

Thesis title      Characterization of rabbit cerebellar  
glutamate binding and effect of dietary  
vitamin E on these binding sites.

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#### ABSTRACT

Indirect evidence implies that glutamate receptor in cerebellum may involve some neuropathological syndromes, one of which is movement disorder. However, extensive studies using rabbit cerebellum have not yet fully identified and characterized the various types of glutamate receptor binding. In the present study, glutamate receptor binding was performed by using 100 nM L-[<sup>3</sup>H] glutamic acid and rabbit cerebellar membrane. The binding was saturable and reversible. It reached equilibrium within 45 min and dissociated from the receptor with a half-life of 21 min. The saturation curve was analyzed by the scatchard plot and the Enzfitter computer program.

It indicated a single binding site with a dissociation equilibrium constant ( $K_D$ ) of  $256.75 \pm 99.55$  nM and a receptor density ( $B_{max}$ ) of  $274.30 \pm 126.80$  p mol/mg protein. The  $IC_{50}$  values for L-glutamate, L-aspartate, GDEE, AMPA, and quisqualate were 0.44, 0.54, 12, 56, 101  $\mu$ M, respectively, whereas values for D-glutamate, D- $\alpha$ -aminoadipic acid, 2 APB, NMDLA, D-aspartate and ibotinic acid were greater than 100  $\mu$ M. NMDA, glycine, kainate and GABA were inactive. Based on these results, the quisqualate receptor subtype in the rabbit cerebellar membrane is indicated.

It has been suggested that cerebellum is more susceptible to damage from vitamin E deficiency than other parts of the brain. The L- $[^3H]$  glutamate binding to rabbit cerebellar membrane was studied in both high vitamin E and low vitamin E dietary conditions. The high (300.56 mg % vitamin E/day) and low (0.56 mg % vitamin E /day) vitamin E diet groups had  $B_{max}$  values (average from four individual experiments.) of  $636.87 \pm 36.67$  and  $265.28 \pm 60.38$  p mol/mg protein,  $K_D$  values of  $232.70 \pm 76.75$  and  $120.19 \pm 15.45$  nM, respectively. Significant difference of the maximum receptor density ( $B_{max}$ ) from control (3 mg % vitamin E / day) was observed only in the high vitamin E group, where it was increased. The  $B_{max}$  and  $K_D$  values of the low vitamin E

group were not significantly difference.

From the histological study, the low vitamin E group showed low affinity of staining with cresyl violet of granule cells and these cells were losely packed, whereas the high vitamin E group remained the same as that observed with normal diet. This result and others indicate that both high and low dietary vitamin E may influence glutamate receptor for rabbit cerebellar function.