

Effect of the CB₁ cannabinoid receptor antagonist AM281 on kissorphin protection against amyloid- β neurotoxicity

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Introduction: Previous *in vitro* and *in vivo* studies demonstrate protective properties of kissorphin (KSO) peptides against amyloid- β (A β) neurotoxicity^{1,2}. Overexpression of the KiSS-1 gene, that encodes the KSO peptides, is also neuroprotective^{1,3}. Endocannabinoids and KSO peptides are neuroprotective against A β 25-35, but not A β 31-35 peptides^{1,4}. The KiSS-1 gene expression is regulated by endocannabinoids⁵. The aim of this study was to determine whether endocannabinoids contribute to KSO protection against A β toxicity using a CB₁ cannabinoid receptor antagonist.

Method: This study employed MTT cell viability assays to investigate the effects of the CB₁ antagonist AM281 on KSO 1-6 protection against A β 25-35 neurotoxicity in human neuroblastoma SH-SY5Y cells. The effects of AM281 on A β 25-35 induced neurotoxicity in KiSS-1 gene overexpressing SH-SY5Y cells (PKiSS)^{1,3} was also investigated^{1,3}. Data was analyzed by one-way analysis of variance (ANOVA).

Results: The CB₁ antagonist AM281 (0.01-10 μ M) promoted a concentration dependent increase in 10 μ M A β 25-35 induced neurotoxicity in SH-SY5Y cells in the presence of 10 μ M KSO 1-6 (Figure 1A). The PKiSS protection against 10 μ M A β 25-35 was reversed by the CB₁ antagonist AM281 (10 μ M) and anti-KSO antibody (1 μ g/ml). In the presence of anti-KSO antibody 10 μ M 2-AG was protective against 10 μ M A β 25-35.

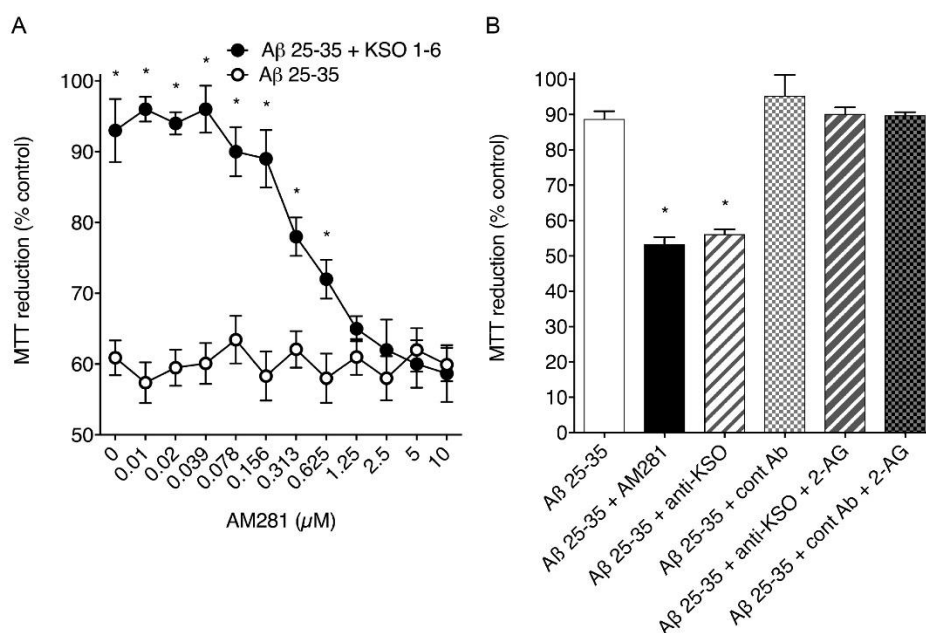


Figure 1. (A) Dose-response curves for AM281 in the presence of 10 μ M A β 25-35 with or without 10 μ M KSO 1-6, on MTT reduction in SH-SY5Y cells. (B) PKiSS SH-SY5Y cells were exposed to 10 μ M A β 25-35 alone; 10 μ M A β 25-35 plus 10 μ M AM281; 10 μ M A β 25-35 plus 1 μ g/ml anti-KSO antibody; 10 μ M A β 25-35 plus 1 μ g/ml control antibody; 10 μ M A β 25-35 plus 1 μ g/ml anti-KSO antibody with 10 μ M 2-AG; 10 μ M A β 25-35 plus 1 μ g/ml control antibody with 10 μ M 2-AG; and cell viability determined by MTT reduction. Results are mean \pm SEM (n=8 for each data point); * = P< 0.05 vs A β 25-35 alone (one-way ANOVA).

Conclusion: In conclusion, protection against A β 25-35 induced neurotoxicity by KSO and KiSS-1 overexpression in SH-SY5Y cells is reversed by the AM281 CB₁ antagonist. Anti-KSO antibodies prevent neuroprotection by KiSS-1 overexpression and 2-AG restores neuroprotection. This suggests KSO neuroprotection against A β involves activation of endocannabinoids.

References:

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