Effects of the cannabinoid receptor antagonist hemopressin on amyloid-β induced toxicity in mouse Neuro-2a neuroblastoma cells

S. J. Werner, S. W. Martin, A. W. Paterson, N. G. Milton. School of Clinical & Applied Sciences, Leeds Beckett University, Leeds, United Kingdom.

Introduction: Pepcans are a group of peptide based cannabinoids which are haemoglobin derived. They act as cannabinoid (CB) receptor antagonists/agonists and are found throughout the CNS[1]. The pepcan hemopressin (Hp) is a nonapeptide antagonist of the CB_1 receptor[1]. Studies have suggested that cannabinoid antagonists, such as the synthetic CB_1 antagonist AM251, have memory enhancing activities following brain trauma[2]. The levels of amyloid-β (Aβ) are elevated during brain trauma and may contribute to tissue damage[3]. Previous *in vitro* studies have demonstrated the protective properties of CB_1 receptor ligands against Aβ induced neurotoxicity[4]. The aim of this study was to determine the effects of Hp on Aβ toxicity.

Method: This study employed MTT cell viability assays to investigate the effects of the peptide CB_1 antagonist Hp, the peptide CB_1 agonist RVD, the lipid CB agonist 2-arachidonoylglycerol (2-AG), the synthetic CB agonist O-2545, the synthetic CB_1 antagonist AM251, the synthetic CB_2 antagonist AM630 plus the synthetic GPR-55 antagonist CID16020046 (10μM for each drug), on 10μM Aβ 25-35 induced neurotoxicity in mouse neuroblastoma Neuro-2a (N2a) cells. Data were analyzed by one-way analysis of variance (ANOVA).

Results: Both Hp and 2-AG had a significant protective effect ($P<0.05$) on Aβ 25-35 induced neurotoxicity in N2a cells. The RVD, O-2545 and AM251 had no effect on Aβ 25-35 induced toxicity in N2a cells (Fig 1A). The RVD, AM251, AM630 and CID16020046 had no effect on Hp protection against Aβ 25-35 induced toxicity in N2a cells (Fig 1B).

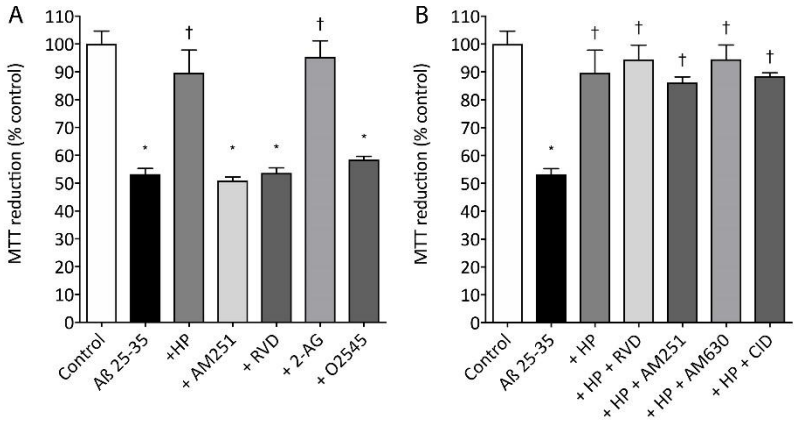

Figure 1. (A) Effect of Aβ 25-35 plus Hp, RVD, 2-AG, O-2545 or AM251 on MTT reduction in N2a cells. **(B)** Effect of Aβ 25-35 with Hp alone or plus RVD, AM251, AM630 or CID16020046 on MTT reduction in N2a cells. Results are mean ± SEM (n=8 for each data point); * = $P< 0.05$ vs control; † = $P<0.05$ vs Aβ 25-35 alone; (one-way ANOVA).

Conclusion: In conclusion, the cannabinoid antagonist Hp is protective against Aβ 25-35 induced neurotoxicity in N2a cells. The Hp protection is not antagonised by RVD or the CB_1, CB_2 or GRP-55 antagonists suggesting either an action via a non-cannabinoid receptor or that Hp is acting allosterically at an alternate binding site within a CB receptor.

References:

(1) Gomes I *et al*. (2009). FASEB J. **23**: 3020-3029.
(2) Sunada H *et al*. (2017). J Exp Biol. **220**:3026-3038.
(3) Edwards G *et al.* (2017). Biochem Biophys Res Commun. **483**: 1137-1142.
(4) Milton NGN (2002). Neurosci Letts **332**: 127-130.