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Short communication

Vitamin E protects against Alzheimer's amyloid peptide (25–35)-induced changes in neocortical synaptosomal membrane lipid structure and composition

Tanuja Koppal [a,c], Ram Subramaniam [a,c], Jennifer Drake [a], M. Renuka Prasad [b], Harbhajan Dhillon [b]

lipid extraction was performed by the method of Bligh and Dyer [1], as modified in Ref. [4]. The lipid extract obtained was then dissolved in 200 μl of chloroform, of which 100 μl was spotted on silica gel G TLC plates for FFA analysis. The respective regions were scrapped into 1.5 ml methanol containing 0.005% BHT (butylated hydroxy toluene). Fatty acids in all the fractions were converted to their methyl esters and then quantitated on a Hewlett-Packard gas chromatograph as previously described [4].

For EPR studies, Aβ (25–35) was first dissolved in PBS (pH 7.4), 1 mg/ml, and divided into two aliquots, one aliquot was then treated with vitamin E (final concentration 5 mM). The peptide solutions were then incubated in a water bath at 37°C for 6 h. Protein concentration in the purified synaptosomes was adjusted to 8 mg/ml, and the membranes were spin labeled with 12-NS as described earlier [3,7]. A 0.5 ml of peptide incubate was then added to 0.5 ml of synaptosomes at the time of acquisition of the spectra. The Student's *t*-test was used to determine *P*-values. P - 0.05 was considered to be statistically significant for comparison between data sets.

A typical spectrum of 12-NS labeled synaptosomal membrane is shown in Fig. 1A. Due to the partitioning of the spin label between the lipid phase and the aqueous phase, one can observe two environments in the EPR spectrum, the lipid-bound (B) and the aqueous-resident, free (F) component. The amplitude of the mid-field line (B_0) was found to be the most sensitive parameter for lipid

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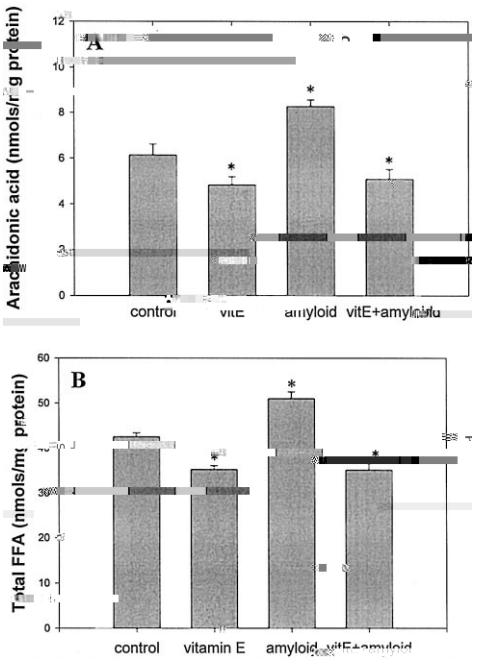

Fig. 2. (A) Effects of vitamin E, Aβ (25–35) and vitamin E + Aβ (25–35) on arachidonic acid (20:4) liberation. Treatment of synaptosomal membranes with Aβ (25–35) did cause a significant increase in the amount of 20:4 as compared to the control (P - 0.04). Preincubating both control and Aβ (25–35) treated membranes with vitamin E lead to a significant reduction in the amounts of 20:4 released (P - 0.04). (B) Effects of vitamin E, Aβ (25–35) and vitamin E + Aβ (25–35) on the total free fatty acid release observed. Treatment of synaptosomal membranesoD 0 T5 ( )

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