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Input-specific targeting of NMDA receptor subtypes at mouse hippocampal CA3 pyramidal neuron synapses

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Abstract

Hippocampal CA3 pyramidal neurons receive synaptic inputs from commissural and associational fibers on both apical and basal dendrites. NMDA receptors at these synapses were examined in hippocampal slices of wild-type mice and GluRε1 (NR2A) subunit knockout mice. Electrical stimulations at the CA3 stratum radiatum or stratum oriens activate both commissural and associational (C/A) synapses, whereas stimulations at ventral fimbria mainly activate commissural synapses. Ro 25-6981 and ifenprodil, the GluRε2 (NR2B) subunit-selective NMDA receptor antagonists, suppressed NMDA receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) at the commissural–CA3 synapses on basal dendrites more strongly than those at the C/A–CA3 synapses on apical or basal dendrites. However, glutamate-evoked NMDA receptor currents were reduced by the GluRε1 subunit knockout to a similar extent at both apical and basal dendrites. The GluRε1 subunit knockout also reduced NMDA EPSCs at the C/A–CA3 synapses on basal dendrites, but did not affect NMDA EPSCs at the commissural–CA3 synapses on basal dendrites. These results confirmed our previous findings that NMDA receptors operating at different synapses in CA3 pyramidal cells have different GluRε subunit compositions, and further show that the GluRε subunit composition may be regulated depending on the types of synaptic inputs, even within a single CA3 pyramidal neuron.

Keywords: NMDA receptors; Hippocampus; CA3 synapse; Ro 25-6981; Gene targeting; NMDA subunits

1. Introduction

The *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor (GluR) plays important roles in synaptic plasticity and in neuronal pattern formation during development (Cline et al., 1987; Kleinschmidt et al., 1987; Bliss and Collingridge, 1993; Malenka and Nicoll, 1993). Combinations of the GluRε (NR2) and GluRζ (NR1) subunits constitute NMDA receptors (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992). There are four GluRε subunit genes (Ikeda et al., 1992; Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992; Nagasawa et al., 1996), although GluRζ subunit variants are derived from a single gene (Moriyoshi et al., 1991; Yamazaki et al., 1992; Hollmann et al., 1993). The molecular composition of NMDA receptors varies depending on brain regions and developmental stages (Watanabe et al., 1992). The four GluRε subunits are also distinct in physiological properties (Seeburg, 1993; Mori and Mishina, 1995). Thus, multiple GluRε subunits are key determinants of the NMDA receptor diversity. The GluRε1 (NR2A), GluRε2 (NR2B) and GluRζ subunit mRNAs are expressed in the hippocampus, but the GluRε3 (NR2C) and GluRε4 (NR2D) subunits are not detected in the hippocampus throughout development (Watanabe et al. 1992, 1993). The GluRε2 subunit is expressed from the embryonic period to adulthood, whereas the GluRε1 subunit is not expressed during the embryonic stage, but increases after birth (Watanabe et al. 1992, 1993).

Hippocampal CA3 pyramidal neurons receive synap-

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tic inputs from associational fibers arising from the ipsilateral CA3 region and commissural fibers from the contralateral CA3 region. These two inputs form synapses on the apical and basal dendrites of CA3 pyramidal neurons (Ishizuka et al. 1990, 1995). Therefore, electrical stimulations in stratum radiatum or in stratum oriens of area CA3 activate both of these inputs at the same time (Harris and Cotman, 1986; Ishizuka et al. 1990, 1995; Castillo et al., 1997; Vignes and Collingridge, 1997). In contrast, stimulations at ventral fimbria mainly activate commissural fibers which make synapses on the basal dendrites of CA3 neurons (Ishizuka et al. 1990, 1995; Katsuki et al., 1991; Ito et al., 1997).

Recently, by examining the effects of targeted disruption of the GluRε1 and GluRε2 subunits on NMDA receptor activities, we have shown that NMDA receptors at the commissural/associational–CA3 (C/A–CA3) synapses on the apical dendrite of CA3 neurons were strongly affected by the GluRε1 mutation, whereas NMDA receptors at the commissural–CA3 (Com–CA3) synapses on the basal dendrite of CA3 neurons were affected by the GluRε2 mutation (Ito et al. 1997, 1998). This suggested that NMDA receptors operating at different synapses might have different subunit compositions, even within a single neuron (Ito et al. 1997, 1998).

The aims of the present study are twofold. First we tried to confirm our previous observations without using knockout mice. For this purpose, we examined the effects of Ro 25-6981 (Fischer et al., 1997; Mutel et al., 1998) and ifenprodil (Carter et al., 1988; Williams et al., 1993; Mott et al., 1998), the GluRε2 subunit-selective NMDA receptor antagonists, on NMDA receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) and long-term potentiations (LTPs) in hippocampal slices prepared from wild-type mice. Next we tried to test whether the GluRε subunits are sorted depending on cell polarity or on the types of synaptic inputs. Our data indicate that input-selective distributions of the GluRε subunits are also found in wild-type mice and that this targeted distribution is dependent on the types of synaptic input but not on cell polarity.

2. Methods

2.1. Animals

C57BL/6 and CBA mice were purchased from Kyudo (Fukuoka, Japan) and Clea (Tokyo, Japan). A mutant mouse lacking the GluRε1 subunit of the NMDA receptor was produced by homologous recombination in TT2 embryonic stem (ES) cells derived from C57BL/6 X CBA mice, using a targeting vector composed of the GluRε1 subunit gene from C57BL/6 mice as described (Sakimura et al., 1995). The chimeric mice derived from the recombinant ES cells were crossed with C57BL/6 mice to yield heteromeric F2 mice with a 75% pure C57BL/6 genetic background. The heterozygous mice for the GluRε1 subunit mutant gene were crossed successively with C57BL/6 mice to yield subsequent generations with a pure C57BL/6 genetic background. The F13 heterozygous mice were crossed with each other to yield homozygous GluRε1 subunit mutant mice (−/−) with a 99.99% pure C57BL/6 genetic background. Their genotypes were determined by tail biopsy and Southern blot hybridization, as described before (Kiyama et al., 1998).

2.2. Electrophysiological experiments

Mouse brains were removed after ether anesthesia and decapitation. Transverse hippocampal slices (≈400 μm) were cut with a Microslicer (DTK-1000, Dosaka, Japan), and were incubated in artificial cerebrospinal fluid (ACSF) (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, saturated with 95% O_2/5% CO_2) to allow them to recover for at least 1 h at room temperature. All recordings were made in a submerged slice chamber perfused with ACSF. Analyses were done with mature mice (9–10 weeks old), using C57BL/6 mice as controls.

For extracellular recording, a recording electrode filled with 0.9% NaCl was used. Low frequency baseline stimuli were given at 0.1 Hz using a bipolar tungsten electrode. An LTP-inducing tetanic stimulus was given at 100 Hz for 1 s. All tetanic stimuli were given at baseline stimulus strength. Synaptic currents were recorded from CA3 pyramidal cells using the blind-patch technique (Blanton et al., 1989) in the whole-cell voltage-clamp mode (Axopatch 1D). Patch electrodes (4–6 MΩ) were filled with an intracellular solution (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). Iontophoretic micropipettes were filled with 0.5 M L-glutamate (pH 8.0). Current pulses of 10–30 ms and 100–200 nA (negative) were used for glutamate iontophoresis. A braking current (positive) of ≈20 nA was routinely used. The amplitudes of NMDA EPSCs and NMDA receptor-mediated currents were estimated as a percentage of the non-NMDA EPSC, because we had to perform cross-slice comparisons (Sakimura et al., 1995; Ito et al. 1997, 1998). We have already reported that the non-NMDA receptor-mediated transmissions are not apparently affected by the GluRε1 mutation (Sakimura et al., 1995; Kutsuwada et al., 1996). All records were filtered at 2 kHz, digitized at 10 kHz and stored on a computer equipped with a Mac Lab 2e A/D converter. Student's *t*-test was used to compare the data and to assess significance levels ($P<0.05$).

2.3. Materials

Ro 25-6981 was a gift from Hoffmann-La Roche (Nutley, New Jersey). Ifenprodil tartrate was purchased from Wako (Osaka, Japan). CNQX, (*S*)-α-Methyl-4-carboxyphenylglycine (MCPG) and glycine were purchased from Tocris Cookson (Bristol, UK). Tetrodotoxin (TTX) was purchased from Sankyo (Tokyo, Japan). (−)-Bicuculline methiodide and all other reagents were purchased from Nakarai (Kyoto, Japan).

3. Results

3.1. Pharmacological properties of NMDA receptors operating at two types of synapses in CA3 pyramidal neurons

Ro 25-6981 is known as a highly selective, activity-dependent blocker of NMDA receptors composed, in addition to GluRζ subunits, of GluRε2 subunits, whereas NMDA receptors composed of GluRε1 subunits are much less sensitive (Fischer et al., 1997; Mutel et al., 1998). Using hippocampal slices prepared from wild-type mice, we compared the effects of Ro 25-6981 on NMDA EPSCs in the Com–CA3 synapse with those in the C/A–CA3 synapses (Fig. 1A and B). To monitor NMDA EPSCs, whole cell recordings were made from CA3 pyramidal neurons using the blind-patch technique (Blanton et al., 1989). In the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and bicuculline (30 μM), NMDA EPSCs were recorded at a holding potential of +30 mV. CNQX at similar concentrations has been shown to cause only marginal effects on NMDA EPSCs, especially in slice preparations (Blake et al., 1988), and we could assume that the subunit dependency of the residual NMDA EPSCs after CNQX reflects more or less the subunit dependency of the total NMDA EPSCs. It was confirmed that the EPSCs measured under these conditions were completely eliminated by 50 μM AP5.

A bath application of Ro 25-6981 (0.3 μM) resulted in a strong reduction of NMDA EPSCs in the Com–CA3 synapse evoked by the stimulations at ventral fimbria (36±5.9% of the control, mean±SEM, n=5, Fig. 1B). In contrast, NMDA EPSCs at the C/A–CA3 synapse evoked by the stimulations in stratum radiatum of area CA3 were less sensitive to Ro 25-6981 (73.4±3.0%, n=5, Fig. 1B). Thus, Ro 25-6981 suppressed NMDA EPSCs in the Com–CA3 synapse more potently than those in the C/A–CA3 synapse (Student's t-test, $P<0.01$).

We also examined the effects of Ro 25-6981 on LTPs at these two synapses. Field EPSPs were recorded in stratum radiatum or in stratum oriens of area CA3, as illustrated in Fig. 1A. We applied 0.1 μM of Ro 25-6981 from 60 min before the tetanic stimulation to 10 min after the tetanus. LTPs of the Com–CA3 synapse were potently suppressed by Ro 25-6981 (potentiation ratio estimated at 90 min after the tetanus was reduced to 33.5±6.1% of the control, n=6) (Fig. 1C). On the other hand, LTPs at the C/A–CA3 synapse were much less sensitive to Ro 25-6981 (potentiation ratios were 88.9±8.1% of the control, n=6) (Fig. 1C). Thus, Ro 25-6981 suppressed LTP in the Com–CA3 synapse more potently than in the C/A–CA3 synapse (Student's t-test, $P<0.01$). A higher concentration of Ro 25-6981 was needed to achieve a similar extent of suppression in NMDA EPSCs (0.3 μM) than in LTPs (0.1 μM). This might be ascribed to the activity-dependent nature of Ro 25-6981 inhibition (Fischer et al., 1997) because the synaptic NMDA receptors are activated strongly and repetitively during the LTP-inducing tetanic stimulation.

We performed similar analyses using ifenprodil, which is another GluRε2 subunit-selective antagonist, but is less potent and less selective than Ro 25-6981 (Fischer et al., 1997; Mutel et al., 1998). Ifenprodil (3 μM) suppressed NMDA EPSCs in the Com–CA3 synapse (31.8±6.0% of the control, n=6) more potently than in the C/A–CA3 synapse (52.4±1.9%, n=5, Student's t-test, $P<0.01$). It also suppressed LTPs in the Com–CA3 synapse (24±4.0%, n=8) more potently than in the C/A–CA3 synapse (46.0±4.2%, n=6, Student's t-test, $P<0.01$). These results strongly support our previous observations obtained using GluRε1 or GluRε2 subunit knockout mice (Ito et al. 1997, 1998).

3.2. Properties of the targeted distribution of the GluRε subunits in CA3 pyramidal neurons

To determine whether the GluRε subunits are localized depending on cell polarity, we examined the effects of the GluRε1 subunit disruption on NMDA receptor-mediated currents in response to glutamate applied iontophoretically. We used a GluRε1 mutant mouse line, produced by a series of backcrosses, with a 99.99% pure C57BL/6 genetic background (Kiyama et al., 1998). To activate NMDA receptors on the apical dendrites or on the basal dendrites of CA3 neurons, an iontophoretic micropipette filled with glutamate (0.5 M, pH 8.0) was placed in the stratum radiatum or stratum oriens of area CA3 (Fig. 2A). Because we had to perform cross-slice comparisons, the amplitudes of NMDA receptor-mediated currents were expressed as ratios to CNQX sensitive non-NMDA receptor-mediated currents. We found that NMDA receptor-mediated currents of the GluRε1 mutant mice were reduced to almost one-half that of the wild-type mice both in stratum radiatum and in stratum oriens of area CA3 (ratios of NMDA current to non-NMDA current were: in oriens, wild-type (WT), 89±5%, n=5; GluRε1 mutant (−/−), 45±4%, n=5, Student's t-test, $P<0.01$; in radiatum, WT, 80±5%, n=8; −/−, 38±8%, n=7, Student's t-test, $P<0.01$) (Fig. 2B

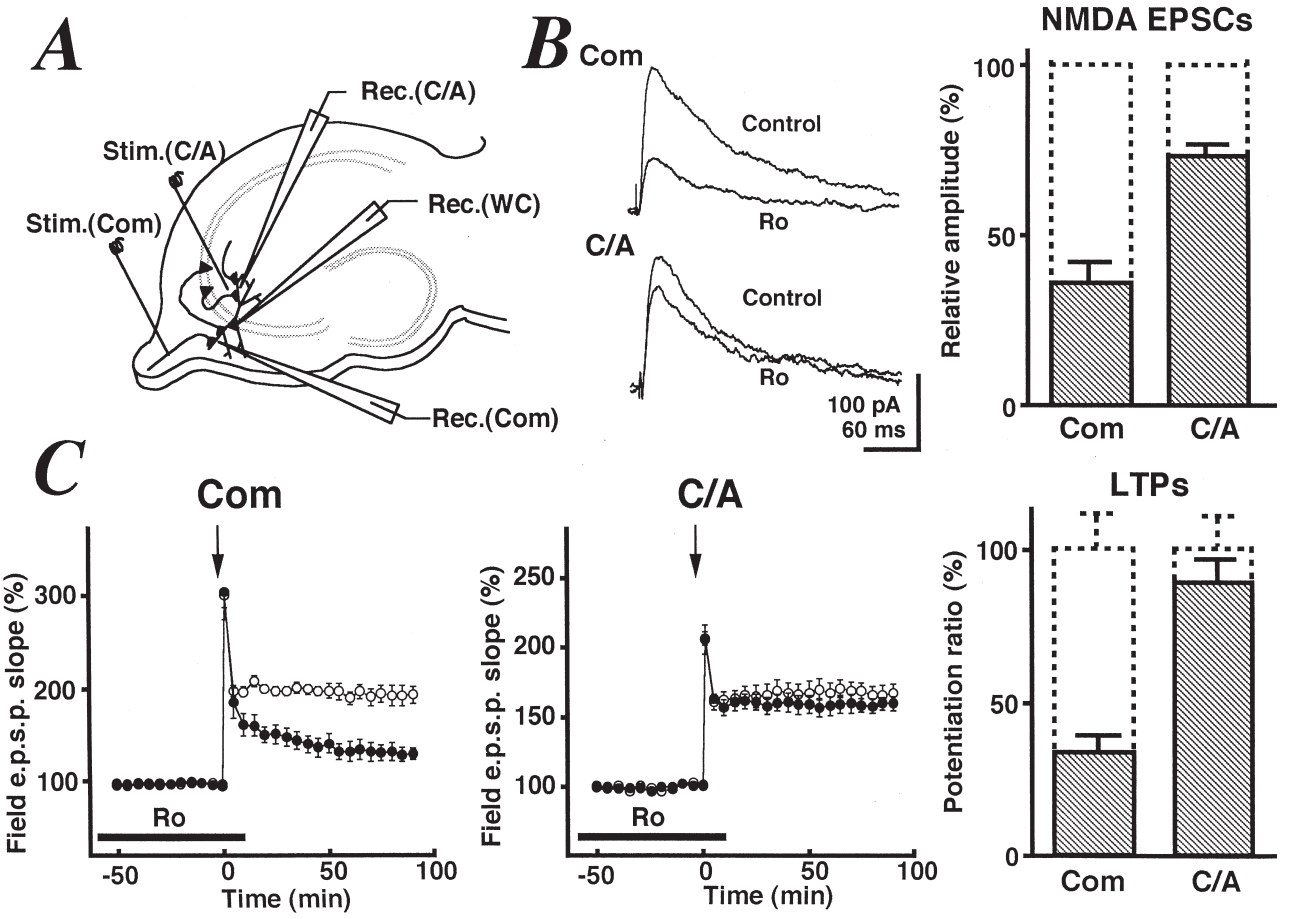

Fig. 1. Effects of Ro 25-6981 on NMDA EPSCs and LTPs in the Com–CA3 synapse and in the C/A–CA3 synapse. (A) Schematic diagram of the arrangement of stimulating and recording electrodes. To activate the commissural inputs, a stimulating electrode was placed in the ventral fimbria [Stim.(Com)]. An extracellular electrode placed in the stratum oriens [Rec.(Com)] was used to monitor the Com–CA3 synaptic responses. To record the C/A–CA3 synaptic responses, stimulating [Stim.(C/A)] and recording [Rec.(C/A)] electrodes were placed in the stratum radiatum of area CA3. To record NMDA EPSCs, whole-cell patch recordings [Rec.(WC)] were made from CA3 pyramidal neurons using the blind-patch technique. (B) Left panel: NMDA EPSC records in the Com–CA3 (Com) and C/A–CA3 (C/A) synapses in the absence (control) and presence of Ro 25-6981 (0.3 μM, Ro). In the presence of CNQX (20 μM) and bicuculline (30 μM), recordings were made by the whole-cell voltage-clamp from CA3 pyramidal cells at a holding potential of +30 mV. The maximal levels of inhibition were observed after 50–60 min exposure to Ro 25-6981. We confirmed the stability of the recordings of NMDA EPSCs over this time period in the control experiments (variations were less than 5% after 60 min). Each trace is an average of five consecutive recordings. Right panel: relative amplitudes of NMDA EPSCs in the presence of Ro 25-6981 were expressed as percentages of the control responses (n=5 each). Error bars indicate the SEM. (C) Left and middle panels: effects of tetanic stimulation (100 Hz for 1 s, arrow) of the commissural afferents (Com, n=6 each) and the commissural/associational afferents (C/A, n=6 each) on the slope of field EPSPs in the presence (●) and absence (○) of Ro 25-6981 (0.1 μM). Ro 25-6981 was applied to the bath from 60 min before the tetanic stimulation to 10 min after the tetanus (thick bars). Field EPSPs were recorded in area CA3, as illustrated in (A). LTP of the field EPSP slope was expressed as a percentage of the mean before tetanic stimulation. Error bars indicate the SEM. Right panel: potentiation ratios of field EPSP slope in Ro 25-6981 treated slices were expressed as percentages of those in the control slices (n=6 each). Error bars indicate the SEM.

and C). The residual NMDA receptor-mediated currents of the GluRε1 mutant mice are ascribed to the remaining receptors composed of GluRε2 subunits. Our results suggest that almost the same number of NMDA receptors composed mainly of GluRε1 or GluRε2 subunits are present on both the apical and basal dendrites of CA3 pyramidal neurons. This indicates that the GluRε subunits are not localized simply by cell polarity. These data do not exclude possible contributions of extrasynaptic NMDA receptors. Thus, we studied direct effects of the GluRε1 disruption on the synaptic NMDA receptors in CA3 neurons.

As described above, electrical stimulations at the stratum oriens of area CA3 activate both the commissural inputs and associational inputs which make synapses on the basal dendrites of CA3 pyramidal neurons. On the other hand, stimulations at the ventral fimbria mainly activate the commissural input, making synapses on the basal dendrites of CA3 neurons (Ishizuka et al. 1990, 1995; Ito et al. 1997, 1998). To determine whether the

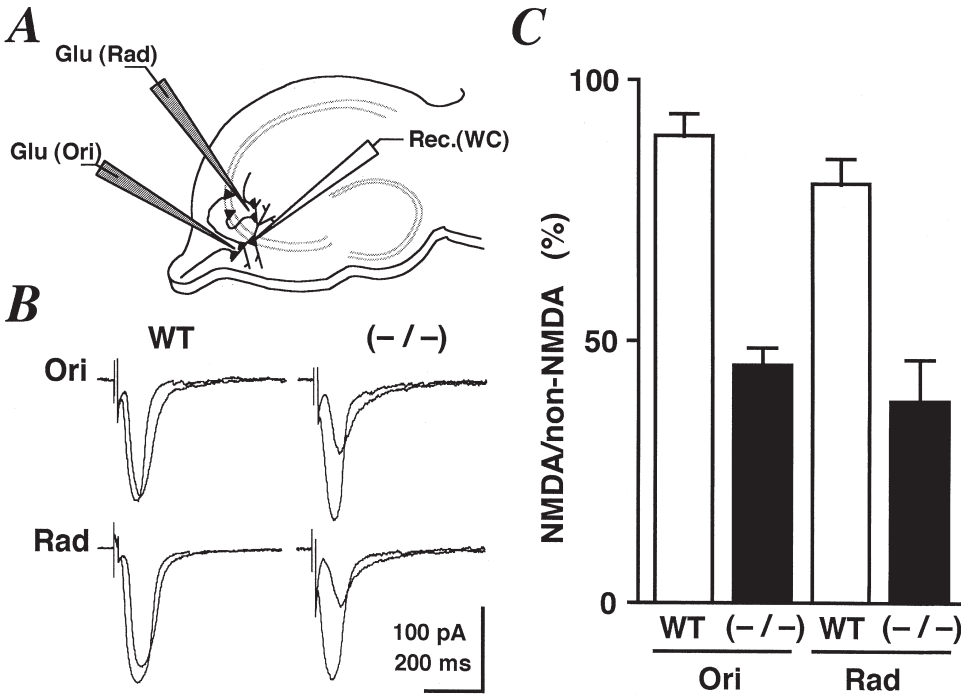

Fig. 2. Effects of the GluRε1 subunit disruption on NMDA receptor-mediated currents in response to glutamate applied iontophoretically. (A) Schematic diagram of the arrangement of an iontophoretic micropipette and a recording electrode. To activate NMDA receptors on the apical dendrites or on the basal dendrites of CA3 neurons, an iontophoretic micropipette filled with glutamate (0.5 M, pH 8.0) was placed in the stratum radiatum [Glu.(Rad)] or in the stratum oriens [Glu.(Ori)] of area CA3. To record NMDA receptor-mediated currents, whole-cell patch recordings [Rec.(WC)] were made from CA3 pyramidal neurons using the blind-patch technique. (B) Representative current records in response to glutamate applied iontophoretically. All recordings were made at a holding potential of −90 mV. Non-NMDA receptor-mediated responses (larger inward currents) were recorded in the presence of TTX (1.5 μM), MCPG (200 μM) and bicuculline (30 μM). NMDA receptor-mediated responses (smaller inward currents) were monitored in Mg^{2+}-free ACSF containing TTX (1.5 μLM), MCPG (200 μM), bicuculline (30 μM), CNQX (20 μM) and glycine (10 μM). Each trace is an average of five consecutive recordings. WT represents wild-type mice, (−/−) represents GluRε1 mutant mice. (C) Ratios of NMDA receptor-mediated currents to non-NMDA receptor-mediated currents. WT represents wild-type mice, (−/−) represents GluRε1 mutant mice. Error bars indicate the SEM (n=5 to 8).

GluRε subunits are sorted depending on the types of synaptic inputs, we examined the effects of the GluRε1 subunit disruption on NMDA EPSCs in the basal dendrites of CA3 pyramidal neurons evoked by the electrical stimulations at these two different positions (Fig. 3A). The amplitudes of NMDA EPSCs were expressed as a ratio to CNQX sensitive non-NMDA EPSCs. We found that the GluRε1 subunit mutation resulted in a significant reduction of NMDA EPSCs in the C/A–CA3 synapse evoked by stimulations at the stratum oriens in the vicinity of the cell under observation (WT, 91±7.5%, n=5; (−/−), 33.1±2.5%, n=7, Student's t-test $P<0.01$, Fig. 3B and C). The residual NMDA EPSC in this synapse of the GluRε2 mutant is ascribed to the GluRε2 subunit. In accordance with our previous reports (Ito et al., 1997), NMDA EPSCs in the Com–CA3 synapse evoked by the stimulations in ventral fimbria were apparently unaffected by the GluRε1 subunit mutation (WT, 51.7±3.8%, n=8; −/−, 45.2±2.8%, n=6; Student's t-test $P>0.21$, Fig. 3B and C). We also performed pharmacological analyses using Ro 256981. In wild-type slices, Ro 25-6981 (0.3 μM) suppressed NMDA EPSCs in the Com–CA3 synapse (36±5.9% of the control, n=5) more potently than in the C/A–CA3 synapse (92±4.2% of the control, n=5, Student's t-test, $P<0.01$; Fig. 3D and E). These results suggest that most of NMDA receptors in the Com–CA3 synapses are composed of GluRε2 subunits. In contrast, the majority of NMDA receptors in the associational–CA3 synapse appear to be composed of GluRε1 subunits, because the GluRε1 subunit mutation resulted in a reduction of NMDA EPSCs only when the associational–CA3 synapses were activated by electrical stimulations at the stratum oriens, and because NMDA EPSCs evoked by the stimulations at stratum oriens were only slightly reduced by a GluRε2 subunit-selective antagonist, Ro 25-6981 (Fig. 3). This indicates that the GluRε subunits are sorted depending on the types of synaptic inputs, even within a single neuron. In wild-type mice, the ratio of NMDA EPSCs to non-NMDA EPSCs in the C/A–CA3 synapse was significantly larger than in the Com–CA3 synapse (Fig. 3C). The explanation might be that the associational–CA3 synapses, formed on the basal dendrites of CA3 pyramidal neurons, were rich in so-called silent synapses (Isaac et al., 1995; Liao et al., 1995) which exhibit exclusively NMDA receptor-mediated transmissions.

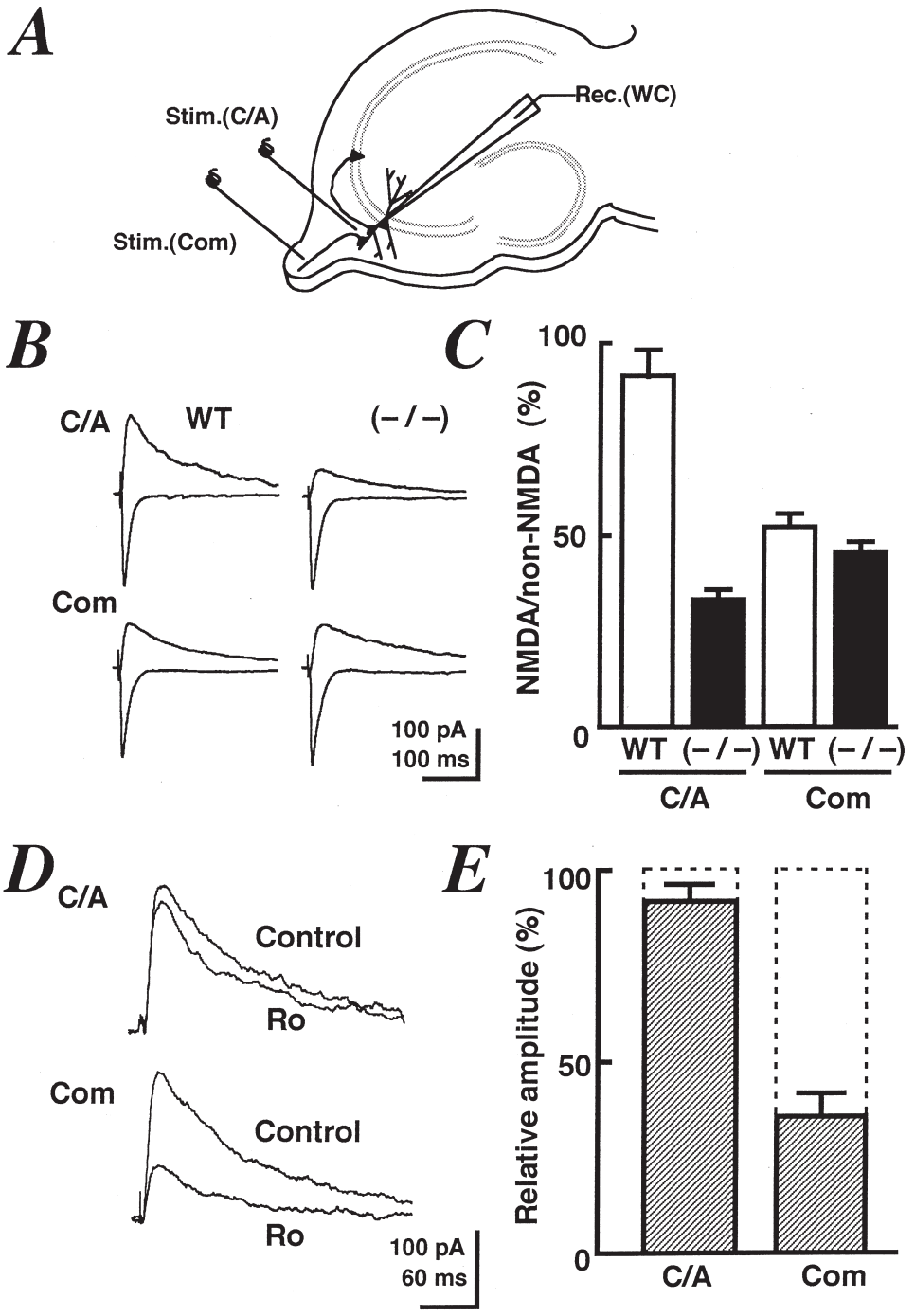

Fig. 3. Effects of the GluRε1 subunit disruption and application of Ro 25-6981 on NMDA EPSCs in the basal dendrites of CA3 pyramidal neurons. (A) Schematic diagram of the arrangement of stimulating and recording electrodes. To activate the commissural inputs, a stimulating electrode [Stim.(Com)] was placed in the ventral fimbria. To record C/A–CA3 synaptic responses at the basal dendrites, a stimulating electrode [Stim.(C/A)] was placed in the vicinity of the recording cell in the stratum oriens of area CA3. Whole-cell patch recordings [Rec.(WC)] were made from CA3 pyramidal neurons using the blind-patch technique. (B) Representative EPSC records in response to the electrical stimulations in the ventral fimbria (Com) and stratum oriens (C/A) of area CA3. In this experiment, a high-Mg^{2+} and Ca^{2+} (4 mM of $MgSO_4$ and $CaCl_2$) containing ACSF was used to increase membrane stability. Upper traces show NMDA EPSCs recorded at +30 mV in a high Mg^{2+} and Ca^{2+} ACSF containing CNQX (20 μM) and bicuculline (30 μM), and lower traces show non-NMDA EPSCs at −90 mV in the presence of bicuculline (30 μM). Each trace is an average of five consecutive recordings. WT represents wild-type mice, (−/−) represents GluRε1 mutant mice. (C) Ratios of NMDA EPSCs to non-NMDA EPSCs. WT represents wild-type mice, (−/−) represents GluRε1 mutant mice. Error bars indicate the SEM (n=5 to 8). (D) NMDA EPSC records in the Com–CA3 (Com) and C/A–CA3 (C/A) synapses in the absence (control) and presence of Ro 25-6981 (0.3 μM, Ro). In the presence of CNQX (20 μM) and bicuculline (30 μM), recordings were made by the whole-cell voltage-clamp from CA3 pyramidal cells at a holding potential of +30 mV. Each trace is an average of five consecutive recordings. (E) Relative amplitudes of NMDA EPSCs in the presence of Ro 25-6981 were expressed as percentages of the control responses (n=5 each). Error bars indicate the SEM. In (D) and (E), the data for the Com–CA3 synapse are the same as in Fig. 1B.

4. Discussion

In a mature mouse hippocampus, three types of NMDA receptors are considered to be expressed, GluRζ/GluRε1 and GluRζ/GluRε2 diheteromers and GluRζ/GluRε1/GluRε2 triheteromers. The receptors functioning in a GluRε1 mutant mouse are therefore GluRζ/GluRε2 diheteromers, and GluRζ/GluRε1 diheteromers are insensitive to Ro 25-6981 or ifenprodil, the GluRε2 subunit-selective NMDA receptor antagonists. Previous studies suggest that the triheteromeric receptors may also be sensitive to these antagonists (Kew et al., 1998; Tovar and Westbrook, 1999). However, the exact numbers of GluRζ and GluRε subunits in each NMDA receptor molecule are not known at present, and the sensitivity might be dependent on the ratios of GluRε1 and GluRε2 in individual receptor molecules. It would be safe to say that receptors sensitive to these antagonists are composed mainly of GluRε2 subunits, and insensitive ones composed mainly of GluRε1 subunits.

Using hippocampal slices prepared from the wild-type mice, we found that Ro 25-6981 and ifenprodil suppressed NMDA EPSCs and LTPs of the Com–CA3 synapse more potently than those of the C/A–CA3 synapse. These results strongly supported our previous observations that NMDA receptors at the C/A–CA3 synapses were strongly affected by the GluRε1 mutation, whereas NMDA receptors at the Com–CA3 synapses were affected by the GluRε2 mutation (Ito et al., 1997). Furthermore, we have reported that the postnatal development of LTPs at the C/A–CA3 synapse closely followed the development of the GluRε1 subunit, whereas the development of LTPs at the Com–CA3 synapse closely resembled that of the GluRε2 subunit (Ito et al. 1996, 1998). Taken together, we concluded that the GluRε subunits are sorted within a single CA3 pyramidal neuron, even in the wild-type mice.

We also examined whether the GluRε subunits are sorted depending on the cell polarity or on the types of synaptic inputs. First, we examined the effects of the GluRε1 subunit disruption on NMDA receptor-mediated currents in response to glutamate applied iontophoretically. We found that NMDA receptor-mediated currents of GluRε1 mutant mice were reduced to one-half that of wild-type mice both in stratum radiatum and in stratum oriens of area CA3 (Fig. 2). This finding suggests that approximately the same number of NMDA receptors are present which are composed, in addition to the GluRζ subunit, of the GluRε1 or the GluRε2 subunit on both the apical and basal dendrites of CA3 pyramidal neurons. This indicates that the GluRε subunits are not localized simply by cell polarity.

Next, we examined the effects of the GluRε1 subunit disruption on NMDA EPSCs in the basal dendrites of CA3 pyramidal neurons evoked by electrical stimulations of two different synaptic inputs. We found that the GluRε1 subunit mutation resulted in a significant reduction of NMDA EPSCs in the C/A–CA3 synapse, whereas NMDA EPSCs in the Com–CA3 synapse were apparently unaffected (Fig. 3B and C). We also performed pharmacological analyses using Ro 25-6981. In wild-type slices, Ro 25-6981 suppressed NMDA EPSCs in the Com–CA3 synapse more potently than in the C/A–CA3 synapse (Fig. 3D and E). These results suggested that the majority of NMDA receptors in the associational–CA3 synapse were composed mainly of GluRε1 subunits, while most of the NMDA receptors in the Com–CA3 synapse were composed mainly of GluRε2 subunits. This indicated that the GluRε subunits were sorted depending on the types of synaptic inputs, even within a single CA3 pyramidal neuron.

Molecular–anatomical analyses have revealed that the GluRε1 and GluRε2 subunits are detected at high levels in the stratum oriens and stratum radiatum of the CA1 and CA3 fields (Fritschy et al., 1998; Watanabe et al., 1998). Consistent with these observations, NMDA receptor-mediated currents induced by iontophoretically applied glutamate were reduced to one-half by the GluRε1 subunit mutation both in the stratum radiatum and the stratum oriens of area CA3 (Fig. 2). These results suggest that a comparable number of associational fiber synapses containing the GluRε1 subunits and commissural fiber synapses containing the GluRε2 subunits may be present on the apical dendrites as well as on the basal dendrites of CA3 pyramidal neurons (Ishizuka et al., 1990).

Associational fibers are the axoncollaterals arising from the ipsilateral CA3 neurons and commissural fibers come from the contralateral CA3 neurons. Therefore, we might be able to generalize our findings and say that GluRε1 subunits are specifically targeted to the synapses formed by the ipsilateral inputs, whereas GluRε2 subunits are specifically targeted to the synapses formed by the contralateral inputs. This idea is supported by the recent findings that stratum lucidum, a mossy fiber-recipient layer of the CA3 subfield, contained GluRε1 subunits and almost excluded GluRε2 subunits (Fritschy et al., 1998; Watanabe et al., 1998), because mossy fibers are the axons arising from the ipsilateral dentate granule cells. We have already reported that the GluRε1 subunit mutation caused significant decreases in NMDA EPSCs and LTPs in the basal dendrites as well as in the apical dendrites of CA1 pyramidal neurons (Sakimura et al., 1995; Ito et al. 1996, 1998). CA1 pyramidal neurons receive Schaffer collateral fibers arising from the ipsilateral CA3 neurons and commissural fibers from the contralateral CA3 neurons on both their basal and apical dendrites (Ishizuka et al. 1990, 1995). According to our idea, NMDA receptors in the Schaffer collateral–CA1 synapses are expected to contain GluRε1 subunits, because Schaffer collateral fibers are the ipsilateral inputs. Therefore, our previous observations in the CA1

region might be accounted for as a selective reduction of NMDA receptor activities in the Schaffer collateral–CA1 synapses.

Our results thus show that the NMDA receptor GluRε subunits are sorted depending on the types of synaptic inputs, which raises several intriguing questions. What are the signals identifying the origins of the presynaptic fibers? How do the postsynaptic pyramidal neurons recognize the presynaptic signals? How do the pyramidal neurons transport the specific subunits to the specific synapses? These processes responsible for input-selective targeting of the GluRε subunits and the physiological relevance of this sorting can only be matters of speculation at present. However, the fact that some kinds of kainate receptors are selectively located in the mossy fiber–CA3 synapses but not in the C/A–CA3 synapses (Castillo et al., 1997; Vignes and Collingridge, 1997), raises the possibility that many other types of glutamate receptors and their subunits are targeted to the specific synapses with similar sorting mechanisms. In the central nervous system, a very large number of synapses are formed on each neuron. Therefore, in addition to axonal guidance (Goodman and Shatz, 1993; Keynes and Cook, 1995), input-selective targeting of glutamate receptor molecules will play some important roles for the construction and the maintenance of neuron networks and for the expression of synaptic plasticity in the brain.

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