Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst051P.pdf

Harnessing The Melanocortin Receptor System To Reduce Leukocyte Recruitment Following Stroke

Paul Holloway[1], Stephen Getting[0,2], Felicity Gavins[1]. [1]Imperial college, London, UK, [2]University of Westminster, London, UK

Introduction

Stroke is a leading cause of mortality worldwide and the largest single cause of adult disability; however, current pharmacological interventions remain extremely limited. A disproportionate inflammatory response exacerbates injury following stroke, thus, resolving inflammation may provide an effective treatment.

The G-protein coupled melanocortin receptors (MCs) have potent anti-inflammatory and neuro-protective activities, making them particularly attractive targets for stroke treatment. However, the relative contribution of each receptor subtype is not fully understood. The melanocortin receptors MC_1, MC_3 and MC_4 have previously been shown to initiate anti-inflammatory processes in a number of different tissues and disease states. This project aims to determine the main receptor subtype/s involved in providing anti-inflammatory protection following cerebral ischemia reperfusion (I/R).

Methods

The bilateral common carotid artery occlusion (BCCAo) mouse model of global stroke was utilised in conjunction with intravital microscopy (IVM), to quantify neuro-inflammatory responses through real time visualisation of cerebral leukocyte recruitment. Investigations employed both pharmacological and genetic approaches using MC selective compounds and KO mice. Briefly; male WT (C57BL/6), recessive yellow (e/e) mice (possessing a non-functional MC_1) or MC_3KO mice (18-30g) were anesthetised (pentobarbital 100mg/kg i.p) and subjected to 5 minutes global brain ischemia followed by 40 minutes or 2 hours of reperfusion. Melanocortin treatments were given (10μg/mouse i.p) at the start of reperfusion. IVM was used to quantify leukocyte-endothelium interactions in terms of rolling cell flux and adherent leukocytes, expressed as cells/mm^2. 40 minute reperfusion WT groups n=6 mice/group, MC_3KO and e/e groups n=4. Two hour reperfusion groups n=3. $P<0.05$ was considered significant using ANOVA with Bonferroni test post hoc analyses.

Results

In comparison to sham-operated animals (rolling: 21.5±6.9 cells/mm^2, adherence: 42.1±14.4 cells/mm^2) BCCAo significantly increased leukocyte rolling (191.0 ±31.49 cells/mm^2) and adherence (282.3 ± 49.4 cells/mm^2) after 40 minutes of reperfusion in WT mice. Both MC_3KO and e/e mice showed no significant differences in sham leukocyte-endothelial interactions and displayed I/R induced leukocyte rolling and adhesion at 40 minutes. However the I/R induced leukocyte rolling observed in e/e mice (539.28± 153.1 cells/mm^2) was significantly greater than in WT mice, suggesting an enhanced inflammatory response and an important role for MC_1.

WT mice treated with the pan receptor agonist, α-MSH. presented a significant reduction in I/R induced leukocyte rolling (58.1±10.4 cells/mm2) and adhesion (51.7±17.2 cells/mm2) at 40 minutes reperfusion. The MC_3 selective agonist, Dtrp^8-γ-MSH, similarly showed reduced

leukocyte adherence, although did not significantly reduced rolling. Antagonism of MC_3 and MC_4 using SHU9119 did not significantly abrogate the anti-inflammatory actions of α-MSH.

Extending the reperfusion period to 2 hours further exacerbated leukocyte-endothelium interactions (rolling: 243.9 ±74.0 cells/mm^2, adherence: 659.7 ±115.6 cells/mm^2). At 2 hours reperfusion α-MSH mediated reduction in leukocyte recruitment remained significant however SHU9119 treatment blunted the anti-inflammatory actions of α-MSH and prevented $Dtrp^8$-γ-MSH mediated reduction in rolling and adhesion.

Conclusions

These results demonstrate that the anti-inflammatory actions of MCs may prove useful in the context of stroke. Furthermore results MC_1 activation may be of particular importance in the early stages of reperfusion. However as the inflammatory reaction progresses the role of MC_3 may become more prominent.