Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst054.pdf

Plasma Membrane And Intracellular CB1 Receptors Modulate Migration Of Oligodendrocyte Progenitor Cells

M Alejandra Sanchez-Rodriguez[1], Oscar Gomez[1], MQ Uyen Le[1], Concepcion Sanchez-Caro[1], Francisco Molina-Holgado[2], Eduardo Molina-Holgado[1]. [1]Laboratorio de Neuroinflamacion, Hospital Nacional de Paraplejicos, Toledo, Spain, [2]Department of Life Sciences, Health Sciences Research Centre, University of Roehampton, London, UK

During CNS development oligodendrocyte progenitor cells (OPCs) migrate from germinal regions to myelinate neuronal axons that form white matter tracts. Among the signals that participate in this complex process, our recent studies reported the expression of a whole endocannabinoid system in oligodendroglial cells that participates in OPC differentiation and proliferation. The present study examines the subcellular location of cannabinoid receptor 1 (CB1) and the role of plasma membrane CB1 receptors versus intracellular sites in the *in vitro* migration of OPCs. Experiments using subcellular fractionation of OPCs showed that CB1 receptors are highly enriched in cytosolic fractions when compared to membrane fractions. The level of CB1 receptors is downregulated during OPC differentiation (1, 2, 3, 5 DIV). Similar results were obtained by immuofluorescence and confocal microscopy using polyclonal antibodies directed against sequences in either N or C-terminal tails of CB1 receptors. Interestingly, anti-CB1 C-terminal antibody mainly stained intracellular receptors, rarely labeled plasma membrane sites. To explore the relationship of intracellular CB1 receptors with the endosomal system we co-localized CB1 with specific antibodies to early (anti-EEA1), recycling RAR11), and late/lysosomes (LAMP) markers of endosomes, as well as with an antibody raised against mitochondria. Our data showed mainly co-localization with anti-mitochondria and to a lesser extent with LAMP1 and RAB11. In addition, we assessed the involvement of CB1 receptors in the migration of OPCs by different assays: agarose drop and Boyden chamber (chemotaxis assay). The CB1 receptor agonist ACEA (Arachidonyl-2-chloroethylamide) increased migration by 80 % in either assay ($p<0.001$ vs. control; $n=4$ by ANOVA followed by a post-hoc Turkey's multiple comparison test) an effect completely blocked by the CB1 antagonist AM281 ($p<0.01$ vs. ACEA; $n=4$). Hemopressin, a peptide antagonist of CB1 receptors that does not cross the plasma membrane reduced by 48 and 55 % ($p<0.01$ vs. ACEA; $n=4$) the number of migrating cells in agarose drop and Boyden assays, respectively. Overall, our data suggest specific functions of cells surface and intracellular CB1 receptors to modulate the migration of OPCs.

Funded: Ministry of Economy and Competitiveness of Spain (Instituto de Salud Carlos III, PI11/01729 to EM-H, CA09/00609 to MQUL) and Gobierno de Castilla-La Mancha (Servicio de Salud de Castilla-La Mancha SESCAM to MAS-R).