Δ^8- AND Δ^9-TETRAHYDROCANNABIVARIN ANTAGONIZE CANNABINOID RECEPTOR AGONISTS BOTH *IN VITRO* AND *IN VIVO*

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Previously, we found Δ^9-tetrahydrocannabivarin extracted from cannabis (eΔ^9-THCV) behaves as a CB_1 receptor antagonist in vitro (Thomas *et al.* (2005). We have now investigated if synthetic Δ^9-THCV (O-4394) behaves in this way not only in vitro but also in vivo. To facilitate future structure-activity studies, synthetic Δ^8-THCV (O-4395) was also investigated, it being easier to synthesise analogues of Δ^8- than of Δ^9-THCV.

In vitro experiments were performed with vasa deferentia and whole brain membranes obtained from MF1 mice (32 to 44 g) (see Thomas *et al.*, 2005 for descriptions of the methods used in these experiments and to determine K_i and K_B values). Nociception was measured 20 min after an injection of Δ^9-tetrahydrocannabinol (Δ^9-THC), O-4395 or O-4394 by subjecting adult male ICR mice (22 to 30 g) to the tail flick test (Varvel *et al.*, 2006). Δ^9-THC was supplied by the National Institute on Drug Abuse (NIDA) and eΔ^9-THCV by GW Pharmaceuticals (Porton Down). For in vitro experiments, WIN55212-2 (WIN) was dissolved in a 1:1 mixture of DMSO and a 0.9% aqueous NaCl solution (saline), while other compounds were dissolved in DMSO. For in vivo experiments, compounds were injected into a tail vein (10 ml kg^{-1}) using a 1:1 mixture of ethanol and alkamuls-620 diluted with saline to a final ethanol to alkamuls to saline ratio of 1:1:18.

In the vas deferens, O-4394 and O-4395 (100 nM) produced parallel dextral shifts in the log dose response curve of WIN, when this was first added 30 min after vehicle, O-4394 or O-4395. Mean apparent K_B values with 95% confidence limits shown in brackets were 4.8 nM (0.3 & 30.3 nM; n=7) for O-4394 and 3.9 nM (0.7 & 13.3 nM; n=7) for O-4395. The corresponding K_B of eΔ^9-THCV is 1.5 nM (Thomas *et al.*, 2005). In brain membranes, O-4394 and O-4395 displaced [^{3}H]CP55940, their mean K_i values with 95% confidence limits shown in brackets being 46.6 nM (31.3 & 69.4 nM; n=5) and 64.4 nM (49.0 & 84.7 nM; n=5) respectively. At 1 μM, O-4394 and O-4395 also produced parallel dextral shifts in the log concentration-response curve of CP55940 for stimulation of [^{35}S]GTPγS binding to brain membranes. Mean apparent K_B values with 95% confidence limits shown in brackets were 82 nM (54 & 124 nM) for O-4394 and 126 nM (83 & 196 nM) for O-4395 (n=5). Corresponding values for eΔ^9-THCV are 75.4 (K_i) and 93.1 nM (K_B) (Thomas *et al.*, 2005). Antinociception induced by Δ^9-THC at 10 mg kg^{-1} was attenuated by O-4394 and O-4395, injected immediately after Δ^9-THC at 0.3 (O-4395 only) or 3 mg kg^{-1} (P<0.01; ANOVA + Fisher's protected least significant difference post hocs; n=6 to 15). O-4394 and O-4395 were not antinociceptive at 3 or 10 mg kg^{-1} but were at 30 and 56 mg kg^{-1} (P<0.05; ANOVA + Fisher's protected least significant difference post hocs; n=6 to 11).

In conclusion, O-4394 and O-4395 exhibited in vitro potency as antagonists of CP55940 or WIN similar to that of eΔ^9-THCV. O-4394 and O-4395 also behaved as cannabinoid receptor antagonists in vivo, as indicated by their ability to antagonize Δ^9-THC.

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