INSULIN-LIKE GROWTH FACTOR I (IGF-I) INDUCES HUMAN MU OPIOID RECEPTOR (MOR) EXPRESSION IN DIFFERENTIATED NEURONS: ROLE OF TRANSCRIPTION FACTORS REST AND STAT3

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Neuronal differentiation is a multifasic process in which the transcriptional repressor REST plays a key role binding a specific sequence (named RE-1) present on regulated genes and capable to block neuron specific gene expression in early stages of neuronal differentiation. In this way, REST allows the proper spatial and temporal expression of neuronal genes. In a previous study we showed that REST is elevated during early steps of neuronal induction by IGF-I in neuroblastoma cells and could contribute to down-regulate genes not yet required by the differentiation program while it declines after five days of treatment for the acquisition of neuronal phenotype. Mu opioid receptor (MOR) has been shown to be a target gene for REST repressive action; its expression, albeit maintained at low levels in undifferentiated neuronal cells, is significantly increased after *in vitro* differentiation by retinoic acid (RA) or phorbol esthers (PMA), suggesting a reverse correlation between REST repressive action and MOR expression levels in differentiated neurons. In the present study we investigated whether IGF-I, which may act in neuron through STAT3 activation, could determine alterations on MOR expression levels in differentiating neuroblastoma cells and which role could play REST in this process. We cloned five different MOR promoter fragments into a Luciferase reporter vector, differing the cloned fragments for bearing both REST and STAT3 binding sites, only STAT3 binding sites or none of them. MOR promoter/Luciferase constructs were used in transient transfection assays on human neuroblastoma cell-line SH-SY5Y, which endogenously expresses IGF-I receptor, MOR and REST, and on rat phaeochromocytoma cell-line PC-12, which lacks of REST expression. In SH-SY5Y, IGF-I increased transcriptional activity only of the MOR promoter fragments without RE-1 sequence; in PC-12 cells, IGF-I determined a transcriptional activation of all MOR promoter fragments, independently on RE-1 presence. Furthermore, in PC-12 cells expressing a recombinant form of REST, only RE-1 lacking MOR promoter fragments were transcriptionally induced by IGF-I. In SH-SY5Y cells, differentiated by treatment with RA and subsequently exposed to IGF-I, RA induced a 50% reduction in REST protein levels while IGF-I induced a 3-fold increase of MOR mRNA and protein levels. Taken together our results show for the first time that IGF-I induces MOR expression in differentiated neurons which lack of REST; thus, suggesting new and interesting perspectives for opioid pharmacology in adult nervous system as well as a new target for IGF-I action.