

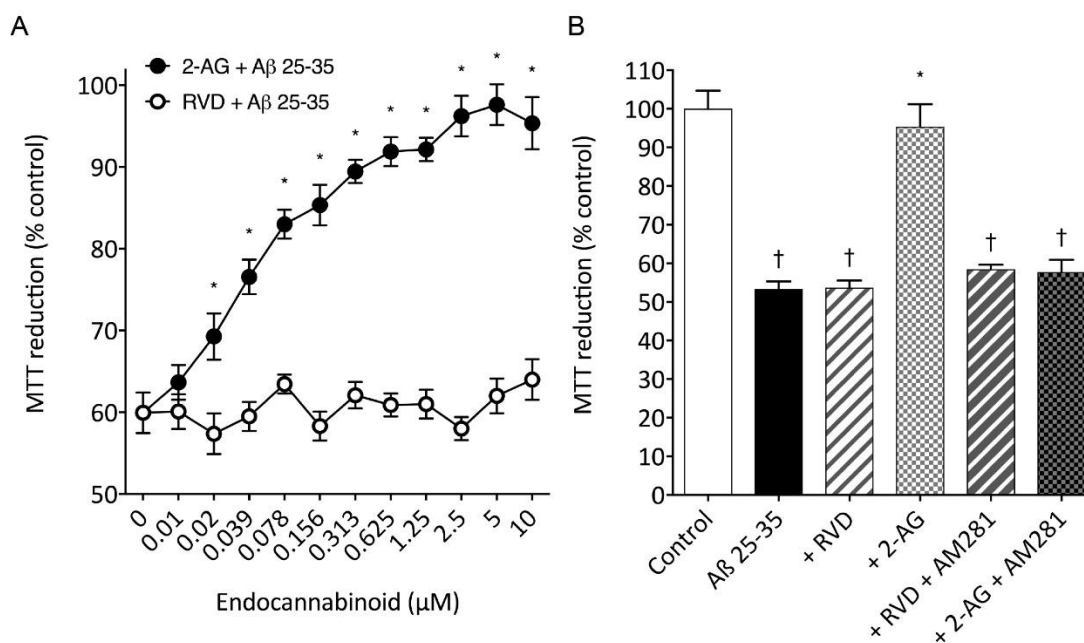
### Effect of RVD-hemopressin on amyloid- $\beta$ induced toxicity in human SH-SY5Y neuroblastoma cells

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**Introduction:** Previous *in vitro* and *in vivo* studies have demonstrated the protective properties of lipid endocannabinoids against amyloid- $\beta$  (A $\beta$ ) induced neurotoxicity<sup>1,2</sup>. Lipid-derived endocannabinoid agonists such as 2-arachidonoylglycerol (2-AG) can exert their effects via both the extra- and intracellular cannabinoid receptor-1 (CB<sub>1</sub>)<sup>3</sup>. Pepcans are a group of haemoglobin derived peptide cannabinoids and are found throughout the CNS<sup>3</sup>. They are cell-impermeant and act on the extracellular CB<sub>1</sub> receptor<sup>3,4</sup> as agonists/antagonists. The pepcan RVD-hemopressin (RVD) is a CB<sub>1</sub> receptor agonist<sup>3</sup>. The aim of this study was to determine whether RVD is protective against A $\beta$  toxicity.

**Method:** This study employed MTT cell viability assays to investigate the effects of the peptide CB<sub>1</sub> agonist RVD and lipid CB<sub>1</sub> agonist 2-AG plus the CB<sub>1</sub> antagonist AM281 on A $\beta$  25-35 induced neurotoxicity in human neuroblastoma SH-SY5Y cells. Data was analyzed by one-way analysis of variance (ANOVA).

**Results:** RVD (0.01-10 $\mu$ M) had no effect on 10 $\mu$ M A $\beta$  25-35 induced neurotoxicity in SH-SY5Y cells, whereas 2-AG (0.02-10 $\mu$ M; P<0.05 vs A $\beta$  25-35 alone) promoted a concentration dependent inhibition (Figure 1A). The CB<sub>1</sub> antagonist AM281 (10 $\mu$ M) had no effect on RVD (10 $\mu$ M) plus 10 $\mu$ M A $\beta$  25-35, however it abolished the protective effects of 2-AG (10 $\mu$ M; P<0.05 vs A $\beta$  25-35 alone) on 10 $\mu$ M A $\beta$  25-35 induced neurotoxicity (Figure 1B).



**Figure 1.** (A) Dose-response curves for RVD plus 10 $\mu$ M A $\beta$  25-35 and 2-AG plus 10 $\mu$ M A $\beta$  25-35 on MTT reduction in SH-SY5Y cells. (B) SH-SY5Y cells were exposed to 10 $\mu$ M A $\beta$  25-35 alone, or plus 10 $\mu$ M RVD alone or 10 $\mu$ M RVD and 10 $\mu$ M AM281 or 10 $\mu$ M 2-AG alone or 10 $\mu$ M 2-AG and 10 $\mu$ M AM281 and cell viability determined by MTT reduction. Results are mean  $\pm$  SEM (n=8 for each data point); \* = P< 0.05 vs A $\beta$  25-35 alone; † = P<0.05 vs control; (one-way ANOVA).

**Conclusion:** In conclusion, the peptide cannabinoid RVD is non-protective against A $\beta$  25-35 induced neurotoxicity in SH-SY5Y cells. Lipid based endocannabinoids, such as 2-AG, are protective against A $\beta$  25-35 induced neurotoxicity<sup>1</sup>. Our results support the suggestion that endocannabinoid neuroprotection against A $\beta$  involves the intracellular CB<sub>1</sub> receptor rather than the extracellular CB<sub>1</sub> receptor<sup>5</sup>.

**References:**

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