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Distribution and development of NMDA receptor activities at hippocampal synapses examined using mice lacking the ε1 subunit gene

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Abstract

The effects of targeted disruption of the gene encoding *N*-methyl-D-aspartate (NMDA) receptor ε1 subunit were examined in hippocampal CA1 pyramidal cell synapses and compared with the effects in the CA3 region. The mutation resulted in the significant reduction of NMDA receptor activities at the synapses in the CA1 stratum oriens, as had been observed in the CA1 stratum radiatum which we reported before. This result was in sharp contrast to our previous observation that in the CA3 region, the ε1 mutation suppressed NMDA receptors at the synapses in the stratum radiatum but not in the stratum oriens. It is suggested that the subunit composition of NMDA receptors may not be determined simply by the location within a pyramidal cell, but by other factors such as properties of synaptic inputs. We also examined the postnatal development of long-term potentiation (LTP) in the CA3 region. The development of LTP at the CA3 stratum radiatum synapses closely followed the development of the ε1 subunit, and the ε1 mutation strongly suppressed this LTP, suggesting that the targeted disruption of the ε1 subunit may not be compensated by other ε subunits. The LTP at the CA3 stratum oriens synapses was not significantly affected by the mutation at any age.

Keywords: Glutamate receptors; NMDA receptors; Long-term potentiation; Gene targeting; Hippocampus; Development

1. Introduction

Some forms of hippocampal long-term potentiation (LTP) are critically dependent on the glutamate receptors of the *N*-methyl-D-aspartate (NMDA) type. The NMDA receptors are thought to be composed of the members of the two glutamatergic receptor subunit subfamilies, the ζ and ε subfamilies (Seeburg, 1993; Mori and Mishina, 1995). However, the precise subunit composition of the individual NMDA receptor molecules has not been established so far (Williams et al., 1993; Sheng et al., 1994; Kirson and Yaari, 1996; Takahashi et al., 1996). Recently, by examining the effects of targeted disruptions of ε1 and ε2 subunits in the mouse hippocampus, we have shown that the synapse on the CA3 pyramidal neurons formed by the commissural/associational (C/A) input was strongly affected by ε1 mutation but not by ε2 mutation, whereas the synapse on the same neurons formed by fimbrial (Fim) input was affected by the ε2 mutation but not by the ε1 mutation (Ito et al., 1997). This suggested that the NMDA receptor molecules functioning at different synapses might have different subunit compositions, even within a single neuron (Ito et al., 1997). However, the significance of the molecular diversification and

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differential distribution are not known. In the present study, we asked two questions related to this observation. The first is whether or not a similar kind of differential distribution is also observed in the CA1 region in the hippocampus. The second is whether or not postnatal developments of LTP at the two types of synapses in the CA3 region follow different developmental time courses, since different ϵ subunits develop in different time courses.

2. Materials and methods

ϵ1 homozygous mutant mice ($-/-$) were obtained, and the experiments were performed using wild-type littermates ($+/+$) as controls, as previously described (Sakimura et al., 1995; Ito et al., 1997). The mice brains were removed after ether anesthesia and decapitation. Hippocampal slices (ca. 400 μm thick) were prepared and maintained as described (Sakimura et al., 1995). The stimulation was given with a bipolar tungsten electrode, and field excitatory postsynaptic potentials (EPSPs) were recorded with an extracellular electrode (filled with 0.9% NaCl). When fimbrial (Fim) regions were stimulated, field EPSPs were recorded at the stratum oriens of the CA3 region (Ito et al., 1997). The commissural/associational (C/A) inputs were stimulated at the stratum radiatum of the CA3 region, and the responses were recorded in the same region, approximately 200 μm apart from the stimulation electrode (Ito et al., 1997). In the CA1 region, the stimulation and field EPSP recording were made at the stratum oriens (Kantor et al., 1996; Son et al., 1996). Baseline stimulation was given at 0.05 Hz, and the LTP-inducing tetanic stimulation was given at 100 Hz for 1 s at the baseline stimulus strength. The artificial cerebrospinal fluid (ACSF) used was (in mM): NaCl, 119; KCl, 2.5; $CaCl_2$, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.0; $NaHCO_3$, 26; glucose, 10.

Synaptic currents were recorded from CA1 pyramidal cells with a patch electrode (4–6 MΩ) in the whole-cell voltage-clamp mode (Axopatch 1D) as described previously (Sakimura et al., 1995; Ito et al., 1996, 1997). The composition of the pipette solution was (in mM): cesium gluconate, 122.5; CsCl, 17.5; HEPES buffer, 10; EGTA, 0.2; NaCl, 8; Mg-ATP, 2; Na_3-GTP, 0.3 (pH 7.2). In the whole-cell patch experiments, picrotoxin (50 μM) was routinely added to the ACSF.

3. Results

3.1. Effects of the ϵ1 subunit mutation on the synapses in the CA1 stratum oriens

In the previous studies, we found that the targeted disruption of NMDA ϵ1 subunit caused a reduction in NMDA receptor-mediated synaptic currents and LTP in the C/A-CA3 pyramidal cell synapse, but not in the Fim-CA3 synapse (Ito et al., 1997). This indicated that the subunit compositions of the NMDA receptors are different at these two synapses. One possibility is that the subunit composition may be regulated by the polarity within the pyramidal neurons expressing NMDA receptors, e.g. the subunit composition may be determined depending whether the receptors are located on the apical or basal dendrite. Therefore, we investigated whether similar diversification occurs among pyramidal neurons in the CA1 region. For this purpose, we examined the effects of the ϵ1 mutation at the pyramidal cell synapse in the CA1 stratum oriens, because we have already reported that the ϵ1 disruption caused a reduction in the NMDA receptor currents and LTP at the pyramidal cell synapse in the CA1 stratum radiatum (Sakimura et al., 1995; Ito et al., 1996).

The NMDA receptor currents were expressed as a ratio to 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)-sensitive non-NMDA receptor currents. We have already reported that no apparent differences were found in non-NMDA receptor-mediated synaptic transmissions between the wild-type and ϵ1 mutant mice (Sakimura et al., 1995), supporting the notion that non-NMDA receptor currents were not significantly affected by the mutation. The present results indicated that the ϵ1 subunit mutation caused the reduction in the NMDA receptor currents at the pyramidal cell synapse in the CA1 stratum oriens (Fig. 1).

We also examined the LTP at this synapse. In accordance with previous reports (Kantor et al., 1996; Son et

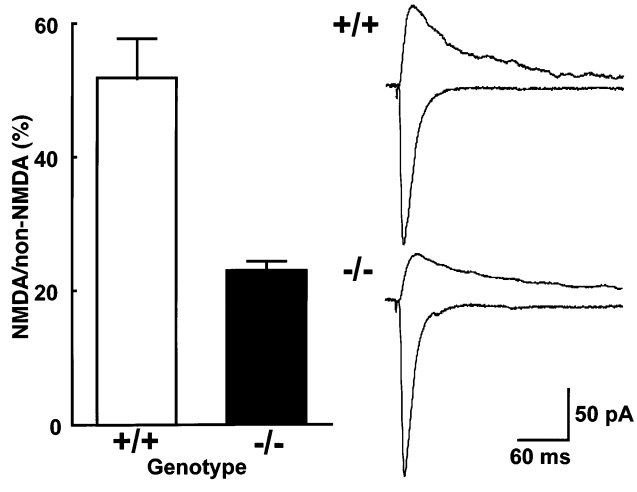

Fig. 1. Effects of ϵ1 subunit mutation on the NMDA receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) at the synapses in the hippocampal CA1 stratum oriens. Left panel: ratios of NMDA EPSCs at $+40$ mV to non-NMDA EPSCs at -90 mV, measured with whole-cell patch recording (mean $\pm$ S.E.M.); right panel: upper traces show NMDA EPSCs in the presence of 20 μM CNQX, and lower traces show non-NMDA EPSCs in the control solution. Each trace is an average of five consecutive recordings.

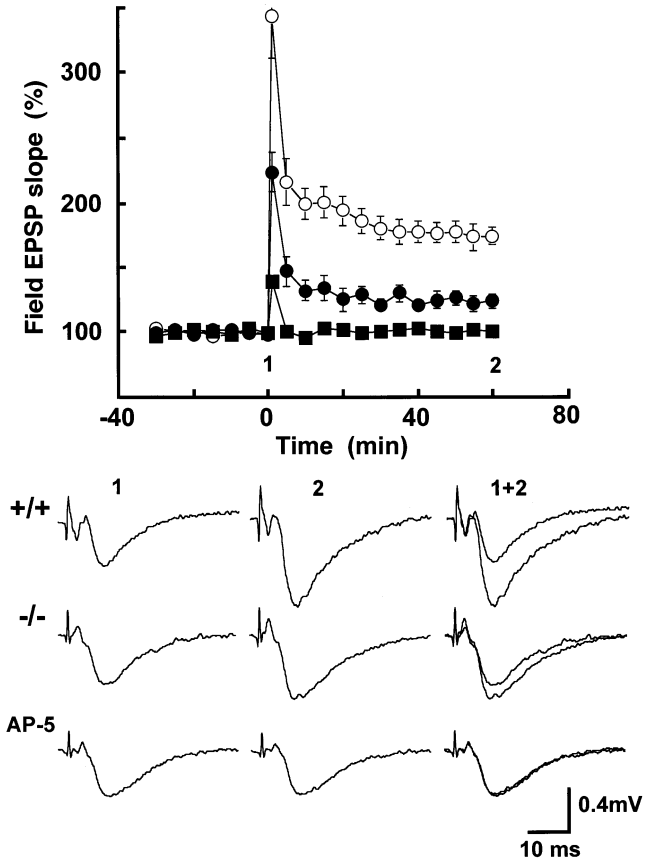

Fig. 2. Effects of $\epsilon 1$ subunit mutation on the LTP at the synapses in the hippocampal CA1 stratum oriens. Field EPSP slopes are expressed as a percentage of the mean before tetanic stimulation. A tetanic stimulus was given at 100 Hz for 1 s at $t = 0$. Open circles represent wild-type slices ($n = 8$); filled circles represent $\epsilon 1$ mutant slices ($n = 8$); filled squares represent wild-type slices in the presence of 50 μM AP5 ($n = 4$). Bars indicate S.E.M. Traces below the graphs represent averages of three consecutive field EPSPs obtained at the times marked by the numbers on the upper panel.

al., 1996), the LTP in the CA1 stratum oriens was dependent on the NMDA receptors (Fig. 2), and this LTP was found to be significantly reduced in the $\epsilon 1$ mutant mice (Fig. 2).

Thus, these results indicated that the homozygous mutation of $\epsilon 1$ subunit ($-/-$) caused the reduction in the NMDA receptor currents and LTP at the pyramidal cell synapse in the CA1 stratum oriens, as well as at the pyramidal cell synapse in the stratum radiatum (Sakimura et al., 1995; Ito et al., 1996). This means that in the CA1 region, NMDA receptors on the apical dendrites of pyramidal cells are dependent on the $\epsilon 1$ subunit to the same extent as the receptors on the basal dendrites. This is in sharp contrast with our previous observation made in the CA3 region (Ito et al., 1997).

3.2. Postnatal development of the CA3 synapses

Next, we examined the development of LTP at the C/A-CA3 synapse and the Fim-CA3 synapse in the CA3 region.

Fig. 3 shows the time courses of the field EPSP slopes of the Fim-CA3 synapse obtained from hippocampal slices of mice at different ages. As can be seen here, the Fim-CA3 LTP induced by tetanic stimulation was essentially unchanged during the course of the postnatal development, and the effects of the $\epsilon 1$ mutation were only nominal or insignificant during this period: At the age of 2 weeks, for example, a Student's t-test for the comparison of the values at 60 min gave $P < 0.26$. The level of the potentiated field EPSP slopes, measured 60 min after the tetanic stimulation, remained at 150–170% of the pretetanic field EPSP slopes at all ages examined, both in the wild-type and mutant slices.

In contrast, the LTP at the C/A synapse showed a strong dependency on the postnatal development, as shown in Fig. 4. In the mice at the age of 2 weeks, the potentiation ratio was low (approx. 130%; Fig. 4d). The LTP of the control mice increased significantly during maturation, and its developmental time course very closely resembled that of the development of the $\epsilon 1$ subunit in the normal hippocampus estimated by immunoblot measurement (Ito et al., 1996). However, the LTP level of the mutant mice at all ages remained at the LTP level of the control mice of the youngest age examined (2 weeks).

Taken together, these results indicated that the LTP in the $\epsilon 1$ mutant slices was essentially age-independent during the period examined at both of the two synapses: the LTP at the Fim synapse was at an increased level (150–170%), whereas the LTP at the C/A synapse was suppressed (120–145%), in all ages examined.

4. Discussion

By examining the effects of targeted disruption of NMDA receptor $\epsilon 1$ or $\epsilon 2$ subunits, we previously found that in the CA3 pyramidal cells, the subunit compositions of NMDA receptors at the C/A synapse, observed in the CA3 stratum radiatum, may be different from those at the Fim synapse in the CA3 stratum oriens (Ito et al., 1997). This suggested the possibility that the subunit composition may be regulated by the synaptic inputs. However, another possibility is that the subunit compositions may be distinct depending upon whether the receptors are located on the apical or basal dendrites. Therefore, here we examined the pyramidal cells in the CA1 region, a functionally different but structurally similar region compared to CA3. The results indicated that, in contrast to the CA3 region, the

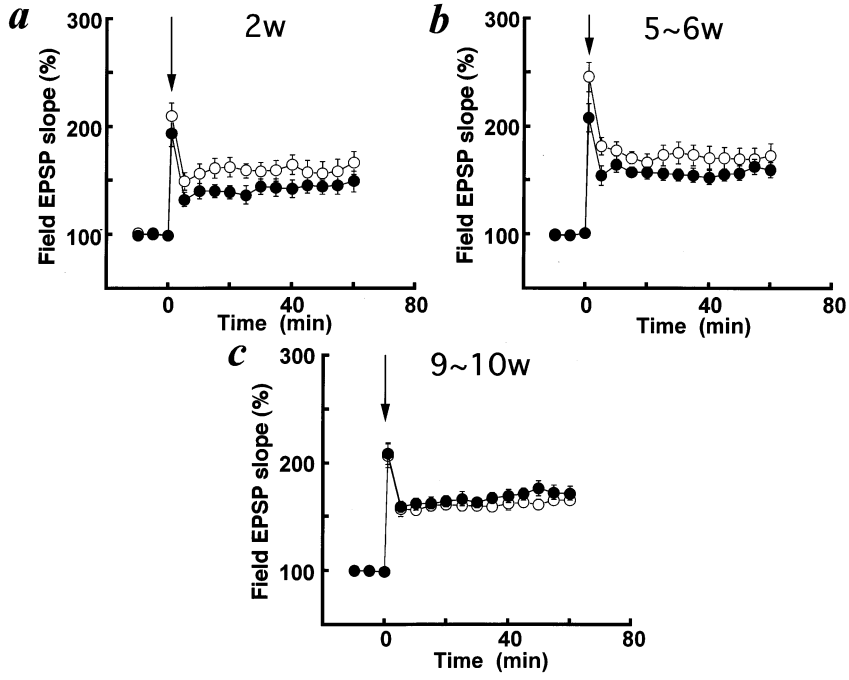

Fig. 3. LTP of the hippocampal field EPSP at the Fim-CA3 synapse. Field EPSP slopes are expressed as a percentage of the mean before tetanic stimulation (100 Hz for 1 s, arrow). Open and filled circles represent wild-type and mutant slices, respectively. Bars indicate S.E.M. ($n = 7–9$).

$\epsilon 1$ mutation caused significant decreases in the activities of NMDA receptors in basal dendrites as well as in apical dendrites of pyramidal cells in the CA1 region (Sakimura et al., 1995; Ito et al., 1996). Thus, different patterns of molecular diversification and differential distribution of NMDA receptors are observed in pyramidal cells in the two closely related regions, CA1 and CA3. This result is consistent with the possibility that the subunit composition may be regulated by synaptic inputs and not simply by its location within a pyramidal cell, although further studies are required to verify this conclusion.

Although the significance of molecular diversification and differential distribution are not known, they may be relevant to the developmental schedules of different synapses. In the hippocampus, the $\epsilon 1$ subunit appears only a few weeks after the birth, whereas the $\epsilon 2$ subunit is expressed early in the fetal stages (Watanabe et al., 1992). Since the NMDA receptors at the C/A-CA3 synapse are more profoundly dependent on the $\epsilon 1$ subunit than the $\epsilon 2$ subunit, the development of LTP at this synapse is expected to more precisely follow the development of the $\epsilon 1$ subunit than that of the $\epsilon 2$ subunit. On the other hand, the NMDA receptors at the Fim-CA3 synapse are more profoundly dependent on the $\epsilon 2$ subunit than the $\epsilon 1$ subunit, and at this synapse, the development of LTP is expected to more precisely follow the development of the $\epsilon 2$ subunit than that of the $\epsilon 1$ subunit. Therefore, we examined the development of LTP at these two synapses, and compared them with the developments of the $\epsilon 1$ and $\epsilon 2$ subunits, in the control and mutant mice.

The results indicated that, in wild-type mice, the LTP level at the Fim-CA3 synapse remained high at all stages of the development examined whereas the LTP level at the C/A-CA3 synapse was low in the mice at the age of 2 weeks and increased with age. These developmental patterns closely resemble those of the $\epsilon 2$ and $\epsilon 1$ subunits. Thus, it seems likely that the LTP at these synapses may primarily be dependent on the $\epsilon 2$ or $\epsilon 1$ subunit. In fact, the targeted disruption of the $\epsilon 1$ subunit caused a strong suppression of LTP at the C/A-CA3 synapse but essentially had no effects on the LTP at the Fim-CA3 synapses, at all ages examined. This suggests that the LTPs at these two synapses are mostly dependent upon the NMDA receptors, either $\epsilon 1$ or $\epsilon 2$ type, and that the targeted disruption of the $\epsilon 1$ subunit may not cause compensatory expression of the $\epsilon 2$ or other ϵ subunits.

The observation that the LTP at either of the two synapses in the CA3 region of the $\epsilon 1$ knockout mice is essentially age-independent is in sharp contrast with our previous observation of the Schaffer collateral/commissural-CA1 synapse, where the LTP level of wild-type mice showed little age-dependency. However, in this observation, the $\epsilon 1$ knockout caused a reduction of LTP which was strongly age-dependent (Ito et al., 1996). Thus, the developmental patterns and the effects of the $\epsilon 1$ knockout observed in the three synapses, the C/A-CA3, Fim-CA3, and Schaffer collateral/commissural-CA1 synapses, are all different from each other. It seems likely that the CA1 synapse may represent mixed

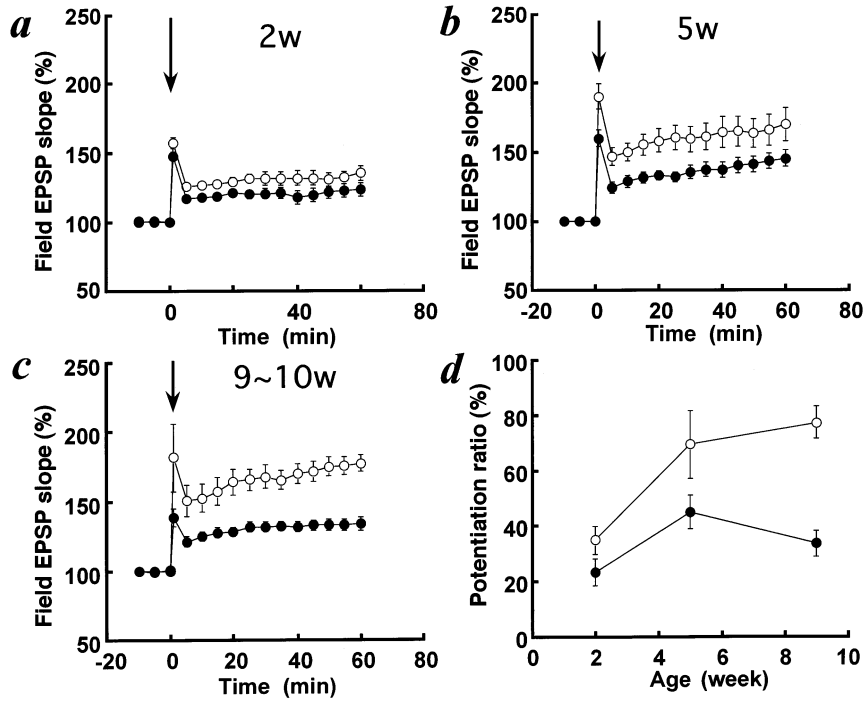

Fig. 4. LTP of the hippocampal field EPSP at the C/A-CA3 synapse. Field EPSP slopes are expressed as a percentage of the mean before tetanic stimulation (100 Hz for 1 s, arrow) in (a–c). Open and filled circles represent wild-type and mutant slices, respectively. Bars indicate S.E.M. ($n = 10–14$). The degrees of the potentiation estimated at 60 min after tetanic stimulation are plotted in (d). Open circles, wild-type slices; filled circles, mutant slices.

properties of both the C/A-CA3 and Fim-CA3 synapses, the ratio of these two components being dependent on age. The CA1 synapse may be similar to the Fim-CA3 synapse at the youngest age, and then become more and more similar to the C/A-CA3 synapse during maturation.

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