The endocannabinoid system controls skeletal muscle cell differentiation via CB1 receptor-dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) and inhibition of K_v7 potassium channels

F.A. Iannotti[1], C. Silvestri[1], A. Martella[1], F. Piscitelli[1], P. Ambrosino[2], S. Petrosino[1], A. Schiano Morello[3], M. Taglialatela[2], V. Di Marzo[1]

[1]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche (CNR) Via Campi Flegrei 34 Pozzuoli (NA), Italy; [2]Department of Health Sciences, University of Molise, 86100 Campobasso, Italy; [3]Institute of Cybernetics, Consiglio Nazionale delle Ricerche (CNR), Via Campi Flegrei 34 Pozzuoli (NA), Italy

Myogenesis is a tightly regulated process in which proliferating myoblasts withdraw from the cell cycle and fuse to form multinucleated myotube [1]. The purpose of this study was to investigate the expression profile and functional role of the endocannabinoid system (ECS) during both skeletal muscle cell proliferation and differentiation.

Using murine C_2C_{12} cells as an experimental paradigm, we found a significant decrease in 2-AG, but not AEA levels during myotube formation. These changes were also corroborated by the expression of all the genes known to be involved in 2-AG and AEA metabolism. qPCR and western blot analysis also showed that the expression of *Cnr1* and *Cnr2* gene, encoding for CB1 and CB2 receptors, was increased during differentiation in C_2C_{12} cells and in primary human myoblasts, with *Cnr1* showing the highest degree of up-regulation. In C_2C_{12} cells, 2-AG (1-3 μM) and ACEA (1-3 μM) (a selective synthetic CB1 receptor agonist), inhibited myoblast differentiation and prevented myotube formation. On the other hand, the 2-AG, ACEA and AEA, stimulated cell proliferation. All these effects were shown to be mediated by CB1 receptors through pharmacological and molecular approaches.

In C_2C_{12} myoblasts, CB1 stimulation by ACEA was found to reduce PtdIns(4,5)P2 (PIP_2) levels and increased $[Ca^{2+}]_i$ in a PLC-dependent manner. Since 'neuronal' K_v7 potassium channels are regulated by PIP2 (2), with $K_v7.4$ channels playing a permissive role for C_2C_{12} differentiation process (3,4), we tested the involvement of $K_v7.4$ in CB1 receptor induced inhibition of myogenic differentiation. In C_2C_{12} cells, biochemical analysis revealed that CB_1 receptor stimulation by ACEA reduced PIP_2 levels and binding to $K_v7.4$ subunits. K^+ currents mediated by overexpressed $K_v7.4$ channels in CB1-co-transfected CHO cells were inhibited in by ACEA in a PLC-mediated manner. Moreover, the pharmacological combination between XE-991 (60 μM), a selective blocker of K_v7 subfamily, with ACEA (3 μM) allowed to further clarify how the ACEA effects during the myotube formation are K_v7-mediated. Taken together all these data indicate a novel role for the ECS in controlling skeletal muscle differentiation, and highlight $K_v7.4$ channels as potential targets for 2-AG/CB1-induced regulation of myogenesis.

References:

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