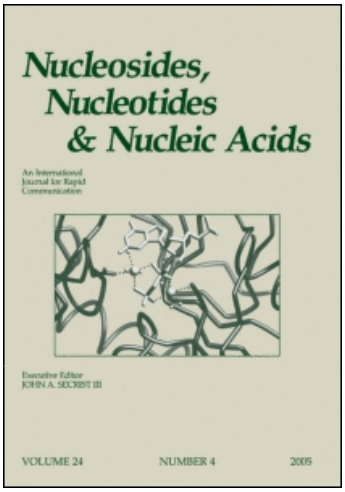

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Ascorbate-Induced Lipid Peroxidation and Inhibition of [^{3}H] Adenosine Binding to Rat Brain Synaptosomes

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ASCORBATE-INDUCED LIPID PEROXIDATION AND INHIBITION OF [^{3}H] ADENOSINE BINDING TO RAT BRAIN SYNAPTOSOMES

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Abstract: Lipid peroxidation induced by ascorbate inhibited [^{3}H] adenosine binding to rat brain synaptosomes, probably via changes in polyunsaturated fatty acid composition.

Ascorbic acid can induce the formation of lipid peroxides in subcellular fractions from brain, and lipid peroxidation by ascorbic acid can lead to large decrements in binding of various ligands to receptors Muakkassah-Kelley et al[1].

In the present work, we studied the effects of ascorbic acid on the binding of [^{3}H] adenosine to rat cortical synaptosomes,and its correlation with lipid peroxidation.

Synaptosomes were prepared from rat brain homogenates according to the method described by Hajós[2]. Binding of [^{3}H] adenosine was performed according Bender et al[3].Lipid peroxidation was induced by incubation,during 10 min (37^{o}C, pH 7.4) with various concentrations of ascorbic acid (0.01mM-2mM). The extent of peroxidation was measured by the thiobarbituric acid test with the method of Kovachich and Mishra[4]. The fatty acid composition analysis was perfomed by gas liquid chromatography with a dual flame ionization detector.

Ascorbic acid (0.1mM) inhibited the specific binding of [^{3}H] adenosine by 53% and 1mM by 78.3%. Lower (0.01mM) and higher (2mM) concentrations of ascorbic acid caused smaller inhibitions in [^{3}H] adenosine corresponding to 22% and 19% respectively, TABLE 1.

This effect of ascorbate on [^{3}H] adenosine binding was temperature-dependent and more evident at 37^{o}C, than at 20^{o}C.

TABLE 1 - Effects of Ascorbic Acid on the specific binding of [^{3}H] Adenosine and on lipid peroxidation

Agent (nM)	[^{3}H] Adenosine Binding (nmoles/mg protein)	Lipid Peroxidation (pmoles malonaldeyde/ /mg protein)
None	869 ± 41	300 ± 43
Ascorbic acid (0.01 mM)	678 ± 32	362 ± 51
Ascorbic acid (0.1 mM)	408 ± 52	505 ± 92
Ascorbic acid (1 mM)	191 ± 20	761 ± 138
Ascorbic acid (2 mM)	704 ± 60	349 ± 5

The [^{3}H] Adenosine concentration was 227 nM
The results are the mean ± S.E.M. of four experiments.

Scatchard analysis revealed that the major effect of ascorbic acid was a reduction in the number of ligand sites. Analysis of fatty acid composition of synaptosomal membranes showed that the membrane contained polyunsatured fatty acids and the ratio (saturated/unsaturated) fatty acids was 2.46 in the control preparation and in treated synaptosomes with 1mM ascorbic acid was 7.14.

The results suggest that ascorbic acid through formation of peroxide intermediate attack double bonds of unsaturated fatty acid components of polar lipids, which are essential to maintain structural integrity of the binding site for adenosine.

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