MECHANISM OF ACETYLCHOLINE-INDUCED CALCIUM SIGNALING, PROLIFERATION AND DIFFERENTIATION EFFECTS DURING *IN VITRO* NEURONAL DIFFERENTIATION OF EMBRYONAL CELLS

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Muscarinic (mAChRs) and nicotinic acetylcholine receptors (nAChRs) are involved in various physiological processes, including neuronal development. We provide evidence for expression of functional nAChRS and mAChRs during differentiation of P19 embryonic carcinoma cells, as *in vitro* model of early neurogenesis. We have detected expression and activity of α_2-α_7, β_2, β_4 nAChR and M1-M5 mAChR subtypes during neuronal differentiation. Nicotinic α3 and β_2 subunit gene expression was induced by addition of retinoic acid to P19 cell cultures. Gene expression of α_2, α_4-α_7, β_4 nAChR subunits decreased during initial differentiation and increased when differentiating cells underwent final maturation. Receptor response of nicotinic agonist-evoked Ca^{2+}-flux was observed in embryonic and neuronal-differentiated cells. Muscarinic receptor response was merely present in undifferentiated cells and increased during neuronal differentiation. The nAChR-induced $[Ca^{2+}]_i$ response in undifferentiated cells was due to Ca^{2+}-influx. In differentiated neurons, besides Ca^{2+}-influx through nAChR and voltage-gated Ca^{2+} channels, nAChR stimulation also induced Ca^{2+}-release from ryanodine- and IP_3-dependent intracellular stores. In both cell states, a7 subtype was the main mediator for Ca^{2+}-fluxes responsible for inhibiting proliferation of embryonic cells and inducing differentiation to neural progenitor cells. M2 and M3 were the principal mediators for Ca^{2+}-mobilization during final neuronal maturation of P19 cell and participate on the neuronal differentiation.
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