Preservation of N-Methyl-D-Aspartate Receptor Binding Sites With Age in Rat Neocortex

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This study used [3H]dizocilpine ([3H]MK-801) binding to examine glycine, polyamine, and zinc subsites of the N-methyl-D-aspartate (NMDA) receptor in well-washed membranes derived from the neocortex of Fischer 344/Norwegian brown rats aged 3, 12, 24 and 37 months. [3H]Dizocilpine binding in the presence of 100 μM glutamate was enhanced by the addition of 30 μM glycine. Binding in the presence of both glutamate and glycine plus zinc modulatory sites, in the long-lived Fischer 344/Norwegian brown strain of rat aged 3, 12, 24, and 37 months. 

METHODS

Male hybrid Fischer 344/Norwegian Brown rats aged 3, 12, 24, and 37 months were obtained from the National Institute on Aging colonies (Bethesda, MD) and were used within 2 weeks of arrival. Animals were maintained on a 12-hour light-dark cycle with free access to food and water. Following decapitation, the neocortex was dissected free of white matter on an ice-chilled petri dish and then frozen (−80°C). Membranes were prepared and assays performed, as described previously (16). Briefly, using well-washed membranes, binding assays were performed in a 0.4 ml final volume of 5 mM Tris-acetate (pH 7.4) for 1 hour at 25°C. Assays used 5 nM [3H]dizocilpine and drugs, dissolved in Tris-acetate buffer, as appropriate. Nonspecific binding was defined by 100 μM dizocilpine and represented 10% of total binding. Curves monitoring the effects of arcaine were performed in the presence of both 100 μM glutamate and 30 μM glycine to preclude actions at glutamate and glycine sites and to reduce the influence of variable amounts of amino acids in the tissue samples. It is unlikely that these conditions measure [3H]dizocilpine binding at equilibrium. However, by monitoring nonequilibrium conditions it is possible to detect the agonist actions of glycine and polyamines as well as the antagonist actions of the competitive polyamine site antagonist arcaine and the divalent cation zinc (5,6,17). Glycine enhancement of binding was determined as fold increase according to the following equation: fold increase = (maximal binding + basal binding)/basal binding, where maximal binding is binding in the presence of 100 μM glutamate and 30 μM glycine, and basal binding is binding in the presence of 100 μM glutamate. Inhibition of binding was quantified by determining values from Hill plots using Accufit-competition software (Lundon Software, Chagrin Falls, OH). Protein concentrations were determined as described previously (16). The effect of age in animals aged 3, 12, 24, and 37 months was assessed by analysis of variance (ANOVA) (css:statistica, StatSoft, Tulsa, OK). Data are presented as
mean ± SEM. Probabilities of p < .05 were considered significant.

RESULTS

[3H]dizocilpine binding was assessed in rats aged 3, 12, 24, and 37 months and the results shown in Table 1. [3H]dizocilpine binding in the presence of 100 μM glutamate was enhanced by the addition of 30 μM glycine as described previously (5,6,16). Binding in the presence of both glutamate and glutamate plus glycine was unaffected by age. The competitive polyamine site antagonist arcaine inhibited [3H]dizocilpine binding in a dose-dependent fashion, and 50 μM spermidine caused a rightward shift in this dose response curve, as shown previously (16,17) (data not shown). IC50 values derived from these plots were not significantly affected by age. Zinc inhibited binding in a dose-dependent fashion, as shown previously (14), but this inhibition was unaffected by age. The absence of a statistically significant effect of age is supported by the absence of any clear trends when mean values are compared across the four age groups.

DISCUSSION

Using [3H]dizocilpine binding, this study has shown that there was no effect of aging on either the glycine co-agonist site or the polyamine and zinc modulatory sites of the NMDA receptor in neocortex of Fischer 344/Norwegian Brown rats aged 3, 12, 24 and 37 months. Binding in the presence of 100 μM glutamate alone was also unaffected by age. Together, these data suggest a preservation of the NMDA receptor with aging. This is in agreement with the study of Shimada and colleagues (18), who showed no change in [3H]dizocilpine binding in the frontal cortex of Sprague-Dawley rats aged 2, 8, and 25 months. However, Tamaru and colleagues (19) found diminished [3H]dizocilpine binding in the presence of both glutamate plus glycine and glutamate plus glycine plus spermidine in neocortex of Fischer 344 rats aged 7 and 29 months when compared with animals aged 3 months. These changes were more pronounced in the presence of spermidine than in the absence of this polyamine. The actions of polyamines at the NMDA receptor are complex and consist of both stimulatory and inhibitory components (6,13,17). It is therefore possible that this confounded the study of Tamaru and colleagues (19). Because arcaine appears to inhibit the stimulatory actions of polyamines (which predominate at lower concentrations) without affecting the inhibitory actions (17), its use in the present study probably provides a more reliable measure of the polyamine site of the NMDA receptor and is consistent with competitive antagonism of the increase in binding produced by spermidine. Age-associated reductions in [3H]CPP and [3H]glycine binding to the NMDA receptor were reported by Kito and colleagues (20), but because these changes reflected differences from animals aged 2 months of age and the process of brain maturation may not be complete until 12 months (21), it seems likely that their results reflect changes occurring as a result of brain maturation rather than senescence. Similarly, the autoradiographic study of Mitchell and Anderson (22) showed reduced [3H]dizocilpine binding in the inner frontal cortex and the entorhinal cortex of animals aged 12 and 24 months when compared with animals aged 6 months. The possibility of such false positive data has been minimized in the present study by examining 3 groups of animals aged 12 months or older. It is also possible that the discrepancy between the autoradiographic study of Mitchell and Anderson and the present study of well-washed cortical membranes is attributable to methodological differences, or it may be a consequence of age-related changes occurring only in discrete regions of cortex and therefore not apparent when the whole neocortex is assessed.

In conclusion, like EAA nerve terminals (15; which were assessed in the same animals), the glycine, polyamine and zinc subsites of the NMDA receptor in rat neocortex are spared in aging.

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REFERENCES

5. Reynolds IJ, Murphy SN, Miller RJ. 3H-labelled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. Proc Natl Acad Sci USA. 1987;84:7744–7748.

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