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Context-dependent control mechanisms of Omega-3 PUFAs and the endocannabinoid system in neural stem cells and microglia: Focus on the interaction between these two players

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Omega-3 polyunsaturated fatty acids (PUFAs) and the endocannabinoid (eCB) system have recently been found to stimulate neurogenesis both in vivo and in vitro. Omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have therapeutic potential in a variety of neurological and neurodegenerative conditions and also have significant neuroprotective potential in acute neurological injury, while the eCB system is widely recognised as a key signalling pathway which regulates axonal growth and drives adult neurogenesis. The aim of this study was to investigate the interplay between EPA or DHA and the eCB system, via CB2 cannabinoid receptor signalling, on neurogenesis in neural stem cells (NSC) and in microglial cultures. Serial dilution assays were used to identify the effect of EPA and DHA on NSC proliferation. Our results indicate that incubation of the NSC with the low doses of both EPA and DHA (one-way ANOVA and Kruskal Wallis post-hoc tests: [$F=15.518$; $P<0.001$ vs. control] and [$F=12.974$; $P<0.001$ vs control] respectively) significantly increased cell proliferation. Specifically, addition of EPA or DHA (both at 0.5 μM, t=7 days) resulted in an increase of 28.9% and 16.9%, respectively. Conversely, addition of higher concentrations of EPA or DHA (both at 8 μM, t=7 days) were seen to significantly decrease cell proliferation (EPA: $P<0.001$ vs control; DHA: $P<0.001$ vs. control). It was concluded that while low doses of EPA and DHA were beneficial to neurogenesis, high doses were seen to be neurotoxic for the NSC. Furthermore, BrdU ELISA analysis was used to identify the relationship between the omega-3 PUFAs and the eCB system in regulating neurogenesis. Blocking the CB2 receptor (AM630, 1μM, t=24h) attenuated the effects of DHA, but not EPA on BrdU incorporation in NSC, suggesting that DHA influences NSC proliferation through the CB2 receptor and its subsequently signalling pathways. Finally, it was found that addition of the omega-3 PUFAs resulted in a dose dependant decrease in the number of microglial cells, as assessed by BrdU ELISA. Indeed, addition of 8 μM of either EPA or DHA was toxic to the microglia (BrdU incorporation decreased by 38.6% ($P<0.005$ vs control) and 57.6% ($P<0.001$ vs. control) respectively). Importantly, the effects were still evident in the presence of AM630, suggesting that both EPA and DHA must act via non CB2 signalling pathways in microglial cells. By further elucidation of these distinct mechanisms it may be possible to increase our understanding of the therapeutic potential of omega-3 PUFAs, and thereby improve treatment strategies in the emerging field of brain repair. These studies are the first to indicate subtle, but important differences in the mechanisms of action of DHA and EPA in neurogenesis, and the mediation of the effects of DHA via the brain endobcannabinoid system.

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