The role of NMDAR subtypes and charge transfer during hippocampal LTP induction

Sven Berberich a, Vidar Jensen b, Øivind Hvalby b, Peter H. Seeburg a, Georg Köhr a,*,

Department of Molecular Neurobiology, Max-Planck-Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany
Molecular Neurobiology Research Group, Institute of Basic Medical Sciences, University of Oslo, N-0317 Oslo, Norway

Received 21 May 2006; received in revised form 10 July 2006; accepted 12 July 2006

Abstract

Activation of NMDA receptors (NMDARs) is a requirement for persistent synaptic alterations, such as long-term potentiation of synaptic transmission (LTP). NMDARs are composed of NR1 and NR2 subunits, and NR2 subunit-dependent gating properties of NMDAR subtypes cause dramatic differences in the timing of charge transfer. These postsynaptic temporal profiles are further influenced by the frequency of synaptic activation. Here, we investigated in the CA1 region of hippocampal slices from P28 mice, whether particular NMDAR subtypes are recruited based on NR2 subunit-specific gating following different induction protocols. For high frequency afferent stimulation (HFS), we found that genetic impairment of NR2A or pharmacological block of NR2A- or NR2B-type NMDARs can reduce field LTP. In contrast, when pairing low frequency synaptic stimulation with postsynaptic depolarization (LFS pairing) in single CA1 neurons, pharmacological antagonism of either subtype modestly reduced the charge transfer during LFS pairing without reducing the LTP magnitude. These results indicate that HFS-triggered LTP is induced by more than one NMDAR subtype, whereas a single subtype is sufficient during LFS pairing. Analysis of charge transfer during LFS pairing in 13 different conditions revealed a threshold for LTP induction, which was independent of the NR2 antagonist tested. Thus, at least for LFS pairing, the amount of charge transfer, and thus Ca²⁺ influx, during LTP induction is a factor more critical than the participation of a particular NMDAR subtype.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Frequency; Temporal signaling; Gene-targeted mouse; NVP-AAM077; Ro25-6981; CP-101,606

1. Introduction

The NMDA receptor (NMDAR) plays a major role in both physiological and pathophysiological processes in the brain (Cull-Candy and Leszkiewicz, 2004; Dingledine et al., 1999), and has been a long-time CNS therapeutic target (Chazot, 2004). The identity of the NR2 subunit (NR2A-D, or ε1–4) strongly influences the biophysical and pharmacological properties of NMDAR assemblies (Vicini et al., 1998). NR2 subunit-specific gating is known to control the kinetics of NMDAR channels, including activation, probability of opening and deactivation (Erreger et al., 2005). These properties produce at the postsynaptic site different temporal signaling profiles, which could contribute to synaptic transmission and plasticity.

Current studies characterizing NMDAR subtypes in synaptic plasticity aim at unmasking NR2 subtype-specific roles. A recent proposal that NR2A-containing NMDARs, also called NR2A-type NMDARs, exclusively induce LTP (for review, see Collingridge et al., 2004) was questioned by other reports showing that NR2B can induce LTP as well (Berberich et al., 2005; Weitlauf et al., 2005; Zhao et al., 2005). At postnatal day 28, hippocampal neurons express comparable amounts of NR2A and NR2B subunits (Sans et al., 2000), forming di- and triheteromeric NMDAR assemblies (NR1/NR2A, NR1/NR2A/NR2B and NR1/NR2B) (Sheng et al., 1994). Their relative contribution to LTP induction is difficult to resolve: i) The available competitive NMDARs antagonists such as d-AP5, d-AP7, d-CPP, Con G and NVP-AAM077 lack...
NR2A/B-selectivity (for review, see Köhr, in press). ii) Although non-competitive NMDAR antagonists have higher preferences for the corresponding NR2-subtypes and are NR2-selective (e.g. Zinc for NR2A; ifenprodil and its derivatives Ro25-6981 and CP-101,606 for NR2B), they maximally inhibit NMDARs by 70–80% (for review, see Neyton and Paoletti, 2006). iii) NMDAR antagonists inhibit diheteromeric NMDARs more than triheteromeric NMDARs (for review, see Neyton and Paoletti, 2006).

Therefore, in this study we investigated influences of distinct gating properties of the hippocampal NMDAR subtypes during LTP induction and focused on the synaptic charge transfer when pairing low frequency stimulation with postsynaptic depolarization (LFS pairing), which reflects the Ca\(^{2+}\) influx during LTP induction. Previous simulations of synaptic responses suggested that the more rapidly gating NR1/NR2A receptors are more effective at mediating charge transfer during high frequency stimulation (HFS), whereas the slower gating NR1/NR2B receptors are better suited during low frequency stimulation (LFS) (Erreger et al., 2005). As Mg\(^{2+}\) unblocks faster from NR1/NR2A than from NR1/NR2B receptors (Clarke and Johnson, 2006) induction protocols involving prolonged postsynaptic depolarizations (e.g., LFS pairing) should reduce the relative importance of the Mg\(^{2+}\) unblock kinetics. Hence, we compared LTP induced by LFS pairing in whole-cell recordings with LTP induced by HFS of afferent fibers in field recordings. Whole-cell recordings allow controlling the extent of postsynaptic depolarization (e.g., 0 mV), while HFS in field recordings can be assumed to depolarize postsynaptic cells less efficiently. These experiments were performed in presence of non-competitive and/or competitive NMDAR antagonists at increasing concentrations to compare selective with unselective NR2 antagonism. In addition, we analyzed two NR2A mutants (NR2A\(^{−/−}\); Sakimura et al., 1995 and NR2A\(^{AC/AC}\); Sprengel et al., 1998) which both make use of NR1/NR2B receptors for LTP induction, but differ in the kinetics of their NMDA EPSCs (Berberich et al., 2005; Kiyama et al., 1998; Köhr et al., 2003), which should affect the charge transfer during LTP induction.

The present study substantiates the evidence for involvement of both NR2A- and NR2B-type NMDA receptors in LTP induction at Schaffer collateral/commissural synapses onto hippocampal CA1 pyramidal neurons. Furthermore, the recruitment of NMDAR subtypes depends on the LTP induction protocols. Finally, the quantitative relationship between charge transfer during induction and magnitude of LTP reveals a critical level of charge transfer via any NMDAR subtype.

2. Methods

All experimental procedures were in accordance with the animal welfare guidelines of the Max Planck Society, and in accordance with the Norwegian Animal Welfare Act and the European Union’s Directive 86/609/EEC.

2.1. Extracellular field experiments

Wild-type (C57Bl/6 strain) mice and mice lacking the carboxy-terminal intracellular domain of the NR2A subunit (NR2A\(^{AC/AC}\); Sprengel et al., 1998) were at P28 killed with desflurane. Transverse slices (400 μm) from the middle portion of each hippocampus were cut with a vibroslicer in the following artificial cerebral spinal fluid (ACSF) containing (in mM): 124 NaCl, 2 KCl, 1.25 KH\(_2\)PO\(_4\), 2 MgSO\(_4\), 2 CaCl\(_2\), 26 NaHCO\(_3\) and 12 2-glucose, 4\% 0.5% CO\(_2\) (pH 7.4). Slices were placed in an interface chamber at 28–32 °C and perfused with ACSF, which in some experiments contained the NR2B-selective antagonists Ro25-6981 (Sigma), CP-101,606 (Pfizer) and/or the NR2A-prefering antagonist NVP-AAM077 (Novartis) at concentrations indicated in the Result section. Orthodromic synaptic stimulation was delivered alternately through two tungsten electrodes, one in stratum radiatum, and the other in stratum oriens. Extracellular responses were monitored in the corresponding layers by two glass electrodes filled with ACSF. Assessment of synaptic efficacy and tetanization procedures were as earlier described (Köhr et al., 2003). Six consecutive responses (1 min) were averaged and normalized to the mean value recorded 4–7 min before tetanic stimulation. Data were pooled and presented as mean ± SEM, and statistical significance was evaluated using a two-tailed, Student’s t-test.

2.2. Whole-cell experiments

The brain was removed from deeply anaesthetized (halothane) P28 mice (wild-type, NR2A\(^{AC/AC}\) and NR2A\(^{−/−}\); Sakimura et al., 1995). Transverse hippocampal slices (250 μm) were prepared and incubated for 30 min at 35°C in ACSF containing (in mM): 125 NaCl, 25 NaHCO\(_3\), 2.5 KCl, 1.25 NaH\(_2\)PO\(_4\), 1 MgCl\(_2\), 25 2-glucose, 2 CaCl\(_2\); bubbled with 95% O\(_2\)/5% CO\(_2\) (pH 7.4). Patch pipettes were pulled from borosilicate glass capillaries and had resistances of 4–6 MΩ when filled with (in mM): 125 Cs-glucuronate, 20 CsCl, 10 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 Na\(_2\)GTP (pH 7.3, 290–305 mOsms). Series resistances (15 to 30 MΩ) and input resistances (100 to 300 MΩ) were continuously monitored at negative holding potentials by measuring the peak and the steady-state current of the instantaneous capacitive transient in response to hyperpolarizing pulses (−5 mV; 20 ms). All patch experiments were performed at room temperature (22°C).

EPSCs were activated by stimulating the Schaffer collaterals about 150 μm distant from the CA1 cell body with a glass electrode filled with 1 M NaCl. NMDA EPSCs were recorded at −40 or +40 mV in ACSF (see above) containing 10 μM bicuculline methiodide (BMI), 5 μM NBQX and 10 μM glycine. For LTP recordings, patch pipettes were filled with (in mM): 120 Cs-glucuronate, 10 CsCl, 10 HEPES, 8 NaCl, 0.2 EGTA, 2 MgATP, 0.3 Na\(_2\)GTP, 10 phosphocreatine. EPSCs were evoked in solutions containing (in mM): 124 NaCl, 26 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 2.5 KCl, 4 CaCl\(_2\), 4 MgSO\(_4\), 10 2-glucose, 0.010 glycine and 0.010 BMI (pH 7.3, 290–305 mOsms). In some experiments, NR2-antagonists were present (see above). LTP was induced by pairing low frequency stimulation (120 pulses, 0.7 Hz) in the test (str. radiatum) but not in the control (str. oriens) pathway with postsynaptic depolarization to 0 mV for 3 min (LFS pairing; Chen et al., 1999). The actual holding potential during induction was rather −11 mV, since liquid junction potentials were not corrected. The charge of each EPSC evoked during LFS pairing was calculated and averaged. Six consecutive EPSCs at −70 mV were averaged and normalized to the averaged responses obtained 5 min before LTP induction. EPSC potentiation was assessed 30 min after induction. All data were pooled across animals of the same genotype and are presented as mean ± SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA), followed by Post-Hoc Fisher’s LSD analysis, or by a two-tailed, Student’s t-test whenever noted.

3. Results

3.1. Tetanic stimulation

In the CA1 region of hippocampal slices from P28 wild-type mice, high frequency stimulation (HFS) of the afferent fibers, in either stratum radiatum or stratum oriens, produced an NMDAR-dependent (data not shown), homosynaptic potentiation of the synaptic responses, characteristic of LTP. The
average field EPSP (fEPSP) slope 40–45 min after tetanization was 1.37 ± 0.06 (mean ± SEM, n = 23), whereas synaptic transmission in the untetanized control pathway was unchanged (1.00 ± 0.02; Fig. 1A). A paradigm with four tetanic stimulations, given at five min intervals, was not more efficient at inducing LTP than a single tetanization (1.43 ± 0.07, n = 14; p = 0.50; Figs. 1B, C), similar to what was observed in adult mice but in contrast to P14 when NR2B-type NMDARs are still more abundant (Körhr et al., 2003). Thus, regarding HFS-triggered LTP, P28 mice have reached a developmental stage comparable to that of adult mice.

LTP induction by HFS in the hippocampus results from NR2A- or NR2B-type signaling (Berberich et al., 2005). The pharmacological support for NR2B contribution during HFS so far rests on experiments using competitive NMDAR antagonists. The NR2B-prefering antagonist Conantokin G (Con G) impaired LTP (Körhr et al., 2003), and the NR2A-prefering antagonist NVP-AAM077 did not block LTP at a high concentration (400 nM; Berberich et al., 2005 but see Liu et al., 2004). Since both antagonists lack NR2A/B-selectivity (NVP-AAM077, Neyton and Paoletti, 2006 and Con G, Wittekindt et al., 2001 but see Klein et al., 2001), we used the ifenprodil derivative Ro25-6981, a non-competitive, NR2B-selective antagonist. When present at 0.5 μM, Ro25-6981 reduced, albeit not significantly, the magnitude of LTP induced by a single tetanization and significantly following repeated tetanization (1 × 100 Hz: 1.22 ± 0.04, n = 23, p = 0.06; 4 × 100 Hz: 1.22 ± 0.07, n = 15, p = 0.03; Figs. 1A, B). This is consistent with a contribution of NR2B-type NMDARs to LTP induction (but see Liu et al., 2004; Massey et al., 2004).

Because another ifenprodil derivative, CP-101,606 at 10 μM, failed to impair LTP induction (Körhr et al., 2003), we expected a stronger reduction of synaptic NMDA currents by Ro25-6981 than by CP-101,606. The opposite, however, was the case (see Section 3.3). Therefore, to examine LTP in the presence of both NR2B and NR2A antagonists, we employed CP-101,606 (10 μM) and NVP-AAM077 (50 nM), at concentrations which preferentially antagonize NR2B- and NR2A-type NMDARs, respectively (Berberich et al., 2005; Mott et al., 1998). This led to a complete block of LTP (tetanized pathway, 1.00 ± 0.05 vs. control pathway, 0.98 ± 0.03, n = 13; p = 0.72; Fig. 1C).

LTP elicited by HFS in wild-type mice appears to involve more than a single NMDAR subtype (see above). In contrast, LTP induction in NR2A−/− mice rests solely on NR1/NR2B receptors and requires repeated tetanization (Sakimura et al., 1995; Kiyama et al., 1998). This observation supports simulations of synaptic responses, suggesting that the slower gating NR1/NR2B receptors should be less effective at mediating charge transfer during HFS (Erreger et al., 2005). Consistently, LTP induction in NR2A−/− mice requires repeated tetanization (Sprengel et al., 1998; Körhr et al., 2003), and LTP at P28 is reduced in NR2A−/− mice compared to wild-type mice following single tetanization (1.16 ± 0.04, n = 30 vs. 1.35 ± 0.05, n = 25; p = 0.004).

In summary, the similar reduction of HFS-triggered LTP in NR2A−/− mice and by the NR2B-selective antagonist Ro25-6981 in P28 wild-type mice is consistent with the involvement of both NR2A- and NR2B-type NMDARs. As HFS-triggered LTP is reduced but not blocked in presence of NR2A or NR2B antagonists, more than one subtype is involved during induction.

### 3.2. Low frequency stimulation

To investigate the contribution of NR2A- and NR2B-type NMDARs during LFS, we recorded from single CA1 neurons...
(P28), and paired low frequency synaptic stimulation with postsynaptic depolarization (LFS pairing: 0.7 Hz, 3 min; 0 mV). This induction protocol potentiated the EPSCs when compared to the preinduction values, in normal solution (2.15 ± 0.17, n = 12) and to a slightly reduced extent in the presence of 10 μM CP-101,606 (1.72 ± 0.03, n = 6), as described (Berberich et al., 2005). Ro25-6981 at 0.5 μM, which reduced HFS-triggered LTP (see above), slightly reduced the amount of LFS pairing-induced LTP, whereas Ro25-6981 at 1 μM strongly reduced LTP (1.78 ± 0.15, n = 3 vs. 1.14 ± 0.05, n = 4, p < 0.01; Fig. 2A). LTP was induced at 0 mV (i.e. −11 mV, see Section 2), and all 120 EPSCs evoked during LTP induction (Fig. 2A, inset) mainly reflect NMDAR-mediated charge transfer, since NBQX reduced the peak amplitude of EPSCs below 15% in separate control experiments (11.2 ± 2.6%, n = 5, except for one recording leading to an EPSC increase). The average charge of each response was slightly reduced in presence of 10 μM CP-101,606 and 0.5 μM Ro25-6981, but was significantly reduced by 1 μM Ro25-6981 (Fig. 2B; p < 0.05). Furthermore, the NR2A/NR2B-non-selective NMDAR antagonist n-AP5 had at 1 μM even no effect on the charge transfer during LTP induction and LTP was induced (Figs. 2B, C). Therefore, LTP was induced in presence of 1 μM n-AP5 or 10 μM CP-101,606, although both antagonists significantly reduce the peak amplitude of synaptic NMDA currents (Berberich et al., 2005; Nishiyama et al., 2000), highlighting the importance of charge transfer during LTP induction.

Interestingly, when comparing the effects of Ro25-6981 at 0.5 and 1 μM, we noted a relationship between the charge transfer during the induction phase and the magnitude of LTP (Fig. 2C). This relationship held also true for earlier experiments (Berberich et al., 2005), which employed NVP-AAM077 at four different concentrations and compared NR2A-preferring with NR2A/2B-unselective antagonism (50–100 vs. 200–400 nM; Fig. 3). Similar to 0.5 μM Ro25-6981, NVP-AAM077

---

**Fig. 2.** Effects of NR2B-selective antagonists and n-AP5 on charge transfer during LFS pairing. (A) Normalized and pooled data of EPSCs evoked at −70 mV in 10 μM bicuculline and 10 μM glycine in slices from P28 wild-type mice in presence of Ro25-6981 (Ro, 0.5 and 1 μM; open and filled squares, respectively). The open triangles show the control pathway with 0.5 μM Ro. The bar indicates LFS pairing at 0 mV in the test pathway. Inset, Representative traces are 120 averaged EPSCs evoked during LFS pairing at 0 mV in presence of 0.5 μM Ro. (B) The columns show the charge of the EPSCs evoked during LFS pairing for CP-101,606 (CP, 10 μM), Ro (0.5 and 1 μM), n-AP-5 (AP5, 1 μM). The EPSC charge was calculated from the 120 EPSCs evoked during LFS pairing. Symbols along the abscissa and the number of experiments do also apply to panel C. (C) Charge transfer during LFS pairing is plotted against LTP magnitude (1 denotes no LTP as in panel A), determined from normalized EPSCs evoked 30 min after LFS pairing relative to EPSCs before induction. For the magnitude of LTP in absence of NMDAR antagonist (no drug) and in presence of CP and n-AP5 see Berberich et al. (2005). Statistical significance was evaluated by ANOVA, followed by Post-Hoc Fisher’s LSD analysis (*p < 0.05 and **p < 0.01). Data are mean ± SEM.
at 50 nM and 100 nM had only minor effects on the charge transfer during induction, and LTP was readily induced (Figs. 3B, C). In contrast, NVP-AAM077 at 200 nM and 400 nM significantly reduced the charge transfer during induction. NVP-AAM077 at 50 nM or CP-101,606 at 10 μM only modestly reduced the charge transfer during induction (Figs. 2B and 3B). Together, however, the two antagonists lowered the charge transfer by about 70% (p < 0.01; Fig. 3B), and blocked LTP (Fig. 3A; CP-101,606 and NVP-AAM077, 0.99 ± 0.19, n = 4; similar to 400 nM NVP-AAM077 alone). Thus, selective or unselective NMDAR antagonism reduce the charge transfer (to around 30%) sufficiently to prevent LTP induction.

We next investigated charge transfer during induction and LTP magnitude in P28 mice expressing genetically altered NMDARs. As in wild-type mice, the stimulation strength was set to evoke EPSC amplitudes between 150 and 250 pA at −70 mV. In NR2A−/− mice neither the charge of the EPSCs at 0 mV nor the magnitude of LTP differed from wild-type mice (Figs. 4A−C; LTP magnitude, 2.1 ± 0.31, n = 5). Comparable charge transfer during LTP induction can be explained by the slower deactivation of the NMDA EPSCs recorded at e.g. −40 mV in NR2A−/− mice (WT, 70.4 ± 3.5 ms, n = 55; NR2A−/−, 272.3 ± 9.7 ms, n = 25; t test, p < 0.0001), although the amplitudes of the NMDA EPSCs were reduced by about 50% when compared to wild type (WT, −96.4 ± 6.1 pA, n = 55; NR2A−/−, −46.5 ± 3.3 pA, n = 25; t test, p < 0.0001). Consequently, the charge of the NMDA EPSCs was higher in NR2A−/− mice at −40 mV (WT, −10.8 ± 0.8 pC, n = 55; NR2A−/−, −15.8 ± 1.3 pC, n = 25; t test, p < 0.005). The latter difference was less obvious at 0 mV, since the EPSC traces in NR2A−/− mice were only acquired for the initial 260 ms (Fig. 4A, inset). However, the charge of the responses during this period was comparable in wild-type and NR2A−/− mice. Furthermore, the charge of the responses during LTP induction in presence of CP-101,606 was insufficient in NR2A−/− mice (about 30%) to induce LTP (0.96 ± 0.05, n = 5; Fig. 4). In P28 NR2AΔC/ΔC mice, similar to NR2A−/− mice, the amplitude of the NMDA EPSCs is reduced but the deactivation is intermediate between wild-type and NR2A−/− mice (about 160 ms, see Steigerwald et al., 2000), explaining the reduced transfer during LTP induction (Fig. 4B) and the failure to reach the magnitude of LTP observed in NR2A−/− mice (1.50 ± 0.27, n = 6, p < 0.01; Fig. 4).
Finally, we examined the time course of the charge transfer during LTP induction. For each of the pharmacological and genetic conditions, we averaged the amplitude and the charge of the initial EPSCs increased and reached plateau after about 80 stimulations. No EPSC increases were observed in those cases which failed to induce LTP (400 nM NVP-AAM077, presence of both 50 nM NVP-AAM077 and 10 μM CP-101,606, and NR2A/C0/C0 mice in 10 μM CP-101,606). However, an EPSC increase during the induction was not a requirement for LTP expression, as indicated by the experiments with NR2A/C0/C0 mice (Fig. 5A). Furthermore in NR2A/C0/C0 mice, but also in other conditions (e.g., NR2A/D0/D0 mice or 200 nM NVP-AAM077, see connecting lines in Fig. 5A), the magnitude of LTP correlated better with the charge transfer during induction than with the amplitude of the EPSCs evoked during the induction phase. This probably reflects the need for sufficient Ca^{2+} influx and is supported by a critical level of charge transfer which became evident when pooling the experimental data (Fig. 5B). Thus, sufficient Ca^{2+} entry during LTP induction is more important than the presence of a distinct NMDAR subtype.

3.3. Effects of Ro25-6981 and CP-101,606 on synaptic NMDA currents in wild-type and NR2A−/− mice

Reduction of LFS-pairing-triggered LTP by 1 μM Ro25-6981 can be explained by reduced charge transfer during LTP induction (Fig. 2). To investigate why Ro25-6981, but not CP-101,606, reduced the magnitude of LTP induced by HFS (Fig. 1), we compared the effects of these two ifenprodil derivatives on pharmacologically isolated NMDA EPSCs recorded from CA1 pyramidal cells of P28 mice. In wild-type mice, Ro25-6981 at 0.5 and 1 μM reduced NMDA EPSCs evoked at +40 mV less than 10 μM CP-101,606 (Fig. 6A; p < 0.05). Notably, Ro25-6981 was even applied for longer periods than CP-101,606 (30 vs. 20 min) taking into account the use- and activity-dependent NMDAR antagonism described for ifenprodil (Kew et al., 1996). The activity-dependent NMDAR antagonism is due to an increase in the NMDAR affinity for glutamate (for review, see Neyton and Paoletti, 2006) and appeared to be more pronounced for Ro25-6981 than for CP-101,606 (30 vs. 20 min) taking into account the use- and activity-dependent NMDAR antagonism described for ifenprodil (Kew et al., 1996). The activity-dependent NMDAR antagonism is due to an increase in the NMDAR affinity for glutamate (for review, see Neyton and Paoletti, 2006) and appeared to be more pronounced for Ro25-6981 than for CP-101,606. Ro25-6981, particularly at 0.5 μM, sometimes increased NMDA EPSCs and slowed down their deactivation kinetics at +40 mV (Fig. 6A, see also Fig. 5F in Zhao et al., 2005), whereas CP-101,606 increased NMDA EPSCs only transiently.
at the beginning of the CP-101,606 perfusion. The similar reduction of NMDA EPSCs evoked at +40 mV by 0.5 and 1 μM Ro25-6981 is consistent with a study reporting that 0.3 and 3 μM Ro25-6981 reduced NMDA EPSCs to comparable extents (Zhao et al., 2005).

To compare the effects of Ro25-6981 and CP-101,606 on NR1/NR2B receptors in neurons, NR2B-mediated NMDA EPSCs were evoked from NR2A−/− mice. At +40 mV, 1 but not 0.5 μM Ro25-6981 reduced NMDA EPSCs more efficiently in NR2A−/− than in wild-type mice (Fig. 6; t test, p < 0.02), whereas 10 μM CP-101,606 reduced NMDA EPSCs even more efficiently in NR2A−/− than in wild-type mice (Fig. 6; t test, p = 0.09). At +40 mV, CP-101,606 reduced NMDA EPSCs even more efficiently in NR2A−/− than in wild-type mice (Fig. 6; t test, p < 0.05), and Ro25-6981 was also in NR2A−/− mice the weaker NMDAR antagonist (Fig. 6B; 0.5 μM, p < 0.01; 1 μM p < 0.05). Thus, CP-101,606, but not Ro25-6981, reduced NMDA EPSCs to an extent as observed for recombinant NR1/NR2B receptors (70–80%; Berberich et al., 2005; Mott et al., 1998). Therefore, impairment of HFS-triggered LTP by 0.5 μM Ro25-6981 in wild-type mice (Fig. 1) can not simply be explained by NMDAR antagonism (see Section 4).

4. Discussion

Our pharmacological and genetic approaches revealed that NR2A- and NR2B-type NMDARs were recruited during HFS-mediated LTP induction, whereas either subtype was sufficient for inducing LTP by LFS pairing. In addition, during LFS pairing, the LTP magnitude at CA3-to-CA1 synapses correlated with the charge transfer during induction. This charge transfer had to reach a critical level to successfully induce LTP. LTP induction by LFS pairing was not prevented when employing NR2A and NR2B antagonists individually and at concentrations acting preferentially on a defined NR2 subtype, as demonstrated here in the hippocampus, and by others in the anterior cingulate cortex (Zhao et al., 2005). Furthermore, blockade of LTP required the combined presence of NR2A and NR2B antagonists [50 nM NVP-AAM077 and 10 μM CP-101,606 in the hippocampus (Figs. 1 and 3), and 400 nM NVP-AAM077 and 0.3 μM Ro25-6981 in the anterior cingulate cortex (Zhao et al., 2005)]. Thus, NR2A- and NR2B-type NMDARs appear to be the major subtypes contributing to LTP induction in the forebrain. In the hippocampus, this conclusion is supported by results from NR2A−/− mice, which...
do not show a compensatory, increased expression of e.g. NR2B or NR2D subunits (Sakimura et al., 1995), and in which LTP induction is mediated through NR2B-type NMDARs, since it was completely blocked by CP-101,606 (Fig. 4). Although NR2D is expressed in the hippocampus (Dunah et al., 1996; Hrabetova et al., 2000; Thompson et al., 2002), NR2D-containing NMDARs are mainly extrasynaptically localized (Lozovaya et al., 2004).

In contrast to LFS-induced LTP, the magnitude of HFS-triggered LTP can be reduced in the hippocampus by pharmacological block of NR2A- or NR2B-type receptors (Berberich et al., 2005 and Fig. 1), which emphasizes the role for both subtypes during HFS. Still, the contribution of these subtypes to the charge transfer during HFS was probably unevenly weighted, as suggested from simulations of synaptic responses (Erreger et al., 2005). Indeed, a higher contribution of the faster gating NR1/NR2A receptors at mediating charge transfer during HFS is indicated by the fact that repeated tetanization generated sufficient charge transfer. Moreover, LTP rescue in NR2A mutants via the slower gating NR1/NR2B receptors required repeated tetanization (Kiyama et al., 1998; Köhr et al., 2003). Easier recruitment of NR2A-type over NR2B-type NMDARs using HFS was further supported by the observation that NVP-AAM077, but not CP-101,606, reduced the amount of LTP induced by repeated tetanizations (Berberich et al., 2005; Köhr et al., 2003). LTP reduction by Ro25-6981 did not involve effects on AMPA EPSCs, which remained unchanged in presence of Ro25-6981 at 1 μM (1.1 ± 0.03, n = 3), and was explained by the reduced charge transfer during LFS pairing (Fig. 2). In contrast, LTP reduction by Ro25-6981 during HFS might have involved mechanisms additional to NMDAR antagonism, e.g. weakly blocking voltage-gated channels (Fischer et al., 1997). Notably, Ro25-6981 compared with CP-101,606 reduced NR2B-mediated NMDA EPSCs less efficiently in wild-type and NR2A−/− mice (Fig. 6). In wild type, the opposite might have been expected, since Ro25-6981, in contrast to CP-101,606, binds with high affinity to triheteromeric

Fig. 6. Effects of Ro25-6981 or CP-101,606 on NMDA EPSCs evoked in hippocampal slices of P28 mice. (A) NMDA EPSC traces recorded from wild-type mice in 10 μM bicuculline, 5 μM NBQX and 10 μM glycine at −40 and +40 mV in absence of drug (black), presence of 0.5 and 1 μM Ro25-6981 (light grey and dark grey, respectively) or presence of 10 μM CP-101,606 (grey). Bars summarize the results using different Ro25-6981 concentrations (black) or 10 μM CP-101,606 (striped). Statistical significance was evaluated by Post-Hoc Fisher’s LSD analysis comparing Ro with CP for each holding potential (*p < 0.05 and **p < 0.01). (B) As in A but in NR2A−/− mice.
NR1/NR2A/NR2B receptors (Chazot et al., 2002). This difference in binding affinity is unexpected in light of the structural similarities between ifenprodil and its derivatives and has so far not been investigated electrophysiologically.

LFS pairing in wild-type mice when 50 nM NVP-AAM077 or 10 μM CP-101,606 was present, and in NR2A−/− mice in absence of drug, demonstrates that the charge transfer via any NMDAR subtype is sufficient to induce LTP. A preference for slower gating NR2B-type NMDARs during LFS pairing is indicated by the ease in inducing LTP in NR2A−/− mice, whose synaptic NMDA currents mediated solely by NR1/NR2B receptors deactivate particularly slowly. Therefore, the faster gating NR1/NR2A receptors appear to be less suited for mediating charge transfer during LFS pairing.

During LFS pairing, the charge transfer correlated with the LTP magnitude (Fig. 5B). Importantly, three conditions compared with control (wild type, no drug) showed that a charge transfer below 30% (about −1 pC) is insufficient to induce LTP (400 nM NVP-AAM077, presence of both 50 nM NVP-AAM077 and 10 μM CP-101,606, and NR2A−/− mice in 10 μM CP-101,606). Furthermore, when reducing the charge transfer pharmacologically to an intermediate level (between −2 and −3 pC using 1 μM Ro25-6981 or 200 nM NVP-AAM077), LTP induction was still prevented (Fig. 5B). In contrast, although the charge transfer during induction was reduced to −2.2 pC in NR2AΔCΔ mice, LTP was induced to a level below that observed in NR2A−/− mice. This difference between the two NR2A mutants is due to the 50% higher charge transfer during LTP induction in NR2A−/− mice (−2.2 vs. −4.4 pC), caused by the slower decaying NMDA EPSCs in NR2A−/− mice (272 vs. 160 ms). Due to the complex phenotype of NR2AΔCΔ mice, i.e. increased formation of triheteromeric NMDARs and impaired NR2A signaling (Köhler et al., 2003), we did not include this genotype in the analysis of our results (Fig. 5B). Fitting the data points by a sigmoid function determined a charge of −3.35 ± 0.26 pC necessary to induce half maximal LTP magnitude. This result is consistent with a threshold for Ca2+ required for LTP induction (Cormier et al., 2001).

As NR1/NR2B receptors in mice older than P21 are not the major NMDAR population in wild-type hippocampal synapses (Kirson and Yaari, 1996; Tovar and Westbrook, 1999), diheteromeric NR1/NR2A and triheteromeric NR1/NR2A/NR2B receptors appear to be the main candidates subserving LTP induction. When both NR2A and NR2B antagonists were present at low concentrations (50 nM NVP-AAM077 and 10 μM CP-101,606), LTP could not be induced. According to a rule derived for recombinant NMDARs (Hatton and Paoletti, 2005), both antagonists may bind to triheteromeric NR1/NR2A/NR2B receptors, but may reduce currents mediated by NR1/NR2A/NR2B receptors to a lesser degree than currents mediated by the respective diheteromers, as described for ifenprodil in cultured neurons (Tovar and Westbrook, 1999). Indeed, the charge transfer during LFS pairing in the presence of both NR2A and NR2B antagonists was reduced more than by the individual antagonists but a sizeable charge transfer remained (Fig. 3). Therefore, the triheteromeric NR1/NR2A/NR2B receptors themselves were either unable to induce LTP, or the two compounds together antagonized di- and triheteromeric NMDARs receptors sufficiently to prevent LTP induction.

In summary, charge transfer via any NMDAR may induce LTP, as long as threshold is reached. The preference for a particular subtype in subserving LTP induction is likely to be influenced by the pattern of presynaptic activity (Aihara et al., 2005) and the actual condition of the postsynaptic cell, but the downstream signaling mechanisms appear to depend more on the integrity of the C-terminal domain of the NR2 subunits than on the channel kinetics of the postsynaptic NMDARs.

Acknowledgements

We thank Dr. M. Mishina (University Tokyo) for providing GluRe1 (NR2A) KO mice, Dr. Y.P. Auberson of Novartis Pharma AG (Basel, Switzerland) for NVP-AAM077, Pfizer Inc (Groton, CT) for CP-101,606, Dr. John Lisman, Dr. V. Pawlak, S. Astori and P. Punakkal for discussions, and Liya Pan for experimental help.

References


