

CHOLINERGIC MODULATION OF EARLY GROWTH RESPONSE FACTOR-1 (EGR-1) EXPRESSION ON HUMAN NEUROBLASTOMA SK-N-BE CELLS

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The transcription factor Egr-1 (Early Growth Response Factor-1, also called Krox24, zif268 and NGF1-A) plays critical, but not fully understood, roles in differentiation, growth, and in response to environmental signals. In our previous *in vivo* studies [1], we described its expression in hippocampus of male rats subjected to spatial memory tests, suggesting a correlation between Egr-1 variations and cholinergic expression. Based on this, our goal in the present work was to *in-vitro* investigate the expression of Egr-1, to verify the link between stimulation of muscarinic receptors and Egr-1 increase. Particularly, we evaluated the existence of an Egr-1 rapid switch-off mechanism due to a two doses administration of an agonist-drug in quick succession (double stimulation).

The experimental system was constituted by human neuroblastoma SK-n-BE cells following retinoic acid-induced neuronal differentiation, and we measured mRNA and protein levels of Egr-1 and its co-repressor NAB-2 (NGFI-A binding protein-2) after administration of the muscarinic receptor agonist carbachol, either alone or in conjunction with scopolamine (non-selective antagonist) or pirenzepine (M_1 -antagonist). Total mRNA was quantified using RT-PCR technique, and western blottings were performed to confirm expression trend on protein levels. The differences versus controls were determined by densitometric analysis and data corrected by expression values of the housekeeping genes GAPDH and tubuline.

Carbachol treatment (50-500 μ M; time-course 30min-24h) stimulated a rapid, large and dose-dependent increase in Egr-1 levels, with a maximum of about 6-fold over basal at 1h (mRNA) and 10-fold at 2h (protein) following administration. This effect was completely blocked by a pre-treatment of 15min with scopolamine (50 μ M) or pirenzepine (100 μ M).

In the double stimulation with carbachol 500 μ M (30min, culture medium removed, next dose for 10min), the second brief incubation with agonist quickly lead to basal the previously-induced increase in Egr-1 mRNA levels. On the contrary, a single (or first) treatment with carbachol solution at higher concentration decreased the NAB-2 expression, while after double stimulation the co-repressor mRNA levels returned to normal values.

These results confirm the correlation between the increase of Egr-1 expression and exposure to muscarinic agonists; the induction seems to be mediated by M_1 receptor, the main subtype in the CNS, because completely blocked by the selective antagonist pirenzepine. A second cholinergic stimulus decreases the Egr-1 over-expression, with a switch-off mechanism that may involve the NAB-2 gene, suggesting that Egr-1 mediates the induction of its own repressor.

1) Casale F, Ghi P, Orsetti M, and Ferretti C. - Behav Pharmacol 2004; vol 15, Nos 5&6: A11.