NEURONAL CELL FATE DETERMINATION IN P19 EMBRYONIC CARCINOMA CELLS IS DEPENDENT ON SPONTANEOUS CALCIUM OSCILLATIONS

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P19 embryonal carcinoma cells can generate *in vitro* progenitors of the three main cell lineages found in the CNS. The signaling pathways underlying the acquisition of differentiated phenotypes are poorly understood. In view of information being transmitted through the frequency of action potentials in the mature nervous system, the effect of different Ca^{2+}-spike frequencies on P2X, P2Y and nicotinic acetylcholine receptor (nAChRs) expression was investigated. Here we tested the hypothesis that Ca^{2+}-signaling controls differentiation of neural precursors to neuronal phenotypes expressing purinergic receptors and nAChRs. Depolarization generating Ca^{2+}-influx, such as neuronal activity does, stimulated expression levels of $P2X_{1,4,6}$ and $P2Y_{1,2,4,6}$ receptors and nAChRs ($\alpha_{3,4,5,6,7}$ and $\beta_{2,4}$). Suppressing elevation of Ca^{2+}-levels in progenitor cells also enhanced expression of $P2X_1$ and α_3 subtypes. Expression of neuronal markers was upregulated in progenitor cells by increasing frequencies of Ca^{2+}-spikes, and expression of these markers was reduced by inhibiting Ca^{2+}-spikes. Oscillations were initiated by activating voltage-operated calcium channels and IP_3-mediated Ca^{2+}-release. Neuronal cell fate determination analysis after inhibiting calcium pathways underlined that IP_3-mediated Ca^{2+}-release was necessary for progress of neuronal differentiation. Thus, spontaneous Ca^{2+}-signals are an intrinsic property of differentiating neurosphere-derived precursors. Their frequency specifies the acquisition of a neuronal phenotype.

Keywords: Spontaneous Ca^{2+} oscillations; Ca^{2+} signaling; purinergic receptors; acetylcholine receptors; neuronal differentiation.

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