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Role Of Endocannabinoid System During Neuronal Development *In vitro*

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Neurons are among the most complex and specialized cells in the human body, with differentiated domains to receive (dendrites) and transmit (axon) a flow of information. Therefore, the acquisition of a morphological and functional polarization represents the basis of the neural network functionality. The neuronal polarization process begins with the axonal specification and subsequent elongation. While the axon is growing the assembly of the axon initial segment (AIS), a crucial domain to generate action potential, takes place. Short after, dendritogenesis and synaptogenesis initiate during neuronal maturation stage (1). One of the regulatory systems that participate in neuronal differentiation is the endocannabinoid (eCB) system (2), and particularly 2-arachidonoyl glycerol (2-AG) signalling has recently been emerged as a molecular determinant of axonal growth and synapse formation (3). However, the role of eCB system during AIS development remains unknown. Thus, in this study we wanted to investigate how eCB system may act over neuronal morphology during different stages of neuronal polarity establishment, specially during AIS development.

Using a well established primary culture model of hippocampal cultured neurons (4) we analyzed successive stages of neuronal development in presence of 2-AG (3 μM) alone, or in combination with the CB1 antagonist SR141716A (1μM), or the inverse agonist AM251 (3 μM). Neurons treated with 2-AG developed significantly longer axons (441.21 ± 19.71 μm) compared with vehicle (ethanol) treated neurons (312.57 ± 13.89 μm, $p<0.001$). This effect was CB1 dependent, confirmed by 2-AG and AM251 combined treatment (279.90 ± 19.11 μm, $p=0.22$), with values similar to control neurons. Regarding to AIS development we analyzed ankyrin-G protein, the AIS master organizer, used in a previous work as integrity indicator of this domain (5). The presence of ankyrin-G was significantly reduced in neurons treated with AM251, where normalized ankyrin-G fluorescence intensity was only 72.98 ± 0.94 % (compared to 100% control value). In more advanced stages, while 2-AG treatment had no effect in dendrite length, neurons treated with AM-251 developed shorter dendrites (37.21 ± 2.13 μm *versus* 64.21 ± 2.13 μm, $p<0.001$). Similar results were obtained with SR141716A treatment (mean ± SEM, n=3 independent experiments, at least 30 neurons/experimental condition of each experiment).

Taken together, these results reveal that eCB system has different roles during neuronal differentiation *in vitro*. Despite the blockage of CB1 receptor has no effect on axonal elongation, in our conditions, exogenous 2-AG can maximize axonal growth at early development stages. Moreover, at later stages, signalling under CB1 receptor is determinant for proper AIS assembly and dendritic maturation.

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