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Cannabidiol enhances microglial phagocytosis via TRPV2 activation

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Introduction: Microglia function as immune-like cells in the central nervous system in the detection and elimination, by phagocytosis, of invading pathogens and inappropriate macromolecules including β-amyloid in Alzheimer's disease (Kalaria *et al.,* 1996). Here we report the effects of cannabidiol (CBD) and other phytocannabinoids on microglial phagocytosis and describe a potential mechanism involved in the process.

Methods: Phagocytosis was assessed in BV-2 and HAPI microglial cell lines, in primary murine microglial cells and in RAW 264.7 monocytes. Cells ($5x10^5$) were cultured on glass coverslips (19 mm) in 12 well plates, and treated with cannabinoids for 24 hours after which the medium was removed and 0.5 µl of fluorescent BSA latex beads (1 µm in size) added to each well for 2 hours. After washing to remove non-phagocytosed beads, the cells were fixed with 4% paraformadehyde, washed with warm PBS and permeabilized with 0.1% Triton X-100. The beads were visualised by confocal microscopy and a phagocytic index calculated by normalizing the number of beads to the number of cells in each field. F-actin was detected by addition of rhodamine phalloidin and cell nuclei with DAPI. Western immunoblotting with an Odyssey imaging system (Li-Cor Bioscience) was used to measure the expression of the transient receptor potential V2 (TRPV2) channel protein. Cannabinoid CB1 and CB2 receptor expression was measured by quantitative PCR. Intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) was measured in FURA-2AM –loaded cells by ratiometric fluorescent imaging. RT-PCR. Data were analysed by ANOVA with Bonferroni *post-hoc* test.

Results: CBD (10µM) enhanced phagocytosis (187% vehicle-treated) in BV2 cells ($p^{**}<0.01$) whereas, other phytocannabinoids (CBG, CBDV, THCV, CBDA and CBGA 10 µM) were without effect ($p>0.05$). Qualitatively similar effects were seen in HAPI and RAW 264.7 types. The CBD-mediated enhancement of phagocytosis was inhibited by the transient receptor potential (TRP) channel blocker ruthenium red (70%) ($p^{**}<0.01$). CBD (10 µM) incubation caused a rapid increase in the expression of TRPV2 protein in BV2 cells ($p^{**}<0.01$) (266% vehicle-treated) measured by Western blotting and an apparent translocation to the cell membrane at 24 hours (immunocytochemistry). CBD (10 µM) also provoked significant increases in TRPV2 mRNA expression at 24 hours ($p<^{***}0.001$) (245% vehicle-treated) in BV-2 cells. CBD (10 µM) also caused a rapid and sustained increase in intracellular Ca^{2+} concentration in Fura-2-loaded BV2 cells over a similar time scale.

Conclusion: The results demonstrate that CBD but not other phytocannabinoids enhances microglial and monocyte phagocytosis. The effect appears to be mediated byTRPV2 channel activation leading to elevated $[Ca^{2+}]i$ and translocation of TRPV2 to the cell membrane.

Reference; Kalaria RN *et al* (1996). *Neurodegeneration.* **5**:497-503.

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