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Age-dependent reduction of hippocampal LTP in mice lacking *N*-methyl-D-aspartate receptor ε1 subunit

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Abstract

The effects of targeted disruption of the *N*-methyl-D-aspartate (NMDA) receptor ε1 subunit gene were studied during the postnatal development of ε1-disrupted mutant mice. Using the mice at the ages of 2–3, 5–6 and 9–10 weeks, we examined NMDA receptor channel-mediated synaptic currents and long-term potentiation (LTP) in CA1 pyramidal neurons of hippocampal slices. NMDA receptor channel currents, expressed as the ratios to non-NMDA receptor channel currents, decreased with the age in both wild-type and mutant mice, but the values in the mutant mice were approximately half of those of the wild-type mice at all ages examined. The LTP in the mutant mice was also reduced, but in contrast to the NMDA receptor channel currents, the extent of the reduction in the LTP was age-dependent. The reduction was marginal at the age of 2–3 weeks, and became progressively prominent to adulthood, with the potentiation being 26 % of that of the wild-type mice at 9–10 weeks.

Keywords: *N*-methyl-D-aspartic acid; Glutamate receptor; Gene targeting; Long-term potentiation; Hippocampus; Slice; Development

The *N*-methyl-D-aspartate (NMDA) receptors play critical roles in synaptic plasticity [1]. They are formed by at least two glutamate receptor subunit families, the GluRε (NR2) and GluRζ (NR1) families [2,4,6,8]. Four members are known for the GluRε subunit family. They are major determinants of the NMDA receptor channel diversity, and distinct in expression profiles and functional properties. On the other hand, only one member is known for the ζ subunit family, which is expressed in virtually all neurons in the brain (ζ1 or NMDAR1 subunit) [9,13]. Recently we reported that targeted disruption of the mouse ε1 (NR2A) subunit gene resulted in significant reduction of NMDA receptor channel currents and long-term potentiation (LTP) at the hippocampal CA1 synapses, as well as a moderate deficiency in spatial learning [10]. These results were obtained from mature animals (9–10 weeks old) [10]. In adult hippocampal neurons, the ε1 and ε2 subunits are the major components of the ε subunit family [7,11,12]. In developmental analyses of these subunits [7,11,12], it has been shown that the ε1 subunit mRNA was hardly detectable during the embryo stage; it increased strikingly over the first 2 weeks after birth. On the other hand, the ε2 subunit mRNA was clearly detected during the embryonic period; it was prevalent at birth and thereafter [7,11,12]. In the present study, at different developmental stages of mutant mice, we examined the effects of the ε1 mutation on the NMDA receptor channel-mediated synaptic currents and on LTP in the hippocampal CA1 region.

Mice lacking the NMDA receptor ε1 subunit were obtained as described previously [10]. Hippocampal slices (approximately 400 μm) were prepared from the animals at the ages of 2–3, 5–6, and 9–10 weeks after birth. Extracellular field potential recordings and whole-cell voltage clamp recordings were performed with the stimulations at Schaffer collateral/commissural afferents as described [10]. The expression of the ε1 and ε2 subunits was examined in the hippocampus of the mutant and wild-type mice at the three ages by Western blot analyses. The mice were decapitated under anesthesia and hippocampi were rapidly removed. Each hippocampus was

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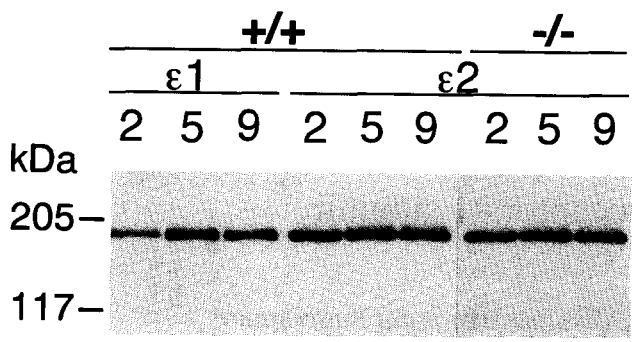

Fig. 1. Western blot analyses of the ε1 and ε2 subunit proteins in the hippocampal homogenates of the wild-type (+/+) and ε1 subunit mutant (–/–) mice. The subunit proteins were detected with anti-GluRε1 or anti-GluRε2 antisera (marked ε1 or ε2, respectively). The numbers represent the ages of the mice (weeks).

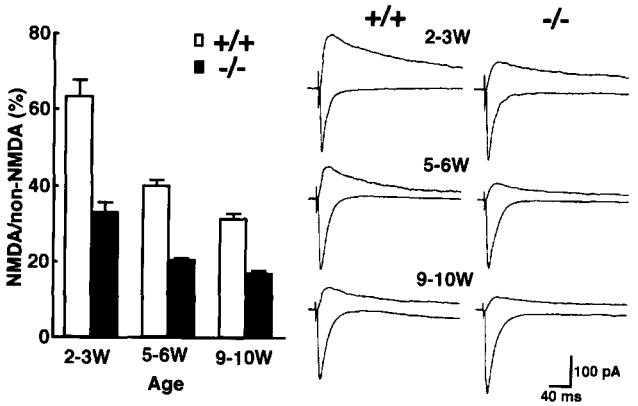

Fig. 2. Synaptic responses in hippocampal CA1 pyramidal cells of mutant and wild-type slices measured with whole-cell voltage-clamp recording. Upper and lower traces (averages of five successive recordings) show typical synaptic currents at +40 mV in the presence of 20 μM CNQX (NMDA receptor-mediated currents) and at –90 mV in the control solution (non-NMDA receptor-mediated currents), respectively. Ratios of the two currents are shown on the left (n = 5–7).

homogenized in 10 vols. of buffer H (10 mM Tris–Cl (pH 7.2), 5 mM EDTA, 0.32 M sucrose, 1 mM phenylmethylsulfonyl fluoride and 10 mg/ml leupeptin) within 3 min of decapitation, and centrifuged at 700 × g for 10 min to obtain a post-nuclear fraction. Protein determinations were done essentially by the procedure of Lowry et al. [5]. Proteins prepared at different postnatal ages were fractionated by 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins in the gels were electro-blotted onto a nitrocellulose membrane (Schleichen & Schnell). The blots were immunoreacted with anti-GluRε1 or anti-GluRε2 sera which were prepared previously [10], and were visualized by chemiluminescence (ECL detection system, Amersham). For quantitative analyses, the immunoreactive bands were scanned using a computing densitometer (Shimazu CS-9300PC). The expression of the ε1 subunit was low at the age of 2 weeks (Fig. 1). It increased during postnatal development and was approximately doubled at the ages of 5 weeks and 9 weeks. In contrast, the expression of the ε2 subunit was high at all three ages examined. The amount of the ε2 subunit in the mutant mice was essentially the same as that in the wild-type mice (Fig. 1).

The synaptic transmission at the CA1 pyramidal cells was indistinguishable between the mutant and wild-type hippocampal slices [10]. The ratios of NMDA receptor channel currents, measured at +40 mV in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), to CNQX-sensitive non-NMDA receptor channel currents, measured at –90 mV in the control solution [10], decreased significantly as a function of age in both the mutant and wild-type mice (Fig. 2). Considering the observation that the expression of the ε1 or ε2 subunit proteins did not decrease during this period (Fig. 1), it is likely that this decrease may largely be due to an increased expression of functional non-NMDA receptors.

The results also indicated that the elimination of the ε1 subunit resulted in the reduction of the relative strength of NMDA receptor channel currents in the mutant slices to approximately half of the control values of wild-type slices at all ages examined (Fig. 2). As has been suggested by the results of in situ hybridization analyses [10], no significant compensation by the other subunits for the lack of the ε1 subunit seemed to occur.

We next examined the effects of the elimination of the ε1 subunit on LTP measured at the CA1 region of hippocampal slices during development. The previous results indicated that 9–10 week-old mutant mice showed reduced spatial learning and hippocampal LTP. The reduction of LTP was already detectable at the age of 2–3 weeks, but was marginal at this age (field EPSP slopes measured 60 min after the tetanic stimulations were 174 ± 11% (n = 9) and 152 ± 8% (n = 10) of pretetanic values for the wild-type and mutant slices, respectively; Fig. 3). It became more evident during the course of maturation (170 ± 6% (n = 8) and 130 ± 6% (n = 13) at 5–6 weeks, 202 ± 19% (n = 10) and 127 ± 5% (n = 10) at 9–10 weeks, for the wild-type and mutant slices, respectively; Fig. 3).

The present results indicated that elimination of the ε1 subunit resulted in the reduction of functional NMDA receptors. However, significant amounts of NMDA receptors are functioning in the mutant hippocampus. In situ hybridization analyses suggest that hippocampal CA1 pyramidal neurons express mainly the ε1, ε2 and ζ1 subunits of the NMDA receptor channel [11,12]. The results of the Western blot analyses (Fig. 1) and in situ hybridization analyses [10] suggested that the expression of the other subunits did not seem to be affected by the ε1 mutation. Thus, the NMDA receptor-mediated currents observed in CA1 pyramidal neurons in the mutant slices may represent mainly the ε2/ζ1 binary complexes; such binary receptors may be capable of triggering LTP.

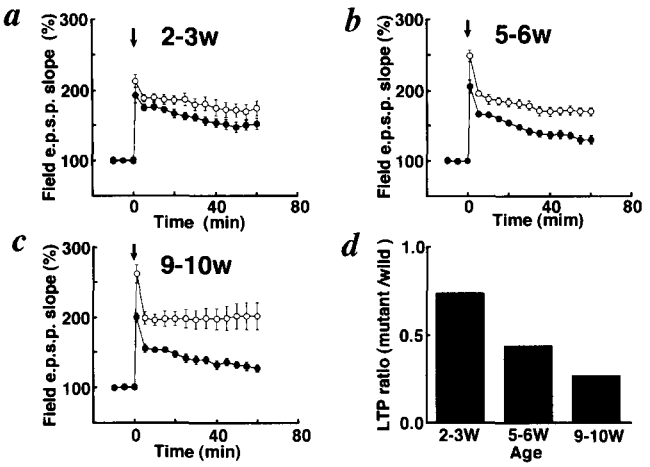

Fig. 3. LTP of the hippocampal CA1 field EPSP expressed as percentage of the mean before tetanic stimulation. Open and filled circles represent wild-type and mutant slices, respectively (a–c). Ratios of the potentiation in mutant slices and wild-type slices estimated at 60 min after tetanic stimulation (100 Hz for 1 s, arrow) are plotted in (d). $n = 8$–13.

It is not clear, at the molecular level, whether the reduction of functional NMDA receptors in the mutant is due to the partial impairment of the entire NMDA receptor population or the complete inactivation of a partial fraction of the receptors. However, at the level of individual cells, the reduction of NMDA receptor activity seemed to be uniform. For example, at the age of 2–3 weeks, five pyramidal cells were examined for both the mutant and wild-type mice; the ratio of NMDA currents to non-NMDA currents ranged from 50% to 74% for the wild-type mice (63 ± 5%) and from 24% to 42% for the mutants (33 ± 3%). No cells were found with the ratio equivalent to the control value, nor with the ratio close to zero. This suggests that most of the individual CA1 pyramidal neurons may express both the $\varepsilon 1$ and $\varepsilon 2$ subunits simultaneously.

In the present study, no correlation was observed between age and the absolute value of LTP, as reported previously [3]. Nevertheless, the effects of the $\varepsilon 1$ subunit elimination became progressively evident to adulthood. The extent of LTP in mutant slices was highest at the youngest age examined, and then decreased. This reduction resembles closely the reduction of the NMDA currents described above. This suggests that, at younger ages, NMDA receptors are expressed more than minimally required for the induction of LTP, at least for the induction of in vitro LTP under our conditions, and these 'redundant' NMDA receptors may be eliminated during the course of subsequent development.

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