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Expansion of Tbr2 Intermediate Progenitors in the Developing Mouse Cortex Via CB1 Cannabinoid Receptor/mTORC1 Signalling

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The CB1 cannabinoid receptor, the major molecular target of endocannabinoids and marijuana active compounds, regulates cortical development and neuronal differentiation. Here we show that CB1 receptor activity drives intermediate cortical progenitor pool expansion by inducing the expression of Tbr2/Eomes via mammalian target of rapamycin complex 1 (mTORC1) signalling. In CB1 receptor-deficient mouse embryos (C57BL/6J), premature cell cycle exit of cortical progenitors, calculated as the fraction of BrdU+Ki67+/BrdU+ (0.49 ±0.03 in WT vs 0.29 ±0.002 in CB1 -/-; n=4, $p \leq 0.01$), and decreased numbers of Pax6-(461 ±39 /VZ+SVZ area in WT vs 334 ±26 /VZ+SVZ area in CB1 -/-; n=8 and 7, respectively; $p \leq 0.01$) and Tbr2-positive (387 ±29 /VZ+SVZ area in WT vs 245 ±32 /VZ+SVZ area in CB1 -/-; n=5 and 6, respectively; $p \leq 0.05$) cells were observed. Evaluation of the mTORC1 signalling pathway, by immunofluorescence and western blot analysis of the phosphorylation status of one of its substrates, the ribosomal protein S6, revealed that CB1 receptor deletion reduced mTORC1 activation in the proliferative ventricular and subventricular zones (0.14 ±0.02 in WT vs 0.10 ±0.02 in CB1 -/-; n=10, $p \leq 0.05$). Likewise, studies using acute CB1 receptor ablation in *Cnr1-floxed* cortical progenitors reduced cell proliferation (0.59 ±0.02 for the control vector vs 0.42 ±0.05 for the Cre-expressing construct; n=4; $p \leq 0.05$) and S6 phosphorylation *in vitro* (0.25 ±0.15 fold-decrease after Cre nucleofection; n=4; $p \leq 0.05$). Pharmacological manipulation of CB1 receptor activity in cortical slices and primary cortical progenitor cell cultures with the CB1 agonist HU-210 (1 μM and 100 nM in DMSO), respectively) increased Tbr2 expression (2.05 ±0,6 fold increase vs vehicle-treated slices; n=4; $p \leq 0.01$) and intermediate progenitor pool size (1.41 ±0.11 fold increase vs vehicle-treated slices; n=4; $p \leq 0.05$) in a rapamycin-sensitive manner, further supporting the involvement of mTORC1 signalling. Luciferase activity assays with Pax6 responsive elements (pCON/P3) and the Tbr2 promoter, together with ChIP analysis, showed that CB1 receptor-mediated regulation of Tbr2 expression occurs downstream of Pax6 induction. Preliminary results derived from ongoing studies aimed at investigating the role of CB1 receptors in human developmental cortical malformations associated to defective progenitor expansion will also be presented. In summary, our results demonstrate that CB1 receptor signalling exerts a crucial role in tuning dorsal telencephalic progenitor expansion by promoting Pax6/Tbr2 transcriptional activity via the mTORC1 pathway.