Abstract

The current study dissected the fascia dentata (FD) and hilar region from the CA and subicular cell fields of the rat and conducted in vitro determinations of the number of binding sites for N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) glutamate receptors across the lifespan. We determined the density of binding of [3H]-glutamate or [3H]-AMPA to NMDA or AMPA receptor sites, respectively. The changes reported might be due to either a change in receptor number or an alteration in the binding characteristics of the receptor site with aging. We found an age-related decline in the number of NMDA receptors in the CA1, CA3 and subicular cell regions of the hippocampus, but not in the FD/hilar region, and an age-related decline in the number of AMPA receptors in the FD/hilar region, but not in the CA fields. The decline in the number of NMDA or AMPA receptors that occurs with aging was not a continuous or homogeneous process. These changes in receptor number might underlie selected age-associated changes in sensitivity to drugs that influence hippocampal function as well as to changes in NMDA-dependent long-term potentiation. A thorough understanding of the mechanisms underlying changes in glutamate receptor function in discrete brain regions, using combined neurochemical and electrophysiological methods, may ultimately provide insight into the fundamental substrates of age-associated memory disorders related to hippocampal dysfunction. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Normal aging is associated with a decline in hippocampal-dependent mnemonic abilities in humans [1], non-human primates [2] and rodents [4]. Glutamate is an important neurotransmitter at hippocampal synapses and is known to be critical for normal learning and memory processes [8]. Glutamate receptors that are sensitive to either N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) are involved in synaptic modification that can be induced in this structure [7,8] that has been hypothesized to reflect a mechanism of information storage [6,16]. Pharmacological blockade of these glutamate receptor subtypes impairs learning and memory performance [17] and can block the induction of long-term potentiation (LTP) in the hippocampus [19]. While facilitation of the function of these receptor subtypes can enhance memory [24], hyperactivity of these neurotransmitter receptors may underlie the neurodegenerative changes and decline in hippocampal function associated with aging [20]. Investigations of changes in the number of these receptors in aging populations have produced conflicting results. Laboratories have reported either no change [23] or a decline [7,15,21,25,26] in numbers of these receptors within the hippocampus. Much of this variation may be related to differences in the species or strain investigated [13,15] or the particular ligand used in the binding assays. In addition, the apparent discrepancy might also be related to regional changes in receptor density within the hippocampus. In the present
study, we dissected the fascia dentata (FD), and hilar region from the CA and subicular cell fields and conducted in vitro determinations of the number of binding sites for AMPA and NMDA glutamate receptors across the lifespan.

2. Materials and methods

2.1. Subjects

Thirty-eight male Sprague–Dawley rats (National Institute on Aging, Bethesda, MD) were fed ad libitum and housed under 12/12 h light conditions until sacrificed. The age (in months) and number (in parentheses) of rats used in this study were as follows: 3 (3), 6 (3), 9 (3), 12 (2), 14 (1), 15 (2), 16 (1), 17 (1), 18 (2), 23 (2), 24 (2), 25 (4), 26 (2), 28 (2), 29 (7) and 31 (1).

2.2. Tissue dissection

The rats were briefly anesthetized using metophane gas and then quickly sacrificed by decapitation. The brains were quickly removed and the hippocampus was dissected out, placed ventral side up (Fig. 1A) on a microdissection tray on ice. Using a microspatula, the FD was dissected free of the CA and subicular fields by gently teasing it away along the hippocampal fissure. Further transection along the margin of the free blade produced a block consisting of subiculum, CA1 and most of CA3 (Fig. 1B) with no granule cells attached, and a block consisting of primarily granule cells of the FD hilar neurons and some CA3c pyramidal cells (Fig. 1C). The tissues were quickly frozen on dry ice and then stored (at −70°C) until all of the samples were collected from all rats.

2.3. Receptor binding assays

Tissues from all age groups were processed at the same time. Membrane fractions were prepared from the tissues by homogenization and repeated centrifugation to produce the P2 fraction; these were followed by hypo-osmotic lysis and repeated washings to remove endogenous neurotransmitters. The left hemisphere’s hippocampus was used for the AMPA assays while the right side was used for the NMDA receptor determinations. Due to the small size of the samples and the desire to avoid pooling tissues only single-point determinations were made.

The NMDA receptor binding assays were performed according to the method of Hall et al. [11]. The P2 fractions were resuspended in buffer containing 500 mM Tris–acetate/50 μM EGTA. The assays were conducted in an incubation volume of 500 μl containing [3H]-glutamate (4.8 nM) and 15–20 μg of membrane protein. Non-specific binding was defined by the addition of 50 μM aminophosphonovalerate (AP-5). Incubation was carried out at 0°C for 60 min and terminated by dilution with 4 ml of ice-cold 50 mM Tris–acetate/EGTA buffer, pH 7.2, followed immediately by filtration through GF/C filters that had been presoaked in 0.03% polyethylenimine.

AMPA receptor binding assays were performed according to the method of Hoffman et al. [12]. The P2 fractions were resuspended in buffer containing 500 mM Tris–acetate/50 μM EGTA/50 mM KSCN. The assays were conducted in an incubation volume of 500 μl containing [3H]-AMPA (4.8 nM) and 15–20 μg of membrane protein. Non-specific binding was defined by the addition of 5 mM glutamate. Incubation was carried out at 0°C for 60 min and terminated by dilution with 4 ml of ice-cold 50 mM Tris–KSCN buffer, pH 7.4, followed immediately by filtration through GF/C filters that had been presoaked in 0.03% polyethylenimine. Age-related differences in the number of receptor binding sites were analyzed by ANOVA.

3. Results

3.1. NMDA receptor changes

There was a significant main effect of Age [F11,34 = 2.89, P = 0.0016] for the number of NMDA receptors in the CA region of the hippocampus. Post-hoc comparisons using Student–Newman–Keuls method found that levels of NMDA receptors in the 25 and 29 month groups differed significantly (P < 0.05) from the 3 month levels. In contrast, there was no significant main effect of Age [F11,34 = 1.14, P = 0.38] for the number of NMDA receptors in the FD region. The results are shown in the top frame of Fig. 2: the data have been grouped as follows: 3–9 month, 12–17 month, 18–24 month, 25–28 month and 29–31 month.

There was a significant main effect of Age [F11,34 = 2.69, P = 0.049] for the number of AMPA receptors in the FD region. Post-hoc comparisons found that levels of AMPA receptors in the 18 month group differed significantly (P < 0.05) from the 3 month levels. In contrast, there was no main effect of Age [F11,34 = 1.06, P = 0.39] for the number of AMPA receptors in the CA1 region of the hippocampus. The results are shown in the bottom frame of Fig. 2.

4. Discussion

The current investigation provides a converging line of evidence with earlier autoradiographic studies of age-related changes in hippocampal glutamatergic receptors [7,13,15] and extends these findings across a wider range of ages. The main findings of this study are that there is an age-related decline in the number of NMDA receptors in the CA1, CA3 and subicular cell regions of the hippocampus, but not in the FD/hilar region, and an age-related
Fig. 1. Hippocampal dissection method (dorsal view) used to provide tissue for the glutamate receptor binding study of hippocampal subregions. (A) Hippocampus from one hemisphere dissected away from the neocortex, thalamic and other regions to which it is attached. The hippocampus of the other hemisphere is dissected into two parts. What remains in (B) are the CA fields and subiculum, and the portion shown in (C) contains the granule cells of the fascia dentata and the hilar region. These are the two regions compared across the lifespan of the F344 rat. Scale bar=1.1 mm.

decline in the number of AMPA receptors in the FD/hilar region, but not in the CA fields. The changes reported might be due to either a change in receptor number or an alteration in the binding characteristics of the receptor site with aging. The results also suggest that changes in the number of NMDA and AMPA receptors that occur with aging are not a continuous or homogeneous process from youth to old age. These results are partially consistent with previous findings, using different methods [7,15], although we did not detect a change in the number of NMDA receptors in FD in our dissected preparation. The present data are consistent, however, with the findings in which the numbers of AMPA receptors were reported to decline within the FD region, the number of NMDA receptors were reported to decline within the CA1 region, and with the observation of no decline in AMPA receptors within CA1. In addition, consistent with a previous analysis of glutamate receptors in hippocampal subfields [21], we found that the overall numbers of AMPA-selective glutamate receptors was higher in the FD as compared to the CA1 region in young rats.

The decline in $[^3H]$-glutamate binding to NMDA re-
and CA1 during aging, a different pattern of results might have been predicted from what was observed in the present study or in previous experiments in which glutamate receptor binding was measured. For example, old, memory-impaired rats show smaller AMPA- and NMDA-mediated responses at the Schaffer collateral-CA1 synapse [3]. This is consistent with the observed decrease in NMDA receptor protein, but not with the finding of a stability in AMPA receptor protein that was observed across the lifespan in the present study. For FD, the AMPA-mediated responses at the medial perforant path-granule cell synapse are larger in old rats, while the NMDA-mediated synaptic responses are reduced in old versus middle-aged rats [5,22]. Part of the explanation for the apparent discrepancy may reside in the fact that the electrophysiological studies assessed specific synaptic connections in CA1 and FD, whereas the receptor binding studies assessed the entire CA1 field, plus CA3 and subicular areas, and the entire FD and hilar regions. Alternatively, the methods used in the present study to assess the existence or absence of receptors, plus the autoradiographic methods used in other studies, may not directly reflect the functional state of those receptors at their synaptic sites. A thorough understanding of the mechanisms underlying the changes in glutamate receptor function in discrete brain regions, using combined biochemical and electrophysiological methods, may ultimately provide insight into the fundamental substrates of age-associated memory disorders related to hippocampal dysfunction.

Fig. 2. Age-related changes in NMDA and AMPA receptor number in two discrete regions of the hippocampus of rats. Thirty-eight rats, ranging in age from 3 to 31 months were used in this study. NMDA receptor number declined with aging in the CA and subiculum fields region (CA) but not in the fascia dentate (FD). AMPA receptor number declined with aging in the FD but not in the CA regions. Values are expressed as mean±S.D.

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References

[5] C.A. Barnes, G. Rao, F.P. Houston, LTP induction threshold change...
in old rats at the perforant path-granule cells synapse, Neurobiol. Aging, in press.


