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Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus

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Abstract

The role of NMDA receptors in the induction of long-term potentiation (LTP) and long-term depression (LTD) is well established but which particular NR2 subunits are involved in these plasticity processes is still a matter of controversy. We have studied the effects of subtype selective NMDA receptor antagonists on LTP induced by high frequency stimulation (100 Hz for 1 s) and LTD induced by low frequency stimulation (1 Hz for 15 min) in the CA1 region of hippocampal slices from 14 day old Wistar rats. Against recombinant receptors in HEK293 cells NVP-AAM077 (NVP) was approximately 14-fold selective for NR2A vs NR2B receptors, whilst Ro 25-6981 (Ro) was highly selective for NR2B receptors. On NMDA receptor-mediated EPSCs from Schaffer collaterals in CA1 neurones, NVP and Ro both reduced the amplitude but differentially affected the time constant of decay. The data are compatible with the selective effect of NVP (0.1 μM) and Ro (4 μM) on native NR2A and NBR2B receptors, respectively. NVP reduced both LTP and LTD whereas Ro reduced only LTP. Thus, LTP was reduced by 63% at 0.1 μM NVP and almost completely at 0.4 μM whereas 5 μM Ro reduced LTP by 45%. These data are consistent with a role for both NR2A and NR2B in the induction of LTP, under our experimental conditions. In comparison, LTD was unaffected by Ro (5 μM) even in the presence of a glutamate uptake inhibitor *threo*-β-benzylaspartic acid (TBOA) to increase the concentration of glutamate at NR2B containing receptors. NVP (0.2–0.4 μM), however, produced a concentration dependent inhibition of LTD which was complete at 0.4 μM. The lack of effect of 0.1 μM NVP on LTD contrasts with its marked effect on LTP and raises the possibility that different NVP-sensitive NR2 subunit-containing NMDA receptors are required for LTP and LTD in this preparation.
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1. Introduction

The central role of NMDA receptors in the induction of long term potentiation (LTP) in the CA1 region of the hippocampus was established by the use of the selective NMDA receptor antagonist, D-2-amino-5-phosphonopentanoate (D-AP5) (Collingridge et al., 1983). Subsequent studies in this brain region also found that the reversal of LTP, known as depotentiation (DP), and long-term depression of baseline transmission (LTD) are also sensitive to D-AP5 (Fujii et al., 1991; Dudek and Bear, 1992; Mulkey and Malenka, 1992). This immediately raised the question as to how the synaptic activation of NMDA receptors could lead to either long-term increases or long-term decreases in the efficiency of synaptic transmission.

One explanation is that the different patterns of synaptic activation of NMDA receptors that are used to induce LTP

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and LTD result in different levels in intracellular Ca^{2+}, with brief but large Ca^{2+} transients favouring activation of kinases leading to LTP and more prolonged but lower Ca^{2+} elevations favouring activation of phosphatases leading to LTD (Lisman, 1989). However, several lines of evidence have suggested that the explanation is more intriguing than this, involving both the subunit composition and location of NMDA receptors. NMDA receptors are believed to comprise two NR1 plus two NR2 subunits, of which there are four possible types (NR2A, NR2B, NR2C and NR2D). NMDA receptors containing, say, the NR2A subunit are often referred to simply as NR2A receptors, and we here use this nomenclature.

Experiments in mutant mice have suggested that LTP involves the NR2A receptors (Sakimura et al., 1995; Sprengel et al., 1998) and LTD is dependent on the NR2B receptors (Kutsuwada et al., 1996). Conversely, experiments that have manipulated the expression of NR2B receptors have suggested that NR2B NMDARs are important for LTP (Tang et al., 1999; Clayton et al., 2002). In these genetic experiments, however, it is difficult to discount an indirect effect on synaptic plasticity resulting from compensatory mechanisms. Pharmacological experiments possibly provide a more direct approach and have also emphasised the importance of the NMDAR subtype, albeit with variable conclusions (Hrabetova and Sacktor, 1997; Hrabetova et al., 2000; Hendricson et al., 2002; Kohr et al., 2003; Sjostrom et al., 2003; Yoshimura et al., 2003; Barria and Malinow, 2005; Mallon et al., 2005; Toyoda et al., 2005; Zhao et al., 2005; Mao et al., 2006). The first studies to address this issue found that NR2A/B receptor selective antagonists preferentially blocked LTP, whilst NR2C/NR2D receptor selective antagonists preferentially blocked LTD (Hrabetova and Sacktor, 1997; Hrabetova et al., 2000).

More recently, the relative role of NR2A and NR2B receptors has been addressed using compounds that selectively antagonise receptors that contain these subunits. For NR2A receptors, the quinoxalinedione, [(*R*)-[(*S*)-1-(4-bromophenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid (NVP-AAM077; Auberson et al., 2002) has been used. This compound, referred to hereafter as NVP, was shown to have good selectivity between human NR2A and NR2B receptors. However, more extensive studies using cloned rat receptors showed less selectivity of NVP for NR2A over NR2B receptors, and also demonstrated effects of NVP at NR2C and NR2D receptors (Feng et al., 2004; Berberich et al., 2005; Neyton and Paoletti, 2006). For NR2B receptors, studies have employed ifenprodil and its more selective derivatives [*R*-(*R**,*S**)-α-(4-hydroxyphenyl)-β-methyl-4-(phenyl-methyl)-1-piperidine propanol] (Ro-25-6981; hereafter referred to as Ro; Fischer et al., 1997) and CP-101,606 (Chazot, 2000). In one study it was concluded that NR2A receptors are required for hippocampal LTP, but not LTD, whilst NR2B receptors are required for LTD, but not LTP (Liu et al., 2004). We independently reached similar conclusions for LTP and LTD of baseline transmission in slices of adult neocortex (Massey et al., 2004). However, other studies have reached different conclusions (for example see Berberich et al., 2005).

In the present study we have assessed the role of NR2A and NR2B receptors in LTP and LTD in the CA1 region of the rat hippocampus. We have restricted our study to rats of two weeks of age. In agreement with previous work, we find a relatively narrow range of selectivity for NVP against rat NR2A vs rat NR2B receptors and that Ro is a highly selective NR2B receptor inhibitor. We find that NVP reduces LTP at concentrations that are selective for NR2A receptors but full block is only achieved with concentrations that also affect NR2B receptors. LTD is also reduced by NVP but only at these higher non-selective concentrations. In contrast, Ro (5 μM), produces a partial block of LTP but has no effect on LTD. These data suggest that at this stage of development NR2A and NR2B receptors may both be involved in the induction of LTP. In contrast, NR2B receptors appear to not be involved in LTD induction. Since LTD is less sensitive than LTP to the actions of NVP it is possible that the effects of NVP on LTD are unrelated to its ability to inhibit NR2A receptors. Whatever the subtype(s) involved, it is clear that different NMDA receptor subtypes are involved in the induction of LTP and LTD in the hippocampus of 2-week old rats.

2. Materials and methods

2.1. Plasmid cDNA

The full-length cDNAs encoding rat NMDA receptor subunits NR1a and NR2B, kindly provided by S. Nakanishi (Institute for Immunology, Kyoto University, Japan), were subcloned into a mammalian expression vector containing a cytomegalovirus (CMV) promoter (pcDNA1/Amp, Invitrogen) and checked by restriction mapping. The NR2A construct, inserted into a CMV promoter-containing vector RK7, was a generous gift from P. Seeburg (University of Heidelberg ZMBH, Heidelberg, Germany).

2.2. Cell transfection

This was carried out as described previously (Collett and Collingridge, 2004). Briefly, HEK293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% dialysed horse serum and 2 mM L-glutamine in a humidified incubator at 37 °C with 5% CO_2. After plating on coverslips, the cells were transiently transfected using SuperFect (Qiagen) with a molar ratio of cDNA for NR1a:NR2 of 1:3, which had been shown to give optimal expression of NMDARs (Chazot et al., 1994). Following transfection the cells were maintained for 24 h in a glutamine-free medium containing 100 μM D-AP5 to prevent activation of expressed receptors (Anegawa et al., 1995).

2.3. Calcium imaging and data analysis

The transfected cells were loaded with 5 μM Fluo3-AM (Sigma) in HEPES-buffered saline (HBS), which comprised (in mM); NaCl, 150; KCl, 3; Hepes, 10; D-glucose, 10; $CaCl_2$, 2; pH 7.35, containing 1 mg ml^{-1} of bovine serum albumen and incubated at 37 °C in the dark. Fluo3-AM-loaded cells were placed in a perfusion chamber and viewed on a confocal microscope (Bio-Rad MRC 1024; objective lens 10×, 520 nm long pass emission filter) equipped with an argon laser (488 nm). Cells were continuously perfused with HBS containing glycine (10 μM) at a rate of 3 ml min^{-1}. NMDA (with 10 μM glycine) was applied in the presence or absence of the antagonists. Responses to NMDA, indicated by increases in fluorescence intensity, identified cells containing functional receptors. During an experiment NMDA (15 μM) was applied at 15 min intervals: two responses in control buffer were obtained, followed by two in the presence of the antagonist and a further two in control buffer.

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