may become more prominent. Further studies will provide a more detailed characterisation of the inflammatory response following BCCAO and assess levels of receptor expression at different time points into reperfusion.