EXPRESSION OF FUNCTIONAL NR1/NR2B-TYPE NMDA RECEPTORS IN NEURONALLY DIFFERENTIATED SK-N-SH HUMAN CELL LINE

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The present study demonstrates that human SK-N-SH neuroblastoma cells, differentiated by retinoic acid (RA), express functional NMDA receptors and become vulnerable to glutamate toxicity. During exposure to RA, SK-N-SH cells switched from non-neuronal to neuronal phenotype by showing antigenic changes typical of postmitotic neurons together with markers specific of cholinergic cells.

Neuronally differentiated cells displayed positive immunoreactivity to the vesicular acetylcholine transporter and active acetylcholine release in response to depolarizing stimuli. The differentiation correlated with the expression of NMDA receptors. RT-PCR and immunoblotting analysis identified NMDA receptor subunits NR1 and NR2B, in RA-differentiated cultures. The NR1 protein immunolocalized to the neuronal cell population and assembled with the NR2B subunit to form functional NMDA receptors.

Glutamate or NMDA application, concentration-dependently increased the intracellular Ca$^{2+}$ levels and acetylcholine release in differentiated cultures, but not in undifferentiated SK-N-SH cells. Moreover, differentiated cultures became vulnerable to NMDA receptor-mediated excitotoxicity. The glutamate effects were enhanced by glycine application and were prevented by the NMDA receptor blocker MK 801, as well as by the NR2B selective antagonist ifenprodil.

These data suggest that SK-N-SH cells differentiated by brief treatment with RA may represent an unlimited source of neuron-like cells suitable for studying molecular events associated with activation of human NR1/NR2B receptors.

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Fig. 1: Expression of NR1 and NR2B proteins in RA-differentiated SK-N-SH cells. NR1 immunostaining was slight in control cells (A) but very marked in neuronal cells of RA-treated cultures (B). Western blot analysis confirmed the expression of NR1 and NR2B proteins in RA-differentiated cultures (RA). Bands corresponding to NR1 (~115 kDa) and NR2B (~180 kDa) were found in extracts from rat hippocampus, run in the same gel as positive control (data not shown).

Fig. 2: RA-differentiated cells (RA) released [$^3$H]ACh in response to depolarizing stimuli. Differentiated cells (RA), but not control cells, responded to NMDA application. The effect elicited by 5 mM NMDA was prevented by 1 µM MK 801, added 5 min before NMDA. Data are means ± standard errors of at least four experiments run in triplicate. Wilcoxon’s rank sum test was used for the statistical analysis of values. * p < 0.01 versus basal value.

Fig. 3: Glutamate-induced excitotoxicity. LDH release was assayed in control (open bars) and RA-treated (black bars) SK-N-SH cells exposed to glutamate (100-500 µM) for 15 min. No modification of LDH release was produced by glutamate in control cultures. In RA-treated cells glutamate caused a dose-dependent increase in extracellular LDH activity. This effect was prevented by 1 µM MK 801 but not by 1 µM CNQX, both added 5 min before glutamate exposure. Application of 5 mM NMDA for 60 min in the presence of 10 µM glycine was also toxic to RA-differentiated cells. NMDA effect was prevented by 1 µM ifenprodil added 5 min before NMDA. Bars represent the means ± standard error of at least three experiments run in quadruplicate. Wilcoxon’s rank sum test was used for the statistical analysis of values. * p < 0.01 versus basal value.