

TRAFFICKING OF $\alpha 3\beta 4^*$ NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

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Neuronal nicotinic acetylcholine receptors (nAChRs) have essential roles in physiological conditions. Alterations in expression of these receptor proteins are involved with several pathological conditions including Alzheimer's and Parkinson's disease. Adenomatous polyposis coli (APC), well-known as a tumor suppressor protein, is present in many cell types. Although the function of APC in the neuronal system has not been established, some evidence suggests that APC is involved with neuronal nAChRs. Recently, APC has been shown to localize $\alpha 3$ nAChRs to neuronal postsynaptic sites.¹

The aims of our studies are to investigate the involvement of APC in the trafficking $\alpha 3\beta 4^*$ nAChRs in adrenal chromaffin and in a stably transfected HEK 293 expressing recombinant bovine adrenal $\alpha 3\beta 4^*$ nAChRs (BM $\alpha 3\beta 4$ cells). First, we wanted to establish the presence of APC mRNA and APC protein in bovine adrenal chromaffin cells and in BM $\alpha 3\beta 4$ cells. Using RT-PCR techniques, we identified cDNA sequences with 97% (BM $\alpha 3\beta 4$ cells) and 93% (bovine adrenal chromaffin cells) homology to the human APC sequence. This confirmed the presence of APC mRNA in both cells. Western blot analyses of cell extracts from both cells confirmed the presence of 282.8 kD and 270.0 kD APC proteins in HEK and chromaffin cells, respectively. BM $\alpha 3\beta 4$ cells were then transfected with APC stealth siRNA for 24 hours. Receptor binding assays, coupled with receptor alkylation, were conducted at 24, 48 and 72 hours posttransfection. We compared these results to the results in puromycin (protein synthesis inhibitor) treated groups. The number of intracellular nAChRs (R_i) was significantly decreased (40.9% and 26.8% at 48 and 72 hours, respectively, $p < 0.05$) whereas that of surface nAChRs (R_s) was significantly increased (270.5% and 274.8% at 48 and 72 hours, respectively, $p < 0.05$). The numbers of R_i and R_s were correlated to the ratios of R_i/R_t and R_s/R_t. Compared to puromycin treated group, we found a reduction in all nAChRs (75.9%, 83.8% and 9.0% of R_t, R_i, and R_s, respectively). Our data suggest that siRNA transfection caused a reduction of APC production within cells which, in turn, led to movement of R_i to the cell surface for maintenance of normal surface expression of R_s. In conclusion, we suggest that APC is involved with nAChR trafficking in bovine adrenal chromaffin cells.

1. Temburni, MK. et al. (2004) *J Neurosci.* 24(30), 6776-6784.

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