Gene regulation of GPR17, a checkpoint receptor in oligodendroglial differentiation

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ABSTRACT

GPR17 is a G protein-coupled receptor activated by both uracil nucleotides and cysteinyl-leukotrienes. We have previously demonstrated GPR17 is a key regulator of oligodendroglial differentiation and myelination. The receptor starts to be expressed in early oligodendrocyte precursor cells (OPCs), it reaches its maximal expression in immature oligodendrocytes and is then progressively down-regulated. In late OPCs, GPR17 forced expression led to impaired maturation, suggesting that its expression needs to be tightly time regulated.
Based on these evidences, this work was aimed at identifying the signaling molecules regulating GPR17 expression during oligodendroglial differentiation. For this purpose, we cloned a putative promoter region of Gpr17 into a reporter vector upstream to a gene encoding for a luciferase. Then, we transfected this construct in Oli-neu cells, an immortalized oligodendroglial cell line, and we set up a reporter assay to evaluate the bioluminescence produced in response to an array of stimuli.
Our results showed that treatment with both dibutyryl-cAMP, an analogue of cAMP, and forskolin, an activator of adenylyl cyclase, led to a significant increase of promoter activity, suggesting that cAMP signaling triggers GPR17 expression. To evaluate if GPR17 could be regulated by neuronal factors, we incubated cells with medium conditioned by cortical neurons. After 48h, we observed a significant induction of promoter activity; this effect was enhanced by heating the medium, suggesting neurons release one or more factors promoting oligodendroglial differentiation via Gpr17 gene, but that an inhibitory thermolabile factor is also present in the neuronal-conditioned medium. In line with this hypothesis, we found that insulin, a component of the medium formulation known to activate the mTOR pathway, strongly inhibited GPR17 promoter activity, whereas rapamycin, an inhibitor of the same pathway, significantly increased it. These data are consistent with the hypothesis that, while a neuronal-derived product activating cAMP is involved in turning GPR17 on, the mTOR pathway, likely activated by insulin-like growth factors, may be responsible for its physiological silencing at later stages of oligodendroglial development. These results may be relevant to the identification of new pharmacological strategies to activate/inhibit GPR17 under dysregulated conditions accompanied by myelination defects.
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