PROTEOLITIC PROCESSING OF THE β-AMYLOID PRECURSOR PROTEIN (APP) IN MEMBRANES OF THE HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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INTRODUCTION

Deposition of β-amyloid peptide, derived from the altered processing of APP, is an invariant feature of Alzheimer's disease. APP, substrate for three different proteases, can undergo two different proteolitic pathways: the non-amyloidogenic pathway involving α- and γ-secretases, and the amyloidogenic pathway involving β- and γ-secretases. Both APP and secretases are membrane-bound proteins, but data concerning their membrane distribution yielded diverse results. Indeed, studies have been often performed using APP- and/or secretase-overexpressing cells and the membrane protein overexpression might lead to altered interactions with membrane lipids. Here we report the proteolitic processing of APP in membranes of non-transfected SH-SY5Y cells.

METHODS

Membrane fractions were isolated by a 5-30% linear sucrose gradient fractionation in the presence of Triton X-100. Every fraction was characterized for its content in lipids and proteins: lipids (cholesterol, sphingomyelin, glycosphingolipid) were analyzed by HP-TLC and proteins (flotillin, actin, transferrin receptor, APP and its proteolitic fragment, α-,β- and γ-secretases) by immunoblotting with specific antibodies.

RESULTS

Membrane fraction analyses show the existence of two distinct pools of APP: whereas the major pool is in the lipid rafts where there is also the fragment β-CTF and β- and γ-secretases, the major pool is in "non-raft" membrane regions where there are also localized α-CTF, α- and γ-secretases.

CONCLUSIONS

These data are consistent with the concepts that APP amyloidogenic processing occurs in raft domains in contrast to non-raft localized non-amyloidogenic process. Moreover, they indicate non-transfected SH-SY5Y cells as valid cellular model to unravel how access of these enzymes to APP is regulated.